

# NIS-Elements AR

## Manual

Publication date 12.05.2014

v. 4.30.00

No part of this publication may be reproduced or transmitted except with the written permission of Laboratory Imaging, s. r. o. Information within this publication is subject to change without notice. Changes, technical inaccuracies and typographical errors will be corrected in subsequent editions.

LABORATORY IMAGING, spol. s.r.o.

Za Drahou 171/17

102 00 Praha 10

Czech Republic



# Table of Contents

1. Command Line Startup Options .....	1
2. Installation and Settings .....	5
2.1. Installation and Updates .....	5
2.2. User Rights .....	11
2.3. NIS-Elements Preferences .....	19
3. User Interface .....	23
3.1. Main Window Components .....	23
3.2. Image Window .....	26
3.3. Arranging User Interface .....	29
3.4. Layouts .....	29
3.5. Layout Manager .....	30
3.6. Modifying Tool Bars .....	32
3.7. Modifying Menus .....	33
3.8. Running a Macro Upon Layout Change .....	35
3.9. Appearance Options .....	35
4. Cameras & Devices .....	37
4.1. Basic Workflows .....	37
5. Image Acquisition .....	55
5.1. Introduction to Image Acquisition .....	55
5.2. Shading Correction .....	56
5.3. Camera ROI .....	57
5.4. About ND Acquisition .....	59
5.5. Time-lapse Acquisition .....	61
5.6. Multi-point Acquisition .....	65
5.7. Z-series Acquisition .....	69
5.8. Multi-channel Acquisition .....	71
5.9. Large-image Acquisition .....	72
5.10. Inputs/Outputs Control .....	74
5.11. Combined ND Acquisition .....	74
5.12. Capturing to Ring Buffer .....	79
6. Displaying Images .....	81
6.1. Opening Image Files .....	81
6.2. Image Layers .....	83
6.3. Navigation in ND2 Files .....	87
6.4. ND Views .....	90
6.5. Large Images .....	92
6.6. LUTs (Look-Up Tables) .....	94
6.7. Synchronizer .....	102
6.8. Organizer .....	103
6.9. Database .....	107
6.10. Saving Image Files .....	110
6.11. Closing Images .....	112
6.12. Supported Image Formats .....	113
7. Image Analysis .....	115
7.1. Preprocessing .....	115

7.2. Histogram .....	116
7.3. Thresholding .....	119
7.4. Binary Layer .....	125
7.5. Mathematical Morphology Basics .....	127
7.6. Regions of Interest - ROIs .....	131
7.7. ROIs in Time - Moving ROIs .....	135
7.8. Analysis Modules .....	135
8. Measurement .....	143
8.1. Calibration .....	143
8.2. Units .....	144
8.3. Rough Measurement .....	144
8.4. Manual Measurement .....	146
8.5. Object Count .....	148
8.6. Automated Measurement .....	153
8.7. Measurement Options .....	154
8.8. Time Measurement .....	155
8.9. 3D Measurement .....	160
8.10. Measurement Features .....	162
8.11. Measurement Features 3D .....	175
8.12. Measurement on Graph .....	177
8.13. Pixel Classifier .....	177
8.14. Exporting Results .....	180
9. Creating Reports .....	183
9.1. Report Generator .....	183
9.2. Report Objects .....	184
9.3. Report Templates .....	186
9.4. Creating Reports from Database .....	187
10. HCA .....	189
10.1. Introduction to HCA .....	189
10.2. Running a HCA job .....	190
10.3. HCA Analysis .....	192
10.4. Viewing Results .....	192
11. JOBS .....	201
11.1. Introduction to the JOBS Module .....	201
11.2. JOBS Explorer .....	201
11.3. Basic Job Rules .....	203
11.4. Job Definition Window .....	207
12. Macros .....	213
12.1. Creating Macros .....	213
12.2. Running a Macro .....	215
12.3. Macro Language Syntax .....	215
12.4. Controlling Cameras by Macro .....	222
12.5. Interactive Advanced Macro (API) .....	222
12.6. Macro Preferences .....	223
13. Movies .....	225
13.1. Capturing AVI Movie .....	225
13.2. Save ND2 as AVI .....	225

---

13.3. Movie Maker .....	225
13.4. About Video Compression .....	227
14. Additional Modules .....	229
14.1. 3D Measurement .....	229
14.2. 4D/6D Acquisition .....	229
14.3. AQI 2D Real Time Deconvolution .....	229
14.4. AQI 3D Blind Deconvolution .....	229
14.5. Bio Analysis .....	229
14.6. Calcium and FRET .....	229
14.7. Database .....	230
14.8. Extended Depth of Focus .....	230
14.9. Filters Particle Analysis .....	230
14.10. HCA .....	230
14.11. HDR .....	230
14.12. Illumination Sequence .....	231
14.13. Interactive Advanced Macro (API) .....	231
14.14. Jobs .....	231
14.15. LIM 2D/3D Deconvolution .....	231
14.16. LIM Legacy Deconvolution .....	231
14.17. Local Option .....	231
14.18. Metalo - Cast Iron Analysis .....	232
14.19. Metalo - Grain Size Analysis .....	232
14.20. ND Advanced Acquisition .....	232
14.21. Single Particle Tracking .....	232
14.22. Time Measurement .....	232
14.23. Tracking .....	232
General Index .....	233



# 1. Command Line Startup Options

See also 2.1.3. Command Line Installation Options [9].

When starting *NIS-Elements* from the command line (or when editing the desktop shortcut properties), you may append some switches with parameters to the main command and thereby modify the startup behavior.

## Note

*Some switches do not have parameters.*

*Example 1.1. Command line switches syntax*

*"NIS-Elements executable" switch#1 parameter#1 switch#2 parameter#2 etc.*

For example, to run *NIS-Elements AR* and open the *starting\_image.jp2* at straight away, run.:

```
"c:\Program Files\NIS-Elements\nis_ar.exe" -f "C:\Images\starting_image.jp2"
```

## **Startup Switches**

**-?** Displays a help screen with the description of switches (basically this page).

**-c "Command"** The application runs the specified *NIS-Elements AR* macro *Command*.

**-f "Filename"** The application tries to open the image pointed to by the *Filename* parameter.

**-g "Grabber Name"** The image grabber (driver) of the specified *Grabber Name* will be used and no camera-selection window will show at startup. Set the parameter value to "last" and the most recently used grabber/camera will be loaded. As the grabber name, you must use internal name of the grabber, not the one you see in the startup dialog window.

Either use one of the following names or search the application log file for the phrase "Grabber Name" (see Fixed Grabber Startup [2]).

**-gn** No grabber (camera) driver will be loaded. Image acquisition will not be possible.

**-h "HW Unit Name"** The specified HW Unit will be used. When using this option you must also specify the Grabber Name. Two HW Units (e.g. two DS-U2 cameras) can be run by one grabber/driver. The available HW Units are listed in the *Acquire > Select \*Camera Name\** window. Set the parameter value to "select" and the selection box will be shown. When using this option you must also use the -g switch. To find the HW Unit Name, please search through the application log file for "HW Unit Name".

**-cam "Camera Name"** The specified Camera will be used. When using this option you must also specify the *Grabber Name* and the *HW Unit Name*. To find the camera name, search the application log file for "Camera Name". It is also possible to use only a part of the Camera Name, but the part of the name must be unique among all the available cameras.

**-i "Config Name"** A user can specify the configuration to be used by *NIS-Elements*. The default configuration is saved in the "C:\ProgramData\Laboratory Imaging\Platform" folder. If you want to create a custom configuration, use the -i switch, e.g.: *nis\_ar.exe -i "My\_Configuration"*. The config-

---

uration of this instance will be saved to the “C:\ProgramData\Laboratory Imaging\My\_Configuration” folder.

This is useful when using two cameras on one microscope for example. You can create a separate program shortcut which uses the -i switch to load a different configuration.

**-s “Settings File”** The application will use the specified *Settings File* to load and save its settings. The settings file can be created from MS Windows Start menu by the *NIS Settings Utility*.

**-l “Language”** The application will run in the specified language (if available). Use three-letter language codes according to the ISO 639 [[http://en.wikipedia.org/wiki/List\\_of\\_ISO\\_639-2\\_codes](http://en.wikipedia.org/wiki/List_of_ISO_639-2_codes)] standard.

**-m “Macro File”** Application will execute the macro file (\*.mac) pointed to by the *Macro File* parameter.

**-passive** The application will not connect to any device on startup (*NIS-Elements AR* will start with blank Device Manager). Camera selection is not affected by this option.

**-p “Command” [“Param”]** The application window will be placed according to the *Command*. Possible values are:

- *left, right, top, bottom* - the window is placed on the corresponding monitor screen.
- *monitor N* - the window is placed on the Nth monitor.
- *rectangle (x0,y0,x1,y1)* - the window is placed in the specified rectangle (in workspace coordinates). The coordinates must be in parenthesis without any spaces.

**-q** A new instance of the application will run. By default, only one instance of *NIS-Elements* is allowed to run.

### **Fixed Grabber Startup**

Each time *NIS-Elements AR* is run or the grabber / camera / HW unit is changed, four parameter values are written to the application log file (C:\ProgramData\Laboratory Imaging\Platform\Logfiles):

- Grabber Name
- HW Unit Name
- HW Unit Connection String
- Camera Name

Let's say we want to set the last used configuration for every *NIS-Elements AR* start:

1. Lookup the parameters in the most recent log file. E.g.:

**Grabber Name** CLxGrabberDriverSim

**HW Unit Name** SimGrabber

**HW Unit Connection String** (empty)

**Camera Name** Sim\_Camera\_Color

2. Modify the shortcut, E.g.:



```
"c:\Program Files\NIS-Elements\nis_ar.exe" -g CLxGrabberDriverSim -h  
SimGrabber -cam Sim_Camera_Color
```

3. Double-click the shortcut. *NIS-Elements* will start without asking for grabber selection



## 2. Installation and Settings

### 2.1. Installation and Updates

#### 2.1.1. The Installation DVD-ROM Content

- *NIS-Elements* software setup file
- Drivers and utilities for the HASP Key
- Drivers for selected cameras
- User's Guide in the PDF file format
- Sample image database
- Sample ND2 image sequences

#### 2.1.2. NIS-Elements Installation Steps

##### Caution

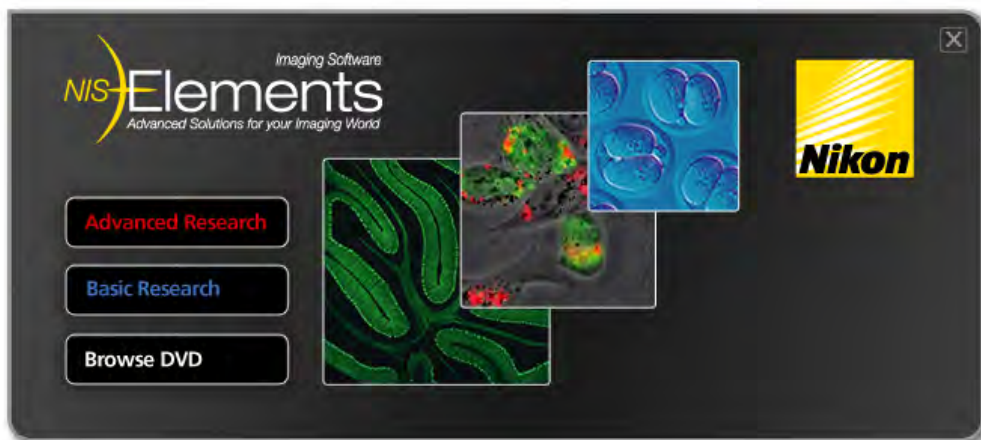
*You have to possess the administrator rights to your computer to be able to install NIS-Elements AR successfully.*

##### **Quick Guide**

- Insert the installation DVD in the DVD-ROM drive. A window automatically appears.
- Install the selected *NIS-Elements* software version, additional modules and device drivers.
- Plug the provided HASP key into the USB port of your PC.
- Run *NIS-Elements*.

##### **Step by Step**

- 1) When the installation DVD is inserted, a selection window appears automatically.



Select the software package to be installed. Select the one you have got the license for and which is properly coded in your HASP key. The installation wizard welcome-window appears. Click Next to continue.

## 2) Install Local Options

Figure 2.2. Local Options installation

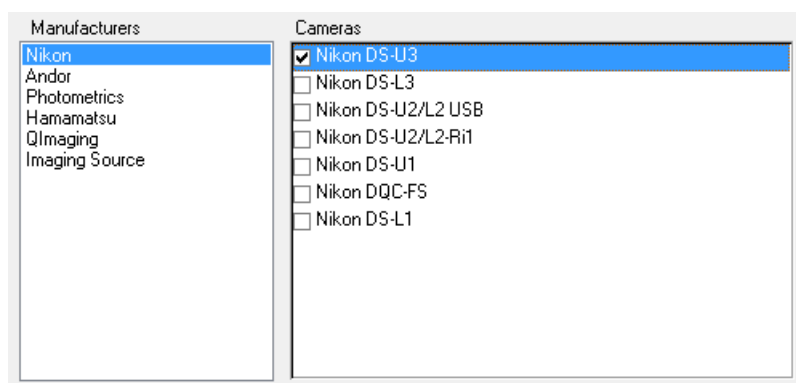


Select whether to install *Local Option* or not. Define the folder where *NIS-Elements AR* should be installed. We recommend to use the predefined directory. If you want to change the directory anyway, press the *Browse* button and select a new one. Otherwise click *Next*.

### Note

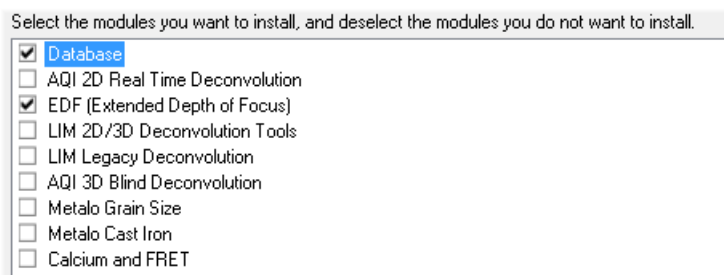
*The Local Option installation provides some advanced features which did not pass the quality assurance procedure yet. We recommend to wait until they are released officially.*

3)



Now, select the cameras which will be used with *NIS-Elements AR*.

4)

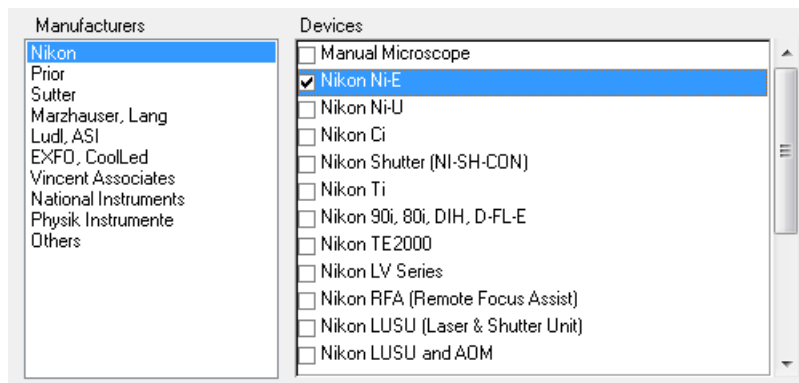


If your licence contains some additional modules besides the *NIS-Elements AR* core software, please select them in this window.

#### Note

*Any module selected will be installed along with NIS-Elements AR automatically. However, you might not be licensed to use it. The module will run after you get the corresponding code registered in your HASP key.*

5)



Select the devices which will be used with *NIS-Elements AR*. Finish the installation by clicking the *Install* button.

#### Note

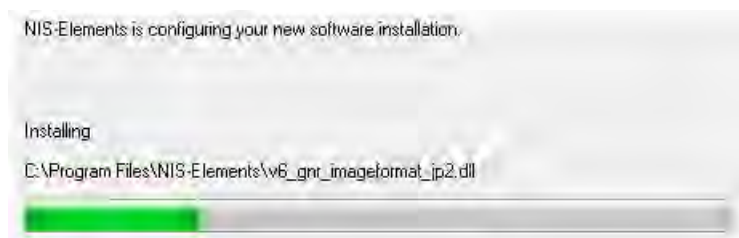
*NIS-Elements may not be connected to devices after installation depending on the PC status at installation. If so, execute the Modify installation command of the Windows Start menu (see the next step) to perform Repair that corrects the previously installed status.*

#### Warning

*For Windows7 users: Some devices do not work correctly after recovery from Sleep mode. Please turn off the Windows 7 Sleep Mode to prevent possible problems:*

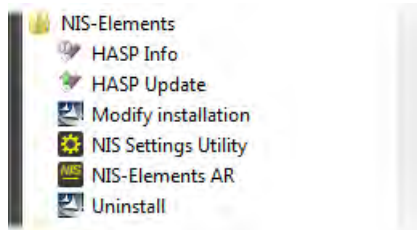
- *Run the Start > Control Panel command on the main Windows tool bar.*
- *Click Hardware and Sound*
- *Select Change when the computer wakes*
- *In the Put the computer to sleep option, select Never*
- *Confirm the settings by the Save changes button.*

6)



The setup creates a new program group in the *Start* menu containing the following items: *NIS-Elements AR* application shortcut, the *HASP* key information shortcut, the *Modify Installation* shortcut (for adding hardware drivers, modules, etc.), the *Uninstall* procedure, and the *Send Info Tool*. A shortcut to *NIS-Elements AR* is created on the desktop too. These changes affect all user profiles of your local Windows operating system.

Figure 2.7. Start menu



#### Note

*Clicking the Uninstall command deletes all installed files from disk, and removes the NIS-Elements AR program group from the Start menu as well as it removes the desktop icon.*

## 2.1.3. Command Line Installation Options

See 1. *Command Line Startup Options* [1] for details about how to use command line switches. The following switches are to be used with the *NIS-Elements* installation file to modify behavior of the installation wizard.

- all** This parameter unhides options in the setup which are hidden by default. It concerns mainly drivers of devices which were formerly supported but are no longer available.
- new** If this command line parameter is appended, the setup checks if any other setup has already been installed. If so, the setup restarts and runs in an independent mode. The update mode is disabled. This enables to install two or more builds together on one operating system.
- xp** This parameter enables the user to install *NIS-Elements* on Windows XP even though this operating system is NOT supported officially.

## 2.1.4. Additional Module/Device Installation

You may need to install a device or an additional module after the *NIS-Elements AR* main system installation.

- Go to [Start menu > Programs > *NIS-Elements AR*] program group.
- Select the *Modify Installation* command.

- Follow the installation wizard instructions. Select the check-boxes by the items you would like to add.
- Finish the installation.

### 2.1.5. Sample Database Installation

If you chose to install the Sample Database, a new subdirectory “Databases” is created inside the *NIS-Elements AR* installation directory (e.g. C:\Program files\NIS-Elements AR \Databases\...). The “Sample\_Database.mdb” file is copied to there along with database images (stored in subdirectories). An administrator username/password to access this database is set to:

- Username: "sa"
- Password: "sa"

### 2.1.6. Software Copy Protection

The *NIS-Elements AR* software is delivered with a hardware key (also called HL = hardware licence).

Figure 2.8. Hardware key



The key contains information about the software licence and allows users to run the corresponding software. A warning message is displayed when user starts *NIS-Elements* with incorrect HASP. Please connect the USB HASP after the *NIS-Elements AR* installation is finished. The utility called HASPinfo is installed to the *NIS-Elements AR* directory. It enables the user to view information about the software licence and is accessible via the *Help > HASP Info* menu command.

### 2.1.7. Device Updates

The main goal of the Device Updates setup is to add new devices/cameras or to fix problems that are localized in the drivers only and do not affect any other *NIS-Elements* functionality.

Device Update setup requires installation of the corresponding *NIS-Elements* version. The setup contains only .dll files supporting a new device or some corrections and upgrades of already existing drivers. This update form keeps *NIS-Elements* stable and the update file size small.



Device Update numbering is consecutive and appropriate to its *NIS-Elements* version until a new full setup of *NIS-Elements* is released (Major version, Service Pack or HotFix) - then the numbering starts from the beginning.

Device Updates are cumulative and contain all the changes from the previous updates. Also full *NIS-Elements* setups (Major versions, Service Packs and HotFixes) will contain all the changes from the previously released Device Updates.

### 2.1.8. Fixes

Fixes represent driver changes which serve for testing purposes or for correcting problems occurring only under special conditions. Fixes are packed into a zip file containing modified driver files. Fixes are usually user requested, therefore they are not considered as Device Update candidates.

### 2.1.9. Installing the Database Module on 64-bit Systems

The *NIS-Elements* installation file contains 64bit MDB drivers which improve the speed of the database module on 64bit systems. However, 32bit version of MS Office 2010 cannot be used along with these 64bit drivers. You can install these drivers, if:

- you run 64bit version of MS Office 2010
- you use other version of MS Office such as Office 2013
- you do not use MS Office at all

Find the drivers in the *NIS-Elements* folder, typically: "C:\Program Files\NIS-Elements\Drivers\Data-base\_MDB\"

## 2.2. User Rights

User management is very useful when a single workstation is shared by number of users. Some user accounts may have administrator rights to *NIS-Elements* while other's privileges are quite restricted. A per-person system may be established or user accounts may be shared. The following principles are utilized within *NIS-Elements AR*:

**MS Windows accounts support** *NIS-Elements AR* can assign privileges to MS Windows accounts. Whoever is logged in the operating system and runs *NIS-Elements* obtains a default set of privileges. System administrator can then further restrict or extend the user's rights.

**NIS-Elements AR password protection** It may be not comfortable enough to log the current user off from MS Windows when a user change is required in *NIS-Elements*. For such case a completely independent list of user accounts, which is not connected with MS Windows users, can be created. Then just restart of *NIS-Elements* is needed to switch the user.

**Private vs. Shared storage** Every user-created item (setting) important in the work-flow can be protected from unwanted change in two levels. First, the creator of the item (optical configuration, layout, objective, etc.) can save it as *private*. Unless its status is changed to *shared*, no other user will even see this item within the lists throughout the application. The second level of protection concerning all *shared* items is provided by assigning users to *Groups* and *Privileges* groups.

**Groups** Every user is a member of a group. The group enumerates shared items (optical configurations, objectives, etc.) which will be visible for its members. *NIS-Elements* administrators can create any number of groups and select the items to be visible.

**Privileges** There are basic groups of privileges which allows users or restrict them to perform certain actions. This concerns for example modifying macros, reports, selecting cameras, etc. There are four levels of privileges by default, but further can be created by an administrator:

- Admin
- Common
- Guest
- Default

### 2.2.1. NIS-Elements authentication

If you have chosen “*NIS-Elements* (Password protected)” user authentication method, a login dialog window appears at every start-up of *NIS-Elements*. Enter a user name and a password for existing user account. If the option *Allow to Create a New User at Start-up* is enabled, you can create a new user account without needing an administrator. This account is assigned to the *Default* group of users and *Default* group of privileges automatically.

### 2.2.2. Creating a Shared Layout

Please read the following example on user management. Step by step, we describe the creation of a shared layout which can not be modified by members of the *Common* and *Guest* groups.

- 1) Run the *View > Layout > Layout Manager* command. Set at least one of the defined layouts as *Shared*. (see 3.3. Arranging User Interface [29])
- 2) In the left column of the window, select *User Rights*.
- 3) Make sure you are logged as a user with the *Privilege to Modify user rights*. If not, use the **Log As...** button to log in under a different account which has the privilege.

**4) Set layout visibility**

Select the *Groups* tab. Here you can see at least one group of items called *Default*. Either select one a group or create a new one by the *New* button and choose *Layout* within the list of items. All layouts set as *shared* will be listed on the right side within the *Accessible items* field. Make sure the check boxes next to the layouts which you allow to be used by the group are selected.

**5) Disable layout modification**

Select the *Privileges* tab. Select the group of privileges which should have not the permission to modify the selected shared layouts. In the *Privileges* field on the right, make sure you de-select the *Modify Shared Layouts* option.

**6) Apply the new policies**

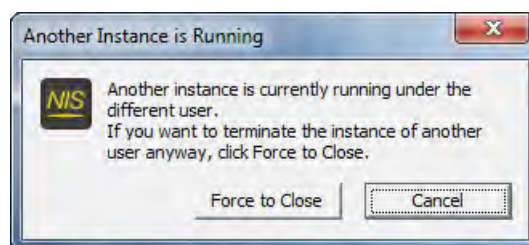
Select the *Users* tab. In the list of users, select the ones which you would like to apply the policy to (users can be selected one by one or by multi-selection with the **Shift** or **Ctrl** key pressed). Then, assign the appropriate *Group* and *Privileges* by selecting them from a pull-down menu on the right side of the window.

**7) Confirm the changes by the **Apply** button.**

## 2.2.3. Closing Another Instance of NIS-Elements

When more users want to work with *NIS-Elements* on the same computer and one of them did not close his *NIS-Elements* running copy, other users cannot use the program, because only one *NIS-Elements* copy can be running at a time. The possibility to close the running program started by a different user during a new *NIS-Elements* launch is available in the following dialog window. This applies only if there are no experiments running.

Figure 2.9. Another instance running



## 2.2.4. User Rights Options

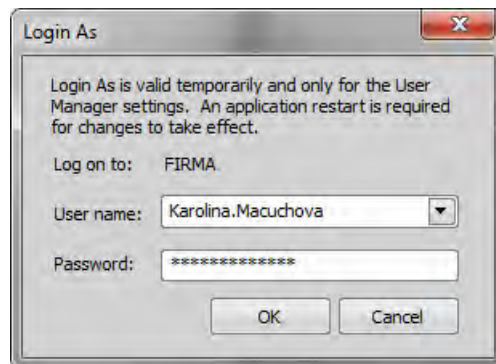
**User Authentication** The *Windows* authentication utilizes Windows accounts to automatically log in to *NIS-Elements AR*.

The *NIS-Elements AR (Password protected)* authentication utilizes application user accounts and passwords at the start-up of *NIS-Elements AR*. Check the *Allow to Create a New User at Start-up* option to enable creating of a new user in the *Login* dialog window.

**Current user** Displays currently logged in user.

**Log As** Opens the *Login As* dialog which enables you to login as a different user e.g. an administrator with more user rights for the purpose of changing User rights settings.

Figure 2.10. Login As



Select the user name you want to log-in as. Enter a valid password and press *OK*. The new user identity is available only while the User manager is opened.

**Statistics** Edit path and file name of a database file for saving statistics.

**Export/Import user rights** These buttons enable anyone to save/load complete settings of user accounts and their privileges to/from an external XML file. Standard *Open* and *Save As* windows appear. Having the data exported may be useful when copying the settings to other computers.

## 2.2.5. Users Tab Options

**New** Enables to create new *NIS-Elements AR* user account. You have to possess the privilege *Modify User Rights*.

Figure 2.11. Creating new user

Enter a new user name, assign him to one of the existing Groups and group of Privileges and set the password. Check the *Enforce user to change his password at start-up* option to make the user change his password at his first login into the *NIS-Elements AR*.

### **User name and password properties**

Length of the user name and password is limited to 50 characters. You can use any combination of characters, symbols or numbers (except the Windows ones: ", :, \*, ?, ", \, /, <, >, |").

**Remove** Removes selected user account. You can select multiple accounts and delete them at once.

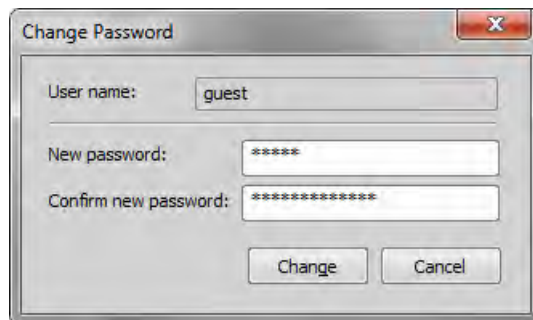
**Copy To** Choose a user from the pull down menu whose group and group of privileges will be changed according to settings of a selected user.

**Duplicate** You can easily create a new user by duplicating the existing. This preserves all account settings under a different name. If you have selected to duplicate more then one user, enter a name prefix, which is added in front of the original name for all selected duplicated accounts. User which was created as a duplicate has an empty password and is forced to change his password at next application startup.

**User Accounts List** This table displays all existing user accounts. You can sort them according to any of four columns. The first column displays type of the account. The second one shows user names. The third one displays assigned group of users. The fourth one displays assigned group of privileges. Select one or more users whose settings needs modification.

**Set password** To change password of a selected *NIS-Elements AR* user account, press the *Set password* button. You have to posses the Privilege Modify User Rights. Otherwise you can change only password of the current user. Following window appears:

Figure 2.12. Reset of password



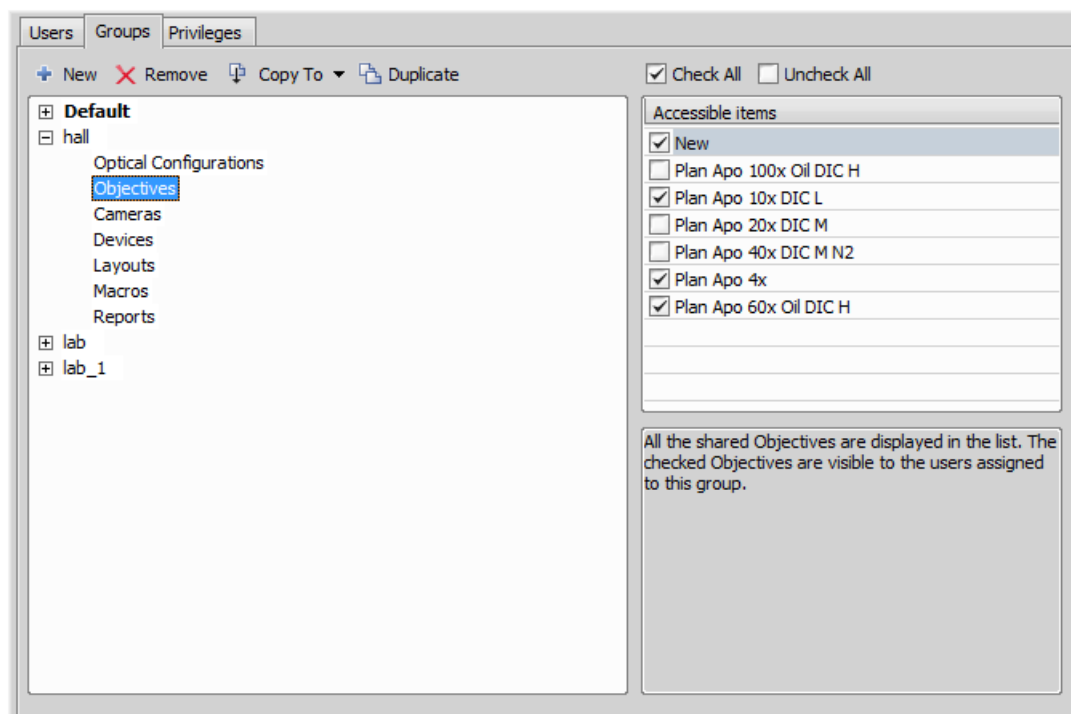
Name of the user is displayed. Enter and confirm new password. When done, press the *Change* button.

**Enforce user to change his password at start-up** Check this option to make the user change his password at his first login into the *NIS-Elements AR*.

**Enumerate All Windows Users** This button starts copying MS Windows accounts to *NIS-Elements AR*. The accounts are assigned to user groups (admin, common, guest) according to their Windows permission settings.

## 2.2.6. Groups Tab Options

Figure 2.13. Groups tab



**New** Enables to create new group of users. You have to possess the privilege Modify User Rights. Enter a name of new group.

**Remove** Removes selected groups. You can select multiple groups and delete them at once.

**Copy To** Choose a group from the pull down menu which settings will be changed according to selected group.

**Duplicate** You can easily create a new group of users by duplicating the existing. This preserves all group settings under a different name.

**List of Groups** All defined groups are listed in this window. Each group contains subsets. If you click on a subset, its items appear in the *Accessible items* list.

**Accessible items** This list shows items of a selected subset. Every item has its own check box. If the check box is checked, all users assigned to the corresponding group will see the item. Otherwise they will not see the item.

**Optical Configuration** Displays all shared optical configurations.

**Objectives** Displays all shared objectives.

**Cameras** Displays all installed cameras. From the pull down menu below select a camera which is used as default for users without the privilege Select Camera.

**Devices** Displays all installed devices.

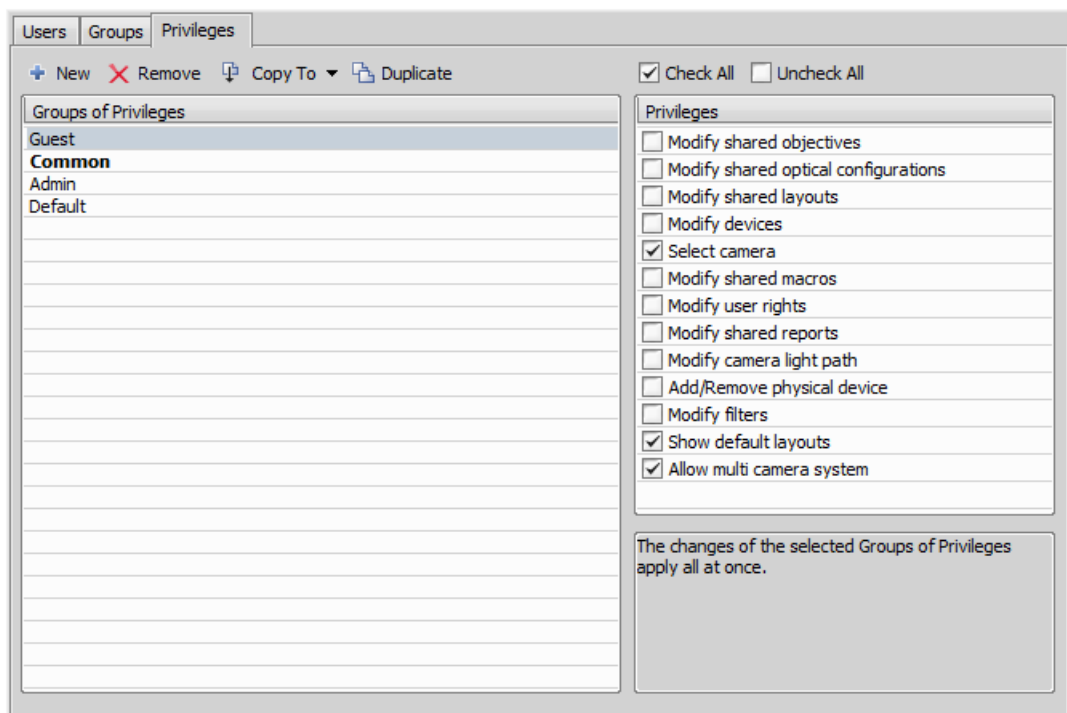
**Layouts** Displays all shared layouts.

**Macros** Displays all shared macro commands. Check a macro to make the content of it visible to this Group.

**Reports** Displays all shared reports and report templates.

## 2.2.7. Privileges Tab Options

Figure 2.14. Privileges tab



**New** Enables to create new group of privileges. You have to possess the privilege Modify User Rights. Enter a name of new group.

**Remove** Removes selected groups. You can select multiple groups and delete them at once.

**Copy To** Choose a group from the pull down menu which settings will be changed according to selected group.



**Duplicate** You can easily create a new group of privileges by duplicating the existing. This preserves all group settings under a different name. If you have selected to duplicate more than one group of privileges, enter a name prefix, which is added in front of the original name for all selected duplicated accounts.

**Groups of Privileges** A list of all groups of privileges is displayed.


**Privileges** A list of privileges is displayed. Every item has its own check box. If the check box is checked, it grants all users assigned to the corresponding group relevant privilege to access, change, modify, etc.

#### Note

*Users that do not have the privilege "Modify Shared Optical Configuration" can temporarily change the brightness settings (e.g. exposure time, gain, etc.) in a shared optical configuration and use such adjusted optical configuration for example in Multichannel acquisition. But all such modifications will not be stored and they will disappear when the application is restarted.*

## 2.3. NIS-Elements Preferences

### 2.3.1. Adjusting Program Preferences

- 1) Run the  *Edit > Options* command. The *Options* dialog window appears.
- 2) Select a tab which contains requested options. Options are sorted into several groups:

**General** Options concerning basic image operations. See 2.3.2. General [20].

**Appearance** Options concerning the graphical user interface. See 3.9. Appearance Options [35].

**Open Next** Options concerning the *File > Open/Save Next > Open Next* menu command. See 6.1.2. Options for the Open Next Command [82].

**Save Next** See 6.10.2. Save Next Options [111] for more information.

**Macro** Configures key shortcuts to macros and sets macros to run automatically on startup See 12.6. Macro Preferences [223].

**Measurement** See 8.7. Measurement Options [154] for more information.

**Data export** See Data Export Options [181] for more information.

**User rights** See 2.2. User Rights [11] for more information.

**Layout Manager** See 3.5. Layout Manager [30] for more information.

- 3) Make any changes you need in preferences and use the following buttons to manage them:

**Defaults for this Page** Restores default settings of the currently displayed options tab.

**OK** Confirms and saves changes made to the preferences. The dialog window is closed afterwards.

**Cancel** Cancels all changes made to the preferences. The dialog window is closed afterwards.



**Apply** Applies changes made in preferences but keeps the *Options* dialog window open. You can apply changes individually on each tab.

**Help** Displays relevant help page.

## 2.3.2. General

### Documents and History


(requires:Local Option)

**Use fix path for images** The defined directory is always used when using the  *File > Open* or  *File > Save As* command.

**Limit number of opened documents** When this options is checked, the user is limited to a single opened image at one time.

**Capture always creates a new document** If checked, every acquisition creates a new image.

**Use last LUTs on image open** When a new image is opened, this option turns the look up tables automatically on, and copies the settings from the current or the last opened image. Some image formats (jp2, ND2) can contain the LUTs settings. If such image is opened, the saved LUTs are loaded instead of the most recently used.

**Use AutoLUTs on image open for images without LUTs information** This option keeps the AutoLUTs feature of the  *File > Open* window always ON, and it applies AutoLUTs to the image after opening. This option is *ignored* if LUTs settings are saved in the image, or if the *Use last LUTs on image open* option is applied.

**Show mapping dialog in organizer after drag and drop** Displays the *Mapping* window every time an image is inserted to a database table. It enables you to check/modify mapping of the image info values to the database table fields.

**Enable saving ND experiment to TIFF series** Displays the *Save As TIFF Series* option within the *ND Acquisition* control panel. It enables you to save single frames of the ND experiment as tiff images. You can modify the order of the dimensions and the images format (mono, color, merged). Also you choose to convert multichannel images to RGB, or to convert images to 16 bit.


**Open new ND views to new window** Displays each newly generated ND view in a separate window.

**Show image info window on save** Displays the *File > Image Properties* window every time an image is saved via the *Save As* command.

**Detect sequence on image open** When you select to open an image that is a part of image sequence such as 001.jp2, 002.jp2..., it is recognized automatically and you are offered to convert it to an ND2 file.

**Show binary layers' contours** Check this item to display contours of binary layers.

**Use zero based time scale for ND documents** (*requires:Local Option*) Select this option to ensure that the first frame of a time sequence will always start at 0.0s.

**State of saturation indicator after start** Select what to do with the saturation indicator settings () after *NIS-Elements AR* is restarted. Turn it ON, OFF, or remember the last setting.

## Rotation Flips and Shifts

**Apply to overlaid binary layer** If checked, in overlay mode both binary and color images are rotated, shifted and flipped.

## Optical Configuration

**Save all camera settings to optical configuration automatically** If the currently selected optical configuration contains Camera Settings and this option is checked, any change of the settings is written to the optical configuration immediately.

**Save brightness to optical configuration automatically** If the currently selected optical configuration contains Camera Settings, the Brightness setting is being updated continuously according to the current state. Also functionality of saving confocal brightness setting(laser power, detector gain and scan frame rate) is contained in this option.

**Select corresponding optical configuration when filter changed...** (*requires:Local Option*) Enabled if "Unselect" in the option below is chosen. Allows NIS-E to automatically change the optical configuration based on the filter selected on the microscope.

### When Optical Configuration setting changed

**Keep Selected** The Opt. configuration name is marked by the "\*" sign and the change is offered to be saved.

**Unselect** Every change of Optical configuration setting results in its deselecting and switching to user settings.

**Save all changes** All changes regarding the Opt. configuration settings are updated automatically according to the current state.

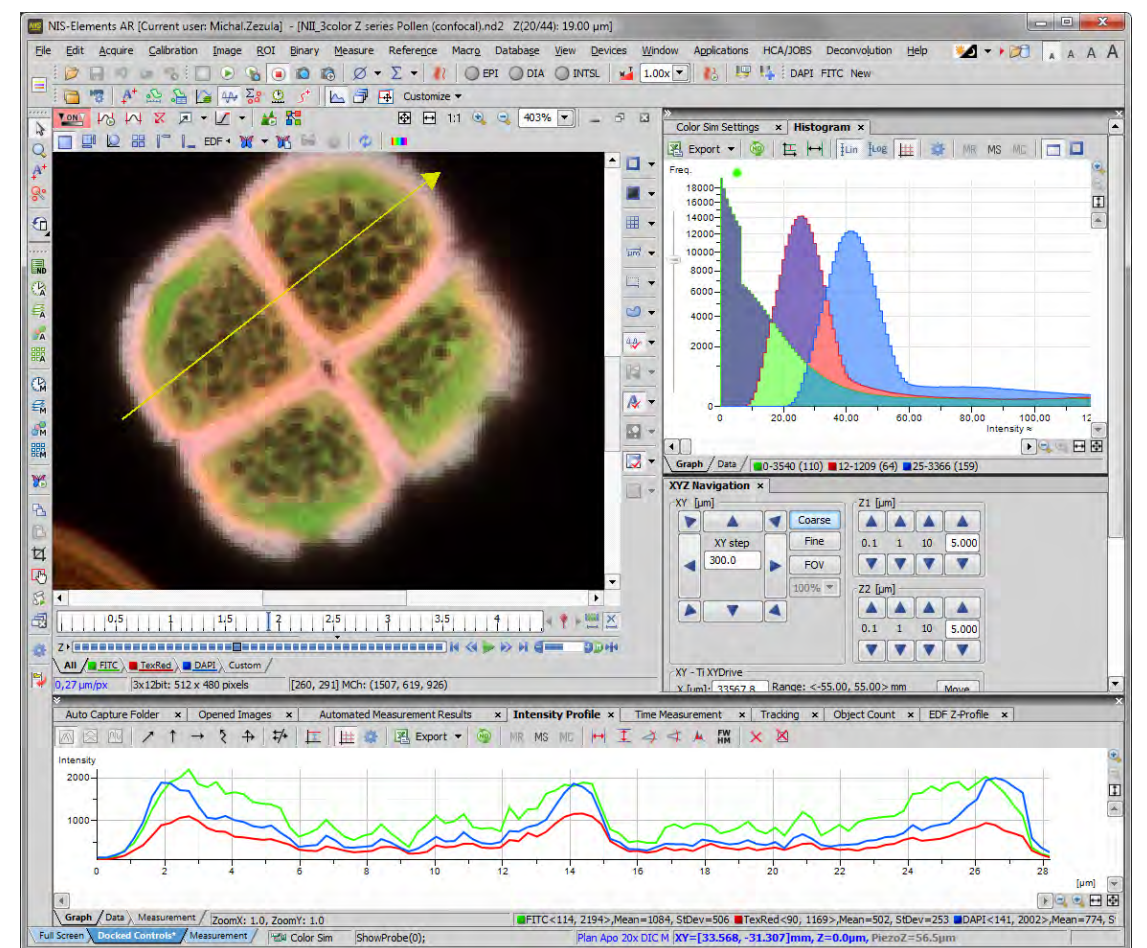
**Temp** *NIS-Elements AR* uses the Temp directory for storing temporary data when there is not enough RAM available. You can redirect the system to use a directory on another harddisk, which may speed up the whole system.

**Defaults for this Page** Restores the default setting for General Options.



### 3. User Interface

Figure 3.1. The NIS-Elements Main Window



### 3.1. Main Window Components

### 3.1.1. Main Menu

All basic *NIS-Elements* functions are accessible from the main menu at the top of the screen. Menu commands are grouped according to their purpose.

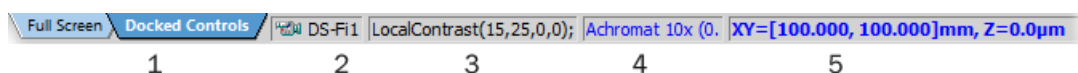
### 3.1.2. Tool Bars

There is a default set of tool bars, each tool bar containing number of buttons. There is also one fully customizable tool bar - the main left tool bar - to which any button can be added. Every button or whole tool bar can be hidden by user. Please see 3.3. Arranging User Interface [29] for further details.

### 3.1.3. Status Bar

The status bar at the bottom of the screen displays the following information:

Figure 3.2. The application status bar



1. This part of the status bar displays available layouts.

#### Note

*The layout Tabs may be hidden when the Show Layout Tabs option in the 3.3. Arranging User Interface [29] window is deselected.*

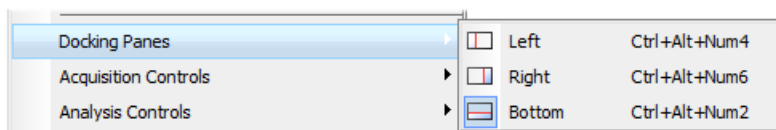
2. This status bar section displays the type of the currently selected camera.
3. Here you can get information about the most recently performed command. The FPS / Exposure / Focus info is shown in case of live image. The black bar indicates the focus rate. Longer black bar represents more of the image in focus.
4. This section show the name of the current objective.
5. Current coordinates of XY (Z) stage are shown in this part of the status bar.

### 3.1.4. Docking Panes

Docking panes are square spaces inside the application window, where you can place (dock) any of the control panels. There is one docking pane available at the Right, Bottom, and Left side of the application screen.

#### **To Display a Docking Pane**

1. Go to the  View > Docking Panes sub-menu and select the pane you would like to display.



#### Note

*The Docking Panes sub-menu can be also displayed by right-clicking into the empty application screen.*

2. The docking pane appears, either empty or with some window(s) docked inside.
3. Repeat this procedure to display more docking panes.


### Handling Control Panels

Various control panels can be displayed docked within the docking panes or they can be floating. See the following picture:

Figure 3.4. The Docked Control Panel Caption



To handle the control panels (CPs), you can:

**Open recently closed CP** Locate the  button on the main left tool bar. When you click it, the list of recently closed CPs appears. Pick one to display it again.

**Add CP to a docking pane** Right click inside a docking pane (3) to display the context menu. Select the control panel to be displayed. If the window is already opened somewhere else (in another docking pane or floating), it closes and moves to the new destination.

**Close CP** Click the cross button (2) in the right top corner of the tab.

**Drag CP** Drag any CP by the tab and drop it somewhere. If you drop it by the edge of a docking pane, it will create another column of this pane. If you drop it over the caption of another CP, it will be docked in the same pane as a new tab. If dropped somewhere else, the CP will be floating.

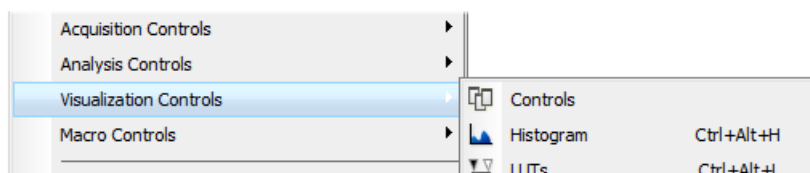
A color frame appears when you place the mouse cursor dragging a CP over the edge of a docking pane or a caption of another CP. It indicates that if you drop it, its placement will be handled automatically.

**Minimize the docking pane** Click the arrows in the top left corner. The pane minimizes to a stripe by the edge of the screen. It can be restored to its original position by double clicking this stripe or by clicking the arrows again.

**Close the docking pane** Click the cross button (4) in the docking pane caption. Or you can right click the pane and unselect the *Docking View* option.

**Dock/undock CP** To dock (and undock) a CP, double click its tab.

**Display CP** Another way to display a CP is to go to the View menu and select the desired control panel. After that, the CP appears on the screen - floating or docked. Positions of the windows are being saved by the system so that each control panel appears in the same position as it was before it being hidden. The controls are sorted to several groups.



**Shrink/Expand dockers** Having more docking panes opened, a situation where there is not enough room for the control panels can occur. In such case, the *Shrink* and *Expand* commands shall be used.



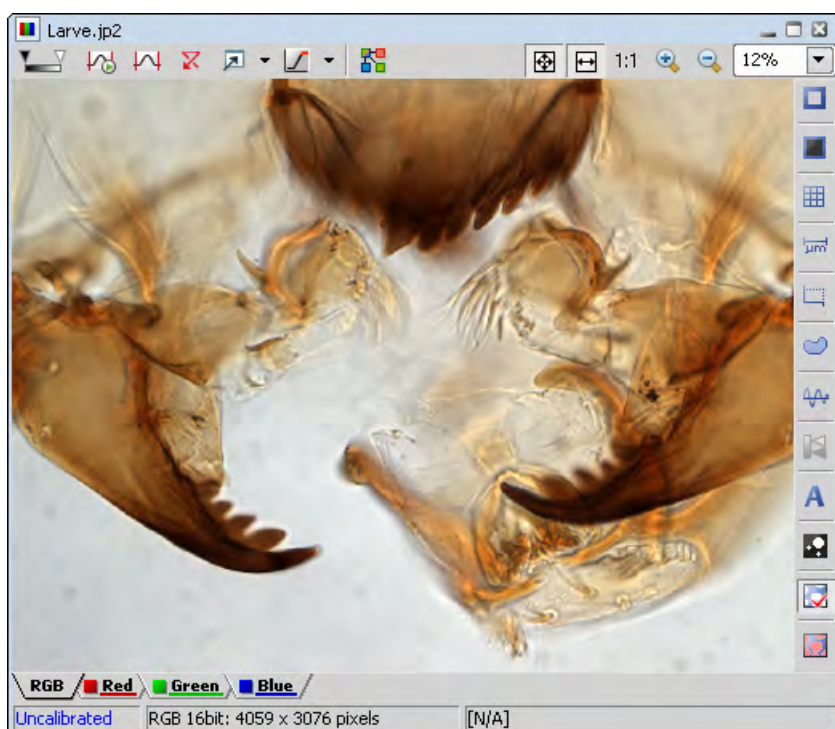
1. Right click the pane you would like to shrink/expand. A context menu appears.
2. Select the *Expand/Shrink* command. When one of the panes shrinks, the neighbouring pane expands to the emptied corner and vice-versa.











## 3.2. Image Window

Tools affecting the appearance of the current image are gathered within the image window tool bars (the top image tool bar and the right image tool bar) . There are the following buttons by default:




Figure 3.7. Image Window





-  **Enable LUTs** Applies LUTs to the image. See 6.6. LUTs (Look-Up Tables) [94].
-  **Keep Auto Scale LUTs** Applies the AutoScale command to the image continuously.
-  **Auto Scale** Performs automatic setting of LUTs.
-  **Reset LUTs** Discards the LUTs settings.
-  **Show LUTs window** Opens the window with LUTs.
-  **Pixel Saturation Indication** Turns on / off pixel saturation indication without setting on / off LUTs. Select the highlighting color from the nearby pull-down menu for *Oversaturated* and / or *Undersaturated* pixels. See 6.2.3. Displaying Image Layers [84].
-  **Split Components** Turns ON a special view, where color channels of the image are displayed separately (tiled).
-  **Fit to screen** Adjusts zoom to view the whole image within the *NIS-Elements AR* screen.
-  **Best Fit** Adjusts zoom to fit the *NIS-Elements AR* image window in one direction but to fill the screen.
- 1:1 Zoom** Adjust zoom so that one pixel of the image matches one pixel of monitor.
-  **Zoom In** Increases magnification of the image.

---

 **Zoom Out** Decreases magnification of the image.

 **Show Probe** This button activates the probe. The probe affects histograms, auto exposure and auto white balance functions.



 **Show Background Probe** Activates the background probe. Some commands use the BG probe data as reference.


 **Show Grid** Displays the grid for rough measurements.


 **Show Scale** Displays the image scale.

 **Show Frame** Displays and applies the measurement frame.

 **Turn ROI On/Off** Displays the Measurement Region Of Interest.


 **Show Profile** Displays the  *Measure > Intensity Profile* control panel. It allows you to specify a linear section in the image of which the pixel intensities graph will be created.

 **View LUT Intensity** Displays the scale of intensities used inside the image. It works on monochromatic images or a single image channel.

 **Show Annotations** Displays the vector layer which typically consists of annotation objects (text labels, arrows) and measurement objects.

 **View Binary** Displays the binary layer of the image.

 **View Color** Displays the color layer of the image.

 **View Overlay** Displays the color layer and the binary layer in overlay.

---

#### Tip

*Right click the icons to invoke a context menu where properties of each tool can be modified.*

### **Channel Tabs**

*Figure 3.8. Channel tabs of an RGB image*

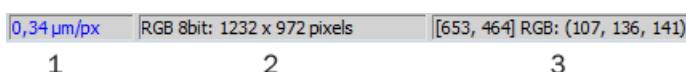


Channel tabs at the bottom left corner of the image window enable switching between image channels. You can also edit their properties using commands available via a context-menu. See also 6.2. Image Layers [83].

### **Status Bar**

The status bar at the bottom of the image window displays the following information:

Figure 3.9. Status bar of the image window



1. The first field of the image window status bar Image displays the calibration. See also 8.1. Calibration [143] , 8.2. Units [144].
2. Image bit depth (8bit, 12bit, 16bit, etc.) followed by Image size. You can change the displayed units from the context menu.
3. Pixel coordinates of the mouse cursor along with channel intensities, Binary layer value (0 or 1) and the Color mode (RGB, Monochromatic, etc.).

### 3.3. Arranging User Interface

Having a well organized application layout can help you make the work with *NIS-Elements AR* very effective. There are the following options on customizing the appearance of *NIS-Elements AR*:

**Custom window placement** All control panels (Camera Settings, Measurement, Histogram, LUTs, etc.) can be arranged inside or outside of the main application window.

**Compact window or multiple windows** The control panels as well as tool bars can be floating or docked on sides of the application screen.

**Multiple monitor support** The *NIS-Elements AR* window can be stretched to occupy two monitors. When you switch from different application, *NIS-Elements AR* will be activated on both monitors.

**Customized tool bars** Tool bar buttons may be added and removed from tool bars. See 3.6. Modifying Tool Bars [32].

**Maximizing the Image Area** You can hide some of the GUI elements which are displayed by default:

- The channel tabs and the layout tabs may be hidden to save some screen-space. Display the 3.9. Appearance Options [35] window and de-select the *Show Channel Tabs* and the *Show Layout Tabs* options.
- Image controls and the image status bar may be hidden. Use the *Auto hide bottom toolbar* option of the 3.9. Appearance Options [35] window.
- When an image is displayed in great magnification, scroll bars automatically appear by the sides of the image window. You can hide them by de-selecting the *Show Scrollbars* context menu option.

## 3.4. Layouts

A layout in the context of *NIS-Elements AR* is a set of options describing the arrangement of control panels, tool bars, and menu items. Blue tabs representing active layouts appear in the application status bar. The following layouts are placed there by default:

- *Full Screen*
- *Docked Controls*
- *Measurement*

Other layouts can be added and managed via the Layout Manager. To hide/show the layout tabs within the status bar, go for *View > Layout > Layout Manager* and (de)select the *Show Layout Tabs* option.

### **To Create a New Layout**

Figure 3.10. Layout tabs



1. Modify the current layout so that it suits your concept of work.
2. An asterisk \* appears next to the layout name (to indicate it has been modified).
3. Right click the layout tab and select the *Save Current Layout As* or *Save As Default* command. If you do not need to create a new layout but would like to save the changes made, just right click the current (asterisk-marked) tab and select the *Save* command from the menu.
4. Write the new layout name and confirm it by .
5. A new tab appears and the layout is saved to the list of layouts.

### **To Reload Previous Layout Settings**

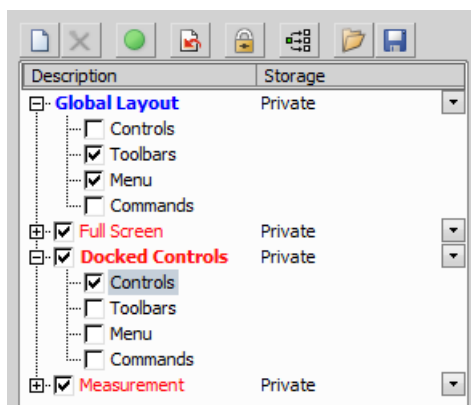
You may want to undo the changes made to the layout. Mostly, it can be done by the *Reload* command. Or by selecting the *Load Default* command.

- Right click the asterisk-marked (recently modified but not saved) tab and choose the *Reload* command. The application restores the last saved state of the layout.
- Right click the asterisk-marked tab and choose the *Load Default* command. The application loads the previously saved default layout.

## 3.5. Layout Manager

Run *View > Layout > Layout Manager* to display the Layout Manager. The list of currently available layouts is placed on the left side of the layout manager. Each layout may contain information about controls, tool bars, menu, and commands to be performed when switching between layouts.

Figure 3.11. List of Layouts





### Modifying the Layout Settings


1. Select items within the *Global Layout* list which you want to be shared by all layouts.
2. The check marks on the left of the layout names indicate the layout visibility. Select the ones you want to display in the application status bar.
3. If an item within some particular layout is not selected, it means you do not want to customize it and the global settings - if selected within the *Global Layout* - will be used. If the item is not selected within the *Global Layout* either, the settings of the most recently active layout will be used.
4. Set whether the layout is *Private* or *Shared*. If set *Private*, it will not be visible for other users. See 2.2. User Rights [11].
5. Customize each item according to your needs (see below).


### Layout Manager Tools

 **New** Adds a new layout to the list of layouts.


 **Remove** Deletes the selected layout. The first two layouts cannot be deleted.


 **Activate** Makes the selected layout active.

 **Load Default** Loads original settings of the selected pre-defined layout (*Full Screen*, *Docked Controls*, *Measurement*) so that it looks just like after the program installation.

 **Lock Layout** Select a layout component and click this button to lock these elements so that they cannot be moved or closed. To unlock these components, click the button again or right-click the locked layout tab at the bottom and click *Unlock Layout*.

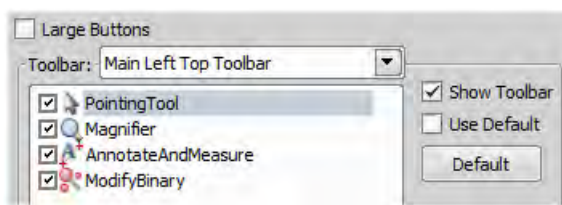
 **Apply to All** Ensures that the executed changes will be applied to all layouts.

 **Import** Enables to load a previously saved set of layouts from an XML file. When you try to import a layout with already existing name, you will be prompted to choose whether: the imported layout replaces the existing colliding layout, or is not imported, or is imported and renamed.

 **Export Layouts** The settings of layouts can be saved to an external XML file. Use this *Export* button. In the windows that appears, define the destination file name and check which layouts will be included in the exported xml file.

## 3.6. Modifying Tool Bars

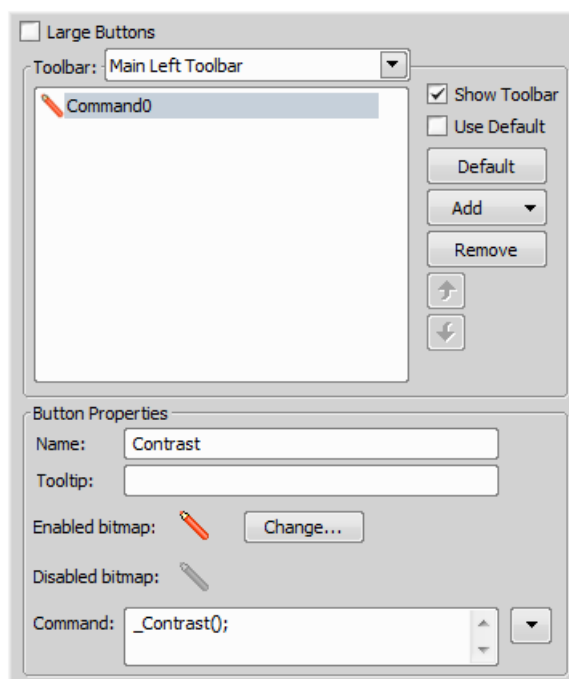
### Hiding Toolbar Buttons



1. Display Layout Manager by the *View > Layout > Layout Manager* command.
2. Select the *Toolbars* item within the list of layouts. The right-side part of the window changes.
3. Choose one of the tool bars which you would like to modify from the *Toolbar* pull-down menu.
4. If selected, de-select the *Use Default* option on the right.
5. Any button of the tool bar may be hidden by de-selecting it. No buttons can be added to any of the tool bars except the *Main Left Tool Bar*.
6. The whole tool bar can be hidden by de-selecting the *Show Toolbar* check box.
7. There are two sizes of buttons available. Select the *Large Buttons* option to use the larger one. This setting is shared by all tool bars.

### Adding Buttons to the Left Tool Bar

Custom user buttons can be added to the main left tool bar. You can define your own buttons which run single macro functions or execute macros . Select the *Main Left Toolbar* from the pull-down menu.



Let's say that we very often use the  *Image > Contrast* command. It is useful to add a shortcut button to the tool bar.

1. Press the *Add* button, and choose *Command* from the pull-down menu:
2. A new command - *Command0* - is added to the list.
3. Now, assign a macro command: Open the pull-down menu on the right side of the *Command* edit box and click *Command List*.
4. A list of commands appears. Choose *\_Contrast()*.
5. Confirm the selection by *OK*.

#### Note

*It is possible to assign a sequence of commands to a single button by repeating this procedure.*

If you are not satisfied with the default icon, you can change it by pressing the **Change...** button. A window for selecting the icon appears. You can select the image from the *NIS-Elements AR* icon set or load some other from any file containing icons (ico, dll). You can define another icon for the command in a disabled state, too.

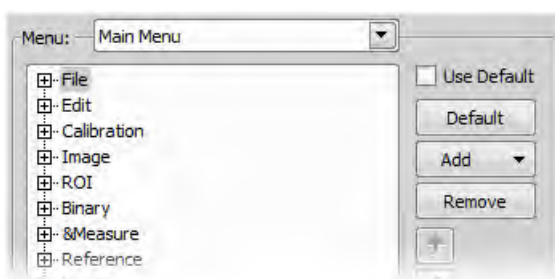
It is handy to define a tool-tip (a text that appears when the mouse cursor is placed over the icon) for your command. Simply write the text into the *Tooltip* box. You can change the position of the command in the tool bar using the arrow buttons. The **Default** button discards your changes and inserts the default set of commands to the tool bar.

## 3.7. Modifying Menus

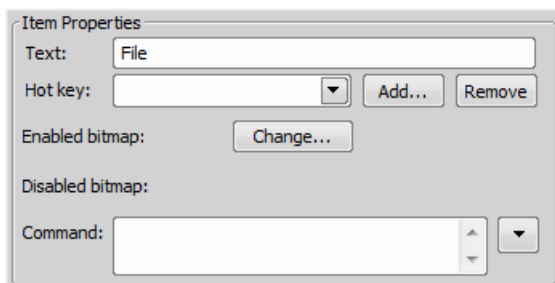
The *Main Menu* and some of the *Context Menus* within the application window may be modified. Items of the context menus can be hidden by de-selecting them similarly to the tool bars. The main menu can be modified as follows:

### **Modifying the Main Menu**

1. Display Layout Manager by the *View > Layout > Layout Manager* command.
2. Select the *Main Menu* in the topmost pull-down menu.



3. Any item may be added to the main menu - a *Separator*, a *Menu Command*, a sub-menu (*Menu Popup*), and even a new menu (*Main Menu Popup*) - by the *Add* button.
4. Select the existing menu item under which you would like to place the new item.
5. Click the *Add* button and select the item to be added from the pull-down menu.
6. Edit the *Item Properties*.



**Text** This is the text which appears in the pull-down menu. You may add "&" before any letter - such letter will be considered a keyboard shortcut when browsing the menu.

**Hot key** One or more hot key shortcuts may be assigned to the command. Just press the *Add* button and press the key combination. Press *Remove* to remove the selected hot key.



**Enabled/Disabled bitmap, Command** These fields serve for assigning a bitmap image and a macro function to the menu command. It works the same way as when modifying the Main Left Tool Bar (described above).

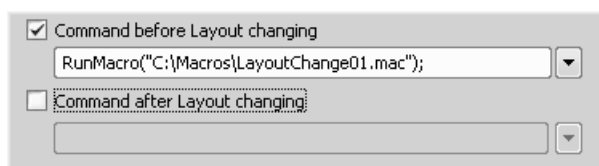
#### Note

*The Default button discards all changes and loads the main menu original configuration. The Remove button deletes the selected item. The arrow buttons move the selected item up/down. The Use Default check box, when selected, applies the default settings to the menu.*

## 3.8. Running a Macro Upon Layout Change


A macro command or a macro can be run upon layout change.

1. Display the layout manager by the *View > Layout > Layout Manager* command.
2. Select the layout by displaying of which the macro function will run and select the *Commands* check box.
3. The following box appears on the right side:



4. Select the timing. The “before” option will run the command when you click on the layout tab, but before actually changing the layout. The “after” option runs the command right after the layout is changed.
5. When the field is enabled, type the command or insert it via the pull-down menu.

## 3.9. Appearance Options

General appearance adjustments can be made in the *Options* window. Run the  *Edit > Options* command and switch to the *Appearance* tab.

**Background** Background of the main screen may use default tiles or custom color.

**Color scheme** There are the following color schemes predefined within the application: Light scheme, Dark scheme, Black scheme.

**Language** Select the language to be used in GUI. The language pack is a part of the 14.17. Local Option [231] feature set.

---

**Prompts on Image Save dialog** Actual words used on buttons of the window which appears if an image has been modified and is about to be closed.

**The Z value displayed in statusbar** Having two Z devices, select whether to display Z1, Z2 or both on the main status bar.

**Close ND Acquisition window after Run** (*requires:Local Option*)

The *View > Acquisition Controls > ND Acquisition* window will be closed automatically upon acquisition start.

**Enable Z intensity control** This option enables the *View > Acquisition Controls > Z Intensity Correction* within *NIS-Elements* user interface.

**Image window tool bars** You can decide whether the image tool bar is integrated to the main tool bar or whether each image has its own. You can also decide the buttons appearance. See 3.2. Image Window [26].

**Auto hide bottom toolbar** This option hides the image status bar and the ND2 control bar (6.3.1. Control Bar [87]) automatically. It is displayed only when the mouse cursor rolls over the bottom part of the image picture.

**Show channel tabs** Show channel names and colors in the image window status bar.

**Show Layout tabs** Show layout tabs in the main status bar.

**Show Task Bar** If unselected, the Windows taskbar will be hidden.

**Default vertical docker on the** (*requires:Local Option*)

Select the preferred side for the default vertical docking pane.

**Keep text size while zooming** When selected, text annotations will not be zoomed with the image. See

 *View > Analysis Controls > Annotations and Measurements*.

**Allow zoom factors lower than best fit** When selected, the image can not be resized to be smaller than displayed using Best fit command.

**Lock camera magnification** If you are used to switch between two camera modes (resolutions), this option ensures that the scene observed does not change its size or position.

**Keep picture window aspect** If checked, the image window respects the image size while zooming.

**Initial Zoom** You can select the zoom factor of the newly opened images. The options are Best Fit, 200, 100, 50, 25%.

**Initial zoom 100% for Live and Capture** Whenever a new Live or Captured window is opened, the zoom will be automatically set to 100%.

## 4. Cameras & Devices

### 4.1. Basic Workflows

#### 4.1.1. Camera Selection on Startup

Let's assume the camera works properly, is connected to the PC with proper system drivers installed and running (if required by the camera).

##### Setting Up the Camera

1)

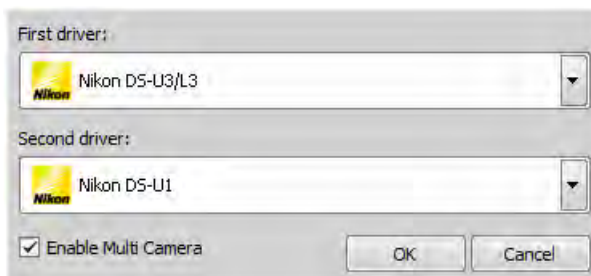
##### Select Camera Driver

You will be asked to select the camera driver every time you launch *NIS-Elements AR*. You can change the driver later using the *Acquire > Select Driver* command. Choose the driver that matches your camera:



##### Multiple Cameras

Two cameras can be connected to one system. If this is your case and you intend to use both cameras, check the *Enable Multi Camera* option and select the *Second driver* from the list.



The following set of buttons appears in the top tool bar when multiple cameras are used:



2)

### **Selecting a Camera**

Color cameras can be used in a monochromatic mode. The actual camera type (color/mono) can be selected by the *Acquire > Select \*Camera Name\** command.

#### Note

*The emulated monochromatic mode is optimized for use with fluorescence specimen where often only a single-color signal is being captured. The following formula is used to compute intensity of the emulated mono image:*

$$I = (I_r * W_r) + (I_g * W_g) + (I_b * W_b)$$

*Where: W is channel weight calculated from the channel histogram and I is channel intensity. W ensures, that channels containing some signal are accentuated while channels without signal are suppressed. As a result, depending on conditions, the brightness of a monochrome image might not change even if the lamp light intensity is changed.*

3)


### **Adjust the Camera Settings**

Exposure time, camera resolution, and other camera-specific features are adjustable from the *Camera Settings* window. To invoke it, use the *Acquire > Camera Settings* command.

## 4.1.2. Optical Configurations

Typically, a laboratory computer image analysis system consists of a computer, a camera, and a microscope equipped with certain accessories (objectives, filters, shutters, illumination, rotary changers, etc). Most of the mentioned microscopic hardware can be motorized and therefore can be controlled via *NIS-Elements AR*. In addition, it is possible to integrate single settings of all these devices into one compact set called *Optical Configuration*. It is recommended to create several optical configurations containing particular devices settings. Then a single click can completely change the current hardware configuration.

### **Creating New Optical Configuration**

- 1) Please check that all the devices (microscopes, cameras, etc.) which you want to associate with the new optical configuration are properly attached to the system and working.
- 2) Choose the  *Calibration > New Optical Configuration* command. In the window which appears, adjust the settings of the devices to match the intended state which will be saved to the optical configuration.

- 3) Type the name of the new optical configuration to the *Name* field. Use a short descriptive name, the name is used on the button in the main tool bar when you select the *Show on toolbar* option.
- 4) In the left column, select which device settings to associate with the Optical configuration.

**Camera setting** A list of the current camera properties appears on the right. It is being updated dynamically.

If you want to use stored ROIs for Camera ROI setting (turn ROI on/off and use the stored ROI value), tick "Use Stored ROI" checkbox in the Camera features box.

**Channel setup** These settings determine how channels of newly captured images will be named and what color will be assigned to them. The available properties depends on the current camera setup (color/mono, triggered acquisition engaged or not, dual view engaged or not, etc.). In the mono camera mode, you can either assign the name, the emission length and color to the channel *Manually*, or leave this task to *NIS-Elements AR* (the *Automatically* option). In such case, information of the light path (emission wavelength) will be used to determine the channel name and color.

**Microscope setting** If there is more than one shutter available and you would like to associate a shutter with the optical configuration, select which one is the *Active Shutter* from the pull-down menu. Select which parts of the microscope shall be included in the configuration by checking them in the *Used devices* dialog box.

---

Note


*Active shutters remember their aperture setting and display it in the Microscope setting section.*

**Objective** An objective mounted to a motorized nosepiece can be included in the configuration. Select the objective from the pull-down menu. Objectives which are currently assigned to any position of the nosepiece are listed. See 4.1.3. Objectives [40].

---


Note

*The objective must be assigned to a nosepiece position via the microscope control pad or the nosepiece control panel beforehand.*

- 5) If some of the device settings still need to be adjusted, click the **Camera & Devices Controls** button and select the appropriate control panel from the pull-down menu. Adjust the settings within the control panel, the optical configuration will be updated automatically.
- 6) Click *Finish* to save the new optical configuration and to close the window.
- 7) You can create more optical configurations by repeating the procedure. The optical configurations are saved to registry immediately. A backup of optical configurations can be made by running the  *Calibration > Optical Configurations* command and clicking the *Export* button.

---

## **Managing Optical Configurations**

To display the optical configurations manager window, run the  *Calibration > Optical Configurations* command. You can make the following actions from the window:

- Create, duplicate, rename, delete, copy settings and switch between optical configurations.
- Modify optical configuration properties.
- Import and export optical configurations to/from an XML file.

## **Operations with Optical Configurations**

Once created, the configuration appears in the list and can be shared with other users by changing the *Private* option to *Shared* (see 2.2. User Rights [11]). The following operations can be performed on the selected configuration:

- It can be deleted by pressing the *Remove* button. A confirmation dialog box appears.
- Its name can be changed by the *Rename* button. A button with the configuration name appears in the tool bar (if the *Show on toolbar* option is selected).
- The configuration settings may be transferred to another optical configuration by the **Copy to** button. Press the button and select the optical configuration to be overwritten with the current one.
- A copy of the configuration can be made via the *Duplicate* button.
- The configuration can be applied to by the *Set As Active* button.
- The settings of all optical configurations can be exported to an external XML file using the *Export* button.
- The previously exported optical configurations settings can be loaded from the XML file via the *Import* button.
- The list of optical configurations can be ordered manually using the arrow-up and arrow-down buttons.
- Each configuration can be arbitrary modified within the right-side portion of the window.

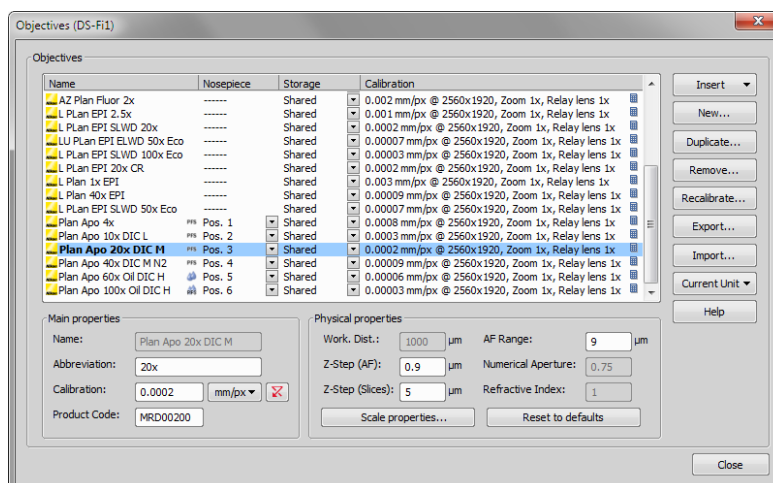
The optical configuration buttons are available in the main tool bar when *Show on toolbar* option was selected during the setting process.


## **4.1.3. Objectives**

If you want to perform measurements on captured images it is wise to calibrate all objectives used to capture images. Whenever an image is captured through a calibrated objective, the image inherits its calibration.

### 4.1.3.1. Managing Objectives

Run the  *Calibration > Objectives* command. The *Objectives* window appears:



A list of used objectives can be created within the Objectives window. For each objective, the objective name, position in the changer, storage status and the calibration are displayed. A *Calculator*  icon next to the calibration value indicates that the calibration has been calculated from objective properties. If the calculator icon is missing, the calibration has been performed manually. Use the buttons on the right side to manage the objectives:

**Insert** Click the button and select one of the objectives from the database (ordered by magnification). Custom objectives can be added to the database via an INI file.

**New** Press this button to create a custom objective. The new objective will be added to the list. Then, define the *Main properties* and the *Physical properties* of the objective.

**Duplicate** If zoom is used, the objective calibration must be re-calculated accordingly. Use this button to make a copy of the objective and define the zoom factor in the window which opens.

**Remove** This button deletes the selected objective.

**Recalibrate** Starts the calibration of the selected objective. See 4.1.3.3. Objective Calibration [42].

**Export** Enables you to export the complete list of objectives to an XML file. The standard Save As window appears.


**Import** This button enables you to import a complete list of objectives from an external XML file previously created by the *Export* button.

**Current Unit** This button invokes a pull-down menu where units for the whole application can be selected.

**Help** Displays a help page to the  *Calibration > Objectives* command.

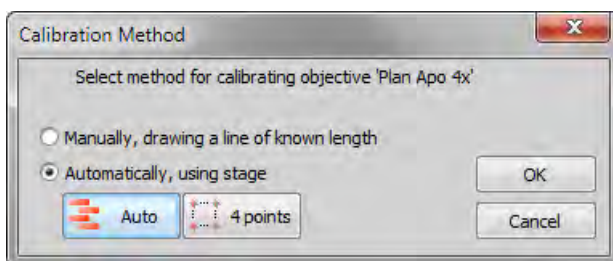
Edit the properties of the selected objective in the bottom part of the window. Press the *Close* button to finish this window.

#### 4.1.3.2. Assigning Objective to a Nosepiece Position

1. To assign an objective to a position or to change an attached objective assignment, click the setup  button in the *nosepiece* section of the microscope control pad.
2. A window appears which enables you to select one of the available objectives to the corresponding position.
3. Each objective has its specifications displayed in the table. These specifications are not editable.

#### 4.1.3.3. Objective Calibration

After you press the *Recalibrate* button in the *Objectives* window or when creating a new custom objective, the following window appears:



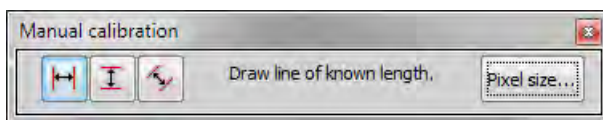
Select one of the calibration methods:

- The *Manual* calibration lets you draw a distance into a picture and assign real length to it (see below).
- If a motorized XY stage is available, the *Auto* and *4 points* automatic methods appear.

Press *OK* to continue...

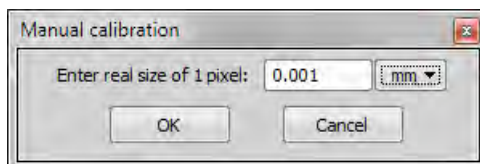
##### **Manual Calibration**

- 1) When performing a manual calibration, the live image starts automatically and the following window appears:





- 2) Select one of the icons to draw a distance to the image. If you know the precise calibration value (px/unit), press the *Pixel Size* button to enter the real size of one pixel:



Enter the calibration value, select units, and confirm the calibration by **OK**. If you do not know the pixel size, you will have to continue with the calibration on live image:

- 3) Insert a calibration slide to the microscope stage.
- 4) The distance is defined by placing lines (Horizontal, Vertical, Parallel) to the image. Choose the orientation of the lines by pressing the appropriate button.

Note

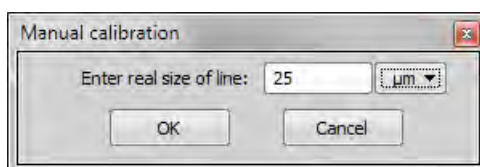
*If you are sure the camera angle is 0, 90, 180, or 270°, it is recommended to select either vertical or horizontal lines. Otherwise, select the parallel lines.*

- 5) Click into the image to place the first line. Place the second line in the intended position by another click.

Note

*You can modify the line position while holding the mouse button. After you release the button no further changes can be made. When "Parallel lines" were selected, draw the first line by clicking twice inside the image. The line can be moved and adjusted arbitrary by mouse. When satisfied, finish the first line by right-click. The second line can be placed by another click to the image, this time only to adjust the distance from the first line. The process is completed by right-click.*

- 6) The following dialog box appears:



- 7) Enter the distance between the two lines and select correct units.

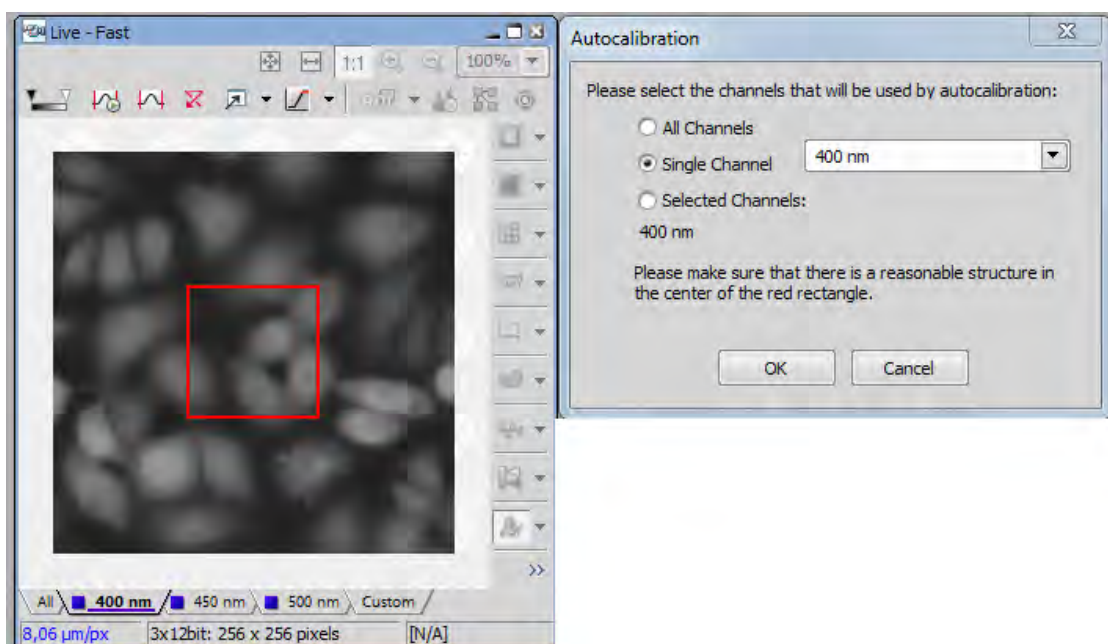
8) Press *OK*. The objective is calibrated now.

### **Automatic Calibration**

The automatic calibration requires a motorized stage. Select one of the following methods:

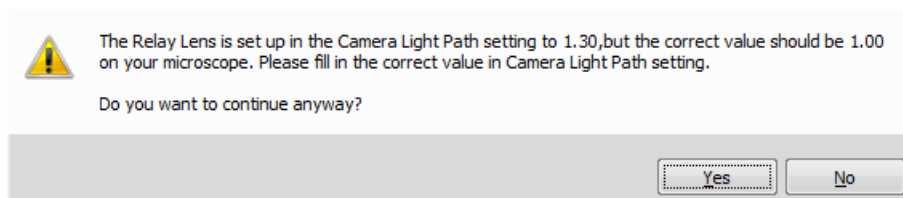
**Auto** The *Auto* method is fully automatic. Calibration is performed on a part of the live image marked by red square that is shown before the calibration is launched. You can also choose the channel to be used by auto-calibration.

*Figure 4.9. Auto-calibration window*



*NIS-Elements AR* moves the motorized stage, acquires two images, and calculates the calibration from the shift of the images. The auto-calibration performs an estimation of the real relay lens zoom factor. If the estimation does not match the value specified within the optical path window, a warning appears.

Figure 4.10. Example of the warning message



It is recommended to cancel the auto calibration (click **No**), check the real zoom factor of your relay lens and check and correct the relay lens settings within the *Acquire > Camera Light Path* command window.

#### Note

*The success of this method depends on the texture of the specimen, its contrast, illumination, etc. If the combination of these factors is unsuitable, the auto calibration may fail. If it fails, try the following:*

- *Move the stage to another area of the specimen to get a better texture.*
- *Improve contrast by setting LUTs.*
- *Check focus, re-focus if needed.*
- *Turn on the *Acquire > Shading Correction > Shading Correction* command ON.*

**4 points** If you select the *4 points* method, the system draws four points on the screen (subsequently) and asks user to move one significant part of the specimen to each position. After all four steps are completed, the calibration is calculated from the moves of the stage.

### **Superresolution Calibration**

This type of calibration is available after successful manual or automatic calibration. It triples the camera's resolution and uses fine stage movements to improve the resulting objective calibration. Click the **Run SR Calibration** button and wait till the objective calibrates.

### **Calibrate Using Objective**

When you have an uncalibrated image, you can use an already calibrated objective to calibrate it. Right-click the *Uncalibrated* field on the image status bar and choose one of the objectives from the *Calibrate using Objective* sub-menu. Of course, the same objective which was used to capture the image shall be selected.

## 4.1.4. Connecting a Device to NIS-Elements

Before you get to work with *NIS-Elements AR*, all hardware accessories should be connected properly to the system. In most cases, the following basic procedure is sufficient to connect a device successfully:

1. Install *NIS-Elements AR*, and select the appropriate device(s) during the installation.
2. Connect the device to the PC and switch the device ON.
3. Run *NIS-Elements AR*, and run the *Devices > Manage devices* command to open the Device Manager.
4. Use the *Add* button to add the device to *NIS-Elements AR*.
5. Select the device from the list of installed devices and press the *Connect* button.
6. Select logical devices to be activated.
7. Configure device-specific settings using the *Configure (physical) Device* and the *(logical) Device Parameters* buttons.
8. Close the Device Manager.

---

#### Note

*MULTIZOOM AZ100M, ECLIPSE LV series, ECLIPSE MA200, or ECLIPSE L200N/300N microscopes can not be connected from NIS-Elements AR while the setup tool of each microscope is active. To connect any of these microscopes to NIS-Elements AR, exit the setup tool and then run the NIS-Elements AR.*

---

#### Renaming of devices

*Either logical or physical device can be renamed by user. Just right-click the device name and select the *Rename Device* command from the context menu. The user-defined names can be handy in two cases:*

- *You are using two logical devices having matching names, but you need to uniquely identify them (e.g. from macro).*
- *You are used to call a device with another name and would like to rename it in order not to be confused by the predefined name any more.*

### 4.1.5. What are “Logical Devices”?

*NIS-Elements AR* handles hardware accessories using the concept of logical devices. There are features of different hardware devices which equal and therefore can be controlled equally. Such features are called “logical devices”. A typical logical device is *Stage XY*. Different microscopes can be equipped with different *XY* motorized stages, although - regarding the user interface - they behave equally. One physical device (a piece of hardware) can contain one or more logical devices the list of which appear in the Device Manager after the connection is established.




#### **Available Logical Devices**

**Analyzer** The analyzer is a polarizing filter placed in the optical path between the specimen and the lamp. The logical device offers two states: ON (inserted) and OFF (extracted).

**Aperture** This logical device is used for controlling apertures in the light path. It is used in complex microscopes rather than as a standalone device. Two parameters can be typically set for aperture devices, the state (ON/OFF) and aperture size.

**Condenser** A condenser is a two-lens combination located next to the illumination source in the optical path. Its purpose is to collect light and direct it to the specimen being examined. The corresponding logical device relates to a changer of different condensers.

**Filter** This logical device controls filter changer movements. There can be several filter changers connected to *NIS-Elements AR* at a time. Each filter changer needs to be set up - filter types shall be assigned to positions of the changer:

1. Display the filter changer control panel ( *Devices > Filters and shutters* or *Devices > Microscope Control Pad* ).
2. Click the settings button , a window appears.
3. Select one of the available positions which the filter will be assigned to.
4. Click the  button, a list of available filters appears.
5. Select the filter name from the list and confirm it by .
6. The filters can be moved within the already defined positions using the *Up/Down* arrow buttons.

#### Note

*When browsing the list of filters, details about the currently selected filter are displayed on the right side of the window.*

**Illuminator** This logical device is used for controlling the specimen illumination remotely. There is no standard dialog box for the illuminator control. Each device handles this logical device via a user interface specially designed for it - typically containing one button for switching it ON/OFF and a slider for regulating intensity.

**Light Path** Some microscopes have more than one port where it is possible to attach a light source or a camera. This logical device can switch the illumination between these ports.

**Microscope** This logical device is used to group standalone logical devices used in certain microscopes. To control the logical devices of a microscope from one control panel, select the *Devices > Microscope Control Pad* command.

**Nosepiece** This logical device serves for controlling microscope objective changers. There can be three nosepiece types attached to a microscope:

- *Manual* - it can not be controlled via the software.
- *Intelligent* - the current nosepiece position is read by the application, but can not be controlled.
- *Motorized* - such nosepieces can be fully controlled via the *Microscope Control Pad* or the *Nosepiece* control panel.

See 4.1.3.2. Assigning Objective to a Nosepiece Position [42] .

**ND Filter** A neutral-density filter is a light absorbing filter whose absorption spectrum is moderately flat. It is used to reduce the illumination intensity within the optical path. The logical device offers two states: ON (inserted) and OFF (extracted).

**PFS** Perfect Focus System - this logical device corresponds to the PFS physical device available with Nikon TE2000/TI microscopes.

**Shutter** This logical device can control shutters installed in your system. This device is handled via the *Devices > Filters and shutters* control panel or straight from the microscope control pad. You can select the type and rename the shutter by running a contextual menu command either within the *Filters & Shutters* control panel, *Device Manager* window, or in the main tool bar.

---

#### Note

*There was a restricted number of shutters which could be connected to the system in previous releases of NIS-Elements AR and they were identified by Type (DIA, EPI, Aux1, etc...). The current version supports identification of shutters by custom Names, but the Type attribute can be still found in some windows (or used in some macro functions). This is to ensure backward compatibility.*

**Zoom** This logical device is used for controlling the zoom factor. Run the *Devices > Zoom Configuration* command to adjust the zoom settings.

**Stage XY** Stage XY enables the movement of specimen within X and Y axes. The system offers user to control stage movements.

**Stage Z** The Z Drive device enables movement throughout the Z axis direction.

**TTL/Analog Input, Output** There are six more logical devices available when the NIDAQ controller set is installed:

TTL Input

TTL Output

Analog Input

Analog Output

Calibrated Analog Input and Calibrated Analog Output.

Each of these logical devices represents a connection to an external device which can receive (send) signals from (to) *NIS-Elements*. The number of available connections depends on the actual NIDAQ card.

## 4.1.6. XY Stages and Z Drives Tips

Please read the following tips for using motorized XY Stages and Z Drives.

### **Motorized Stage Initialization**

A motorized stage being initialized could strike the objective. Before initializing the stage, make sure that the objective is away from the stage. If a motorized Z drive is available, use the *Devices > Objective Clearance* command to prevent this.

## **Setting Software Limits to Stage Movement**

Some microscopes enable you to reduce the range of movement of the motorized stage by setting limits within the configuration window.

- 1) Display the configuration dialog window (within the *Devices > Manage devices* window).
- 2) Move the stage to the position where the limit shall be set.
- 3) Click the appropriate button.
- 4) Set all the limits by repeating this procedure.

### Caution

*This procedure can not be used if you already set some limits and would like to broaden them at the same time. NIS-Elements AR will not allow you to move the stage to any out-of-limits position. What you need to do is to reset the limits within the configuration window beforehand.*

## **Using two independent Z Drive devices**

Workstations can be equipped with two independent Z drive systems, one coarse (slow) and the other fine (fast). Typically, the first one is used for specimen manipulation in Z axis, when changing objectives, etc. , the second one is used for auto-focusing. The following text explains how *NIS-Elements* handle the two Z drives.

**Absolute Z** The current positions of both Z drives (Z1, Z2) and the absolute Z position (sum of Z1 and Z2) are displayed in the main status bar. If there is not enough space to display all these values, only the *absolute Z* value is displayed (Z1 and Z2 still appear in a tool-tip). The absolute Z value appears in the Z Series Setup.

### **Active Z**

The concept of *Active Z* enables user to select the preferred Z drive:

- The active Z drive is used when performing auto-focus.
- The *Devices > Enable Mouse Joystick Z in Live (\*current value\*  $\mu\text{m}$ )* command applies to the active Z.

You can select the Active Z device in the *Devices > Mouse Joystick and Auto Focus Z: \*Z Drive Name\** menu.

### **Regular Z drive + Piezo Z drive**

If you have a regular Z drive and a Piezo Z drive installed, some functionality is added:

- The *Move Piezo Z to* button is added to the *Z Series Setup* window when Piezo Z is selected.
- The *Devices > Keeps Z position and centers Piezo Z* and the *Devices > Move Piezo Z to Home Position* commands appear in the *Devices* menu

The button and the *Devices > Keeps Z position and centers Piezo Z* command provide corresponding functionality. They move the Piezo Z device to its home position, and compensate this shift by moving the second Z drive in order to maintain the absolute Z position.

## 4.1.7. Camera Settings

A digital camera mounted to a microscope port or a macro-scope stand records images of the observed scene on a light-sensitive sensor, and transfers them to a computer. *NIS-Elements AR* supports various cameras differing from each other in resolution, frame-rate, sensor type, etc. Despite these differences, controlling different cameras is similar. The following features are included in the *View > Acquisition Controls > \*Camera\* Settings* window depending on a particular camera type.

### Note

*The complete list of cameras and devices supported by NIS-Elements is available in a separate document.*

**AE Compensation** In automatic exposure modes, the compensation affects how optimum exposure settings (Exposure time and Gain) are calculated. The Compensation value is expressed in Exposure Values (EV). Setting the compensation to + 1.0 EV makes the image twice brighter (e.g. doubles the Exposure time or Gain).

**AE Lock** This option makes the automatic exposure mode to lock the current exposure settings (Exposure time and Gain).

**Analog Gain** Controls strength of the camera analog signal before it is digitized). This setting affects image brightness.

**Auto White Balance (AWB)** The AWB button performs an automatic white balancing. It adjusts the red, green, and blue image components in order to get a neutral white color.

**Averaging** Averaging is a commonly used technique of decreasing noise in the image. In this method 2, 4, 8 or 16 consecutive frames are averaged together.

**Binning** The binning mode provides considerably enhanced camera chip sensitivity by integrating more elements (pixels) together. E.g.: *binning x4* integrates the signal from the area of 4x4 chip elements to one pixel of the resulting image. Smaller resolutions and faster frame rates are achieved using the binning modes.

**Contrast** Affects dynamics of how the luminosity is rendered. There are several modes for different illumination (contrast) scene situation.

**Exposure Mode, Exposure** *Exposure* or *Exposure time* is the time of charge accumulation in a camera chip between two adjacent frames. Prolonging the exposure time increases brightness of the image as well as its quality (there will be less noise).

Exposure mode determines how the Exposure time is calculated. Generally, automatic and manual modes are used:

**Auto Exposure, AE** Calculates *Exposure* time and *Gain* in order to achieve optimal brightness of the scene. Image quality is the priority, therefore longer exposure times are being preferred. In



most cases, the auto exposure can be run once by pressing the *Auto Exposure* button, or continuously by selecting the nearby check box.

---

#### Note

*Depending on particular light conditions, the result of Auto Exposure may not be optimal for image acquisition.*

---

#### Warning

*You can use one optical configuration when working with live image and switch to another optical configuration just for the image acquisition, very often, this is performed automatically i.e. by a macro command. If the "on-acquisition" optical configuration has the Auto Exposure camera mode turned ON, it may produce over- or under-saturated images. Since the Auto Exposure algorithm estimates the optimal exposure time by analyzing several last frames, it is not able to make the estimation correctly if the change of optical configurations would cause significant change in image brightness. To prevent this, we recommend to use Manual Exposure mode in the optical configuration used for capturing.*

**Manual Exposure** User selects both *Exposure* time and *Gain* manually.

**Format - Fast (Focus), Format - Quality (Capture), Resolution** Usually, cameras can provide several image resolutions. *NIS-Elements AR* enables to create two presets of the resolution settings - *Fast* and *Quality*. The formats then differ in image size, and consequently in frame rate (number of frames per second - fps). The higher the resolution is, the lower frame rate can be achieved. Available resolutions depend on the camera type.

---

#### Note

*When switching between the formats while observing the live image, the size of the image on the screen is maintained - the zoom setting is changed instead. Only in some special cases, the behavior changes and the image size is changed instead of the zoom setting which is maintained.*

**Gain** Controls the sensitivity of a camera. Increasing the gain increases brightness of the image, but decreases quality (the more random noise, the more streaky noise and color unevenness), and increases the frame rate indirectly by enabling shorter exposure times.

**Gamma** The gamma correction maps the intensity of live signal exponentially to the gamma parameter. For  $\gamma < 1$ , dark portions of the image are enhanced whereas for  $\gamma > 1$ , image parts of higher intensities are enhanced.

**Hue** Hue shifts image colors across the rainbow.

**Live Acceleration** Would you like to increase the frame rate of the live signal? Move the slider to a desired multiplier. The system then automatically shortens the exposure time (so the frame rate rises), and the loss of intensity is compensated by gain (software multiplication).

**Maximum Exposure** This is a safeguard of the time of the Auto Exposure. For quick exposure, it is convenient not to set this value too high.

**Metering Mode** If this option is available, auto exposure can be calculated with an emphasis on over-exposed peaks (*Peak*) or average pixel intensity (*Average*).

---

**Offset** Sets the brightness of the image. It is a constant additive (positive or negative) changing all pixel values of the image. With negative offset value the dark image areas become pure black. Considering fluorescence microscopy, an appropriate offset setting can create continuous black background and thus help to enhance (together with gain or illumination enhancement) the contrast.

**Overillumination Tolerance** Sets how many pixels should be white after the Auto exposure is performed. Use lower values (0.01%) for very bright (shining) objects (fluorescence). For common bright field, even 1% may be a good value. Optionally, you can define the absolute number of white pixels.

**Saturation** The amount of saturation determines how colors are rendered. More saturation produces richer colors. Less saturation makes the colors gray.

**Scene Mode/Preset** There are presets of camera settings optimized for the specific usage. You can reset the settings by the *Reset* button.

**Asbestos** Asbestos.

**Bioscience/Biological microscopy:**

**B** Bright field.

**D (DIC/PH)** DIC or phase contrast.

**E** Enzyme labeled antibody method,

**F (DF/FL)** Dark-field fluorescence.

**H** Hematoxylin and Eosin stain.

**HAL-Bright Field** Halogen illuminated bright field.

**HAL-ELA** Halogen illuminated enzyme labeled antibody method.

**HAL-HE** Halogen illuminated Hematoxylin and eosin stain.

**LED-Bright Field** LED illuminated bright field.

**Industrial microscopy:**

**C** Circuit boards.

**FPD** Flat panel displays.

**M (Metal)** Metal or ceramic.

**W (Water/IC)** Wafer IC-Chip.

**Neutral** Neutral preset.

**Separate Channel Settings** Having a color camera, influence of the settings described above on each color channel can be adjusted. In such case, three edit boxes with the default values set to 1.0 are displayed below the feature setting. These values determine the influence of the feature on each color channel.

**Sharpness** Some camera settings provide the sharpness control which affects how sharp edges in the image appear. Too much sharpness leads to over-saturated edges.

**Target Maximum Intensity** Restricts the maximum of image intensity after the auto exposure was applied. The value represents a percentage of the whole camera dynamic range.


**Trigger Mode** Sets the exposure method.

**Bulb** The exposure time and frames timing are controlled by external signal connected to the camera.

**Internal** The exposure time and the beginning of each frame acquisition is controlled by the settings in *NIS-Elements*.


**Strobe** In this mode, the beginning of each frame exposure is being controlled by external signal.

**Use Current ROI** The currently defined region of interest, set by the *Define ROI* command, can be switched ON/OFF by checking this item.

**Using Probe** The probe  enables you to determine a small image area that serves as the data source area for LUTs, histogram etc. It also affects the AWB (auto white balance) and the AE (Auto Exposure) features of the cameras which support it. When the probe functionality is not supported, the AWB and AE algorithms are computed from the whole image.

**White Balance** There are usually Red, Green and Blue Gain properties that control how colors are rendered. It is used to eliminate a color cast from white areas.

## 4.1.8. Controlling Illumination Devices

The substance of an illumination device is the light source. The light emission is produced mainly by a laser, diode or a light bulb. *NIS-Elements AR* does not distinguish between wavelength modules of these light sources and all are controlled in a similar manner. Basically you select which wavelength(s) you want to use for illumination and set its light intensity. The selected wavelength modules are turned off until you activate the  shutter button usually placed in the bottom left corner of the control panel.



## 5. Image Acquisition


### 5.1. Introduction to Image Acquisition


Having *NIS-Elements AR* installed and all hardware accessories set up, you can start capturing images. Let's begin with the simplest case.

#### How to capture a single image

- 1) Turn the connected camera and other devices ON and start *NIS-Elements AR*.
- 2) Run *Acquire > Camera Settings* to display the *Camera Settings* control panel.
- 3) Switch camera to the *Live-Fast* mode ( *Acquire > Live - Fast* )
- 4) Adjust resolution of the *Live Fast* mode to get a continuous Live image. It is recommended to set a low resolution in order to achieve high frame rates. This is good when searching the specimen or when focusing manually.
- 5) Adjust *Exposure time* to get a nice image of the scene.
- 6) Focus on the scene.
- 7) Optionally, turn on the camera ROI. The *Define ROI* button appears on the tool bar if the camera ROI function is supported by your camera. See also 5.3. Camera ROI [57].
- 8) Capture the image by running the *Acquire > Capture* command
- 9) A new image is opened automatically and named "Captured".


#### Tools for Handling the Live Image

 **Live - Quality**, **Ctrl** + **+** If a high frame rate is not crucial or if you would like to view the live image exactly as it will be captured, run the *Acquire > Live - Quality* command. It displays the live image in the *Quality (Capture)* resolution. This resolution is used whenever an image is captured.

 **Capture**, **Ctrl** + **-** When pressed, the camera exposure runs till the end of the current frame, and the next frame is captured and displayed on the screen (it is the first frame with the complete exposure after you have pressed the *Capture* button).

If you are using the *Live-Fast* mode and click *Acquire > Capture*, *NIS-Elements* will automatically switch to the quality mode to capture the image. When the image is captured, it is opened on the screen in a new image window.

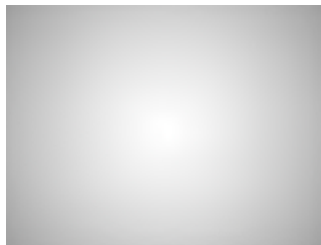
---

 **Freeze**, - The *Freeze* button interrupts the camera exposure, and displays the very last complete frame.

## 5.2. Shading Correction

Shading correction is a method which can correct illumination inhomogeneities of the captured images. How does this work? First of all, a “correction image” must be captured. It is an image which represents illumination intensities within the field of view. Such image is created by capturing a blank scene and can look like this:

*Figure 5.1. Inhomogeneous illumination*



As you see in the image, the illumination intensity is 100% in the center but gets darker near the edges. This can be corrected by applying the shading correction which will - based on the correction image - equalize intensities of the resulting image:

*Figure 5.2. Image acquired without the shading correction*

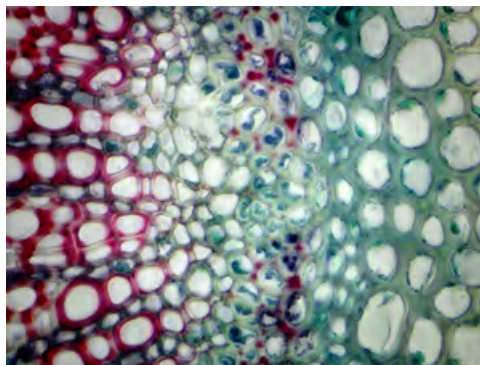
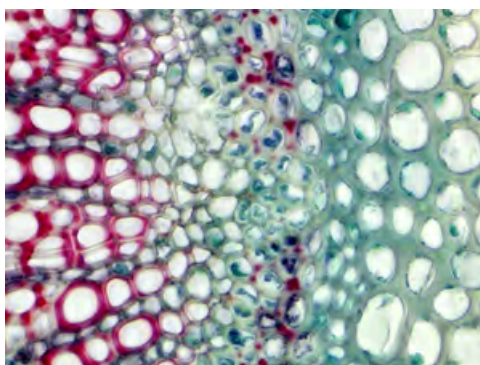


Figure 5.3. Image acquired and corrected



The shading correction can minimize the illumination and background inhomogenities by subtracting a blank scene from the current image.

### **How to setup a shading correction**

- 1) Decide whether to use just a single shading correction image or different image for each optical configuration. Make the selection via the *Acquire > Shading Correction > Shading per Optical Configuration* commands.
- 2) Get the correction image. You can either acquire a new image by the *Acquire > Shading Correction > Capture Correction Image* command or use an existing one. To use some existing image, open it and call the *Acquire > Shading Correction > Use Current Image As Shading Correction* command. In both cases the correction image will be kept in memory. To view the correction image, run *Acquire > Shading Correction > Show Correction Image*.
- 3) In case of “per optical configuration” shading correction, repeat this procedure for with each optical configuration turned ON.
- 4) The *Acquire > Shading Correction > Shading Correction* is enabled if only the correction image is available for the current acquisition settings. For example, if the *Shading per Optical Configuration* is chosen but the correction image for the current OC has not been captured yet, the *Acquire > Shading Correction > Shading Correction* stays disabled.

## 5.3. Camera ROI

You can specify a camera ROI on a live image by selecting the *Acquire > Camera ROI > Define ROI* command. The live image is then restricted to the defined area whenever the **ROI** button is pressed.

Figure 5.4. Turn Camera ROI ON/OFF

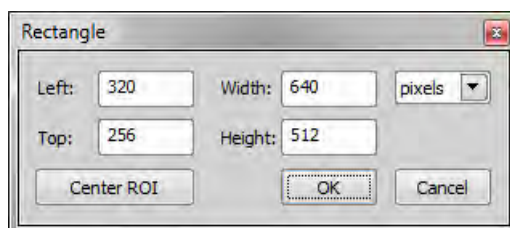


The camera ROI can be also saved to a file for later use. This can be done by the *Acquire > Camera ROI > Save ROI* command. When the time comes, load the saved camera ROI by the *Acquire > Camera ROI > Load ROI* command. The current ROI will be overwritten.

The same **Define ROI** button appears in the toolbar of a captured image too. It does not matter whether you define the ROI on the captured or live image, the setting is shared.

### **Camera ROI Definition**

- 1) Run *Acquire > Camera ROI > Define ROI*. The following window appears:



- 2) A ROI frame appears in the image at the same time. You can either change ROI dimensions or/and position using a mouse cursor, or you can enter precise numerical values in the dialog window.
- 3) If you would like the ROI to be placed in the center of the camera chip, click **Center ROI**. The ROI will move to the center of the live image.
- 4) Confirm the action by the **OK** button.

---

#### Note

*NIS-Elements may adjust the ROI size automatically after the user sets it. Such automatic adjustment is needed in order for the ROI to work in different resolutions of the Live Fast and Quality Capture formats.*

---

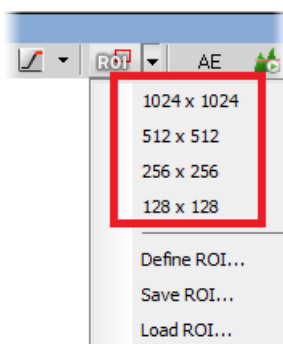
#### Caution

*Nikon DS cameras have fixed-sized camera ROI, therefore width and height of the ROI cannot be changed. Only its position can be adjusted within this window.*



### Predefined Camera ROI

If you are using Andor Neo/Zyla or Hamamatsu Flash 4.0, 2.8, you can choose the Predefined Camera ROI in the ROI pull down menu, offering several predefined frame sizes.



## 5.4. About ND Acquisition

*NIS-Elements AR* being a multi-purpose imaging system can be used as a handy tool to study objects, live organisms, processes, etc. The universal ND2(N-dimensional) file format is what makes this possible. One ND2 file can contain multiple images organized according to what type of acquisition they came from. There are the following acquisition types:

#### Note

*Each acquisition type gives a name to the corresponding dimension of the resulting ND2 file. For example, we can speak about two-dimensional TZ ND2 file, which means the file contains the Time-lapse dimension and the Z-series dimension.*

**Time-lapse - T** A sequence of images can be captured over a period of time and create a time-lapse image. See 5.5. Time-lapse Acquisition [61].

**Multi-point - XY** Images from different areas of the slide can be acquired. See 5.6. Multi-point Acquisition [65].

**Z-series - Z** Several techniques utilize Z-stacks of images. Such Z-stack can be for example converted to a 3D model of the specimen. See 5.7. Z-series Acquisition [69].

**Multi-channel - λ** Fluorescence signals can be captured selectively using excitation and emission filters, each of them creating a different color channel. See 5.8. Multi-channel Acquisition [71].

**Large image - M** (requires:4D/6D Acquisition)

When the area of interest exceeds the camera field of view, you can capture a large image composed of multiple image frames and let it be stitched by an automatic algorithm. See 5.9. Large-image Acquisition [72].

An nd2 file can be created (captured) either containing one dimension or combining several of them.

- Single-dimensional documents can be created automatically (using motorized accessories) or manually using commands from the *Acquire* menu.
- To create a multi-dimensional image, use the *Applications > 6D > Define/Run ND Acquisition* command (ND module required).

### **Common ND Experiment Options**

The following options can be applied to all types of ND experiments:

**Experiment** You can enter a custom name of the experiment. Also name of the phase is editable.

**Path** Browse to a folder where your ND2 images will be stored.

**Save to File** Please see 5.11.2. Save to File [75].

**Custom Metadata** (*requires:Local Option*)

Check this option to add custom metadata. The created metadata can be changed after acquisition. Display image properties ( *File > Image Properties*), display the *Custom Metadata* tab and click the **Modify Description** button.

**Record Data** Please see 5.11.3. Recorded Data [76].

**Auto Focus** Automatic focusing can be used during the experiment. You can select the auto-focus method that best meets your needs. It can be determined whether the focusing shall be performed at the beginning of the whole experiment or at the beginning of each phase. The *Define* button shows a window where you can define parameters of the selected focusing method.

#### Note

*If the auto-focus is set to be performed Before each Phase and the ND Experiment contains just a single time phase, the automatic focus will not be performed. To run auto focus, use the At the Beginning option.*

**Closing Shutter** The active shutter can be closed between single image acquisitions. Just select the *Close active Shutter...* option in the experiment window.

**Running Macro Commands** You can define a command (or a macro) to be run in various stages of the experiment. Select the timing in the *Advanced* section of the experiment window and enter the command to be run.

**In/Out signals** (*requires:4D/6D Acquisition*)







An ND2 experiment can control some accessory devices and can be controlled by another accessory devices at the same time. This can be achieved by sending/receiving Analog/TTL signals through an NIDAQ card. If the NIDAQ PCI card is installed, an extra tab called *Inputs/Outputs* appears within the ND experiment window.

**Load** Use this button to for a configuration XML file to be loaded. The file with the current configuration can be created by the *Save* button.

**Save** Click this button to save the current configuration of this dialog to an XML file. A standard *Save As* window appears.

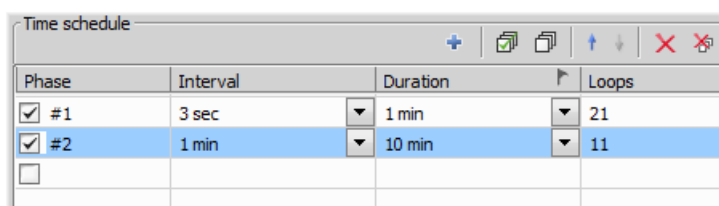
## ND Sequence Options

The following options can be applied to time, multipoint and multichannel sequences in ND experiments:

- + New** This button adds new step into the sequence.
-  **Select All** Use this button to select all steps in the sequence.
-  **Clear Selection** Use this button to clear all steps selection.
-  **Move Up**,  **Move Down** These buttons moves the current step up/down in the sequence.
-  **Remove Current** This button removes current step from the sequence.
-  **Remove All** This button removes all steps from the sequence.

## 5.5. Time-lapse Acquisition

Detailed studying of long-lasting processes is enabled by the time lapse acquisition mode of *NIS-Elements* AR. The achievable experiment duration is limited by the hardware abilities of your PC only. Invoke the *Acquire > Capture Timelapse > Capture Automatically* command to set the experiment up:



Phase	Interval	Duration	Loops
<input checked="" type="checkbox"/> #1	3 sec	1 min	21
<input checked="" type="checkbox"/> #2	1 min	10 min	11
<input type="checkbox"/>			


The *Time schedule* table enables you to define consecutive time phases where duration, interval between single images, and number of images of the phase can be adjusted. The *Interval*, *Duration*, and *Loops* settings are bound together, so you just need to set two of these parameters. The remaining parameter is calculated automatically. Only the selected time phases will be captured.

The flag mark indicates the Duration/Loops priority. For example: if the camera exposure time exceeds the defined *Interval* between frames, then the experiment settings are not achievable - only the marked column will be pushed to be correct. You can set the priority column by clicking on its caption.

### Special Options

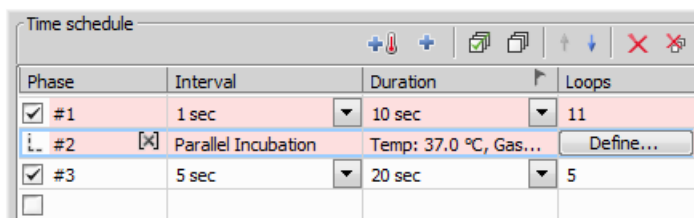
The following options can be used with this type of ND acquisition. Options common to all types are described here.

#### Incubation *(requires:Devices)*

This option is available when an incubator is properly connected. Press the  *Add New Incubation* button to add incubation phase to the time schedule list. You can connect the incubation phase to the previous time phase which makes the incubation run parallel with the selected time phase. To connect

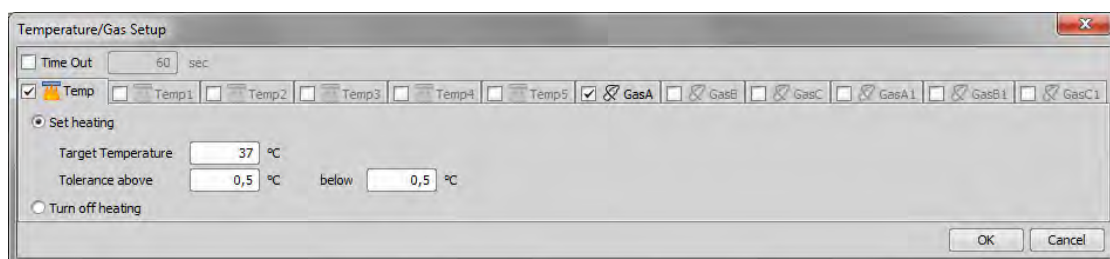
an incubation phase, press the up arrow button that appears in the Phase column next to the phase name. To cancel the connection, press the cross button that has replaced the arrow button.

Figure 5.8. Connected Incubation Phase



Phase	Interval	Duration	Loops
<input checked="" type="checkbox"/> #1	1 sec	10 sec	11
<input checked="" type="checkbox"/> #2	Parallel Incubation	Temp: 37.0 °C, Gas...	Define...
<input checked="" type="checkbox"/> #3	5 sec	20 sec	5
<input type="checkbox"/>			

The *Duration* column displays value defined in the Incubation Phase Settings window that appears after you press the *Define* button. Incubator parameters (temperature and gas) can be set within this window.



**Switch Transmitted Illuminator off When Idle** If this option is selected, the lamp is turned off whenever there is a long-enough gap between two acquisitions.

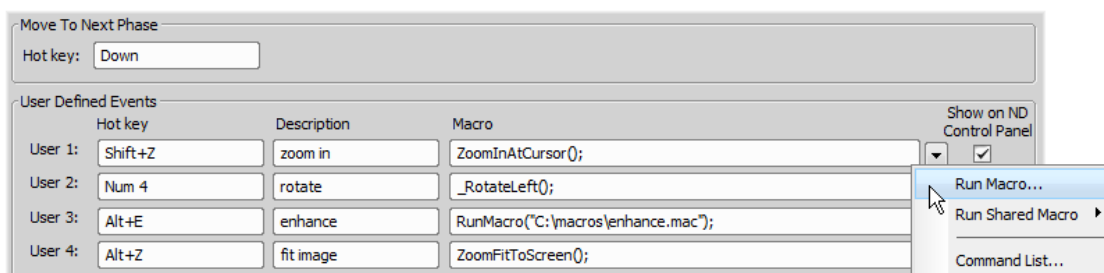
#### Warning

*It takes a little time for some lamps to reach the set intensity. In such case, we recommend to use the `Wait()` ; macro function before the capture is performed.*

**FRET** Checking this item turns on the FRET View for nd2 file which will be acquired. You can open the FRET View dialog by pressing the *Define FRET* button.


**Use HW sequencer** This option is available only for A1 confocal microscope. If checked, A1 HW sequencer will be used for acquisition. It provides the ability to define two experimental setups for HW Sequencer on and off.

**Events** Press the **Events...** button to display the following window:



Define user hot keys which run user events during time-lapse acquisition. Simply click inside the *Hot* key field of the User events window and press any key combination you want to assign to the event. Then you can enter the event description. A macro command can be specified which will be executed when the event occurs (triggered by user). When the event occurs, the specified command is run and a marker is placed to the image sequence.

#### Tip

- Check the *Show on ND Control Panel* option for any event to be displayed it in the *View > Acquisition Controls > ND Control Panel* window.
- In the *Selected User Event* pull down menu at the bottom of this window, choose one of the events which will be inserted to an ND2 image sequence every time the  *Insert Default User Event* button is pressed. See *Event and Selection Controls* [89].

#### Note

When the time measurement runs on live image, an ND acquisition is in progress, or the Time ND document is active, the hot keys of the defined events take priority over all other hot keys.

### Keep Object in View (requires:Local Option)

This function automatically finds the object of interest based on the correlation between the previous and the next frame and always keeps it in the center of view using the microscope stage to compensate the object's motion.

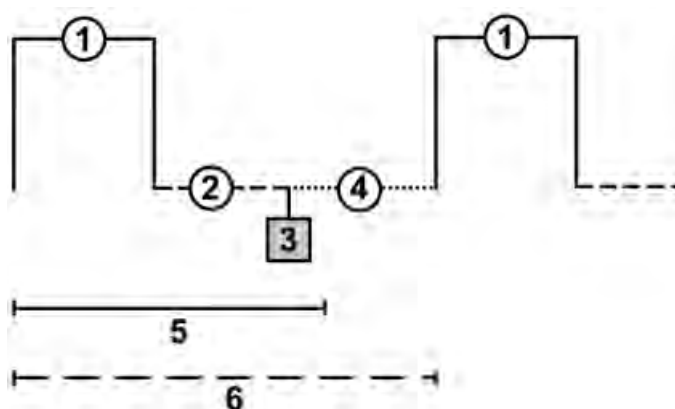
Start by checking the *Keep Object in View* check box and clicking **Settings** to specify the channel on which the object of interest is visible and will be followed. Move your object into the center of your field of view. Adjust your camera and illumination setting to highlight the object. High contrast and low noise image bring the best results. Now you can run the ND Acquisition. The stage is automatically moved in between capturing frames so that the object of interest is always centered.

## 5.5.1. Timing Explanation

When performing fast Time-lapse acquisition (capturing images in short intervals), it is important to understand how the acquisition with digital camera works.

**Case #1: No delay** If the interval in the experiment is set to "No delay", the camera runs in "internal hardware trigger mode" (i.e.: Timed mode, Streaming mode). The camera sends maximum frames to the computer. This may result in a situation where the computer is not capable of receiving all the frames the camera sends. If so, depending on the camera type, the overflowing frames may be omitted.

**Case #2: Interval is too short** The time needed to receive one frame consists of three phases: exposure time, readout time and software overhead delay.

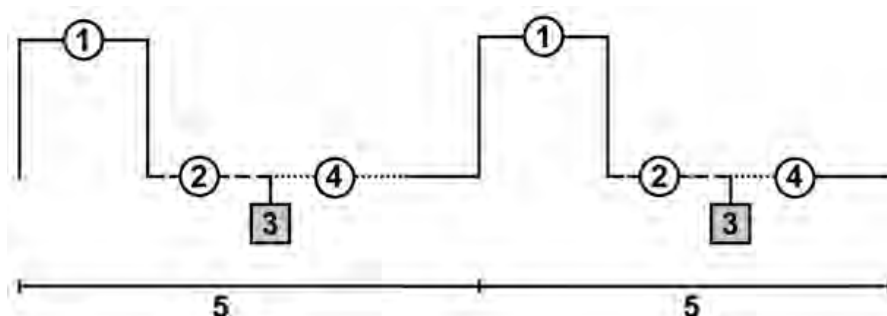


#### **Legend:**

1. exposure time
2. readout time
3. camera sends data to PC
4. software overhead time
5. specified interval
6. resulting interval

The camera runs in a "software triggered mode" where it gives the frames much slower than in "no delay" because it is being synchronized with other events. In this case it is synchronized with timing of the time-lapse acquisition. If the interval between frames is set to a shorter time than is the sum of the three phases (e.g. 50 msec), some latency will occur. As a result, the interval in the image sequence is prolonged to (exposure time + readout time + SW overhead).

**Case#3: Interval is sufficient** The camera runs in a "software triggered mode". If the interval is sufficient, the timing in the resulting image sequence will equal the timing specified within the acquisition window.

**Legend:**

1. exposure time
2. readout time
3. camera sends data to PC
4. software overhead time
5. specified interval

## 5.6. Multi-point Acquisition

This window serves for defining XY(Z) points to be scanned during the multipoint capture experiment. This feature is available when a motorized stage XY(Z) is present in the system. Check the *Include Z* box to display the Z column. The list of defined points can be saved (and loaded later) to an XML file by the *Save (Load)* button. Run the *Acquire > Capture Multipoint > Capture Automatically* command.

Points ☐ Move Stage to Selected Point

+ Add

Point Name	X [mm]	Y [mm]		PFS	Camera	Z-Stack
<input checked="" type="checkbox"/> #1	-22.080	36.500		N/A		
<input checked="" type="checkbox"/> #2	-> -21.080	35.500	Offset All X,Y	N/A		
<input checked="" type="checkbox"/> #3	-18.080	36.500		N/A		
<input type="checkbox"/>						

☐ Include Z
 ☐ Include Z-Stack
 ☐ Include Labels

Optimize Copy Paste Load... Save...
 ☒ Include Camera Settings
 ☐ Include ROIs Definition

### 5.6.1. Point by Point (Manual) Multi-Point

1. Move the stage to the first position.
2. Press the **+** *Add New* button. A new line containing current coordinates of the stage appears in the list.
3. Move to the next position and repeat the steps until you have all the intended points defined.

### 5.6.2. Well Plate (Rectangular) Multi-Point

You can insert a pattern designed to cover a wellplate. Of course it can be used to create any rectangular pattern.

- 1) Click the **Custom** button. The following window appears
- 2) Select *Manual* if you know the distances between wells. Otherwise select the *Interactive* and continue.

**Manual** This method will create a pattern which will scan *Rows* x *Columns* fields. The *Distance X* and *Y parameters* specify distances between two fields. The scanning will begin at the current position.

**Interactive** The interactive method lets you specify the number of fields to be scanned and starts a wizard where you will specify the top-left and the bottom-right corner of the well plate. The distances between wells are calculated automatically.

- 3) Click **Finish**.

### 5.6.3. Large Image (Covering) Multi-Point

A Multi-point which will cover certain area can be created:

- 1) Click the **Custom** button and select the *Large Image* tab
- 2) Set the large image settings:

**Scan Area** Select whether to define the *Scan Area* by number of fields or the actual field size.

**Camera** Select the camera to be used. This field is enabled only if you have a dual-camera setup.

**Objective** Select the objective for which the multi-point will be calculated. The resulting number of fields / area size changes depending on the selected objective magnification.



**Overlap** Overlap of neighboring images in %.

- 3) Click **Finish**.

### 5.6.4. Random Multi-Point

(requires: *Local Option*)

Specify the area where a random set of points will be generated.

- 1) Click the **Custom** button and select the *Random* tab.
- 2) Select shape of the area to be filled with random points

**Rectangle** The area will be defined by a rectangle. Define X and Y coordinates of left top corner of the area, width and height of the area.

**Radius** The area will be defined by a circle. Define X and Y coordinates of center of the circle.

- 3) Specify *Number of points* in the multi-point set.
- 4) Click **Finish**.

### 5.6.5. To Change a Single Z Coordinate:

1. Make sure the *Move stage to selected point* is pressed. This button ensures that the motorized stage moves to the coordinates of the currently selected point.
2. Click inside the line you would like to change.
3. Move the Z drive to the new position.
4. Click the <- button.

Note

*The XY coordinates of one point cannot be adjusted (unless you delete it and add a new point).*

### 5.6.6. Special Options

**Autofocus** None, Steps in Range, Steps in Continuous.

**Custom** Displays a tool for creating predefined multi-point patterns.

---

See 5.6.2. Well Plate (Rectangular) Multi-Point [66], 5.6.3. Large Image (Covering) Multi-Point [66], 5.6.4. Random Multi-Point [67].

**Execute Command after Capture** You can either Run Macro, or select a command from the Command list. The command is executed after capturing.

**Execute Command before Capture** You can either Run Macro, or select a command from the Command list. The command is executed before capturing.

**Include Z** If selected, also the Z coordinate will be taken into account during XY(Z) acquisition.

---

Note

*This option is disabled if the Relative XY option is used.*

**Leave PFS offset ON between points** Check this item to keep PFS on while moving between the points.

**Move Stage to Selected Point** This button ensures that the motorized stage moves to the coordinates of the currently selected point.

**Offset All**

The **offset all** button can shift the XY coordinates of all points in the same way:

1. Make sure the *Move stage to selected point* is pressed. This button ensures that the motorized stage moves to the coordinates of the currently selected point.
2. Select one point of the list.
3. Move the XY(Z) stage to a new position (define the offset).
4. Press the *Offset All* button.
5. The coordinates of all points change - the same shift which you made with the stage is applied to them.

**Optimize** If the *Optimize* button is pressed, the system will re-order the defined points in order to minimize the XY stage trajectory.

**Point name** Displays the name of the point. You can change the name (default names are #1, #2 #3, etc.).


**Redefine reference Z after Auto Focus/PFS** Check this option to redefine reference Z position after performing autofocus or PFS (if AF or PFS is turned on).

**Relative XY** Check the *Relative XY* item to consider all coordinates as relative with respect to the current stage position. Any of the points may be used as the reference point, just right-click it and select *Set this point as a reference position*.

**Split Multipoints** Select this box to perform the *File > Import/Export > Split Multipoints* command directly after the ND2 experiment. (*requires:Local Option*)

---

Note

*When you apply the Split Multipoints command on ND acquisition that contains only the XY dimension, the information about the ND dimensions in the  File > Open dialog becomes unavailable.*


**Use Focus Surface** Uses Focus Surface for capturing.

**X, Y, Z** Displays X, Y and Z coordinates of the point. The arrow button assign current position of Z stage to the point. The *Offset All Points* item shifts the X and Y (Z) coordinate of all points in the same offset which is defined as a difference between current stage coordinate and coordinate of the current point.

## 5.7. Z-series Acquisition

Automatic capturing of images from different focal planes using a motorized Z drive can be performed. Run the *Acquire > Capture Z-Series > Capture Automatically* command to display the setup window. There are three different approaches of how to set the experiment:

### Top and Bottom, Step

- 1) Press the  button, the cube turns blue.
- 2) Run the Live camera signal. In case you have two Z drives, select the Z device from the pull-down menu.
- 3) Set the Z range: move the Z drive to the top position and press the *Top* button, move it to the bottom position and press the *Bottom* button.
- 4) Define the *Step* size in  $\mu\text{m}$  or the number of steps to be captured.

#### Note


*In this mode the Home position is assigned automatically to the middle step (e.g. the third step out of the whole 5 steps). The Reset button discards the Top, Home, and Bottom positions settings.*

*Depending on the direction of Z-acquisition, the last Z position (top or bottom) may slightly differ from the user setting. However, you can select which position will be preserved exactly. Right-click one of the Top/Bottom buttons and select Keep exact Bottom/Top Position. The setting is indicated by underlining the button text.*

### Home Position, Range

- 1) Press one of the following buttons, the cube turns yellow. The range is defined by the home-position and the scanning range:

 **Symmetric mode** Define the *Range* by inserting a value in  $\mu\text{m}$ .

 **Asymmetric mode** The range is specified by two values, *Below* - the distance below the home position, and *Above* - the distance above the home position.

The  **Relative** button resets the *Home* position value and the Z range is calculated relatively.

- 2) Run the Live camera signal. In case you have two Z drives, select the *Z device* from the pull-down menu.
- 3) Move the Z drive to the position which you would like the Z drive to move around. Press the *Home* button to define the home position.
- 4) Specify the scanning *Range* .
- 5) Define the *Step* size in  $\mu\text{m}$  or the number of *steps* to be captured.

---

Note

*When you set the Home Position in the Top/Bottom or Symmetric mode, its value is kept after switching to the Asymmetric mode.*

### **Special Options**


**Defining TIRF position** When the TIRF system is enabled, the TIRF option appears in the right portion of the window. To set the TIRF position:


- Select the *TIRF* check box to enable TIRF.
- Move the Z drive to the correct TIRF Z position.
- Click the *Set TIRF Pos...* button.

### **Using TIRF**


- Enable TIRF by selecting the *TIRF* check box within the Z-Series experiment settings
- At least one value of the *Z Pos* column of the Multi-channel acquisition setup has to be set to *TIRF* (See 5.8. Multi-channel Acquisition [71].).

**Piezo Z** If a Piezo Z drive device is connected, the *Piezo* button appears in the panel. Use the pull-down menu to select the action which will be performed upon pressing the button:

 **Keeps Z position and centers Piezo Z** This option moves the Piezo Z drive to the home position, but compensates the movement by the second Z drive in order to keep the original absolute Z position (sum of Z1 and Z2).

 **Move Piezo Z to Home position** Moves the Piezo Z drive to the home position regardless of the absolute Z drive position.

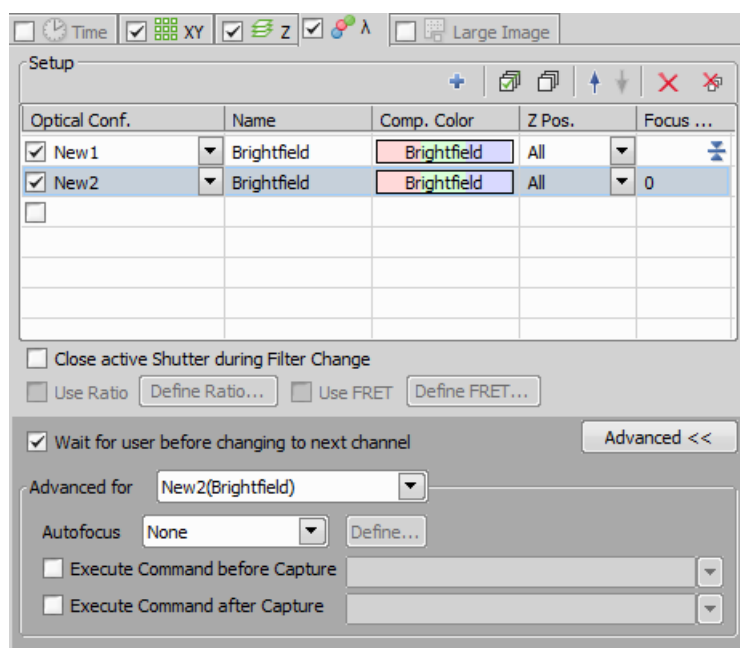
**Direction** You can define the Z-stack scanning direction - either from *Top to Bottom*, or vice versa (*Bottom to Top*).

**Two Z devices** Two Z devices (Main Z + Piezo Z) can be used for Z-Series acquisition and  Autofocus task in Jobs. Z device combination is used automatically if AF/Z-Stack range exceeds the range of Piezo Z. Combining two Z devices speeds up the Z stack acquisition, however the user can still decide whether or not to use the Z device combination. Only the Main Z device is used if it is selected in the Z device combo box. Only the Piezo Z device is used if it is selected in the *Z device* combo box and the

defined *Range* is within the device range. Combination of both Main Z and Piezo Z is used if Piezo Z is selected in the *Z device* combo box and the defined *Range* is bigger than the device range.

## 5.8. Multi-channel Acquisition

Fluorescence (multi-channel) pictures can be acquired. Run the *Applications > 6D > Define/Run ND Acquisition* command and select the *Lambda* tab. There you can set the multi-channel experiment.



### Channels Setup

Specify how many channels are to be captured. Set a descriptive name of each channel and select the optical configuration that is to be used for its acquisition. If no suitable configuration exists, you can create a new one by selecting the *<define new>* option from the pull-down menu in the Optical Configuration column. The *Comp. Color* column specifies the color tone, in which the channel will be displayed. The *Focus Offset* column enables parfocality adjustments for different filters (wavelengths).

### Special Options

**Camera** When you are in a multi camera mode, you can select the camera to capture each channel.

**T pos.** If there are T dimensions engaged in the experiment, another column called T Phase appears in the table. You can reduce the number of captured channels by selecting *First* or *n-th* (the channel is included in the first or every n-th time phase only).

**Z Pos.** If there is a Z dimension engaged in the experiment, another column called *Z Pos.* appears in the table. You can reduce the number of captured channels by selecting the *Home* option - the channel will be captured within the home position of each Z series only. Also, if a *TIRF* system is available, the *TIRF* option appears as an option. If selected, the channel will be captured within the TIRF position of each Z series only (the TIRF position can be set within the Z-Series acquisition setup window).

**Merge Cameras** Available only in multi camera mode. When using two cameras, two nd2 files will be created by default. If the *Merge Cameras* option is selected, only one nd2 file is created. This nd2 file contains the maximum parameters from the two cameras (width, height and bit depth per component).

**Stretch Camera images to Same Size** Available only for multi camera mode. When the two cameras differ in resolution, the camera images will be stretched to the same size.

---

Note

*The settings of the T phase and Z Pos. columns can be combined in any way, but at least one channel must be included in every T or Z loop. In other words, the All option must be selected at least once in each of the columns.*


### **Advanced Options**


**Wait for user before changing to next channel** The application waits for the user confirmation before changing to next channel.

**Advanced for** Choose the color components.

### **Manual Capture**

The *Acquire > Capture Multichannel Image > Capture Manually* command runs the live image and displays a new toolbar with the *Capture* button. After pressing the *Capture* button one channel is acquired. The number of channels depends on the settings in the *Acquire > Capture Multichannel Image > Multichannel Setup* window. After all channels are acquired, the *Recapture* button replaces the *Capture* button. It enables you to capture the multichannel image again. All previously obtained multichannel data will be lost. To recapture a single channel, select it by mouse and press the *Recapture* button. When satisfied, press the *Finish* button.

 **Capture >>** This command opens a new image window with the live image and the *Capture* button. After pressing the *Capture* button one channel is captured. The number of channels depends on the settings of the *Acquire > Capture Multichannel Image > Multichannel Setup* window.

 **Recapture** After all channels are acquired, the *Recapture* button replaces the *Capture* button. It enables you to capture the multichannel image again. All previously obtained multichannel data will be lost. When a single channel is selected, it can be recaptured separately.

**Finish** Press the *Finish* button to complete the multichannel capturing.

## **5.9. Large-image Acquisition**

*(requires:Local Option)*

There is a special option in the ND2 acquisition window, to capture a Large Image. A motorized XY stage is required to have this option enabled. When set to work, it captures several frames within a defined area and stitches them together, so you get an extra large image.

#### Note

When camera ROI on Live Image is on (see: 5.3. Camera ROI [57]), only its frame defining the sensor area is used during the scanning.

### Scan Area

The area can be defined in three ways:

**Define by setting an array of frames** Check the first radio button and define the number of rows and columns of the scanning grid.

**Set the Area Size** Check the second radio button and define the size of the scanned area in millimeters.

**Pattern** Click the **Browse** button and select an XML file containing a large-image pattern. This XML can be created using the **Acquire > Grab Large Image Free Shape** command.

#### Caution

Do not mistake it with similar XMLs created by other functions. For example the XML files saved from the **Acquire > Capture Multipoint > Capture Automatically** command window would not work.

### Stitching

Define the stitching method:

**Stitch** The system automatically stitches the images together to form a large image using *precise stitching (Image Registration)*. If there are more channels available, select which one to use from the pull down menu.

**Do not Stitch** The system will stitch the images together without using the *Image Registration* algorithm. Stitching can also be done manually after the acquisition finishes via the **File > Stitch Large Image from Files** command.

**Use ... for Stitching** Select which channel will be used for Large image stitching.

**Overlap** Enter the percentage of image overlapping which is used for image stitching.

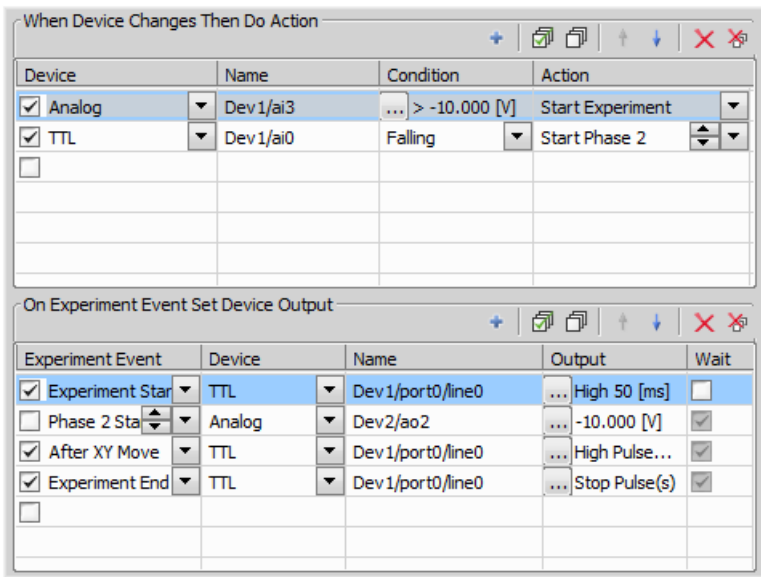
**Close Active shutter during stage movement** Check this item to close the shutter during stage movement.

## 5.10. Inputs/Outputs Control

(requires:Local Option) (requires:Devices)

This special option in ND acquisition enables to control the experiment from outside (section *When Device Changes Then Do Action*) and also allows the experiment to give feedback (section *On Experiment Event Set Device Output*). A special device (NI DAQ controller set) is required to run this option.

Figure 5.16. Input/Output Example



An example of an input: When voltage in dev1/ai3 line of an analog input is higher than -10 V, then the experiment starts.

An example of an output: When experiment starts, a high TTL type of signal which lasts 50 ms is given through Dev1/port0/line0 line.

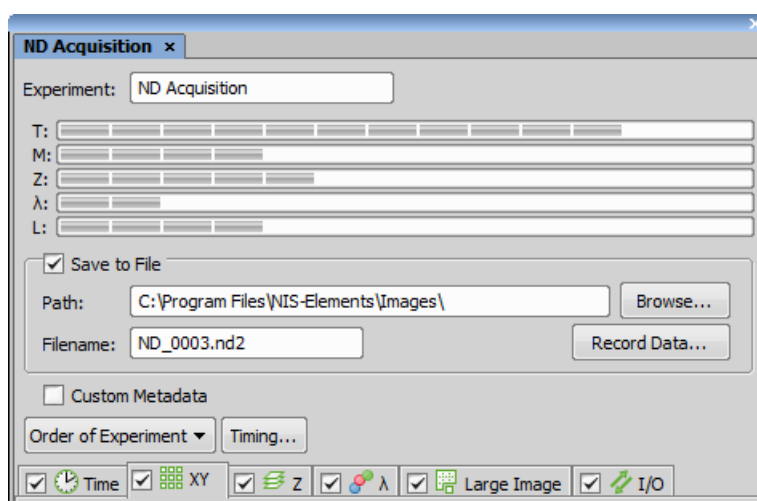
## 5.11. Combined ND Acquisition

The dimensions can be combined together creating an N-dimensional image as the result:

1. Run the *Applications > 6D > Define/Run ND Acquisition* command.



2. The following control panel appears:



3. Select one or more dimensions (tabs) which should be included in the resulting ND2 file. Above the tabs, there is a preview of how the nd2 file structure will look like.
4. Press the *Order of Experiment* button and select the item which defines the desired order of loops in the nd2 file.
5. Set the parameters of each dimension acquisition.
6. Select whether to save the nd2 file directly to a file or just to keep it in memory to be saved afterwards. To do so, select the *Save to File* check-box. The path and the file name shall be defined if saving to a file is preferred.
7. Click the *Run now* button to start the acquisition.

#### Note

*When the experiment is finished, a window may appear saying "Please wait. Finishing ND experiment, writing image data to the harddisk". The Cancel button within this window enables you to abort saving of the file. Only the data which has already been physically written to the resulting ND2 file will be available (this also concerns the Recorded Data).*

### 5.11.1. Z Intensity Correction

The **Run Z corr** button works similar to the **Run now** button, but the acquisition uses Z intensity correction Defined within the *View > Acquisition Controls > Z Intensity Correction* window.

## 5.11.2. Save to File

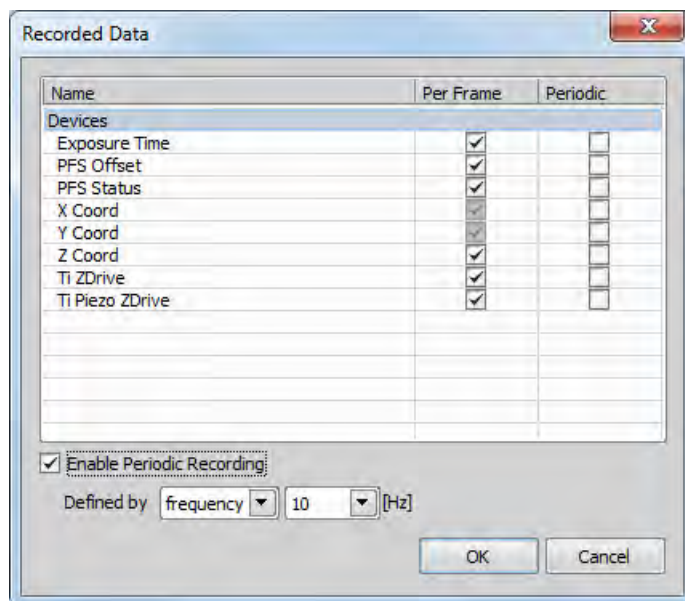
An image can be captured and saved manually after the experiment is finished, or it can be saved to harddisk “on the fly”:

- Check the *Save to File* box. The window reveals an extra section.
- Insert the *Path* or use the *Browse* button to define the destination folder.
- Specify the *Filename*.

## 5.11.3. Recorded Data

The nd2 file contains various *Recorded Data* which carry e.g. acquisition times of single frames. Some of them can be recorded optionally:

- Press the *Record Data* button. The following window appears:

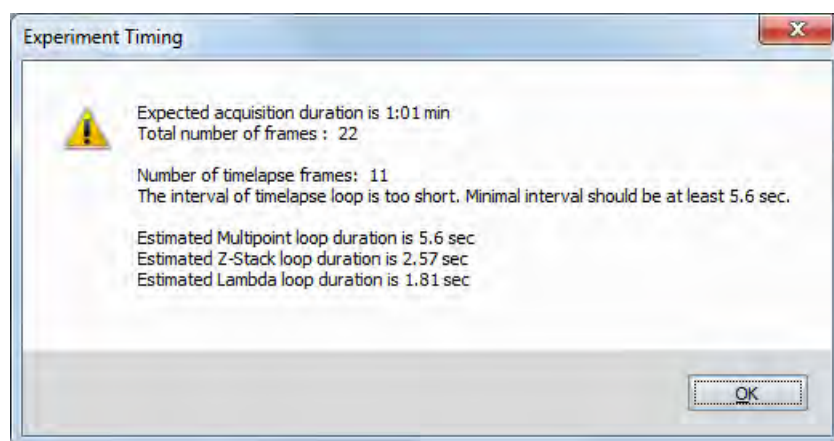


- Select whether to record the data for each frame (*Per Frame*) or with a certain *Frequency* or *Time*.
- The data to be recorded shall be check-marked.
- To view the recorded data of an opened ND2 file, invoke the *File > Image Properties* command and switch to the *Recorded Data* tab.

### 5.11.4. Experiment Timing (*requires:Local Option*)

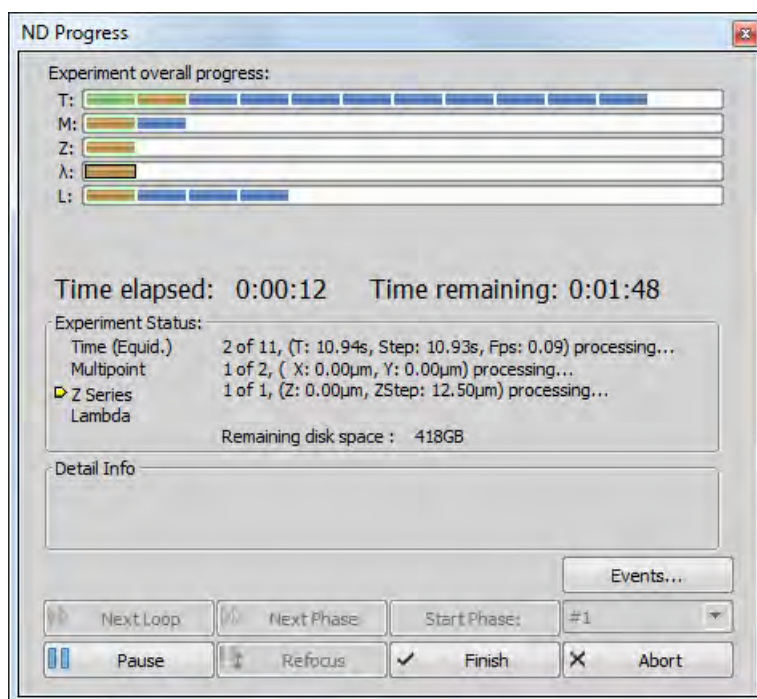
This button shows the window with expected acquisition duration, total number of frames and number of timelapse frames.

Figure 5.19. Experiment Timing window



### 5.11.5. ND Experiment Progress

The following window appears while the experiment is running:



The window displays the experiment overall progress, time elapsed from start of the experiment, estimated remaining time, remaining free space on the disk where the captured nd2 file is being saved, and detailed messages about the experiment work-flow such as: “waiting for next loop”, “stage moving” etc.

You can control the running experiment by the following buttons:

**Next Loop** When the time interval of one time loop is long enough, the system captures all the included dimensions and waits for the scheduled beginning of the next loop. In such case the *Next Loop* button becomes enabled and you can click it to start the next time loop ahead of the schedule.

**Next Phase** After you press this button, the experiment jumps to the beginning of the next time *Phase* after the current loop is finished.

**Start Phase** Use this button to start the time phase selected in the combo box. Select the phase first and press the *Start Phase* button.

**Pause/Continue** The experiment can be paused by the *Pause* button. Let it go on by pressing the same button.

**Refocus** If there is a need to focus manually, press the *Refocus* button. It pauses the ND experiment and runs *Live* camera signal.

**Finish** This button stops the experiment and saves captured data.

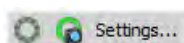
**Abort** Use this button to stop the experiment without saving the captured data.

**Events** If a remarkable event occurs during the experiment, the user can press a shortcut key and the information will be saved to the resulting nd2 file. You can define the key-shortcuts after you press the **Events** button.

See Special Options [61].

## 5.12. Capturing to Ring Buffer

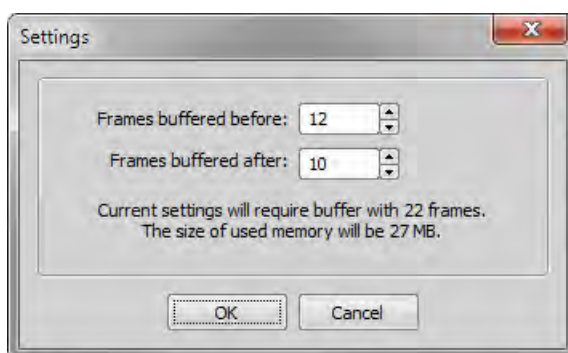
The Capturing-to-RAM technique enables user to record sequences displaying very quick actions - lasting tens of milliseconds. Make sure that the RAM Capturing buttons are displayed on the *Grabber & Devices* ToolBar (see 3.3. Arranging User Interface [29]), or use the corresponding commands.




- *Acquire > RAM Capture > Circular Buffer ON/OFF*
- *Acquire > RAM Capture > Capture*
- *Acquire > RAM Capture > Settings*

The technique uses a ring buffer to store temporary data. The ring buffer is a part of virtual memory that is being constantly (and repeatedly) filled with the live image data. Depending on how fast the action to be captured is, you should set the ring buffer size (in seconds).


1. Run the *Acquire > RAM Capture > Settings* command. The following window appears:




2. Set the *Time buffered before/after* values. These values represent the time interval before and after you run the *Acquire > RAM Capture > Capture*  command. Image frames from this time interval will be included in the resulting image sequence.
3. Confirm the settings by the *OK* button.

---

### **Turn the Buffer ON**


- Turn the circular buffer ON by the *Acquire > RAM Capture > Circular Buffer ON/OFF* command to enable buffering of the Live image to RAM.
- The ring buffer functionality gets activated. The circular buffer button  (if displayed on the toolbar) indicates the buffer activity.

### **Capture the Image Sequence**

- Press the *RAM Capture*  button or run the *Acquire > RAM Capture > Capture* command.
- A new image window containing the captured image sequence opens.

#### Note



---


*If you press the **Ram Capture**  button while in the Frozen mode or with the circular buffer turned OFF, only the frames of the Time buffered after interval will be captured.*

## 6. Displaying Images

### 6.1. Opening Image Files


*NIS-Elements AR* offers several ways to open an image file, using either:

**The  File > Open command** To invoke the Open dialog box where you can select the file to be opened, run the  *File > Open* command. This command is also called when you click the *Open* button on the main tool bar

**Organizer** An image can be opened by double clicking its filename within *Organizer*. Run the  *View > Organizer Layout* command **F10** to switch to the Organizer. See the 6.8. Organizer [103].

**Recent Files List** You can quickly access the last opened images using the *File > Recent Files* menu.

**Open next/previous/first/last Commands** These commands enable you to continuously open the subsequent images from a particular directory or a database table. The *File > Open/Save Next > Open Previous*, *File > Open/Save Next > Open Next*, *File > Open/Save Next > Open First*, *File > Open/Save Next > Open Last* commands may be used.

**Auto Capture Folder** The *Auto Capture Folder* is a control panel that can be displayed by calling the *View > Acquisition Controls > Auto Capture Folder* command. It displays images within a selected folder. To change the folder click the  button in the top left corner of the control panel and browse for another folder. Any image of this folder can be opened by a double-click.

**Any File Manager** During installation, *NIS-Elements AR* creates file associations to files that are considered its native format for storing images (JPEG2000 , ND2). The JPEG2000 (JP2) and ND2 image files can be then opened in *NIS-Elements AR* just by double clicking their names within any file manager or the desktop.

#### 6.1.1. Switching Between Loaded Images

Commands for managing the opened images are grouped in the *Window* menu. The recently opened files are listed in its bottom part. The currently displayed image is indicated by the selected radio button. To change the current image, select it from the list or use the *Next* or the *Previous* commands (represented by **Ctrl + Tab** and **Ctrl + Shift + Tab** shortcuts).

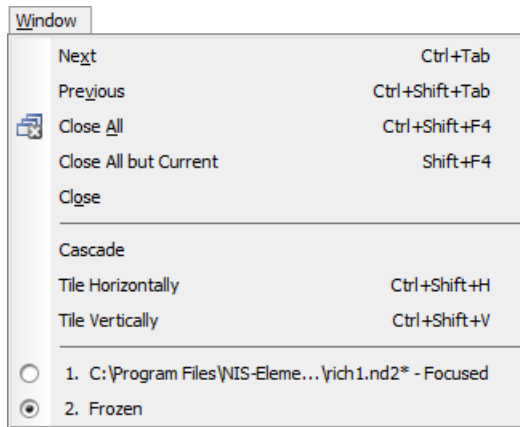


Image windows can be also arranged automatically using the *Tile horizontally* or *Tile vertically* commands. This will change the size and position of the opened documents and they will be arranged next to each other in the selected direction.

## 6.1.2. Options for the Open Next Command

This window enables you to configure the *File > Open/Save Next > Open Next* command properties. Run the *Edit > Options* command and switch to the *Open Next* tab.

### Open from file

**Directory** Specifies the directory with files to open.

**Files of Type** Filters the files to open by the image format.

**Order By** This pull-down menu enables you to select one of the image properties as the ordering criterion. The button toggles the alphabetical order.

**Prefix** Filters the files to open by prefix. You can click to show advanced filtering options window. The button turns the advanced filter on/off.

**Next file** Defines the name of the file that will be automatically opened after pressing OK. You can see its name on the right side of the box.

**Open from Database** These options equals the ones described above.

**Limit Number of Image** Limit the number of documents, which will be opened and accessible in the Opened Images tab. The maximum is 24 opened documents.

**Defaults for this Page** Pressing this button restores the default settings of this window. All your changes will be lost.

**OK** Confirms all the changes and closes the window.

**Apply** Saves the changes which are applied, but the window remains opened.



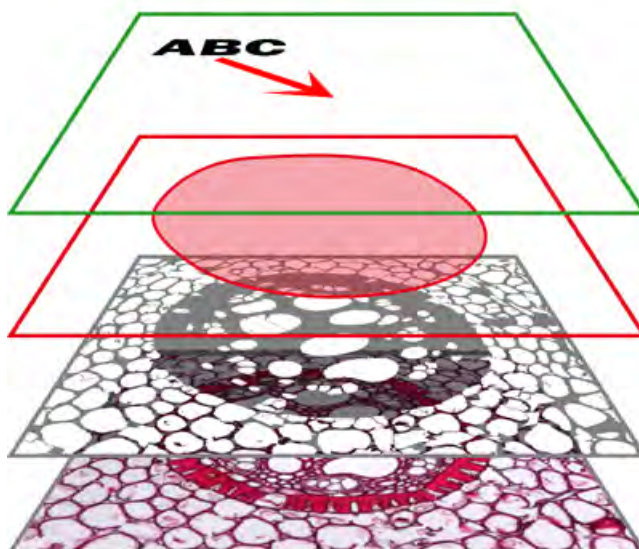
**Cancel** Discards all the changes and closes the window.

**Help** Displays relevant help page.

## 6.2. Image Layers

### 6.2.1. Introduction to Image Layers

The following image layers can be saved within a *NIS-Elements AR* image.



**Annotation layer** In this layer, vector objects are stored. The results of manual and automatic measurements, text labels and other annotations can be included.

**Binary layer(s)** The binary layer is usually the result of thresholding. By thresholding, you distinguish objects of interest from background. A picture of night sky can make a nice example - shining stars may be “thresholded” and compose the binary layer. Then, by means of automatic measurement, you can e.g. count them. More than one binary layer can be placed over one image.

**ROI layer** ROIs (regions of interest) is a strong tool for distinguishing objects from background similar to the binary layer. The advantage over the binary layer is that ROIs are vector objects and therefore provide different way of manipulation. See 7.6. Regions of Interest - ROIs [131].

**Color layer** The color layer contains the image data captured by a camera. It can handle images with the depth of up to 16 bits per color component. The dimensions of this layer determines the dimensions of the other layers.

---

#### Note

*When saving an image, only some file formats are capable of saving all the layers. The other image formats will save the content of the color layer only. See 6.12. Supported Image Formats [113].*

## 6.2.2. Image Types

### **RGB Images**

Images acquired by a color camera typically consist of three components that represent red, green and blue channel intensities. You can display a single color channel using the tabs located in the bottom-left corner of the image window. Or, an arbitrary combination of them can be selected while holding the Ctrl key down.



### **Multi-channel Images**

These documents usually arise from fluorescence microscopy. Instead of 3 color components (RGB), multichannel images can be composed of arbitrary number of user-definable color channels.

---

#### Note

*If there is a image that contains more than 8 components, the tabs in the bottom left corner of the image are replaced by the wavelength dimension, similarly to how other dimension loops of nd2 files are displayed.*

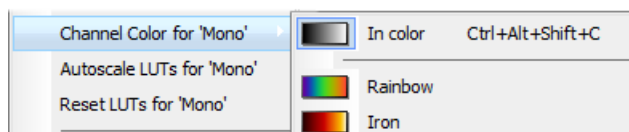
### **ND2 (multi-dimensional) files / datasets**

An nd2 file is usually a sequence of images. One ND2 file can contain multiple images organized according to what type of acquisition they came from. There are the following acquisition types, also called dimensions available: Time-lapse, Multi-point, Z-series, Multi-channel, Large-image. The dimensions can be combined together.

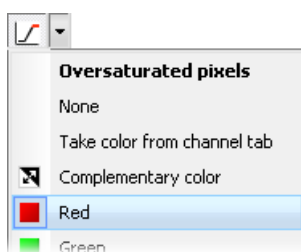
Please see 6.3. Navigation in ND2 Files [87], 5.4. About ND Acquisition [59]

## 6.2.3. Displaying Image Layers

- Single channels and monochromatic images can be displayed using a predefined color scale. Right click the “channel tab” in the bottom left corner of image window and select a color scale from the *Channel Color for...* sub-menu.



- Over-saturated and under-saturated pixels may be highlighted in the image. Color of these pixels may be selected from the context menu of the *Pixel Saturation Indication* button. The button is placed on image tool-bar. Select one of the following options for each group of pixels referring to *Oversaturated* and *Undersaturated* pixels:



**None** The pixel saturation will not be indicated.

**Take color from channel tab** Color which has been assigned to each channel will be used for display. See 6.2.4. Assigning Colors to Channels [85].

**Complementary color** Colors complementary to the display color of each channel will be used in order to ensure good visibility of the highlighted pixels.

**“Color Name”** Several basic colors are pre-selected in the menu.

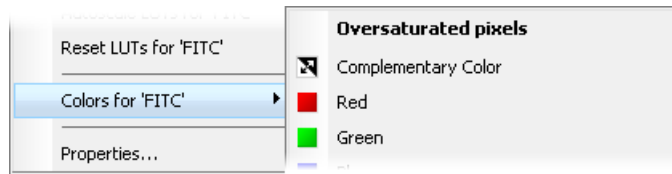
You can turn the saturation indication ON and OFF by clicking the button.

## 6.2.4. Assigning Colors to Channels

Despite the color in which a channel is displayed, other per-channel colors can be assigned to it. This regards colors for:

- Indication of over-saturated pixels (the color will be used with the *Take color from channel tab* option).
- Indication of under-saturated pixels (the color will be used with the *Take color from channel tab* option).
- Display of a binary layer “attached” to a particular channel. (the color will be used until the binary layer is detached from the channel).

To select these colors for each channel, right-click the channel tab and select it from the *Colors for '...'* sub-menu.



## 6.2.5. Copying Channels by Drag and Drop

Drag one of the channel tabs and drop it:



- to another image, so it will become a multichannel image;
- inside the *NIS-Elements AR* application window in order to create a new image

### Note

- *Even the All and RGB tab can be copied.*
- *When extracting a channel from Live image, it does not freeze the camera signal.*

### **ND2 files**

- 1)** Drag one of the channel tabs and drop it by the *left mouse button*. It behaves the same way as if performed on a single image.
- 2)** Drag one of the channel tabs by the *right mouse button* and drop it to the application screen. A context menu appears that enables you to select whether to create the new image from all frames or just a single frame.
- 3)** Two *nd2* files that have matching structure can be *merged into one* easily. E.g. two single channel Z stacks with the same number of Z positions can be merged into a single two component ND2 Z-stack by dragging one into the other.
- 4)** Drag one of the channel tabs of a *single image* and drop it to an *nd2 file*. The channel will be copied to every loop of the ND2 file.

### Note

*This behavior, too, takes place when copying channels that do not have matching structure (just the current frame is copied to every position of the target ND2)*

## 6.2.6. Shifting Image Channels

The currently selected channel(s) can be shifted using the **CTRL + SHIFT + Arrow** keys. All channels are visible during the operation so you can see the complete image.

The procedure also works on nd2 files equally to other image processing performed on ND2 files:

1. Select the image frame and the channels to be shifted.
2. Perform the shift using **Ctrl+Shift+Arrows** keys.
3. A standard ND2 image processing window appears (see 7.1.2. ND2 Files Processing [115]).
4. Select which part of the nd2 file to apply the shift to, and confirm it by **OK**.

## 6.3. Navigation in ND2 Files

### 6.3.1. Control Bar

When an ND2 file is opened, its structure is pictured at the bottom of the image window. There is a time line with all captured images indicated by gray markers. The blue-highlighted marker indicates the currently observed image. Below the time line, loops of each dimension are indicated by rectangles. In case the multi-channel dimension is included and the number of channels does not exceed 10, the channels are indicated by the color tabs at the very bottom of the image window.

Browse the nd2 file by clicking inside the time line. You can also display a single loop by selecting the corresponding blue rectangle. There are some examples, how the nd2 file control bar can look like in different cases:

Figure 6.8. A T/Z/multi-channel image with some loops selected.

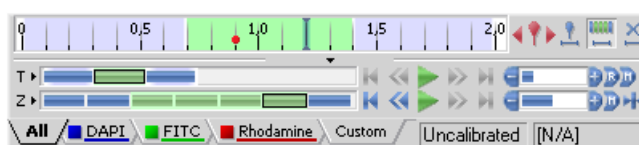


Figure 6.9. A T/Z/multi-channel image containing 11 channels and the time line hidden



Figure 6.10. An HDR multi-exposure image



## Playing Controls

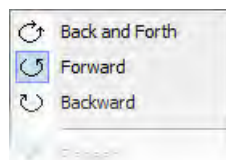
- ▶ Play Sequence** Plays all images of the dimension at selected speed. If a selection is applied, only the selected images will be included in the playback.
- ⏏ Stop Playing** Stops playing the sequence at the last displayed frame.
- ◀ Previous Position** Displays the previous frame of the dimension.
- ▶ Next Position** Displays the next image of the dimension.
- ⏮ Decrease/Increase Playing Speed** Changes the playback speed by one step down/up
- ⏭ Real Time Playing Speed** Sets the playing speed to real-time (as the image was captured).
- ⏮ Maximum Playing Speed for Every Frame** Sets the playing speed to maximum while the display of every frame is guaranteed (when the speed is set to maximum by the + button, some frames are usually omitted when playing the sequence depending on your graphic card).
- 🏠 Home Position** Displays the frame of the Z dimension that was set as “home” during the acquisition.

## Tips



- Right-click the selection to invoke a context menu. The selection can be adjusted, deleted, or the nd2 file can be cropped.
- Detailed info about dimensions will be displayed after you click the leftmost button of the control bar (T>, Z>, ...).
- Place the cursor over one of the dimensions. A tool-tip which displays statistics of the dimension appears.

## Playing options


Right click the speed bar to display the following context menu and select the playing mode.




*Backward* selects the direction of playing the image sequence. When you check the *Stop on Events* option and then press the ▶ *Play Sequence* button, a dialog window appears once the first user event

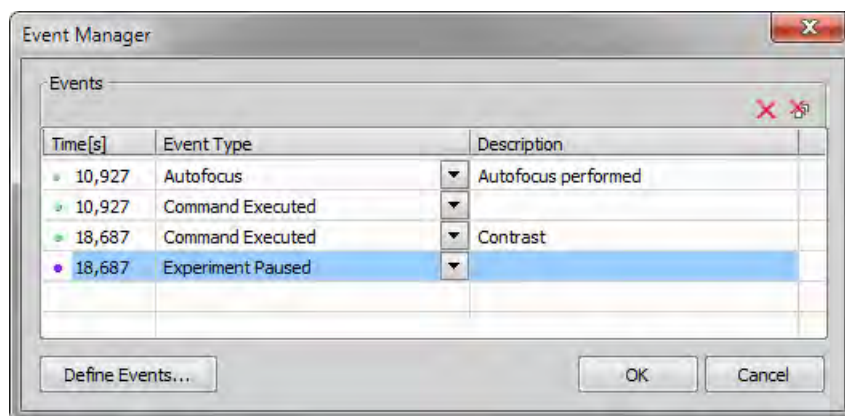
is reached. The dialog window displays information about the reached user event. Use the *Continue* button to continue playing to the next event, or press the *Stop* button to stop the playback at the current frame. Within the window, you can also select the *Do not ask again in this session (Always stop)* option, the playing will stop automatically on every user event but the window will not appear. *Repeat* command sets the repetitive infinite playback. *Fast Advance* command corresponds to the  **Maximum Playing Speed for Every Frame** button described above. *Real Time* command corresponds to the  **Real Time Playing Speed** button described above.

## 6.3.2. Events




User events can be pinned to any of the frames of the image in order to highlight a remarkable scene or action. There is number of event types. Some of them are inserted automatically while the ND experiment is running, others must be defined by user (by the  button). You can manage the events from several context menus which appear when you right-click:


- The time axis
- The  *Insert Default User Event* button
- One of the event markers


All the context menus contain the *Event Manager* command. Open the *Event Manager* to manage events within the current nd2 file. The time can be put in, and the event type selected from a combo box. See 5.5. Time-lapse Acquisition [61].



### Event and Selection Controls


-  **Insert Default User Event** This button inserts an event marker to the current frame.
-   **Move to Previous/Next User event** Click these arrows to browse through the frames where user events occur.

 **User Event Selection** This button selects frames of the ND2 file according to the *Event Playing Range* settings - right click this button and pick the *Event Selection Range* command from the context menu to view/modify them. A simple window appears. Define the number of frames/seconds which will be selected around each event.

 **Range Selection** Clicking this button selects a user defined range of frames. The selection is done by mouse. A range can be selected by mouse while holding the **Shift** key down. Single frames can be added/removed while holding the **Ctrl** key down. Right-click the navigation bar to invoke the context menu. A selection can be adjusted, deleted, or the nd2 file can be cropped.

#### Note


*ND2 files may contain extremely large number of frames so it may be helpful to omit some of the frames from the selection. Right click the Range Selection button and a context menu appears. It enables you to select every 2nd, 5th, 10th, 20th, 50th, 100th frame. The Select All Frames option selects the whole range of frames as if you have selected the first and the last time phase with the **Shift** key down, but preserves the Select Every... setting.*


 **Disable Selection** Press this button to de-select all frames of the nd2 file.


## 6.4. ND Views


There are several views which can display the nd2 files in various ways. Some views are available for some dimensions only. If a view is available for two or three dimensions of the nd2 file, a pull-down menu appears in the top image tool bar. There you can select the dimension to be displayed.


#### Note


*When you switch the view, a new image window opens by default. This behavior can be changed within the  **Edit > Options** command window so that only one view of an image will be opened at a time.*

 **Main View** When you open an ND2 file, it opens in this view.

 **Maximum/Minimum Intensity Projection View** These projections analyse all frames of one dimension and pick pixels with the maximum/minimum intensity values. These pixels are used in the resulting image. (Requires Z or T dimension)

 **Slices View** This view displays orthogonal XY, XZ, and YZ projections of the image sequence. (Requires Z or T dimension).

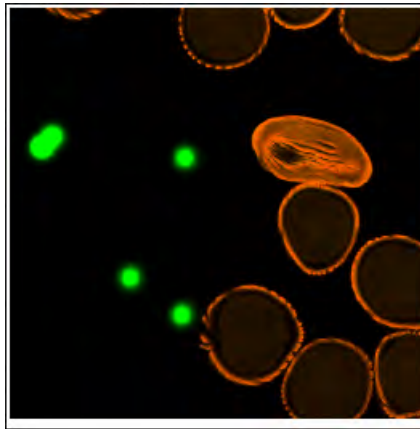
 **Volume View** This view creates a 3D model of the acquired object. (Requires Z dimension)

 **Tiled View** This view displays frames of the selected dimension arranged one next to other. (Requires Z, T or XY dimension)

One or two dimensions can be viewed at a time.



*Figure 6.13. Main View, Max/Min Intensity Projection View*



*Figure 6.14. Volume View*

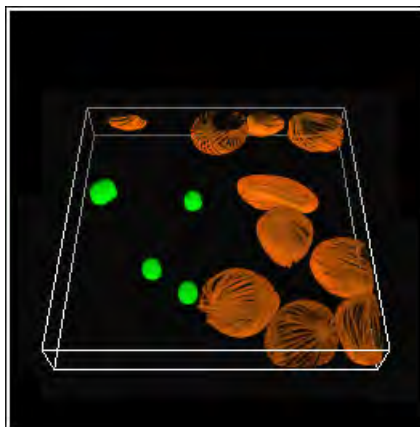


Figure 6.15. Slices View

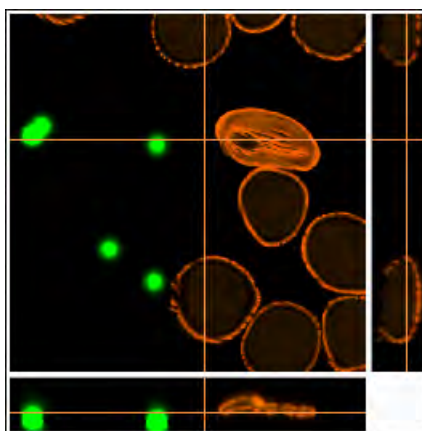
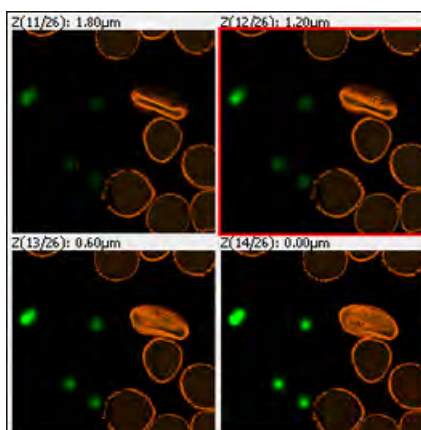


Figure 6.16. Tiled View



### 6.4.1. ND2 Information

Information about current frame of an ND2 document or live image can be displayed using the *Show ND Information* command from the image contextual menu. It displays information about current frame of the ND2 document or live image. All possible information (metadata) are displayed by default.

You can edit which information is displayed in the *Label Properties* dialog window: Right click the ND2 information field and select the *ND Info Properties* command. Use options and tools in this window to customize the display of the ND information - you can change text properties, type of displayed information, accuracy of the displayed number up to three decimal places, format of the information, duration of display and other.

## 6.5. Large Images

### 6.5.1. Opening Files in Progressive Mode

If a single image (or one ND2 frame) is too big and it can not be loaded to RAM in one piece - you will be asked whether to open it in a *Progressive Mode*. This means that only a thumbnail of the image will be loaded to the RAM. When zooming-in to view image details, the image data of the particular portion of the image will be loaded to RAM progressively (piece by piece).

Many image processing functions and commands are disabled in this mode. This problem usually occurs with *Large Images* created by stitching several frames together. In case you need to process such an image, a smooth solution is to split it into tiles and create a multi-point ND2 file out of it. Please see 6.5.2. Splitting Large Images [93].

### 6.5.2. Splitting Large Images

You can split an existing large image to tiles easily.

- 1) Open the large image to be split.
- 2) Run the *Image > Split Image* command. A dialog window appears.
- 3) Set split options:

**Split to separate files - output format** Select the output format (ND2 or TIFF). A separate file will be saved for each tile. Define *Output folder* and *Prefix* where the files will be saved.

**Create multipoint ND Document** Only one multi-point ND2 file will be created and opened after the split.

**Tile Size, Number of tiles** Define width and height of a single tile in pixels or set the number of columns and rows to which to split.

**Overlap** Set overlap value of neighboring tiles either in % or the current calibration units (presumably  $\mu\text{m}$ ). Overlap up to 50% of the tile size is allowed, higher values are reduced to the maximum

**Fill background** Select a color to fill empty spaces which will appear the total area of tiles exceeds the image. Use the **Optimize** button to prevent the empty spaces.



- 4) If you do not require the tiles to have exact size, click the **Optimize** button. The system will arrange/resize the tiles automatically so that the whole image is covered precisely.
- 5) Click **OK**.

### 6.5.3. Locating XY Positions Between Images

If two images have matching XY coordinates you can locate the exact position in one image inside the other. This would be the case especially after using the *Image > Split Image* command. Right click the location within the first (multi-point) image and select *Find this Point in Paired Document* from the context menu.

If you do this right after splitting the large image (the two images are “paired”), the system displays it and highlights the XY position by a flashing cross. If it is not clear to the system which images shall be paired (e.g. three or more images with matching coordinates are open), the user will be prompt to select the second image to the pair.

## 6.6. LUTs (Look-Up Tables)

LUTs is a useful tool for image color and brightness modifications. You can use LUTs to enhance images for observation purposes so the color modifications will be non-destructive to the image data. LUTs settings are saved along with the image file. If required, the LUTs settings can be applied to the image data by pressing the  button. Use the  *Show LUTs window* button in the image tool bar to display the LUTs window.

The following actions are available within the LUTs window:

- Adjusting the *Gamma parameter*. The gamma curve is drawn in gray over each graph. You can drag the middle point and move it up or down, or enter an exact value to the G: field at the top of the graph area.
- Adjusting the input intensity range. The input intensity range can be restricted by moving the black and white triangular sliders to the center. All pixels with values outside of the modified input range will be set to maximum/minimum values and the remaining pixels will be approximated in order to fill the output (full) range.

#### Note

*This is the way how images can be equalized. For example, if the image is very dark and most of the histogram is located to the left while the right part of it is flat, you may move the white slider(s) to the position where the histogram line starts to rise. This will incredibly brighten the image and reveal details which were previously hidden in a dark.*

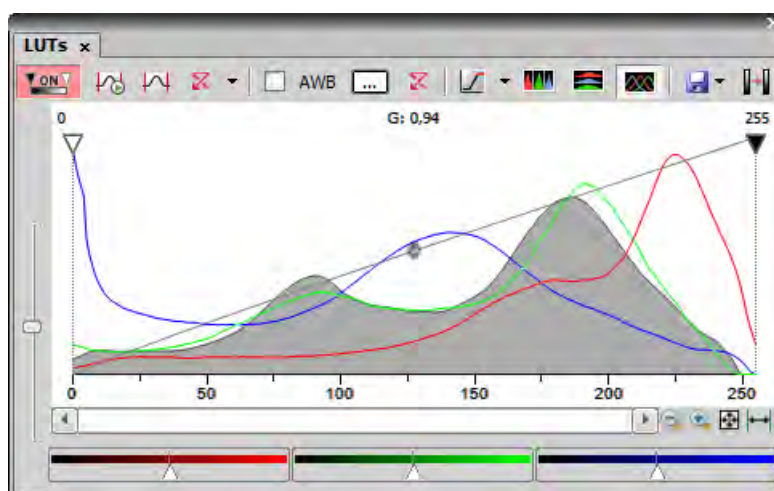
- You can adjust the histograms height non-proportionally by moving the slider on the left side of the window up and down.

#### **Tips**

- Right-click the graph area and (de)select *Draw trend style*. When ON, the LUTs curves will be smoothed to display the data trends rather than represent the actual image data values.
- The position of the black, white and Gamma sliders can be reset by a double click.

Different controls will be available when applying LUTs to monochromatic, RGB, multi-channel or spectral image. When LUTs are active, the LUTs button in the top-left corner of the image window is highlighted red.

### 6.6.1. LUTs on RGB Images



There are 3 separate windows for each of the RGB channels. All channels are controlled at the same time by default. To control each channel separately, press and hold the **Shift** key while you drag the sliders or adjust Gamma.

#### LUTs Tools

**ON** **Enable/Disable LUTs** This button applies LUTs to the current image.

**Keep Auto Scale** Press this button to run the auto scale procedure permanently (on the live image). When you turn this button OFF, the settings remain as if the *Auto Scale* button was pressed only once.

**Auto Scale** This button adjusts the white slider position of all channels automatically with the purpose to enhance the image reasonably. If you have selected the *Use Black Level* option from the *Settings* pull-down menu, the black slider will be affected too.

**Reset All Components** Discard all LUTs settings and turn LUTs OFF by pressing this button.

**Settings** Display the pull down menu and select one of the following commands:

- *Brightfield Opacity* - You can change opacity of a brightfield channel. Define the opacity in the *Brightfield Opacity* dialog window that appears after you run this command.
- *Use Black Level* - Check this item to ensure that the black slider will be affected by the auto scale functions.


- **Settings** - opens the *Auto Scale Settings* window. See *Auto Scale Settings* [96].


**Keep Auto White Balance Check Box** Check this option to switch the Auto White Balance functionality permanently (on the live image).


**AWB** Press this button to perform the Auto White Balance operation once.


**Auto White Balance Color (...)** This button Opens the *AWB Color* window and enables you to select a color shade which the system shall eliminate (make white). See *AWB* [97].


 **Reset AWB** Discard all AWB settings with this button.

 **Color Oversaturation** Switch this button ON and the system highlights all pixels with values reaching maximal values. Select colors to highlight the pixels with in the pull-down menu. See also 6.2.3. Displaying Image Layers [84].


 These buttons arrange histograms of single channels next to each other horizontally or vertically.

 This button arranges histograms of single channels overlapped. Use sliders at the bottom of the graph window to amplify or reduce the green/red/blue component display in the view.

 **Save/Load LUTs** This pull-down menu enables user to handle LUTs settings in various ways. They can be saved to a \*.lut file and loaded later. Or, LUTs settings saved along with an image image can be loaded directly from this image by the *Reuse LUTs from File* command. Or you can copy/paste the settings within the documents opened in *NIS-Elements AR*.

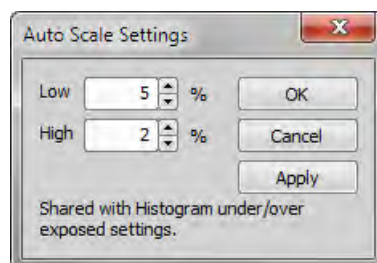
**Modify Image Through LUTs** Press this  button to apply the LUTs settings to the image data - the original image will be overwritten. Until you press this button, no changes are made to the image data.

 **Reset Zoom** Zooms the histogram to fit the preview window.

 **Auto Range** zooms the histogram so that the “high” and “low” limits are distinguishable. For example, if a small intensity range is defined on a 16bit image the low and high lines are displayed as one-pixel line. Pressing this button will stretch the histogram in order to display the lines separately.

## **Auto Scale Settings**

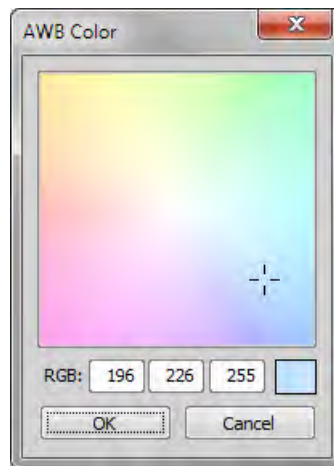
Press the arrow next to the *Reset LUTs* button and a pull-down menu appears. Invoke the *Settings* command.



The *Low* and *High* fields determine how many of all pixels of the picture are left outside the sliders when *Auto Scale* is applied (0-10%).

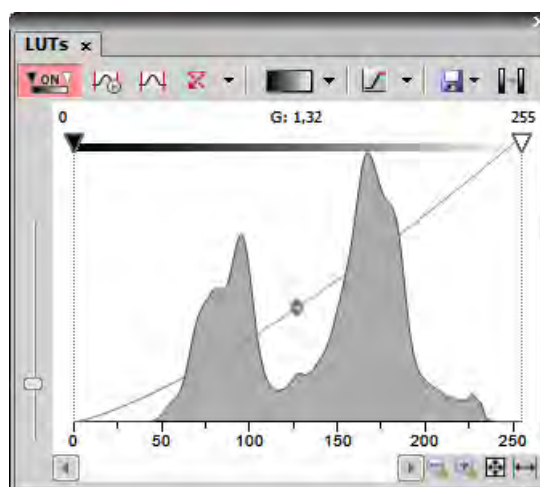
## AWB

White color in some images may suffer from having a color tint. The AWB (Auto White Balance) mode adjusts the image to get pure white instead of this tint. Similarly to LUTs auto scale, the AWB function can be used once, or permanently on the live image. If you know the tint your “white” color has, you can select this color by the color picker that appears after pressing the ... button:



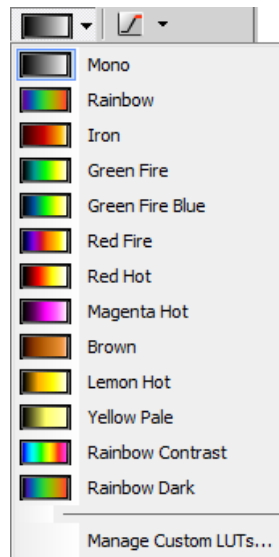
Choose the color by mouse. You can also enter the RGB values to the fields below. A preview of the chosen color appears in the rightmost rectangle.

## 6.6.2. LUTs on Monochromatic Images



All features mentioned above are also valid for monochromatic images, except that the *AWB* function is not available. There are some other features added when in “mono” mode.

**Gradient** Mapping monochromatic images to pseudo-color gradients is often used to enhance details in the image which would otherwise not be obvious. This button indicates the currently selected gradient. When pressed, a pull-down menu appears where you can select the color scheme you would like to use. Try a few of the schemes to see which one suits best.



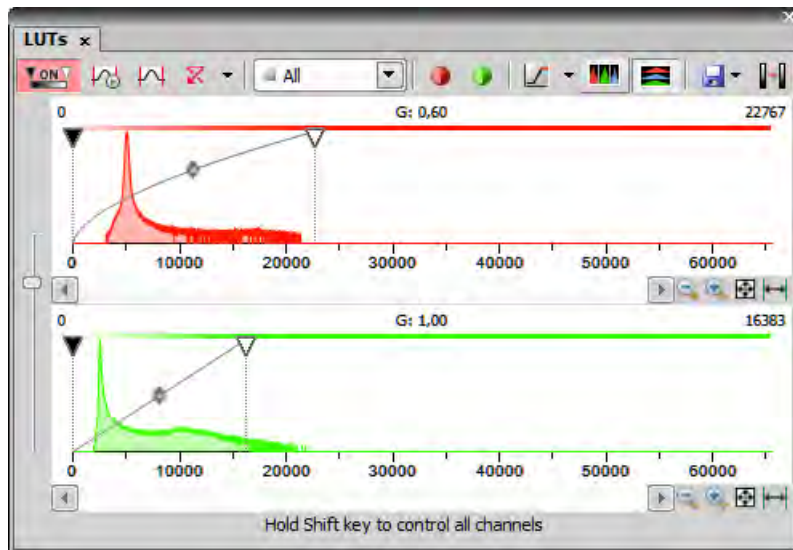
### **Manage Custom LUTs** (*requires:Local Option*)

The *Manage Custom LUTs* command in the pull down menu enables to define a custom LUTs gradient. Use the tools available in the dialog window to define your custom gradients. They then appear in the pull down menu ready to be applied. Please see *View > Image > LUTs > Create Custom LUTs* for details about the custom LUTs definition.



### 6.6.3. LUTs on Multichannel Images

Figure 6.22. LUTs window on two-channel image



Most of features mentioned above are also valid for multi-channel images. There are some other features added when in “multi-channel” mode. Each channel is controlled separately by default. To control all channels at the same time, press and hold the **Shift** key while you drag the sliders or adjust Gamma.

When the image contains up to three channels, all channels can be visible at one time and you can handle them similarly to the RGB mode. When there are more than three channels, only one channel is displayed at one time. A pull-down menu in the tool bar enables you to select the channel to be displayed.

Next to the channel selection pull-down menu, there is this *Auto Scale* button. By pressing it, the system will automatically adjust only the current channel settings.

### 6.6.4. LUTs on Spectral Images

When observing a spectral image, the graph inside the LUTs window does not show the image histogram(s), but reveals another information about the image. Intensity values are on the Y axis and spectra is on the X. There are two curves in the graph. The top (white) curve displays the maximal intensity and the color curve displays the mean intensity of each channel.

#### Note

*Spectral image consists of many channels, each representing a single wavelength.*

The LUTs behavior depends on the selected displaying mode. There are four displaying modes. You can select the mode either by a pull-down menu in the LUTs tool bar, or within the image top tool bar.

- *True Color*
- *Custom Color*
- *Grouped Color*
- *Gray Scale*

In the bottom part of the LUTs window, *Gain* and *Black Level* settings can be adjusted either for all channels together or for each group separately - depending on the displaying mode. Gain brightens the image and the Black Level works just like the left (black) on RGB/mono images.

#### Note

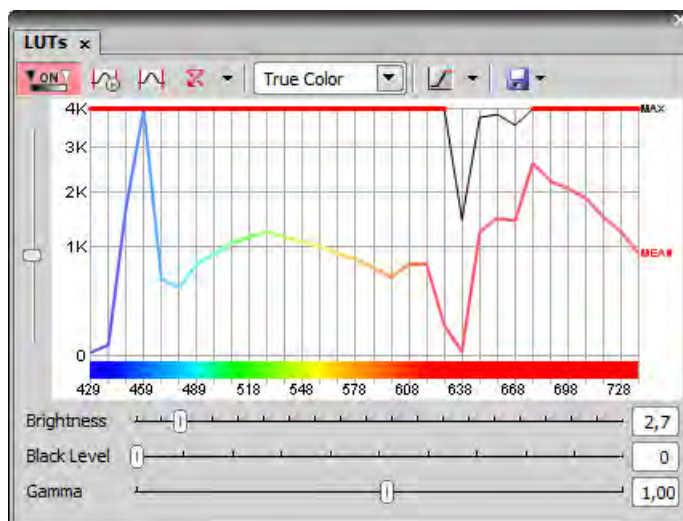
*Each slider (gain or black level) offers a range of values to be set by moving it, but any higher value can be entered in the field next of the sliders.*

### **TD channel**


A spectral image can contain additional TD (transmitted detector) channel. Its basically a standard monochromatic image. LUTs settings of this channel appear on the right edge of the LUTs window.

### **True Color Displaying Mode**

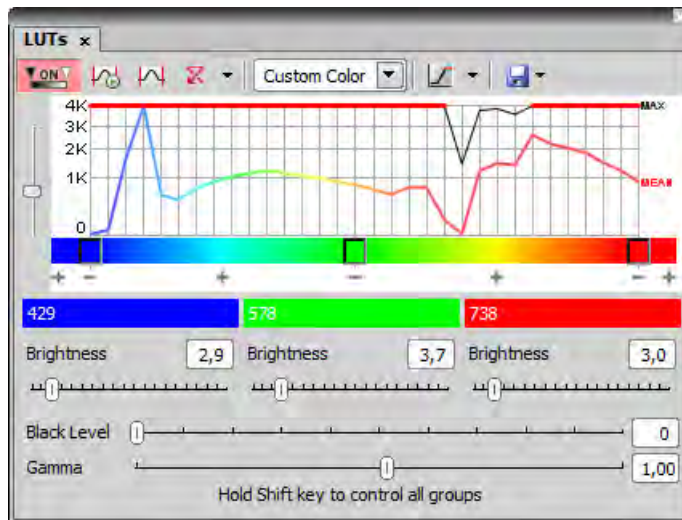
You can switch to this displaying mode by clicking the  button located in the image tool bar.




### **Custom Color Displaying Mode**

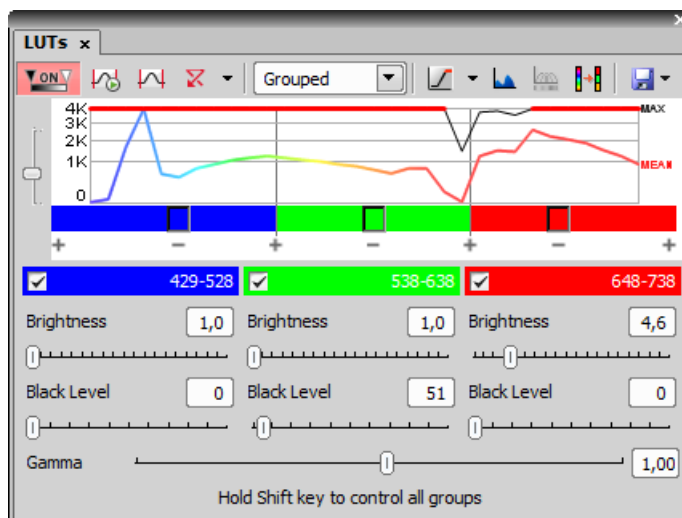
You can switch to this displaying mode by clicking the  button located in the image tool bar. You can assign custom colors to sections of the spectrum. Sections (colors) can be added/removed by clicking

the + and - buttons placed below the spectral graph. To change the section color, click the button located in the color stripe. A window appears where you can select the color from a palette or define it by *Hue* or *Wavelength* settings.




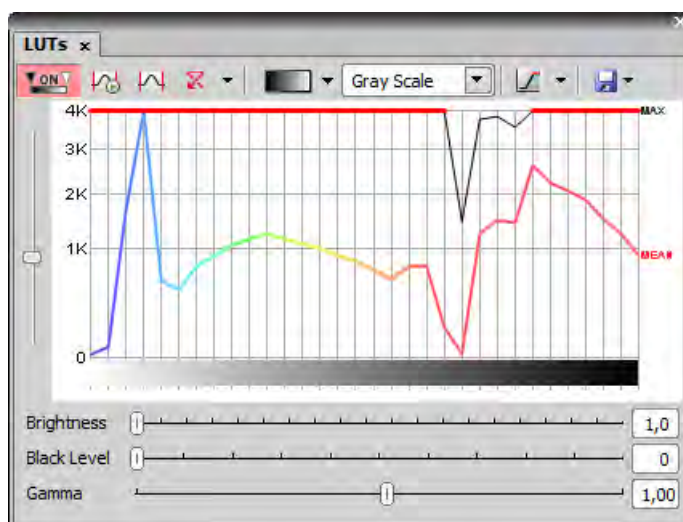
### **Grouped Displaying Mode**

You can switch to this displaying mode by clicking the  button located in the image tool bar. This view enables you to reduce the number of channels multiple-times. You can adjust the number of groups by the + and - buttons and also change each channel width by moving the white horizontal dividers. Gain and Black Level for each group can be adjusted.



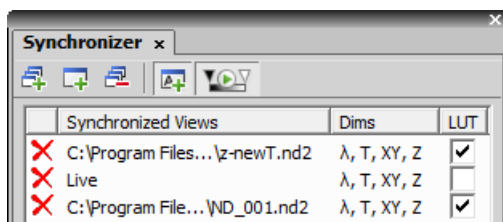
## Gray Scale Displaying Mode




You can switch to this displaying mode by clicking the  button located in the image tool bar. The image is displayed in gray scale, but various color gradients can be applied to it.



## 6.7. Synchronizer


Synchronizer enables to compare (run and view) two or more nd2 files at the same time. It automatically synchronizes view of all documents added to the list. Run *View > Visualization Controls > Synchronizer*:





- Open at least two nd2 files you would like to observe.
- Use the *Add All Views*  button to add them to the synchronizer table. In case there are more images opened which you do not want to add, use the *Add Current View*  button on each of the images. You can also select the *Add New Views Automatically*  button to have all newly opened ND2 files synchronized.
- The names of all added images appear in the *Synchronizer* window.

All documents added to the Synchronizer are automatically being synchronized.

- Set one of the selected images active.
- Any action regarding the view (Z position, displayed frame, etc.) is performed on the synchronized images as well as on the active one.
- Besides synchronization of dimensions, you can also synchronize LUTs settings of the images by checking the *LUT* check box. LUTs synchronization works only between LUT-compatible images (images must have the same number of channels etc.). If the images are not LUT compatible, only the *Keep AutoScale* and *AutoScale* actions are synchronized.

You can select the *Enable LUT synchronization by default*  button. All newly added documents will have the option to synchronize LUTs selected by default.

- To remove an image from synchronizer, click the reset button  placed before its name. To remove all images from synchronizer, press the *Remove All Views*  button.

## 6.8. Organizer

### 6.8.1. About Organizer



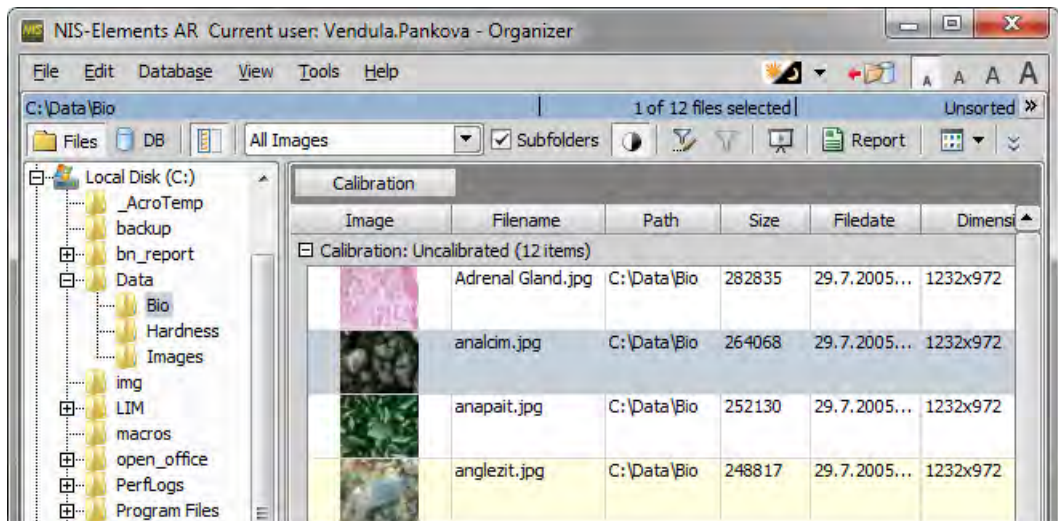
Apart from the main application layout used for capturing and image analysis, *NIS-Elements AR* provides an extra layout called *Organizer*. Organizer makes the work with image files and databases as easy as possible. To activate it, run the  *View > Organizer Layout* command or click the  button located in the top right corner of the application window. The screen opens divided into two identical panes. To switch from one pane to the other, use the *View > Next Pane (F6)* command. To copy files between the panes, simply drag the images from one side to the other side.

Figure 6.28. The organizer layout.



- This button toggles the display of the directory tree. You can switch it off to get additional space to display images.
- In the nearby pull-down menu, it is possible to set the file type and only the one will be displayed. Or, *All Images* can be displayed.
- If the *Subfolders* check-box is selected, all images from included sub-folders are displayed.

## 6.8.2. Image Filter

You can define a filter which enables you to display only images which fit the defined conditions. One or two conditions may be applied.

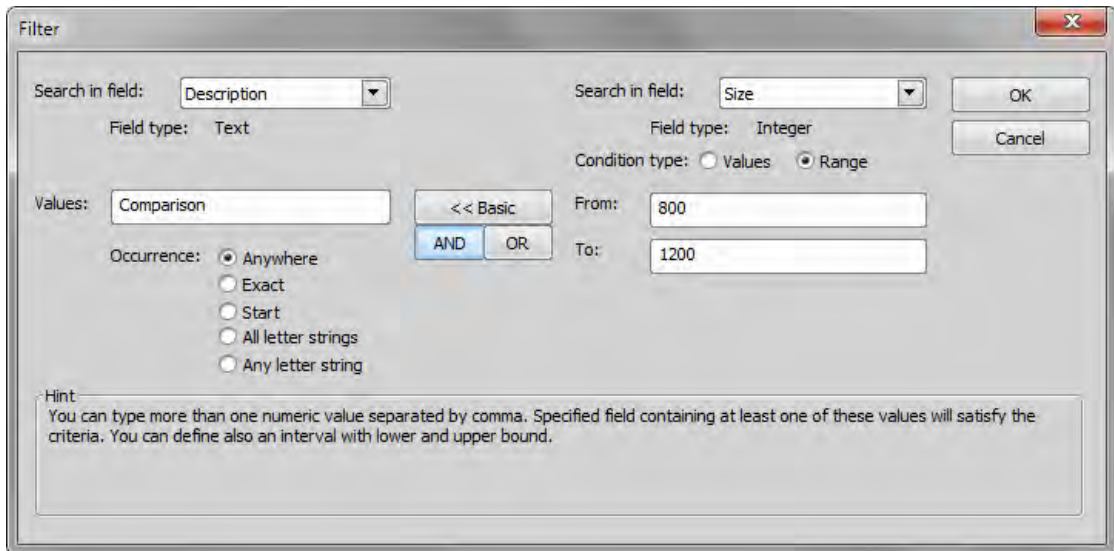
- Pressing this button invokes the filter setup window.
- This button activates the filter.

### **Basic Mode**

This mode enables you to filter files according to one condition.

### **Advanced Mode**

This mode enables you to define two conditions with a relationship between them. Either select *OR* to display files matching at least one condition, or select *AND* to display files which match both conditions.



1. *Search in field* - select the field where *NIS-Elements AR* should search for a given expression.
2. If the selected field is of a numerical type (e.g. Size, Calibration, File date etc.) you can specify, whether you want to find the exact *value* or a value in a given *range*. This is selected by the *Condition type* radio button.
3. If the field type is *Text* , the *Occurrence* setting determines the way of evaluating the expression:
  - *Anywhere* - If the given sequence of characters is found anywhere in the field, the system will evaluate it as a match. For example: you have entered “set” to the *Values* field. The filter will select records with the following field values: “set”, “reset”, “settings”, “preset”, etc.
  - *Exact* - If the given sequence of characters exactly equals to the content of the field, it is evaluated as a match. Fields containing the “set” value only will match.
  - *Start* - If the entered string is found at the beginning of a field, it passes the condition. For example: fields containing “set”, “settings”, “setup” will match.
  - *All letter strings* - It is possible to search for more expressions. These should be entered separated by commas. If you want to enter an expression with a space, insert it quoted. If this option is selected, only records where all of those expressions appear (anywhere) will match.
  - *Any letter string* - This option is for entering multiple expressions as above, but this time every field with an occurrence of at least one from the given expressions is matched.

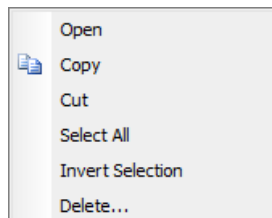
### 6.8.3. Operations with Images within Organizer

- To open an image double click its thumbnail. *NIS-Elements AR* will close *Organizer* and display it in the main window.




- To select multiple images, either click on the first and the last image holding the **Shift** key (continuous group selection) or click individual images with the **Ctrl** key down.
- You can copy one or more images from one folder to another by “drag and drop”.
- To delete selected images press the **Delete** key.


All these operations and some other can be invoked also from the context menu, which appears after you right-click on the image thumbnail:




### **Thumbnail Displaying Options**

You can adjust the way images are shown in the organizer.

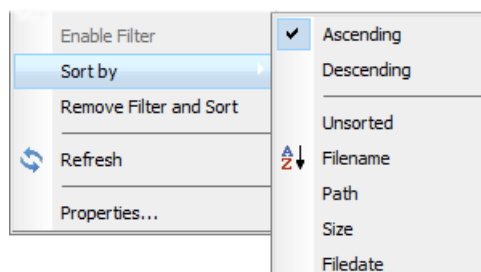
 **Thumbnail View** Press this button and select the size of displayed image thumbnails. Selecting the *Details with preview* option will display images below each other with all available information aside.

 **Rotating Images** There is a possibility to rotate images from within the organizer. It affects not only the image thumbnails, but the image data too. Press the corresponding buttons.

 **Autocontrast** Press this button to turn the *Apply autocontrast to thumbnails* option ON. It enhances the image thumbnails automatically. Dark images gain details.

### **Sorting of Images**

To order displayed images, right click anywhere in the pane - a context menu will appear. Move to the *Sort by* sub-menu which offers several ordering criteria. If the ordering is turned ON already, the icon is displayed on the left side of the applied criterion.





### Grouping of Images



To arrange the view of images efficiently, you can use the capability of grouping of images. Drag the column name bar to the grouping bar (right above the column name bars). All files with matching field values of the selected column will be grouped together. This can be undone by dragging the column caption back to the others. See Figure 6.28, “The organizer layout.” [104] (the Calibration column is grouped).

#### Note

*If you have the Database module installed, the Organizer becomes switchable between two modes: the Files View and the Database View. See description of the Database View.*

## 6.8.4. Resizing the Organizer Panes

The pane size is adjustable. To resize it, you can either:

- 1. Place the mouse cursor over the dividing line in the middle.
- 2. The cursor becomes an arrow with two tips.
- 3. Drag it left or right to the new position.
-  Resizes the panes to achieve the same size for both of them.
-  Resizes the pane to its maximal/minimal size (one pane is then displayed on the whole screen).

## 6.9. Database


After you install the 14.7. Database [230] module, the *Database* menu appears in the main tool bar.

#### Note

*Viz. 2.1.9. Installing the Database Module on 64-bit Systems [11].*

### 6.9.1. New Database

Before you can connect to a new database the structure of tables and fields, or at least the protection level shall be set:

1. Run *Database > New Database* .
2. Specify the file to be created.
3. Select one of the database templates. Each template consists of several predefined tables. If you choose *Blank* , no tables will be created (later you will have to use the  *Database > New Table* command). Click **Next** .

4. Select the default protection of the database. The user accounts can be based on MS Windows accounts, or arbitrary number of database user accounts protected by password can be created. Or, the database can be fully accessible for all users - if the *Not Protected* option is selected. Click **Finish**.
5. The database connection will be automatically created and connected. You can browse the database with the *Organizer*.

## 6.9.2. New Connection


If there is an existing database created by *NIS-Elements AR*, you can connect to it via the *Database > New Connection* command:

1. Run *Database > New Connection*. A window appears.
2. Locate the database (\*.MDB) file.
3. Confirm the action with **OK**.

Connections to databases can be managed via the *Database > Manage Connections* command.

## 6.9.3. Database Tables

A new table can be added to an existing database. Every database must contain at least one table. If you have created a blank database or you would like to add a table to any of the connected databases, do as follows.

1. Run  *Database > New Table*. A window appears.
2. Select one of the connected databases to which the table will be added.
3. Select one of the table templates. Table templates can be imported from the other connected databases. If you choose *Blank*, you will have to create the table structure from scratch (in the next step). Click **Next**.
4. Define the field properties in the window that appears.
5. **Finish** the table creation.

## 6.9.4. User Permissions

Database access rights can be set for individual users. As we mentioned above, there are two types of protection:

**Password protection** Any number of database user accounts can be created. Then you can connect to a database by the user name and password.

**Windows account protection** User permissions to databases can be set for different MS Windows users. The access to a database is then granted if only the user is currently logged in Windows.

Both protection types can be combined in one database. To manage the user accounts and permissions of a connected database:

#### Note



*You have to be connected to the database under an account with sufficient user rights.*


1. Run *Database > User Accounts and Permissions* . A window appears.
2. Select the database of your interest. All user accounts of the database will appear in the list below.
3. Create new or select an existing user account.
4. Use the buttons on the right to manage the account properties, duplicate it (along with the permissions settings), change the password, modify permissions (**Change properties**), or even delete it.


## 6.9.5. Database Backup

The capability to backup the database is essential for serious work. The Database Backup Scheduler enables to backup the MS Access database automatically once in a precisely specified time interval, so you do not have to be afraid to loose any data. The Database Scheduler uses the standard Windows Scheduled Tasks tool (Start > Control Panel > Scheduled Tasks). Once you schedule a backup, there is only one condition to perform it successfully: The computer must be turned ON at the scheduled time. Configure the backup via the *Database > Schedule Database Backup* command.

## 6.9.6. Database View within Organizer

You can browse the database using the built-in *Organizer*. Use the  *View > Organizer Layout* command (or the to Press the  button located in the top right corner of the screen) to display the *Organizer*. Here you can either browse images saved on the hard disk or browse the database. Switch between the two views by the following buttons:

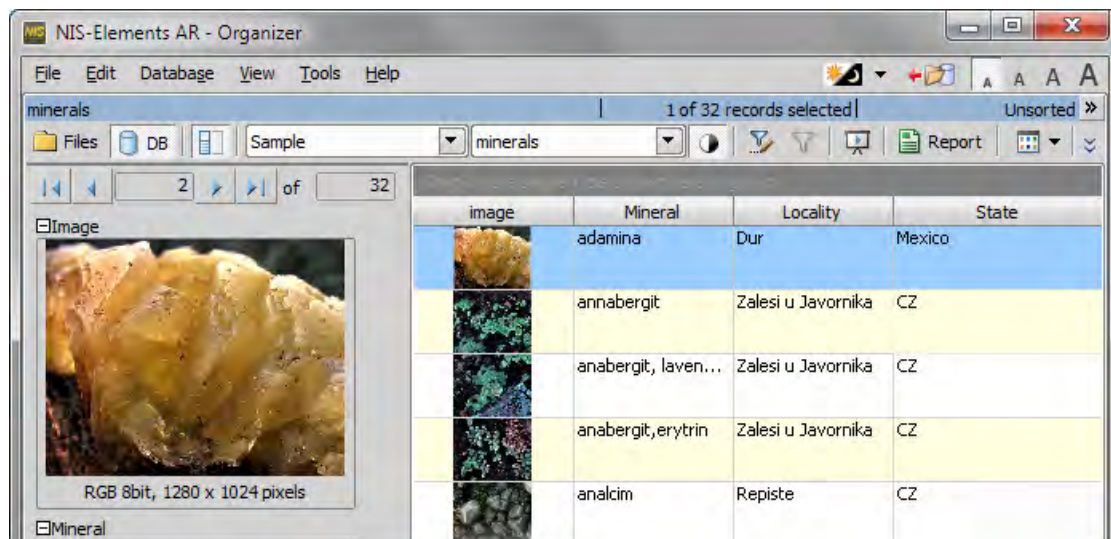
 **Files** This button switches the pane to show a directory tree and images from the selected folder (and optionally its sub-folders).

 **Database** This button switches the pane to show the database structure and lists images from the currently selected database table.

See 6.8. Organizer [103] for general information about how to use the Organizer.

## Features Available in the Database View

Figure 6.32. The Database View



- This button displays the detailed information about the selected image. You can switch it off to get additional space to display images.
- The nearby pull-down menu displays the database connection name and enables to switch between active connections.
- The next pull-down menu enables you to select a database table to be displayed.

## Operating with Images

You can insert images to a database by “drag and drop”. Simply drag the image from a folder and drop it onto the pane, where the database table is opened.

## 6.10. Saving Image Files

*NIS-Elements AR* offers several ways to save an image file, using either:

**The File > Save As command** The most common way of saving the current image. You can select image format in the Save as type pull-down menu. See also 13.2. Save ND2 as AVI [225].

**The File > Open/Save Next > Save Next command** This command saves the current image (live or static) automatically according to the settings defined within the general options window. See 6.10.2. Save Next Options [111].



**During image-capturing experiments** Images and image sequences can be saved automatically during experiments such as ND Acquisition (5.4. About ND Acquisition [59]) or a JOB run (11. JOBS [201]).

### 6.10.1. Saving Images with UAC

Because of the security enhancement with the UAC (user account control) of *Windows Vista* and *Windows 7*, it is not possible to save images in those folders that require Windows administrator user rights. Those folders are:

- C:\Windows and subsequent folders
- C:\Users and subsequent folders excluding C:\Users\[login-user-name]
- C:\Program Files and subsequent folders excluding C:\Program Files\NIS-Elements AR\Images

### 6.10.2. Save Next Options

This window enables you to configure the  *File > Open/Save Next > Save Next* command properties. Run the  *Edit > Options* command and switch to the *Save Next* tab.

#### Save to File

**Directory, Prefix, Digits, File Format, Compression** Defines name of the file that will be automatically generated. Let's assume, that you have selected - Directory: c:\images; Prefix: seq; Digits: 4; File Format: JPEG2000. Then, by pressing *Enter* (calling the *Save Next* command), an automatic file named "seq0001.jp2" is generated and saved to the "c:\images" directory. When pressing *Enter* again, the file "seq0002.jp2" is saved to the same directory etc.

**Define Image Info** Displays a dialog box, where you can enter some description (jp2 meta-information), that will be saved with every image:

**Overwrite/Skip already existing files** Allow or deny rewriting of the images already existing in the default save directory.

**Save to Database** When selected, images are being saved to a chosen database table, instead of saving them directly onto disk.

**Database** If you are already connected to some database, select it from the pull-down menu. Else a dialog window appears, which enables you to connect to any database.

**Table** Select the database table which the images will be stored to.

**Autoincremental field** Choose which field of the database will be used to store the generated image descriptions.

The image descriptions are generated according to the *Prefix, Digits, Number* values. Let's assume, that you define - Prefix: seq; Digits: 4; Number: 20. Then, by pressing *Enter*, the current image is saved to the selected database table and the automatic image description is put into the *Autoin-*

cremental field of the table. Its name would be "seq0020" in our case. When pressing *Enter* again, the second record signed "seq0021" is created.

**External mapping** You may create an external \*.txt or \*.ini file containing mappings that assign table field names to some particular values. These values are automatically filled into the database records when the *Save Next* command is called. Please, see the comments below for an example.

---

#### Note

*External mapping example - The \*.ini file content may be:*

```
[Table 1]
Author=Jack Sparrow
Experiment number=12
Sample=Malus silvestris
```

*It means: When saving images to the database table named "Table 1", the fields of Author, Experiment number, and Sample will be filled with the specified values.*

### Options

**Show grabbing dialog before saving** Before an image is saved the Grabbing dialog box is shown. Selecting this option enables you to define the way of grabbing (with/without a shading correction, averaged etc).

**When saving, display Image Info dialog box** You can change the default image information (jp2 meta-information) right before saving.

**Sound Alert** If checked, a short tone from your PC speaker is played every time the *Save Next* command is used.

**Save with annotation and binary layers** If checked, images are saved together with the binary and annotation layer.

**Change to live after saving an image** After saving a single image, a Live image is displayed immediately.

**Defaults for this Page** Pressing this button restores the default settings of this window. All your changes will be lost.

**OK** Confirms all the changes and closes the window.


**Apply** Saves the changes which are applied, but the window remains opened.

**Cancel** Discards all the changes and closes the window.

**Help** Displays relevant help page.

## 6.11. Closing Images

- The currently displayed image can be quickly closed by pressing the cross button in the top-right corner of the image window.

- The image can be also closed by invoking the *File > Close* command.
- If you want to close all images, use the  *Window > Close All* command.
- If you try to close an image that has been changed, *NIS-Elements AR* will display a confirmation dialog box, offering to save the changes.
- Use the *Window > Close All but Current* command to close all opened documents but keep the current one opened.

## 6.12. Supported Image Formats

*NIS-Elements AR* supports a number of standard file formats. In addition, *NIS-Elements AR* uses its own image file format (ND2) to fulfil specific application requirements.

**JPEG2000 Format (JP2)** An advanced format with optional compression rates. Image calibration, text descriptions, and other meta-data can be saved together with the image in this format.

**ND2 Format (ND2)** This is the special format for storing sequences of images acquired during ND experiments. It contains various information about the hardware settings and the experiment conditions and settings. It also maintains all image layers of course.

**Joint Photo Expert Group Format (JFF, JPG, JTF)** Standard JPEG files (JPEG File Interchange Format, Progressive JPEG, JPEG Tagged Interchange Format) used in many image processing applications.

**Tagged Image File Format (TIFF)** This format can save the same amount of meta-data as JPEG2000. TIFF files are larger than JPEG2000 files but are loaded faster. TIFF files have several ways to store image data, therefore there are many versions of TIFF. *NIS-Elements AR* supports the most common TIFF modalities. TIFF image format in *NIS-E* supports also floating point images.

**CompuServe Graphic Interchange Format (GIF)** This is a file format commonly used on the Internet. It uses a lossless compression and stores images in 8-bit color scheme. GIF supports single-color transparency and animation. GIF does not support layers or alpha channels.

**Portable Network Graphics Format (PNG)** This is a replacement for the GIF format. It is a full-featured (non-LZW) compressed format intended for a widespread use without any legal restraints. *NIS-Elements AR* does not support the interlaced version of this format.

**Windows Bitmap (BMP)** This is the standard Windows file format. This format does not include additional image description information such as author, sample, subject or calibration.

**LIM Format (LIM)** Developed for the needs of laboratory image analysis software. Nowadays, all its features (and more) are provided by the JPEG 2000 format.

**ICS/IDS image sequence** ICS/IDS image sequences are generated by some microscopes and consist of two files: the ICS file with information about the sequence; the IDS file containing the image data. The ICS file must be stored in the same directory together with the IDS file.

---

### Caution

*ND Images containing multi-point XY dimension cannot be saved to a file in the ICS/IDS format. See also 5.4. About ND Acquisition [59].*

---

**NanoZoomer files (NDPI, VMS)** *(requires:Local Option)*

We can open these files produced by Hamamatsu devices and save the image information to an ND2 file.

**ShuttlePix files** *(requires:Local Option)*

Nikon ShuttlePix Digital Microscopes produce standard TIFF and JPEG files and save the calibration info to a separate file. *NIS-Elements* recognizes the calibration file and loads it along with the image data.



## 7. Image Analysis

### 7.1. Preprocessing

#### 7.1.1. Processing On: Intensity/RGB/Channels

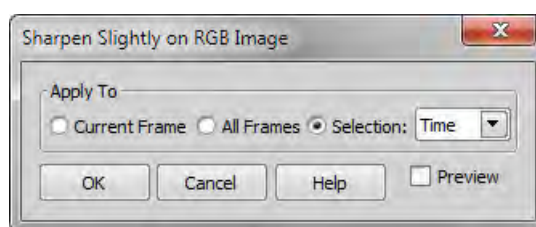
You can decide whether to perform the processing on single RGB channels of the image, or on the intensity component of the image only. The RGB/Intensity setting is global so it is applied to all the other processing commands automatically. When processing a multichannel image, this option is disabled - the operation is applied to channels automatically.

##### Note

*In case the command does not open a window where it would be possible to make the choice, the setting from the last performed processing command (the global setting) is applied.*

#### 7.1.2. ND2 Files Processing

Most of the image processing and binary image processing commands can be applied to one frame, all frames, or selected dimension of the nd2 file. When you use such command with an ND2 file, the following options are added to the command dialog window:



**Current Frame** The operation will be performed on the current frame of the nd2 file.

**All Frames** The operation will be performed on all frames of the current nd2 file.

**Selection** The operation will be performed on all frames of the selected dimension. If you selected some of the frames/loops of the nd2 file by mouse, the *Selection* option appears in the pull-down menu. Opt for it to process only these selected (green highlighted) frames/loops.

When you process an image containing T and Z dimensions, selecting the Z-Series dimension performs the operation on all Z frames of the current T loop.

### 7.1.3. RGB/Mono/Multichannel Image Processing

Options of some of the image processing commands may vary slightly depending upon the type of image being processed. See 6.2.2. Image Types [84] for the more detailed explanation of the image types.

#### RGB

- When the image processing function works with the image components, you can select whether the *Red*, *Green*, or *Blue* component is used.

#### Mono

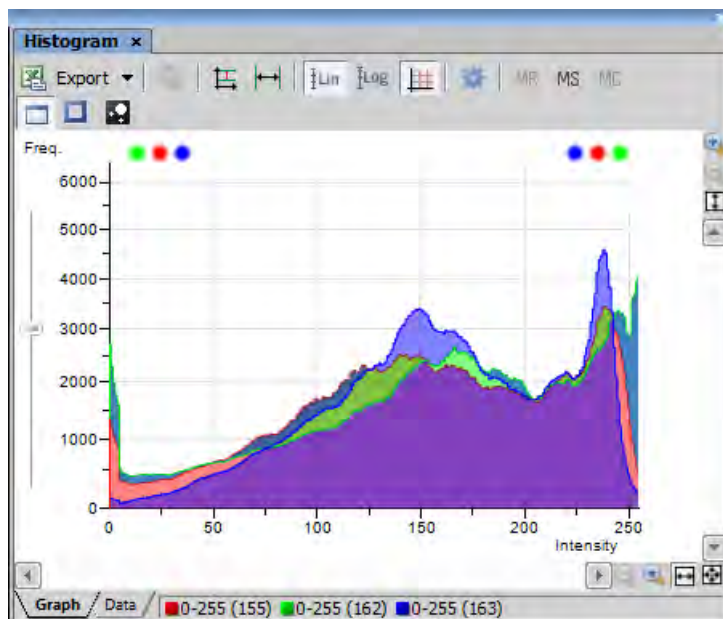
- You cannot select which component is used, because the image contains only one component, which is then selected automatically.

#### Multichannel images

- When the image processing function works with the image components, you can select which component is used from the pull down menu or other selection tool.





## 7.2. Histogram

A histogram displays frequencies of pixels of a certain intensity value. The intensity values range from 0 to 255 (on 8bit images). A separate curve is created for each color channel. Run the [View > Visualization Controls > Histogram](#) command to display the histogram control panel:





## 7.2.1. Source Data

Source data of the histogram can be viewed if you swap to the *Data* tab in the bottom-left corner of the window. The area from where the histogram is calculated can be restricted according to the selected histogram mode:

-  The data are obtained from the whole image.
-  The data are obtained from within the probe.
-  The data are obtained from within the current ROI.
-  The data are obtained from the image parts under the binary layer.

## 7.2.2. Export

 The source data or the histogram image can be exported to an external file. Display the *Export* pull-down menu and select a suitable destination. Click the button to perform the export. The *Export ND histogram*  button enables to export histogram data of all frames of the current nd2 file. The same destination which is selected in the *Export* pull-down menu is applied.


### Note


*Please, see the 8.14. Exporting Results [180] chapter for further details.*

## 7.2.3. Histogram Scaling

The histogram can be zoomed in and out using the zoom buttons on sides of the window. There are also other options to adjust the graph appearance:

**Left slider** There is a slider along the Frequency axis. Drag it in order to stretch the view of either lower or upper values of the axis.

 **Auto Scale Vertical** Zooms the graph of each channel separately to fit the available area. When this function is ON, the histogram is not proportional.

 **Auto Scale Horizontal** Zooms the graph so that the marginal zero frequencies, if there are some, are excluded from display.

 **Graph Linear** Displays the linear scale on the Y axis.

 **Graph Logarithmic** Displays logarithmic scale on the Y axis.

 **Show Grid** Turns ON/OFF the grid in the background.

## 7.2.4. Overrun Indication

If certain amount of pixels with maximum/minimum intensity values (black/white pixels) are spotted in the image, color dots appears above the graph. The color indicates which channel is affected. When you place the mouse cursor over the dots, a tool tip message appears with details about the percentage of under/overexposed pixels. The percentage limit setting is loaded from the LUTs settings (the color dots do not appear unless the set percentage of black/white pixels is exceeded).

### Note

*If the overrun concerns more than three channels (considering multi-channel images), only one white dot is displayed instead of many color dots.*

## 7.2.5. Graph Memorizing

You can save the current graph to memory and display it later for comparison with another graph.

**MR** Displays memorized graph.

**MS** Memorizes the current graph. Only one graph can be memorized at a time. If any graph has already been memorized before, it will be overwritten by the current graph.

**MC** Clears the memory.


## 7.2.6. Histogram Options

### Drawing Style

There are two ways the histogram can be drawn:

- *Raw Data* - tries to draw the source data precisely to the graph
- *Trend Style* - interpolates the data so that the histogram lines appear smoother.

### Histogram Options Window

The graph appearance can be modified. Press the **Options**  button - a window appears where the following settings can be adjusted:

**Colors** Graph background and axes colors can be selected.

**Pen Width** Set the width of the histogram(s) line to 1, 2, or 3 pixels.

**Fill Graph Area** The area below the histogram line can be filled with the channel color.

**Graph Area Opacity** Select the opacity of the Graph Area color in %.

**Vertical/Horizontal AutoScale, Show Grid** These options equals the corresponding buttons of the histogram tool bar.

**Interpolation method** Select the way of drawing the graph line. The *Linear* (smooth) and *Quick* (precise) options are available.

**AntiAlias** Smooths the edges of the graph line.

**Horizontal axis always visible** If checked, the axis does not leave the graph area while zooming in the graph.

## 7.3. Thresholding

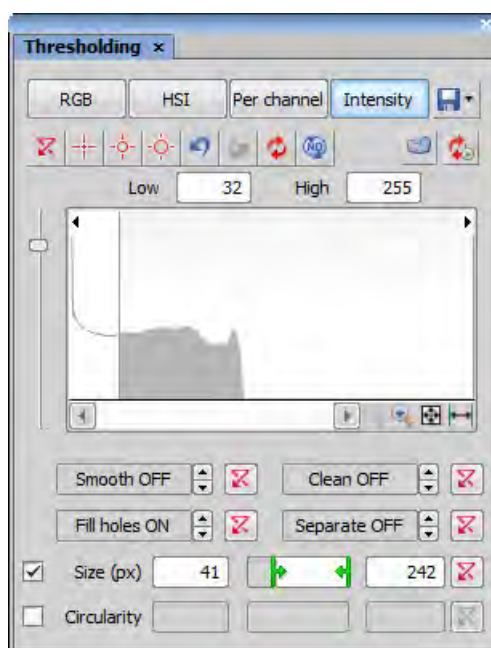
Specifying correct threshold limits is a crucial procedure of the automated image analysis. The point is to determine which pixels will and which will not be included in the binary layer and thereby distinguish objects to be analyzed from background. Thresholding can be performed in the following modes:

### Note

*To display the thresholding control panel, run the View > Analysis Controls > Thresholding command.*


### 7.3.1. RGB Mode


The *RGB* thresholding mode is available only if an *RGB* image is opened. There are two ways how to define the threshold limits, by choosing reference points within the image, or by defining the limit values for each color channel.



---

To threshold the image use the following tools:


 **Reset** This button erases the threshold settings (no binary objects are created).


 **Single point threshold tool** Select it and click inside the image to define the threshold. The threshold ranges will be adjusted so the selected pixel will fit inside.


 **3 point circle tool** Picks threshold from the radius of 3 pixels.


 **6 point circle tool** Picks threshold from the radius of 6 pixels.


 **Undo** Reverses the previous threshold operation.


 **Redo** Returns the threshold as it had been before the *Undo* button was used.

 **Re-threshold image** Use this button to update the binary layer according to the current settings. The purpose of this button is to obtain the original binary layer in case it has been modified e.g. by a command from the binary layer.

 **Keep Updating Binary** Press this button to keep the binary layer up-to-date all the time. You may find this function useful especially when thresholding is applied to the live image.


 **Threshold ND image** When an nd2 file is opened, this button appears. Define the threshold limits on the current frame and use the button to apply it on the whole nd2 file.

 **Full Image/Use ROI** If selected, threshold is defined only on the area of the Region Of Interest.


 **Save / Load Threshold Settings** This button invokes a pull-down menu which enables to load/save the current threshold settings from/to an external file (\*.threshold).

 **Zoom** Zoom the histogram in/out.

 **Auto Zoom** Zooms the histogram to fit the preview window.

 **Auto Range** Zooms the histogram so that the “high” and “low” threshold lines are distinguishable. For example, if a small intensity range is defined on a 16bit image the low and high lines are displayed as one-pixel line. Pressing this button will stretch the histogram in order to display the lines separately.

### **How to use the picker tool to threshold an image**

1. Select one of the cross hair  tools.
2. Left click on the image over a pixel of the image that should be considered part of the threshold/ binary layer. All pixels with similar intensity values of the image will also be highlighted. An outline will also display around the object(s) as well as the building solid colored threshold/ binary layer.
3. Continue to click on areas of the image that should be part of the threshold/ binary layer.

---

#### Note

*Right-clicking inside the image can be used to swap between the color mode (without the definition window) and overlay mode (with the definition window).*

### **How to use the histogram to threshold an image**

1. Move your mouse cursor over the histogram and hover over the vertical line labeled *L* (lower limit). Move the vertical line left or right to set the lower limit of the threshold. Use the image and its building threshold to provide feedback on whether the limit is set in the correct place. Accepted areas of the histogram for the threshold are colored gray.
2. Repeat first step with the vertical line labeled *H* (higher limit)
3. You can combine the histogram with picker tools to create the thresholded image.

### **Threshold Adjustments**

Thresholding parameters can be adjusted very precisely in the following way:

- Threshold limits of each channel can be adjusted by rewriting the values in the top-left/top-right corner of each channel histogram.
- Threshold range of each channel (the colored stripe) can be shifted by mouse. Place the cursor in the middle of the range (a circle appears) and drag it left or right.
- Threshold limits of each channel can be adjusted by mouse. Place the cursor on the edge of the thresholding range and drag it left or right.

The two last behaviors can be further modified by pressing additional keys:

- Move the threshold using the right mouse button - the binary layer in the image is not updated continuously, but is updated only once after the mouse button is released. Such operation saves some computing and therefore is quicker.
- Hold **Shift** while moving the threshold limits - the threshold adjustment is performed on all channels together (RGB images only).
- Hold **Ctrl** while moving the threshold limits - both the low and the high threshold limits move in opposite directions.

### **Binary Operations**

Four basic operations can be performed on the binary layer before it is displayed on screen. To turn the operation on, click on the up arrow button in order to define the number of its iterations:

**Clean** Removes small objects from binary image.

**Smooth** Smooths the binary image contours.

**Fill Holes** Fills holes within binary objects.

**Separate** Separates objects.

### **Restrictions**

**Size** Define size range using min/max value in the edit boxes or using the slider.


---

**Circularity** Define circularity range using min/max value in the edit boxes or using the slider.

### **Thresholding Large Images**

If thresholding images larger than 5000 x 5000 pixels, a new check box called *Preview on selected area only* is displayed. This feature enables to display the threshold preview just on a limited area to speed up the thresholding adjustment.

### **Changing Appearance of the Threshold Layer**

To change the color or transparency of the layer, right-click the  **Overlay** button in the general controls toolbar in the document window. A contextual menu appears, where you can select one of the predefined transparency levels, or run the *Colorize Binary Objects* command. This command displays the binary objects in several different colors. The algorithm ensures that two neighboring objects are never colored by similar colors.

See 7.5. Mathematical Morphology Basics [127].

## 7.3.2. HSI Mode



The *HSI* thresholding mode is available only if an RGB image is opened. The thresholding procedure works the same way as in the RGB mode except that the pixel values are displayed in the HSI (Hue, Saturation, Intensity) color space and that the *Saturation* and *Intensity* channels can be switched OFF. Therefore you can threshold over the *H*, *HS*, or *HI* channels only. Switch the channel off by de-selecting the check box in the top-left corner of the channel histogram.

## 7.3.3. Intensity Mode

The thresholding procedure works the same way as in the RGB mode except that it is performed on the intensity pixel values.

## 7.3.4. MCH Mode

The *MCH* mode is not available when working with a monochromatic (single channel) image. This mode is dedicated to perform threshold on multichannel images, but can be applied to RGB images as well.

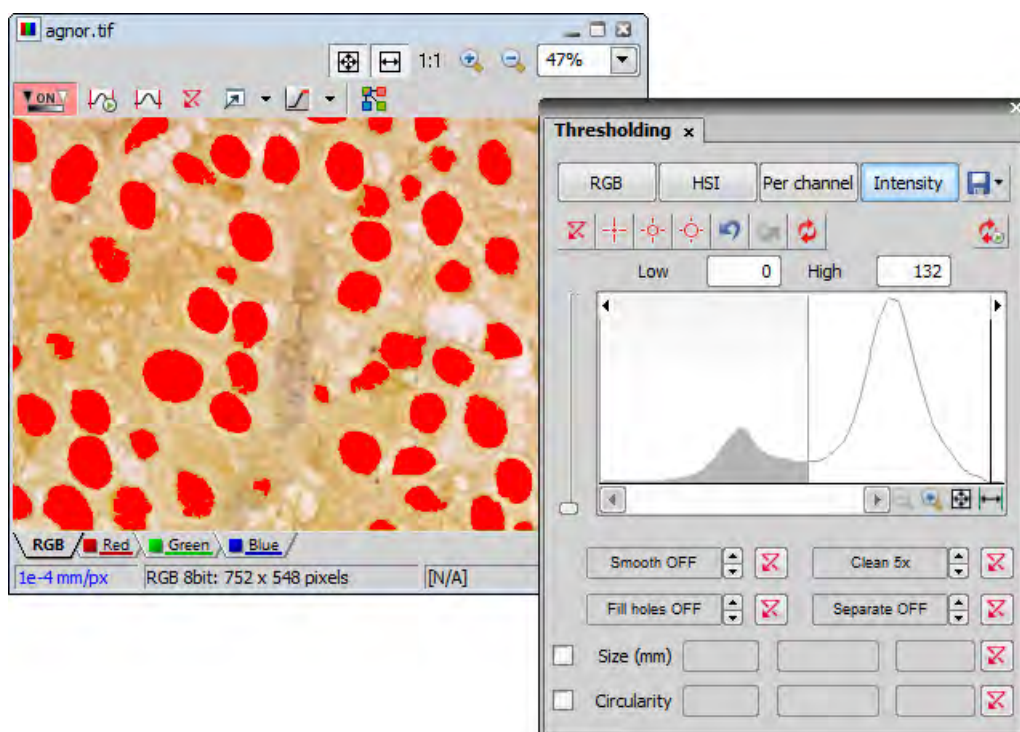
- If there are many channels, only one channel histogram is displayed. Switch between the channels by selecting their names from the bottom pull-down menu.
- The binary layer(s) can be displayed in two modes:
  1.  Each channel can create its own binary layer
  2.  One layer can be created as an intersection of all channels binaries.




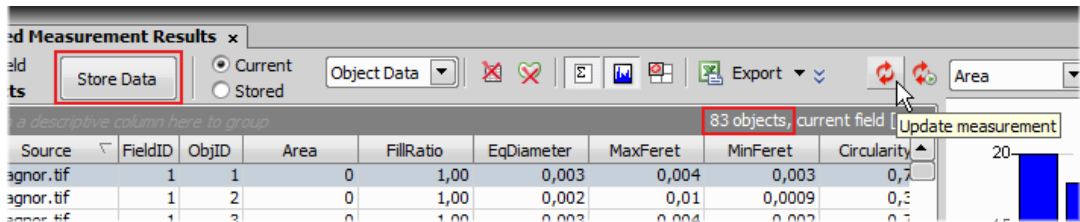
## 7.3.5. Thresholding Example

### Step by Step

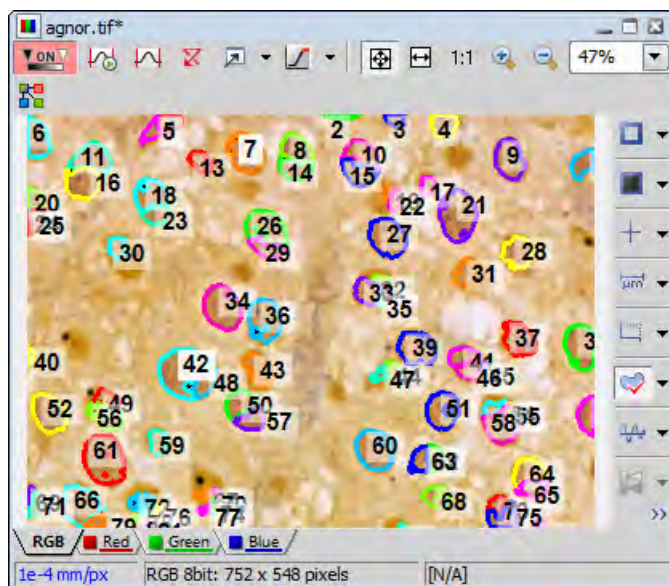
1. Threshold the image using the *Intensity Measurement* option in the *Thresholding* control panel.



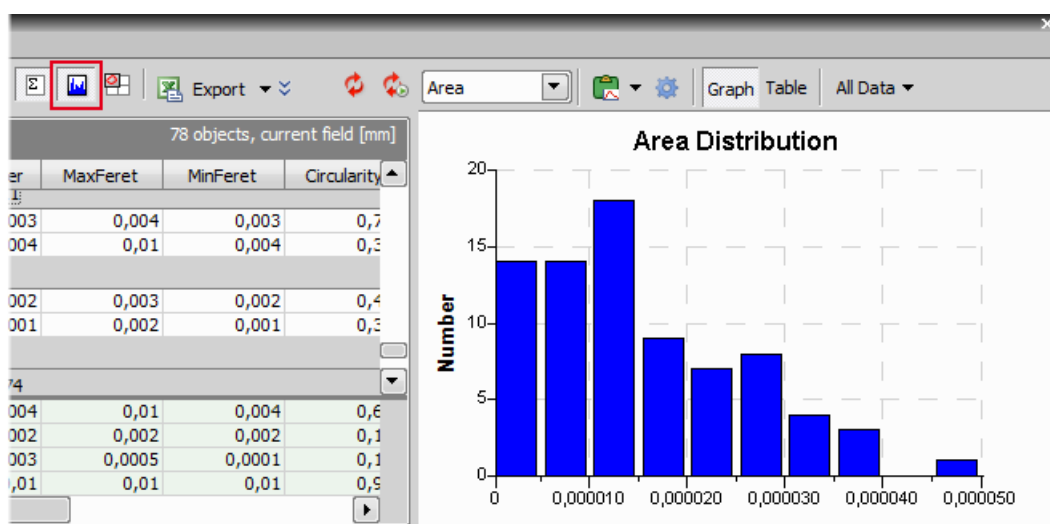
2. *Clean* the image to remove detected small non-nuclei areas.
3. Select features to be measured in the *Automated Measurement Results* control panel. Press the *Options* button and choose the *Select Object Features* command. An *Object Measurement Setup* window appears. Add or remove features you want to measure.
4. Press the  *Update measurement* button in the *Automated Measurement Results* control panel to measure image features. Overview the results. Press the *Store Data* button to save the data for future reference.



- Right click the Turn ROI On/Off button in the right document toolbar. Choose the ROI > Move Binary to ROI command. Each binary object turns into separate ROI.

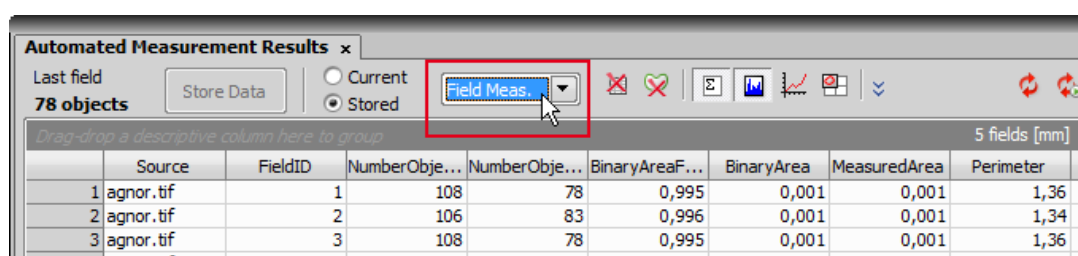


- Threshold the cell nucleoli in the nuclei the same way as was already described above using the *Thresholding* control panel.
- Measure the thresholded nucleoli using the *Update measurement* button in the *Automated measurement Results* control panel. Press the *Store Data* button to save the data.
- Group results per ROI: drag the title of the column called *RoiID* into the grey area above the columns. You can drag and drop any combination of features as well. Results always group according to such selected features.
- Turn on the graph using the *Show Histogram* button.



- 10 To get the information about area percentage, select the *Field measurement* option in the top toolbar of the *Automated measurement Results* control panel.

Figure 7.8. Example



Field 1 displays results of measurement when measured without ROI. That means % of nucleoli in the entire image.


Field 2 displays results of measurement when measure with ROI on. That means % of nucleoli within the nuclei.



- 11 You can export the result to various locations using the *Export* command.


## 7.4. Binary Layer

### Working with the Binary Layer

There are the following buttons at the bottom of the right image tool bar.

 **View Binary** This button displays the binary layer only of the image. It can be edited by hand using the binary editor ( *Binary > Binary Editor* ).





 **View Overlay** The binary layer can be displayed together with the color layer using an overlay mode. Several binary layers can be created for one color image. Manage them using the  *View > Analysis Controls > Binary Layers* control panel.

 **View Color** This button switches to view the color image (only).

The binary layer, as a result of thresholding, can be modified by hand using the binary layer editor. It is a built-in application providing various drawing tools and morphology commands. Go for the *Binary > Binary Editor* command or press *Tab*. New buttons appear on the tool bars:


### 7.4.1. Drawing tools

The binary image can be modified using various drawing tools. Although the way of use of some tools differs, there are some general principles:

- Make sure you are in the right drawing mode (drawing background /foreground 
- Drawing of any object which has not been completed yet can be canceled by pressing *Esc* .
- The polygon-like shapes are drawn by clicks of the left mouse button. The right button finishes the shape.
- The automatic drawing tools (threshold, auto detect) have a changeable parameter. It can be modified by  and  keys or by mouse wheel.
- The scene can be magnified by the UP/DOWN arrows when mouse wheel serves another purposes.
- You can drag a magnified image by right mouse button.
- A line width can be set in the top tool bar.
- Hints are displayed in the second top tool bar.


### Binary Layer Color and Transparency

When in overlay mode:



- The *Insert* key switches between predefined overlay colors.
-  increases/decreases the binary layer transparency.

### **Erasing Single objects**

Single binary objects can be erased in the following way:

- Run *View > Analysis Controls > Binary Toolbar*.
- Select the *Delete Object*  tool from the toolbar.
- Click inside the objects to be erased.

### **Multiple Binary Layers**

An arbitrary number of binary layers can be created within one image. Click this *Create New Binary Layer*  button in the image tool bar to add a new binary layer. The binary layer that you are currently editing can be selected in the nearby pull-down menu. Binary layers can be managed from the  *View > Analysis Controls > Binary Layers* control panel.


## 7.5. Mathematical Morphology Basics


The binary image as a result of thresholding often needs to be modified before a measurement is performed. Edges of the objects can be smoothed, holes in the objects filled etc. by using the mathematical morphology commands.


### Note


*“Image Analysis and Mathematical Morphology” by J. Serra (Academic Press, London, 1982) was used as a reference publication for the following overview.*


The basic processes of mathematical morphology are: erosion, dilation, open, close and homotopic transformations.


 **Erosion** After performing erosion, the objects shrink. Marginal pixels of the objects are subtracted. If an object or a narrow shape is thinner than the border to be subtracted, they disappear from the image.


 **Dilation** After performing dilation, the objects enlarge. Pixels are added around the objects. If the distance between two objects is shorter than twice the thickness of the border to be added, these objects become merge together. If a hole is smaller than twice the thickness of the border, it disappears from the image.

 **Open** Open is erosion followed by dilation so the size of the objects is not significantly affected. Contours are smoothed, small objects are suppressed and gently connected, particles are disconnected.


 **Close** Close is dilation followed by erosion so the size of objects is not significantly affected. Contours are smoothed, small holes and small depressions are suppressed. Very close objects may be connected together.

 **Clean** This transformation is also called geodesic opening. The image is eroded first so small objects disappear. Then, the remaining eroded objects are reconstructed to their original size and shape. The advantage of this algorithm is that small objects disappear but the rest of the image is not affected.

 **Fill Holes** Fills the holes inside objects. This transformation is handy when objects have a rich inner structure with intensities typical for background. After applying the *Fill Holes* transformation, objects become homogeneous.

 **Contour** This transformation converts objects into their contours.

 **Smooth** Smooth affects rough edges of the objects makes them smooth.

 **Morpho Separate Objects** This transformation detects standalone objects that are connected together and isolates them.

### Homotopic Transformations

Homotopic transformations preserve topological relations between objects and holes inside them. Using a homotopic transformation, an object with 5 holes will be transformed to another object with 5 holes. Two objects without any holes, even if they are very near each other, will become again two objects without holes, but likely with a different shape and size. Opening, Closing, Erosion and Dilation are not homotopic transformations. Typical homotopic transformations in *NIS-Elements AR* are: *Skeletonize*, *Homotopic Marking* and *Thickening*.

## 7.5.1. Connectivity

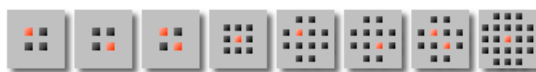
Applying the above mentioned transformations has some limitations due to digital images rasterization.

When speaking about binary image processing, a binary image is a set of pixels where values 1 represent objects and values 0 represent background. In the square grid of the image, two possible connectivities can be used for processing - the 8-connectivity or the 4-connectivity. Look at the picture below. If the 8-connectivity is used, the two pixels represent one object. If the 4-connectivity is applied, there are two objects in the picture. *NIS-Elements AR* works with the 8-connectivity model, so all pixels neighboring by the corner belong to one object.



## 7.5.2. Structuring Element = Kernel = Matrix

When applying Erosion, Dilation, Opening or Closing, one of the parameters which determines the transformation result is the selection of kernel (structuring element, matrix) type. There are the following kernels used in *NIS-Elements AR*:



The bright pixel in the center or near the center of the kernel represents its “midpoint”.

*Example 7.2. Erosion*

Let's assume 1 and 0 represent object(1) and background(0) pixels of the binary layer. You can imagine the erosion as the following algorithm:

Move the midpoint of the kernel to every point of the image. Each time, look at the neighboring pixels of the kernel and make the following decision:

- If there are object(1) pixels in all the positions of the kernel, set the midpoint to object(1).
- If there is at least one background(0) pixel in the kernel, set the midpoint to background(0).

*Example 7.3. Dilation*

You can imagine the dilation as the following algorithm:

Move the midpoint of the kernel to every point of the image. Each time, look at the neighboring pixels of the kernel and make the following decision:

- If there is at least one object(1) pixel in any position of the kernel, set the midpoint to object(1).
- If there are background(0) pixels in all the positions of the kernel, set the midpoint to background(0).

*Example 7.4. Open and Close*

Open is performed by eroding the image and then applying a dilation to the eroded image. On the contrary, Closing is performed as a dilation followed by erosion.

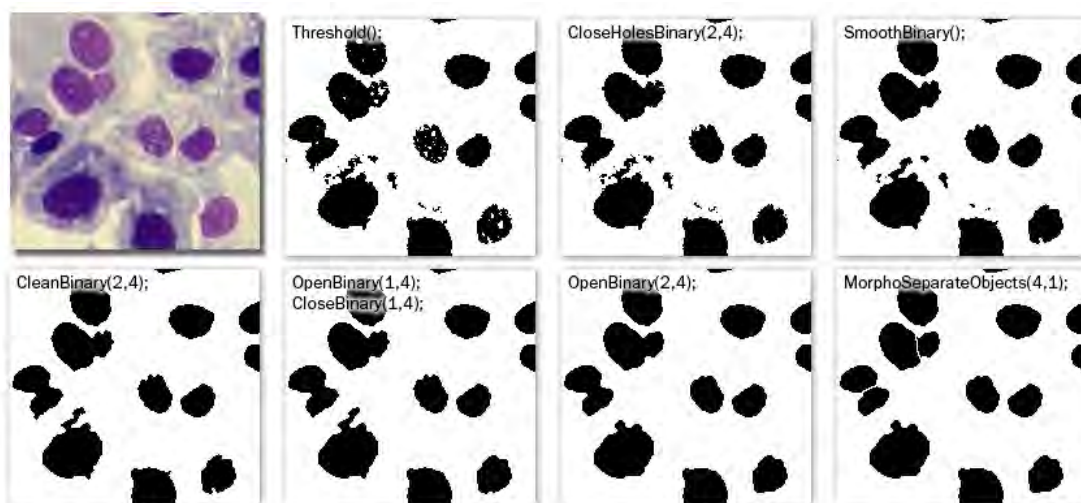
**Repetition issues**

If the midpoint is not in the center, applying erosions or dilations by odd number of iterations causes image to shift by 1 pixel. Normally, the total image shift would be determined by the number of iterations (in pixels). *NIS-Elements AR* eliminates this shift: it changes the position of the midpoint 1 pixel down-rightwards within the kernel for even operations. For opening and closing it is possible to eliminate this shift totally. However, if you run the erode or dilate processes again and again using the kernel with even dimensions and odd number of iterations, then the shift becomes significant.

## 7.5.3. Mathematical Morphology Examples

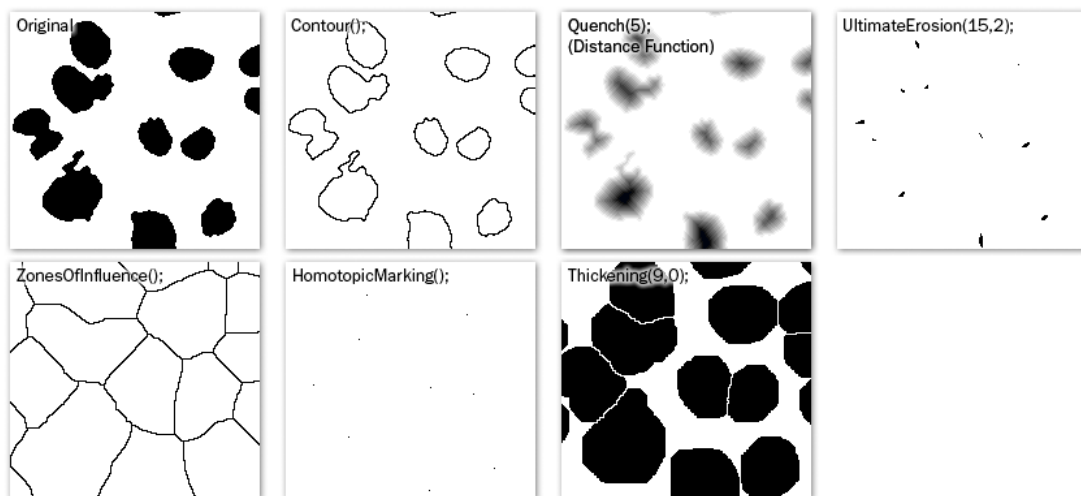
Please see the following examples of some of the binary functions applied to an image. In the following sequence of images, the functions were applied subsequently:

Example 7.5. Functions applied subsequently



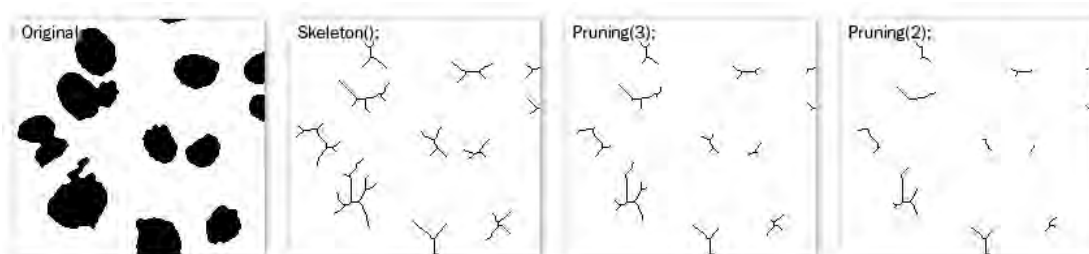
Example 7.6. Single function applied to the original

The following examples were created by calling each of the functions separately:





Example 7.7. Other functions applied subsequently



## 7.6. Regions of Interest - ROIs

In laboratory imaging experiments, users are often interested in just a part of the image. To define such an interesting part of the image we use one or more Regions of Interest (ROIs). These regions are used later in analysis and measurements.

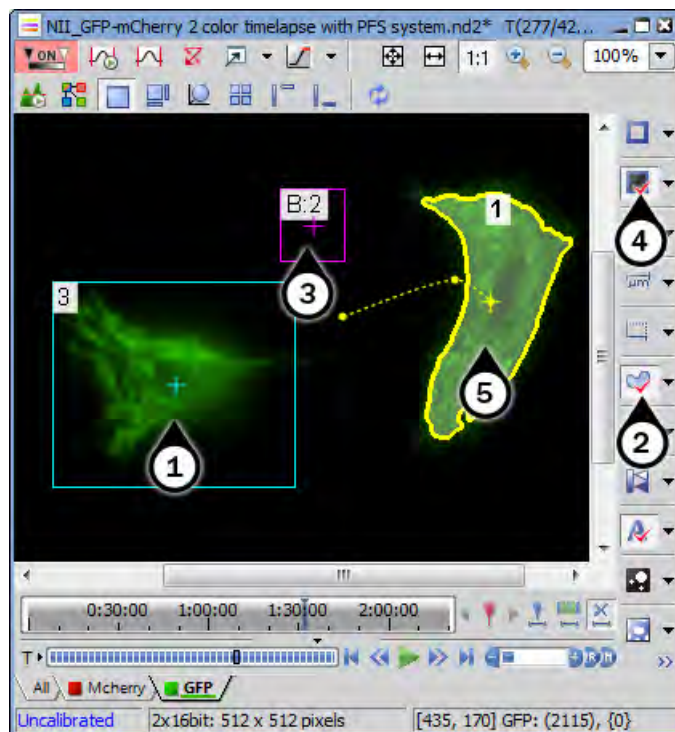
### Note

*Do not confuse image ROIs with Camera ROI which is used to reduce the active area to just a part of the camera sensor (CCD or CMOS). Such reduction usually results in higher frame rate. See 5.3. Camera ROI [57].*

### 7.6.1. Creating and editing ROIs

ROIs can be created and edited using commands gathered in the ROI menu. ROIs can be also created from binary objects, graticules (for equally spaced shapes) and from binary layers created by tracking.

Figure 7.14. Available ROI tools



1. Global ROI
2. Turn ROI On
3. Background ROI
4. Turn Background ROI On
5. Moving ROI

## 7.6.2. Types of ROI

Individual ROIs (ROI objects) are 2D objects. They can have different shape: rectangular, elliptical polygonal or bezier. One shape can be created with one or more tools. For instance polygon ROI can be defined using both “Draw Polygonal ROI” and “Autodetect ROI”.

In case of an ND file which has time axis (e.g. time-lapse) individual ROI objects can be defined as “Global” or as “Changing over time”. Global objects do not change any of their characteristics over time. Objects that are defined as changing over time can change their position and shape in time (however objects cannot change their shape - e.g. from ellipse to polygon). This feature is useful for track moving or warping objects.

To track moving objects use the “Edit ROIs in Time” editor (available as a Local Option) or just change any global ROI into changing over time from the objects' context menu and move it or change its' shape (see interactive edit) on two or more frames. Such frames are called “key-frames” (2 shows trajectories with little boxes representing the key-frames). All frames in between key-frames are interpolated from these two key-frames. Frames before and after first and last frame respectively are taken from that first or last frame. Therefore it is not necessary to reposition or redraw the ROI on every frame.

In case of Multi Point nd2 file there is one more option: ROI can be defined per Multi Point (which is the default). It means that ROIs from one point are completely independent of ROIs defined on the other point (even if they have the same number). This applies also to ROIs defined as “Changing over Time” (in case the image has time axis too). On the other hand if the ROI is changed to a “Global” it will be shared among all points of the Multi Point set.

Depending on the application purposes ROI objects can have different usage. NIS can handle “Background ROIs”, “Reference ROIs”, “Stimulation ROIs” and “Standard ROIs”.

### 7.6.3. Interaction with ROIs

ROIs can be manipulated anytime directly in the image window. Many of the following techniques work in ROI editors too. Objects cannot be moved or resized when they are Locked (some analysis tools or users may lock them to prevent accidental modification). They must be unlocked (from the ROIs' context menu) before any manipulation. Note that the mouse cursor changes over the ROI to indicate which action will be started after mouse button is pressed (it changes when modification keys like Alt and Ctrl are pressed).

**Selection** ROI objects can be selected by clicking on an unselected object (other previously selected are unselected). Clicking on a ROI object with Ctrl key pressed results in altering the selection state. More than one object can be selected in this way. Double-clicking on any object results in only that object being selected. All objects can be selected by pressing Ctrl+A or by choosing “Select All ROIs” from the menu over any ROI. Alternatively, it is also possible to select a set of objects by dragging a rectangle around them with Ctrl key being pressed.

**Moving** Objects are moved by simply dragging them (cursor indicates that). When more objects are selected only the one under cursor is moved by default. This can be changed by pressing the Alt key and move all selected objects. When object is changing over time, any moving results in a new key-frame. Individual dots representing the key-frames can be moved too. It is possible to duplicate existing objects by Shift dragging. It works on selected objects. In case of a key-framed object, object is duplicated with all its' key-frames unless Alt is pressed.

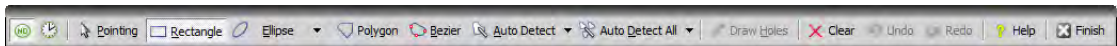
**Resizing** Resizing a ROI is done by dragging its' contour (the cursor changes accordingly). Note that resizing hit-zones extend towards outside of the object (as opposed to moving hot-zones which extend towards inside of the object). Resizing always affects only one object (selection is not taken into account). The behavior slightly varies depending on the object shape. By default resize does not hold aspect of the object - width and height can be changed independently. By holding Shift key aspect is maintained (useful for circles) and the center is not moved. Rectangle can be also rotated by using Alt key while dragging.

Exact values can be entered in “ROI Properties” dialog which is accessible from the ROI context menu.

## 7.6.4. Simple ROI Editor

When creating more than one ROI, the Simple ROI editor is the tool of choice. With this tool it is possible to make all possible shapes with different methods. All interaction techniques work here as well.

*Figure 7.15. Simple ROI Editor toolbar*



Note that many actions have a hot-key (P for Pointing tool, R for Rectangle, E for ellipse, L for Polygon, B for Bezier, A for Auto detection, H for drawing holes and others) to speed up the process of interaction.

## 7.6.5. Auto-Detect Tool

Auto-detect tool is available from ROI menu, Simple ROI editor, Edit ROIs in time editor and some other places. It produces a Polygon ROI. Using the tool is two step process: The first click on an image structure results in preliminary object contour, that is drawn on the screen. After that, user can further tweak the object contour and then finish whole auto-detect by confirming with right mouse button. User can always cancel auto-detect by pressing Esc key.

The very first click is important, because these pixels under the cursor determine intensities from which the algorithm guesses the whole object. As a general rule it is better to choose brightest pixels in the bright objects (fluorescence) and dimmest in the dark object. It is also good to click near the center of the object.

After the click the algorithm presents its guess of the object contour and goes into the interactive phase. In this phase it is possible to:

**Redefine the first point** by clicking again in the image

**Cancel auto-detect** by pressing Esc key

**Grow or shrink the object area** (changes the intensity range of pixels making the object) by scrolling mouse wheel or pressing PageUp/PageDown (for finer steps use Ctrl + mouse wheel)

**Erode or Dilate** morphologically the object by 'E'/'D' or Down/Up keys

**Open or Close** morphologically the object by 'O'/'C' keys

**Morpho-separate** portions of the object by 'S' or 'P' key (if it is not possible to separate the key does nothing)

## 7.6.6. Using ROIs for analysis

ROIs are used mainly in Time Measurement to measure varying image intensity inside the ROIs, Automated Measurement to measure ROIs features or number of binary objects inside each ROI, Object Count to restrict binary objects to areas of interest only, ROI statistics to measure intensities interactively.

In all these cases ROI selection is taken into account as well as intersection options in Measurement Options.

Additionally the Time measurement interprets the ROI usage type. Background ROIs are used for offset subtraction and Reference ROIs can be used for bleaching correction.

## 7.7. ROIs in Time - Moving ROIs

### 7.7.1. Introduction to Moving ROIs


#### **To define a moving ROI:**


1. Open or capture a time-lapse nd2 file.
2. Display the first frame of the image and open the vector ROI editor by the *ROI > Simple ROI Editor* command.
3. With one of the editor tools, draw the ROI around an object. Close the ROI editor.
4. Right-click the ROI and select *ROI Changes Over Time* from the context menu.
5. Play the image sequence and if the object of interest starts to move out of the ROI, pause the replay.
6. Now it is possible to drag the ROI to the new position. If the ROI is created by the vector editor, its size and rotation can be also modified.
7. Play the sequence further and drag the ROI to a new position each time the object in the image moves.

#### Note

*ROI positions between two user-defined frames are interpolated. If the object of interest was moving at constant speed with a linear trajectory, then it would be sufficient to define the ROI positions only in the first and the last frame of the image sequence.*

#### **Tips on Moving ROI**

**ROI Tracking** Motion characteristics of a manually defined moving ROI can be measured using the *View > Analysis Controls > Tracking* control panel. Just click the *Add Moving ROIs*  button to add the current ROIs to the table of objects.

**Automatic ROI Definition** Automatic *Tracking* features may be utilized to define moving ROIs. Already tracked objects may be converted to moving ROIs by clicking the *Binary to ROI*  button.

**ROI Multiple Selection** Use the Ctrl key to select/unselect more than one ROI. When you have selected more ROIs, press and hold the **Alt** key while dragging a single ROI. Other selected ROIs will not be moved but remain selected.

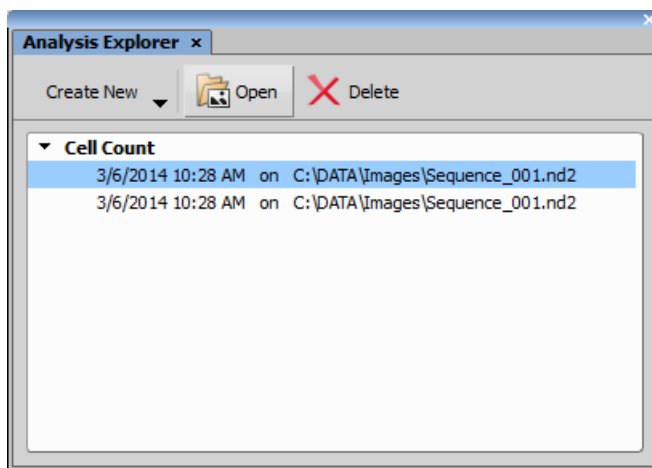
## 7.8. Analysis Modules

### 7.8.1. Analysis Explorer

(requires:Local Option)

#### 7.8.1.1. Analysis Explorer Basics



The *Analysis Explorer* control panel enables the user to manage and run analysis recipes. Choose *View > Analysis Controls > Analysis Explorer* to display it.




There is a button with a pull-down menu in the top left corner where all available analyses are listed. Once you save the analysis, a new item is created in the explorer. Each time the analysis is run, a new record is added under the analysis name. It is linked to the image file to which the analysis was applied.

#### 7.8.1.2. Usage


##### Creating an analysis

1. Open an image to be analyzed.
2. Select the analysis from the  *Create new* pull-down menu. The analysis definition window appears.
3. Adjust the analysis settings - live preview of the segmentation is displayed in the image so you can play with the segmentation parameters. Save it using the  **Save As** button. Now the named analysis is listed in *Analysis Explorer* and is ready to be run on other images.

### Running analysis on the current image

1. Open an image.
2. Select the analysis name within *Analysis Explorer*.
3. Click the  **Run** button. The analysis will be applied to the current image.

### Batch analysis

1. Select the analysis name within *Analysis Explorer*.
2. Click the  **Batch...** button. A dialog window appears.
3. In the *Analysis Recipe* portion of the window select the level of user interaction:
  - Run the batch without use interaction** The analysis will be applied to all images “as is”.
  - Show definition for each image** After opening each image in the batch, the analysis definition window appears allowing you to adjust the settings for each image separately.
  - Show definition once** The definition window appears only after opening the first image of the batch.
4. When the batch is finished, a confirmation window appears. Click **OK**.


**Reviewing measurement results** All measurement results are saved to the global *NIS-Elements* results table. Run the *View > Analysis Controls > Automated Measurement Results* command to display the appropriate control panel.



#### Caution

*Each run or batch run clears the automated measurement results table.*

### 7.8.1.3. Managing Recipes


Particular settings of your analysis may be saved as a recipe so that you can re-use the settings anytime in future. Moreover, each run of the analysis recipe saves the settings to the database so you can create a recipe out of the analysis run. Here are the options:

**Export to a file** Right-click the analysis name and select  **Export** command. Browse for the location to save the analysis definition to and click **Save**. The file will be saved with an extension appropriate for the analysis type (such as \*.cellcount)

**Import from a file** Click the  **Create new** button and select  **Import Recipe from File**. Locate the file containing the analysis definition (e.g.: “c:\recipes\definition.cellcount”) and click **Open**.

**Import from JOBS** (requires:Jobs)

Click the  **Create new** button and select  **Import Recipe from JOBS**. A list of recipes saved from the JOB definition window appears. Select one and click **OK**.

**Applying local changes to a definition** Local changes (e.g. different threshold value) may have been applied to each run of one analysis. You can replace the original analysis definition by one of these locally changed. Just right-click the analysis run and select  *Revert to this definition*.

**Editing, Renaming or Duplicating analysis definition** All these actions are accessible from a context menu which appears when you right-click an existing analysis name.

## 7.8.2. Cell Count Analysis

*(requires:Local Option)*

This analysis is designed for counting organic cells. It operates on a single channel and produces one binary mask and a set of predefined features. It has similar functionality as the Object Count in *NIS-Elements AR*.

Start by choosing your optical configuration, selecting a color that is well visible in the preview image (check the *Preview* check box) and thresholding. The threshold bar enables selecting low and high intensity thresholds. Only the pixels within this interval will be taken to make objects visualized as a binary mask.

Under the main Threshold bar there are four post-processing controls to further tweak the objects (*Smooth, Clean, Fill holes, Separate*). If the objects are too rough it is a good idea to try some smoothing, but not too high values in order to avoid merging of nearby objects. Then use little bit of Clean to get rid of the smallest objects. Set Fill holes if it makes sense in your sample. And finally play with Morphological separate (higher numbers separate less – produce bigger objects). You can filter the objects based on their Size (EqDia – Equivalent Diameter: calculated from object area as if it was a circle) or *Circularity*. Then you can check each feature you want to measure. Following features are available: *Area, Eq. Diameter, Circularity, Elongation, Length, Width, Max Feret, Min Feret, Mean Intensity, Sum Intensity, Perimeter*. For their detailed description please see 8.10. Measurement Features [162]. If your object size does not differ, you can *Use object standard size*. *Object count* field shows the number of objects detected on the current frame using the current analysis settings. Finally you can choose whether to *Save features for each object* and confirm the analysis setting by clicking **OK**.

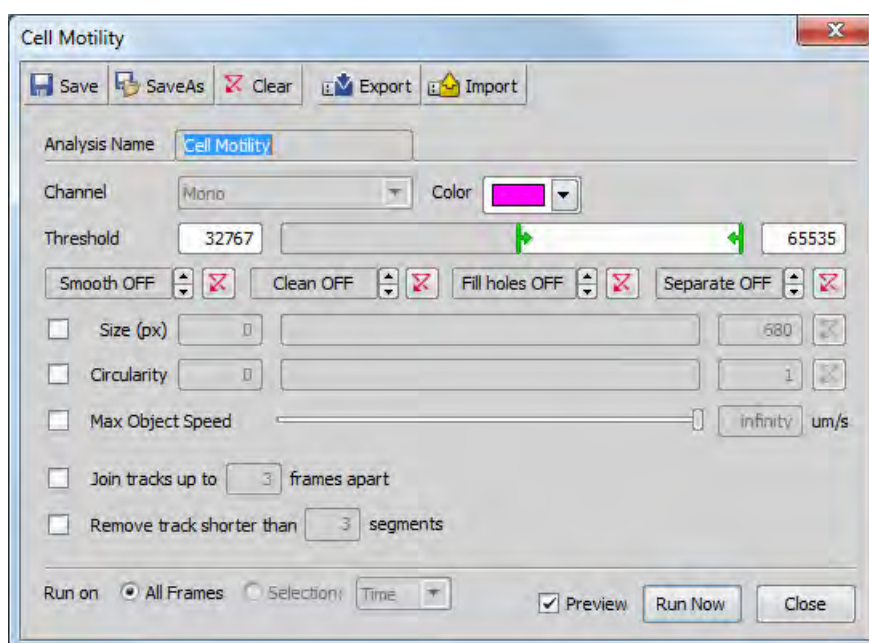
Where CaptureName is the name of the capture task, CellCountName is the name given to the CellCount analysis, FieldFeature is Count, Area or AreaFraction and ObjectFeature is ObjectArea, ObjectEqDia, ObjectMeanIntensity, ObjectSumIntensity.

## 7.8.3. Cell Motility

This analysis measures cell motility. A simple timelapse-only ND2 file must be open for this command to be enabled.



## Options



**Recipe** Select which recipe (a collection of settings) is used for the task from the pull down menu. You can also *Load* another recipe you have previously created or get. When you made some changes to the recipe, you can either *Save* them to the currently selected recipe, or *Save As* a new recipe.

**Channel** Select which channel is analysed.

**Color** Choose color of the binary layer.

**Threshold** Use the sliders to define the thresholded objects. You can also overwrite manually the values of thresholding limits.

**Binary operations** You can apply some of the binary operations to improve the thresholded binary image.

**Restrictions** You can filter the detected binary objects using the *Size* or *Circularity* restriction filters.

**Run on** Select which dimension is used for the analysis.

**Preview** Check to display preview of the result.

**Run Now** Starts the task and displays the *View > Analysis Controls > Automated Measurement Results* window and the *Measure > Time Series Graph* with the results.

**Cancel** Cancels the task and closes the window.

## 7.8.4. Cell Proliferation

This analysis measures cell growth in time. A simple timelapse-only ND2 file must be open for this command to be enabled.

## 7.8.5. General Analysis

### 7.8.5.1. Introduction to General Analysis

With *General Analysis*, it is possible to make multiple binary masks on a single channel as well as to combine multiple masks by means of generic expression with powerful operators. It is also possible to calculate custom features. High interactivity enables viewing your result at any time. Check boxes enable viewing just the desired operations or analysis steps. Complicated sequences can be created in few minutes - e.g. color image processing, segmentation, binary image processing, object filtration and measurement, etc. Binary masks can also be mixed using a general expression. Any binary mask can act as a ROI (Region Of Interest) inside which measurement can be done (organelles, markers in cells, etc.).

*General Analysis* exists in two forms - as a part of the Jobs module local option (results go to the Jobs database and are automatically saved as *Job Runs*) and as a local option of *NIS-Elements* available from the *Image* menu (results are shown in the *Automated Measurement Results* tab and are automatically deleted after closing unless they are saved). Except small details, the graphical user interface is almost the same in both forms.


See also: 10.3. HCA Analysis [192].

### 7.8.5.2. How To Set General Analysis

#### 1) **Insert analysis info**

You start with an empty dialog with the *Info* tab selected. Here you can insert description of the analysis.

#### 2) **Add relevant color channels to the analysis**

Decide on which image channels the segmentation will be performed. Add these channels to the *Analysis steps* tab by the  *Add Channel* button.

#### 3) **Define analysis of each channel**


Adjust the preprocessing, segmentation, binary processing parameters and measurement features for the added channels. The resulting binary layers are displayed in the current image window. You can turn each layer ON/OFF by the check box in front of the channel name.

If preprocessing has been applied, you can always display the original image by selecting the *View original* check box.

#### Note

*Any changes made to the color image are temporary (only for Thresholding and Spot Detection) and all measurements are made on the original (unaffected) image data as well as saving.*


### 4) Combine binary layers (optional)

You can perform logical operations processing and measurements on multiple binary layers. To do so, create a “combined” channel by clicking the  *Add Combined* button and specify the parameters.

### 5) Define ROI

Binary mask of one channel can be used as a ROI to restrict measurement on another channel. If this is the case, click the *Add ROI* button. In the ROI channel, again, specify the measurement features.

### 6) Define calculations

Click the  *Add Calculations* button to add the calculation step in the analysis. In this step you can define any mathematical expression using the features measured in previous steps of the analysis.

### 7) Run the analysis

Click the **Run Now** button to apply the analysis to the current image. A new record will be appended to the list of performed analyses in the *Analysis Explorer*. The measured results are then accessible in the *View > Analysis Controls > Automated Measurement Results* window.

## 7.8.6. Image Intensity Analysis

(requires:Jobs)

The Image Intensity analysis evaluates the intensity of the whole Field of View. It is purposefully simple. The example of usage for this analysis is when deciding whether there is a signal in the field or not - i.e. if you want just to decide if to capture (or not to) the whole well. If the field is the whole well, there are some options to restrict the well outline (rectangular or circular) and center portion if necessary. It is also possible to restrict the range of intensities. Image Intensity analysis window enables defining the Image Intensity as shown on the following picture. If you click the first Preview icon you can see your changes directly on the live image.

This analysis evaluates the image data intensity. It is purposefully simple. Image Intensity is useful if you want just to decide if to capture (or not to) the whole well. You can evaluate the whole image, circular / rectangular region of the image or the thresholded portion.

---

### 7.8.7. Live/Dead Analysis

*(requires:Jobs)*

This analysis is used for counting live and dead elements in one sample. Two optical configuration channels are required - one channel is used for counting Live elements and one for Dead elements.

### 7.8.8. Wound Healing

This analysis measures changes of cell distribution in time. A simple timelapse-only ND2 file must be open for this command to be enabled.

## 8. Measurement

### 8.1. Calibration

Calibration of the image is of crucial importance to measurement. On calibrated images, realistic measurements can be performed and objects of different images could be compared to each other. A correct calibration has to be made before you begin to measure.

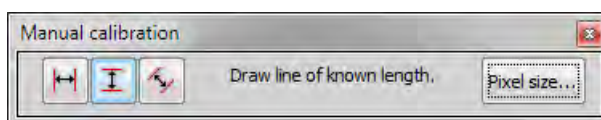
There are two ways how to obtain a calibrated image:

- To capture images with a calibrated system (objective). Please see the 4.1.3.3. Objective Calibration [42] chapter which explains how to calibrate the *NIS-Elements AR* system.
- To calibrate an uncalibrated image manually:

#### **Calibrating an Uncalibrated Image**

1) Run the *Calibration > Recalibrate Document* command.

2)



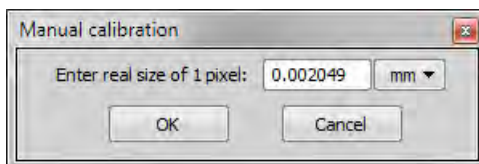
3)

- a) If you know the size of one pixel, click the **Pixel size...** button.
- b) If you do not know the pixel size, draw a distance to the image and set its real length. For this purpose, you should use an image of a calibration slide or a ruler captured with your system.

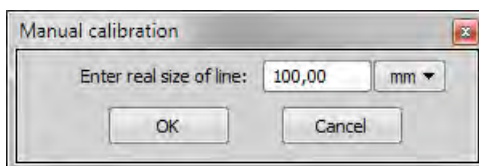
There are three modes: horizontal, vertical and parallel lines, select one of them. Click into image to place first line, then second line appears on your mouse cursor. Place this line and the following dialog box appears.

4)

*Figure 8.2. Pixel Size Calibration*



*Figure 8.3. Line-length Calibration*




5) Click **OK** to finish the calibration.

## 8.2. Units

*NIS-Elements AR* supports the following units:

pixels  
nanometers  
micrometers  
millimeters  
centimeters  
decimeters  
meters  
kilometers  
inches  
mils

If the image is uncalibrated, pixels are the only units available. In case of a calibrated image, it is possible to select other units which are then used to display all values (e.g. measured length/area). There are two ways of how to select the desired units:









- Right click the image status bar where the calibration is displayed. Select the units and precision from the context menu.
- Or, click the *Current unit* button located in the  *Calibration > Objectives* command window and select the units.

## 8.3. Rough Measurement

Quick and approximate measurements can be performed utilizing graticules. They behave like adjustable floating rulers. User can simply align a graticule with the measured object and read the distance value (e.g. the diameter). To activate the graticule, press the *Graticules* button.

### 1) Select Graticule Type

The type of the graticule ruler is indicated by a picture on the *Graticules* button placed on the right image tool bar. To change the graticule type, click on the graticules button with the right mouse button and select the appropriate item from the context menu:

-  Rectangular Grid
-  Circle
-  Simple Circle
-  Cross
-  Industrial Cross
-  Simple Cross
-  Vertical Ruler
-  Horizontal Ruler


### 2) Define Graticule Properties

Right click the *Graticules* button and select the *Graticules properties* command from the pull-down menu. A window appears where display parameters (shape, color, line width, density of lines) of all graticules can be adjusted.

#### Graticules Density

The density value - the closest distance between two line intersections of the graticule - can be set, or you can let *NIS-Elements AR* adjust it automatically according to the current zoom factor. The units selection depends on the image calibration (calibrated/uncalibrated).

#### Note

*Concerning the Cross  graticule. The distance defined by Density is divided into smaller sections automatically. However, for odd density values greater than 10, the markings remain hidden because it is not possible to display them accurately.*

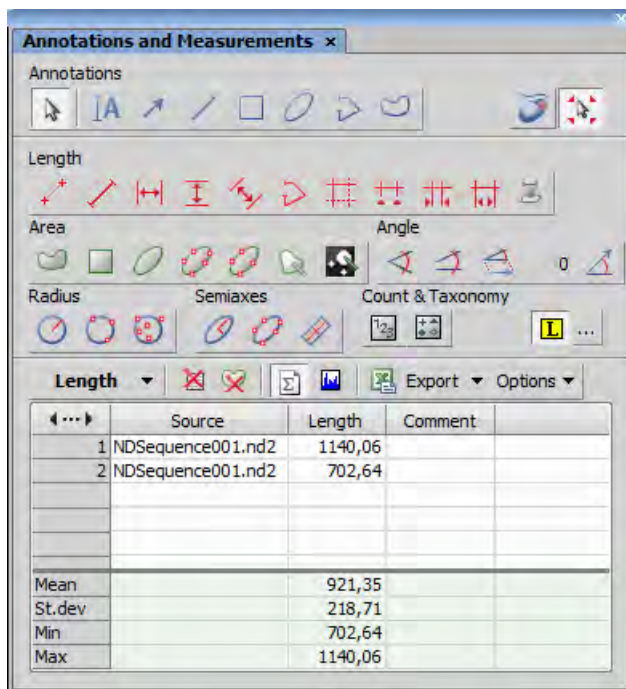
### 3) Measure the Image

The graticule measurement provides the following options:


- The graticule can be moved by mouse arbitrary.
- The graticule position can be reset by using the *Move Graticules to Center* command from the context menu.

- A binary layer can be created from the current graticule using the *Copy Graticules to Binary* command.
- A *Graticule Mask* can be created from the current binary layer using the *Copy/Add Binary to Graticule Mask* command.
- A *Graticule Mask* can be stored/loaded to/from an external file via the *Save Graticule Mask As* and *Open Graticule Mask* commands.
- A new image containing graticules can be created by the *Edit > Create Full View Snapshot (8bit RGB)* command.

## 8.4. Manual Measurement



Length, area, angles, taxonomy, counts, circle radius, and ellipse semiaxes can be measured manually over an image. The results are being recorded to a simple statistics table, which can be exported to a file or clipboard. Also, the data can be presented as a graph.

- Run the  *View > Analysis Controls > Annotations and Measurements* command. The manual measurement control panel appears.
- Select a tool corresponding to the feature you are going to measure. There are several tools for measuring each feature.




- Measure the objects in the image using mouse.
- Select where to export results in the *Export* pull-down menu.
- Export the results using the *Export* button.

### **Example Procedure**

Measurement of the image of a grain:



- 1) Select the *Vertical parallel lines*  tool.
- 2) Place the first line on the top edge of the crystal by clicking into the image. The position of the line can be adjusted while you hold the left mouse button down. After you release it, the line is positioned.
- 3) Repeat this to place the second line on the bottom edge of the crystal.
- 4) When finished, an arrow is drawn between the lines, and the result of the measurement is attached. A record with the measurement type and the measured value is added to the results table.

### **Working with the Measurement and Annotation Objects**

There are two types of vector objects which can be placed over the image: annotation objects and measurement objects.

**A** This button of the right image tool bar turns the visibility of annotation layer ON/OFF. Right-clicking the button displays a context menu with the following options:

- *Clear All Objects* will delete annotation and measurement objects.
- *Clear Annotation Objects* will delete all annotation objects.
- *Clear Measurement Objects* will delete all measurement objects.
- *Select Annotation Objects* will select all objects that are not the measurement ones.
- *Select Measurement Objects* will select all measurement objects.
- *Select All Objects* and *Deselect* will select/deselect all annotation and measurement objects.

---

**Visibility of annotation/measurement objects** In an ND document that contains time dimension, you can set visibility of any annotation object by the following rules:

- object is visible globally means in all frames in all dimensions - select the option *Object is always visible*
- object is visible in all T, Z dimensions, but only in current multipoint - select the option *Object is always visible* and check the *Object belong only to Current Multi Point* option
- object is visible only in selected time frames in all multipoints - select the option *Object is visible in range*
- object is visible only in selected time frames in current multipoint - select the option *Object is visible in range* and check the *Object belong only to Current Multi Point* option

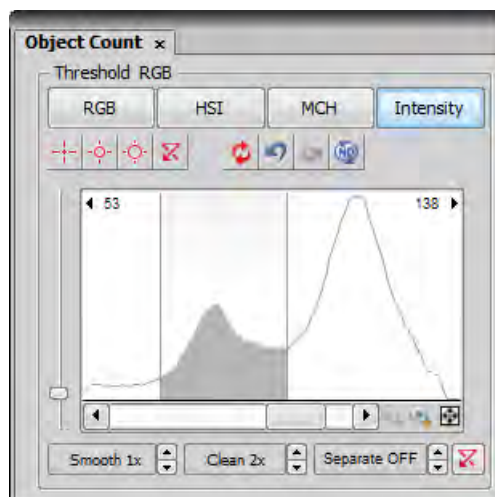
Select one of these rules in the context menu that appears when you click with right mouse button on an annotation object in the ND document. Detailed definition of object visibility can be done using commands in the *Visibility in Time* sub-menu.

## 8.5. Object Count




The *Object Count* tool is designed for automated object detection and counting. It enables users to threshold the image, automatically measure the binary objects, and export the measured data to a file in a straightforward way. Object count can be performed even on Live image. Run the *View > Analysis Controls > Object Count* command to display the control panel.

The recommended workflow through the dialog is to move from left to right, starting with thresholding, then using restrictions and finally reviewing the result and export out of *NIS-Elements AR* to a text file or an MS Excel spreadsheet.

## 8.5.1. Thresholding the Image



The threshold limits shall be defined by picking object-typical colors from the image. Select one of the following tools:

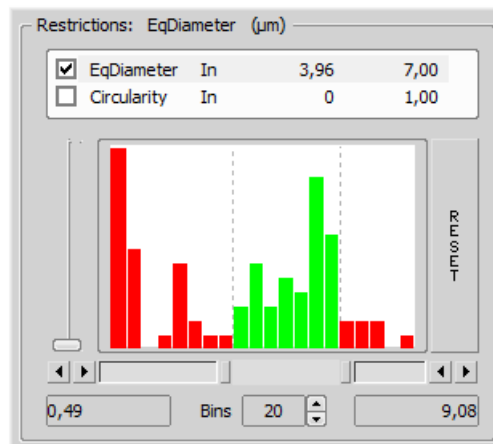
-  *1 point threshold tool*
-  *3 points circle threshold tool*
-  *6 points circle threshold tool*

Click inside the image to define typical object areas. The system will detect similar parts of the image and highlight it by color. The threshold limits are indicated in the histogram and can be further modified by dragging the limit lines by mouse.

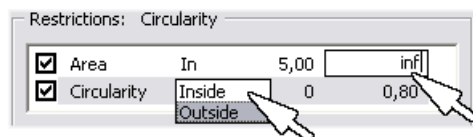
Please, refer to the 7.3. Thresholding [119] chapter for further details on thresholding.

## 8.5.2. Applying Restrictions

The number of objects that are included in the results table can be restricted by defining limits. Objects which do not fit these limits will be left out of the result table.



- Right click to the restrictions field to select one or more of the available measurement features.
- Select the restriction feature you would like to define.
- Move sliders below the histogram to set the limits. The limit values are indicated next to the feature name, and can be modified by double clicking the indicated value.
- Decide whether the defined interval will be excluded or included - this is done by setting the *Inside/Outside* option next to the feature name.
- The nearby check box indicates whether the restriction is currently being applied or not. If applied, the histogram below is color, otherwise it is gray.



#### Note

*Infinitude can be defined by entering "oo" or "inf".*

**Reset** The *Reset* button on the right side discards restriction settings of all features.

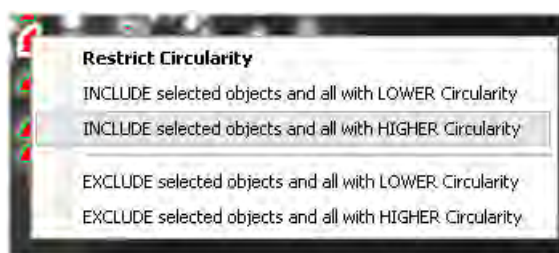
**Bins** This setting determines the number of columns in the histogram.

### Restrictions from the Image

A reference object can be picked from the image and used to set the limit values.

- Select the restriction feature you would like to set by mouse. Let's assume you have selected *Circularity*.

- Right click the thresholded object which is going to be used as source. A context menu appears.
- Select whether to *INCLUDE* or *EXCLUDE* the selected object and all others with higher/lower circularity.
- The restriction will be applied and indicated by colors within the image.



### 8.5.3. Results

The following results appear in the results table:

- *Total Area* is the area of the ROI. It is shown in square pixels or square *calibration units*.
- *Measured Area* is the area of the binaries inside the ROI.
- *Number of objects* - number of objects after applying restrictions.
- *Table of results* - measurement results for each object are displayed in this table. Select the columns to display/hide from the context menu which appears when you right-click one of the column captions.
- *#* - number of selected objects is displayed in the left part of the tool bar. One or more objects can be selected by mouse while holding the Ctrl or the Shift key.


Result


Total Area: 6534,13  $\mu\text{m}^2$


Area Fraction: 16,55 %


55


0












 Export





☒ Use Standard EqDia


5  $\mu\text{m}$



ROI ID	Binary ID	#	Area	Perimeter	Circularity	MeanIntensity
Whole Image	Threshold	1	22,40	24,60	0,47	97,68
Whole Image	Threshold	4	17,05	15,73	0,87	95,32
Whole Image	Threshold	5	23,99	19,31	0,81	90,72
Whole Image	Threshold	9	31,39	33,82	0,34	93,19
Whole Image	Threshold	10	32,77	36,45	0,31	78,24
Whole Image	Threshold	11	31,28	20,59	0,93	97,34
Whole Image	Threshold	12	35,06	32,44	0,42	88,56
Whole Image	Threshold	14	12,94	13,90	0,84	119,15
Whole Image	Threshold	15	15,94	16,37	0,75	86,34
Whole Image	Threshold	16	23,23	21,40	0,64	110,55

## Tool Bar

There is a tool bar above the results table which contains the following buttons:


 **Delete selected objects** Removes the selected objects. The objects can be selected by mouse while holding `Ctrl`.


 **Invert selection** Deselects objects selected by mouse and vice versa.

 **Generate binary** Erases binary objects which do not meet the restrictions settings.


 **Show Object Catalog** Opens the *Object Catalog* control panel with data source automatically set to "Object Count".

**Export** The measured data can be exported to clipboard or a file using the *Export* button. Please refer to the 8.14. Exporting Results [180] chapter for further details.



 **Export ND data** When an ND2 file is opened, the thresholding, measurement, and restrictions are applied to a single frame. Pressing this button will apply it to all frames of the ND2 document and export the results to a file or clipboard according to the current export settings.

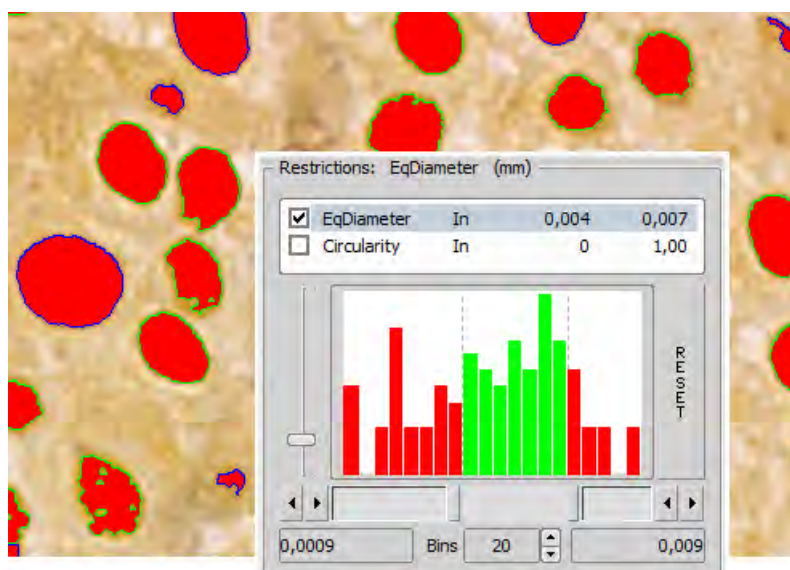
 **Save/Load Object Count configuration** Invokes a pull-down menu which enables you to load/save the current object count settings from/to an external file (\*.counting).

**Use Standard EqDia** Check this option to enable counting of objects using standard EqDiameter. Value of the standard EqDiameter parameter can be edited in the field nearby.

 **Keep updating count** Refreshes the measured results each time the binary layer changes (e.g. when the threshold is modified).

## 8.5.4. Object Count Procedure Example


1. Open the *Object Count* control panel. See 8.5. Object Count [148].
2. Threshold the image. Use the thresholding picking tools and click on the image to sample areas of interest. These pixels selected will determine what intensity/ part of histogram is considered accepted. The threshold will detect several specs or smaller objects such as noise or background. If desired, the *Clean* and *Smooth* filters will exclude smaller objects.
3. You can also measure using the defined region of interest (the  button) or measurement frame (the  button). Check the options are properly set in the *Measure > Options* dialog window.
4. Apply restrictions. Select *Area* from the list of available restriction features. Use the sliders in the histogram to specify the lower limit and the upper limit. The lower and upper limit will update in the display.



The green area of the histogram represents the accepted range areas. Any object with an area in the green range will be considered an object. Alternatively, any object with an area in the red ranges will not be considered an object.

The outlines of objects on the image differ according to whether the object is restricted or accepted. In this case, accepted objects have a green outline and restricted objects have a blue outline.

To ensure that that count is updating with any change, click the  icon:

- Remove any unwanted object from the object count by selecting the **Delete Selected Objects**  icon from the Result section. The object will lose its thresholded overlay and will be excluded from the measurement.

#### Warning

*You cannot Undo this action. To restore an object repeat the original threshold procedure or load a saved Object Count configuration.*

- View the result data and export them to a file or spreadsheet application. See 8.14. Exporting Results [180] for further information.

## 8.6. Automated Measurement

Automated measurement is the most powerful of image analysis features. In combination with user macros, *NIS-Elements AR* can become a semi- or fully-automatic image analysis tool. There are several key procedures which a proper automated measurement should involve:

- Optical system calibration (see the 4.1.2. Optical Configurations [38] chapter)

- Image acquisition (see the 5. *Image Acquisition* [55] chapter)
- Defining threshold, creating the binary layer (see the 7.3. *Thresholding* [119] chapter)
- Performing the automated measurement
- Results presentation

### **Image Layers Involved in Measurement**

The the binary layer and also the color image can get involved in the automated measurement:

- *Binary layer* - is typically used for shape and size measurements (area, perimeter, surface fraction).
- *Color layer* - intensity or hue measurements are carried out on this layer. The area covered with the binary layer is used as the source data.

### **Object/Field Measurement**

*NIS-Elements AR* distinguishes two types of automated measurements: object and field.

**Object Measurement** Groups of neighbouring pixels of the binary layer are called objects. Results of the object measurement are usually the object data such as Length, Area, CentreX/Y (the X,Y position of the central pixel of an object), etc.

**Field Measurement** Field measurements produce information regarding the whole measurement area (measurement frame, ROI). Such information can be Area Fraction, Mean Brightness, Density Variation, etc.

Please see the complete list of measurement features in the 8.10. *Measurement Features* [162] chapter.

### **Restricting the Area of Measurement**

The area of measurement can be restricted by the measurement frame or by a user defined region of interest (ROI).

**Region of Interest - ROI** ROI is a user defined area of the image. Whenever the ROI is displayed (by the *View > Layers > ROI* command), it is also applied to restrict the area of measurement.

**Measurement Frame** The measurement frame is a re-sizable rectangular frame which serves for bounding the area accepted for measurement. Turn the measurement frame ON via the *Measure > Use Measurement Frame* command.

Binary objects touching edges of ROI or the measurement frame can be treated differently (excluded from the measurement or included). This behavior can be specified within the *Measure > Options* window.

### **Statistics and Data Presentation**

*NIS-Elements AR* calculates basic statistics - mean value, standard deviation and distribution of all measured features. The features to be measured can be selected in the *Measure > Object Features* and the *Measure > Field and ROI Features* windows. Results of all automated measurements are presented and can be exported from the *View > Analysis Controls > Automated Measurement Results* control panel.



## 8.7. Measurement Options

Run the  *Edit > Options* command and select the *Measurement* tab.

**Rules for Excluding Objects** This section regards automatic measurement. Select the options how to treat the objects touching the area borders when you are performing the automatic measurement:

**Using Measurement Frame** Pick up the option how to treat the object touching the measurement frame. Run the automatic measurement by the *Measure > Use Measurement Frame* command.

**Using ROI** Pick up the option how to treat the object touching the ROI frame. Run the automatic measurement by the *View > Layers > ROI* command.


**Without Measurement Frame and ROI** Pick up the option how to treat the object touching the image border. Turn off the measurement frame and ROI and run the automatic measurement.

These options do not affect the field measurement results (field measurement is always applied inside the defined area without exceptions).

**Object Colors** After the automatic measurement is performed, all measured objects are highlighted by color borders. Here you can select colors for the objects excluded from measurement according to the ROI/measurement frame settings. The *Out of limits* color will be used to highlight objects which do not fit the applied restrictions ( *View > Analysis Controls > Automated Measurement* ).

### Manual Measurement

**Automatically zoom in Length manual measurements** This option zooms the image while placing the measurement points to the image. Only the indicated measurement tools are affected.

**Copy manual measurement objects from live image to captured** *NIS-Elements AR* enables to measure manually on live camera signal (  *View > Analysis Controls > Annotations and Measurements* ). After the *Acquire > Capture* command is used, the measurement objects in the image are copied (or not) to the captured image according to this setting.

**Label** The manual measurement objects can be labeled. Select what information will be attached to every manual measurement object in an image.

## 8.8. Time Measurement

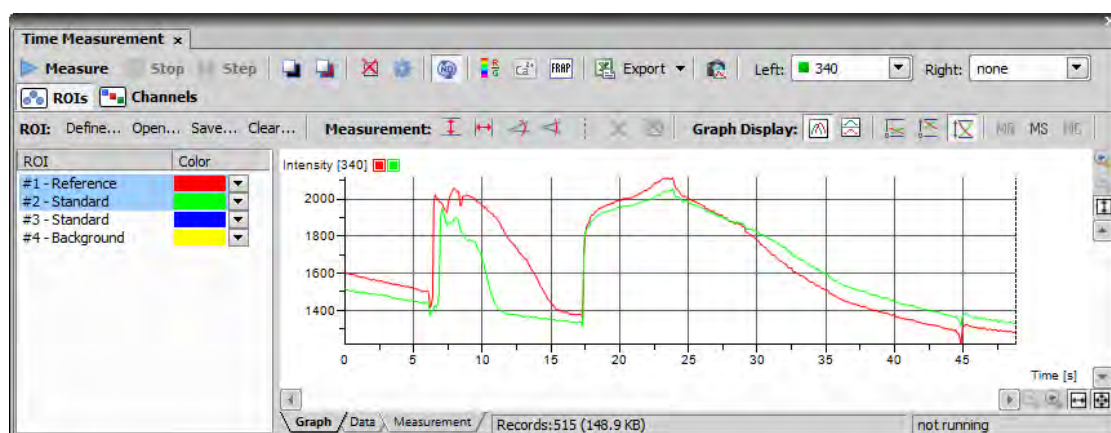
The time measurement tool enables to record average pixel intensities within Regions Of Interest (ROIs) during a time interval. The measurement can be performed on Live camera signal or on a saved nd2 file. Before the measurement is started, regions of interest shall be defined and turned ON. When no ROI is defined, Time Measurement is performed on the whole image automatically.

### Note

*Time measurement is not supported for multichannel ND documents containing a brightfield channel.*

Run the *View > Analysis Controls > Time Measurement* command to open the Time Measurement control panel.

Figure 8.12. Time measurement window



The control panel consists of three parts: measurement tools, ROI definition tools and data display.

## 8.8.1. Quick Guide

- Enable the Measurement ROI by the *View > Layers > ROI* command.
- Run the *Acquire > Live - Fast* command to display the live camera signal.
- Define ROI(s) within the image. Time measurement will be performed under the ROI areas. Use the *ROI > Simple ROI Editor* command or the *Define* tool bar button to open the vector ROI editor and use the *ROI > Raster ROI Editor* command to display the raster ROI editor. See 7.6. Regions of Interest - ROIs [131].
- Once the ROIs are defined, press the *Measure* button - the measurement will start. When the current ND document contains combination of time dimension with Z dimension or multipoints, press the *Measure All* button to measure all frames across all multipoints and/or Z stacks at once.
- Press the *Stop* button to finish the measurement.
- Export the results via the *Export* button. See 8.14. Exporting Results [180] for further details.
- A snapshot of the graph will be created if you press the *Create Snapshot* button.

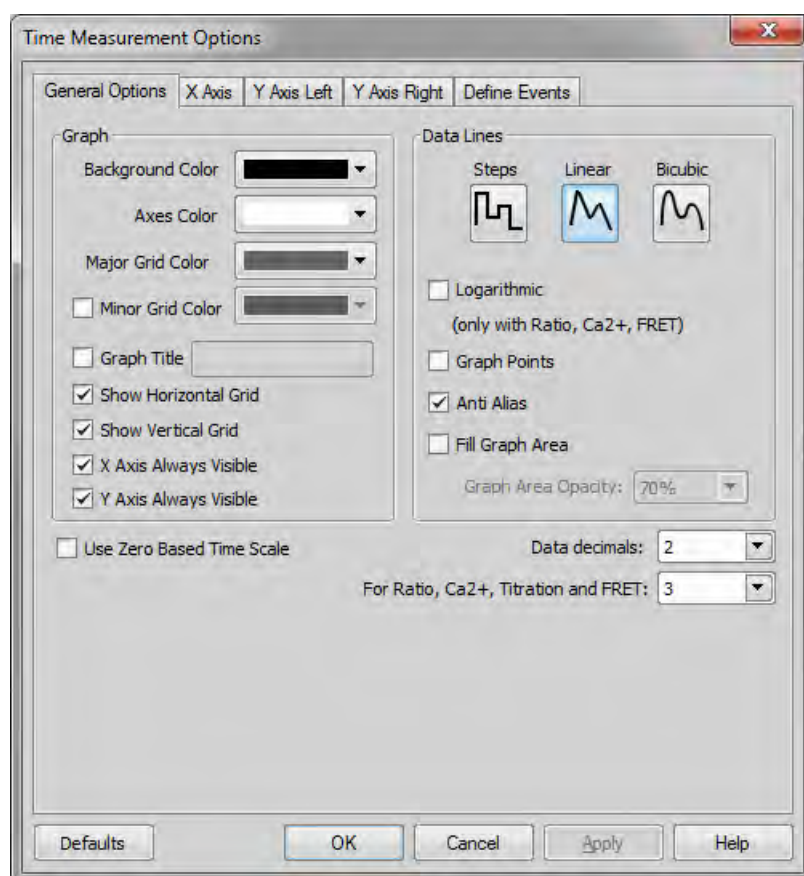
### Note

If there are too many ROIs defined and the graph gets confusing, a single ROI (and the corresponding graph) can be highlighted. Right click the ROI name and select *Bookmark this ROI* from the context menu.

## 8.8.2. Options

Click the *Options*  button. A dialog appears where you can specify the time measurement details:

### General Options tab



**Graph** Choose background, axes and grid colors from the palette. Check the *Graph Title* option to display a title, whose text you can enter in the adjacent field. Then check the *Show ... Grid* items if you want to make them visible in the graph. If *.. Axis Always Visible* item is checked, the axes do not leave the graph area while zooming in the graph.

**Data Lines** Select interpolation method or drawing the graph lines by pressing the relevant button. You can choose Steps (rough), Linear (smoother), or Bicubic (really smooth) interpolation method.

**Logarithmic** Check to use logarithmic scale in the graph.

**Graph points** Check this item to display data points. Small dots indicating the actual data values position can be displayed on the graph line. The points appear only if the distance between them is big enough for them to be recognized (they usually appear when you zoom in the graph).

**Anti Alias** Turning this option ON will make the graph lines look smooth.

**Fill Graph Area** Check this item to fill in the area under the line chart with a color. Select the amount of opacity used from the list of predefined values.

**Show point coordinates** Check this item to display or hide X, Y coordinates of pixels in the Data tab of the *Intensity Profile* control panel.

**Display During Measurement** Select one of two options:

**Fit graph to measured data** If you select this option, displayed area of graph during measurement is fit to measured data.

**Fixed time range** If you select this option, displayed area of graph during measurement is fixed to defined values. Then define time range of displayed area of graph.

**Use Zero Based Timescale** Select this option to ensure that the first frame of a time sequence will always start at 0.0s.

**Data decimals** Define decimal precision of the results in the data table.

**For ratio, calcium titration and FRET** Define decimal precision of data in data table for ratio, calcium titration and FRET.

## X axis

The screenshot shows the 'X Axis' tab of a control panel. It contains two main sections: 'Graph Range' and 'Zoom'. The 'Graph Range' section has four rows, each with a label, two radio buttons ('Auto' and 'Fixed'), a text input field, and a unit label '[μm]'. The 'Zoom' section has two radio buttons ('Best Fit' and 'Fixed'), and two rows with labels, text input fields, and unit labels '[μm]'.

Section	Label	Auto	Fixed	Value	Unit
Graph Range	Minimum:	<input checked="" type="radio"/>	<input type="radio"/>	0.00	[μm]
	Maximum:	<input checked="" type="radio"/>	<input type="radio"/>	0.00	[μm]
	Major Ticks:	<input checked="" type="radio"/>	<input type="radio"/>	50.00	[μm]
	Minor Ticks:	<input checked="" type="radio"/>	<input type="radio"/>	25.00	[μm]
Zoom	<input checked="" type="radio"/> Best Fit		<input type="radio"/> Fixed		
	Minimum:			0.00	[μm]
	Maximum:			0.00	[μm]

**Graph Range** The *Auto* option enables that the range values are set automatically. On the contrary the range values can be set on *Fixed* values also. The range values can be set for minimum of X axis, maximum of X axis, major and minor grid of X axis.

**Zoom** If you select the *Best Fit* option, zoom of the X axis will be set to best fit. Otherwise you can set the *Fixed* range.

## Y axis left/right

The screenshot shows a software window with five tabs: 'General Options', 'X Axis', 'Y Axis Left' (selected), 'Y Axis Right', and 'Define Events'. The 'Y Axis Left' tab contains three sections:

- Graph Range:**
  - Minimum: ☒ Auto ☐ Fixed
  - Maximum: ☒ Auto ☐ Fixed
  - Major Ticks: ☒ Auto ☐ Fixed
  - Minor Ticks: ☒ Auto ☐ Fixed
- Zoom:**
  - ☒ Best Fit ☐ Fixed
  - Minimum:
  - Maximum:
- Left data line pen:**
  - Width:  px
  - Style:
  - Color:

Applies in cases when it cannot be taken from ROI or Channel. (e.g. Ratio, Ca+, etc. in Channel mode or Recorded data).

The *Graph Range* and *Zoom* options are described above.

**Left/Right Data line pen** Set the line appearance - line color (in case color of image component is not set), thickness and style (solid, dot, dashed, dash-dot).

## Define Events

This tab enables to define user hot keys which run special user events during time measurement. Simply click inside the *Hot key* field of the User event and press any key combination you want to assign to the event. Then you can arbitrary enter an event description. Finally select or enter command or macro which should be executed when you press the set hot key.

General Options
X Axis
Y Axis Left
Y Axis Right
Define Events

Move To Next Phase  
Hot key: -

User Defined Events

	Hot key	Description	Macro	Show on ND Control Panel
User 1:	Num *		_Sharpen();	<input checked="" type="checkbox"/>
User 2:	Num 6			<input type="checkbox"/>
User 3:	Num /	enhancement	RunMacro("C:\macros\enhar	<input checked="" type="checkbox"/>
User 4:	.			<input type="checkbox"/>
User 5:	Num 7			<input type="checkbox"/>
User 6:	None			<input type="checkbox"/>
User 7:	None			<input type="checkbox"/>
User 8:	None			<input type="checkbox"/>

Selected User Event: User 1

Reset all hot keys
Load...
Save...

When these events are performed on a document, they are also displayed in the graph. These events are marked on the time axis and identified by their name. For more information see Special Options [61].

## 8.9. 3D Measurement

2D binary layers on a Z-series can be connected to 3D objects. Spatial characteristics (8.11. Measurement Features 3D [175]) of these objects can be examined within the *Measure > 3D Object Measurement* window. This window allows you to create 3D binary objects and measure their features. It also allows you to export the results to Excel.

Figure 8.17. 3D Object Measurement Window

Color	ND.M	ND.T	Obj ID	Tree ID	Track ID	Binary Layer	Channel	X	Y	Z	Volume	Surface	EqDiameter	SumIntensity	MeanIntensity
Red	1	2	1	N/A	N/A	Threshold DAPI	DAPI	137468.44	10332.33	9.63	6442367...	7357569...	2308.60	689563.00	110.37
Green	1	2	2	N/A	N/A	Threshold DAPI	DAPI	152220.60	1317.32	9.82	8279802...	1093750...	1165.04	82911.00	103.25
Blue	1	2	3	N/A	N/A	Threshold DAPI	DAPI	169213.75	2612.90	9.39	1113597...	1404603...	1286.01	143419.00	132.80
Yellow	1	2	4	N/A	N/A	Threshold DAPI	DAPI	187157.76	1113.48	10.26	4897766...	7470402...	977.99	44023.00	92.68
Cyan	1	2	5	N/A	N/A	Threshold DAPI	DAPI	101395.95	3340.48	10.26	6217585...	6319127...	1058.95	66483.00	110.25
Magenta	1	2	6	N/A	N/A	Threshold DAPI	DAPI	269419.44	12073.48	10.35	9537755...	9781807...	1221.28	100266.00	108.40
Orange	1	2	7	N/A	N/A	Threshold DAPI	DAPI	225660.68	49810.95	9.54	8477878...	9414895...	5450.41	10369494.00	126.12
Light Blue	1	2	8	N/A	N/A	Threshold DAPI	DAPI	107211.22	14146.14	9.73	8919090...	1021445...	1194.28	103778.00	119.97
Dark Blue	1	2	9	N/A	N/A	Threshold DAPI	DAPI	175744.43	21998.11	9.15	2625202...	3691497...	1711.54	281544.00	110.58
Light Green	1	2	10	N/A	N/A	Threshold DAPI	DAPI	285838.85	22913.05	10.68	1185775...	2042003...	609.54	10993.00	95.59
Dark Green	1	2	11	N/A	N/A	Threshold DAPI	DAPI	67814.63	28995.89	10.51	6104163...	6129705...	1052.47	65358.00	110.40
Light Yellow	1	2	12	N/A	N/A	Threshold DAPI	DAPI	103655.52	33408.89	9.64	1762164...	2078713...	1498.59	216596.00	126.74
Dark Yellow	1	2	13	N/A	N/A	Threshold DAPI	DAPI	123307.55	34066.31	9.10	1943639...	2869646...	1548.36	263327.00	139.70
Light Cyan	1	2	14	N/A	N/A	Threshold DAPI	DAPI	143026.83	33733.10	9.01	1511605...	2114018...	1423.90	210932.00	143.88
Dark Cyan	1	2	15	N/A	N/A	Threshold DAPI	DAPI	172091.98	49212.40	9.46	4036067...	8493410...	3264.08	731864.00	103.49
Mean								153915.93	117006.25	11.33	1583611...	1877780...	889.32	187320.50	102.59
St.dev								74069.81	75403.03	1.43	8059796...	9009847...	697.36	984608.05	16.15
Min								9879.52	1113.48	8.65	1546663...	3814247...	309.12	1353.00	86.61
Max								285838.85	281139.28	14.70	8477878...	9414895...	5450.41	10369494.00	154.85

## Options

**Define threshold 3D** You can define the threshold to create a 3D binary objects according to the color channel and/or the size of objects. The appearing window also provides the possibility to use Smooth, Clean and Fill holes functions, as well as to highlight or choose just one object.

Figure 8.18. Defining 3D Threshold

3D Threshold

Active channel:

☐ Smooth OFF ☐ ☒ Size (µm)

☒ Show color together with thresholded binary

**Connect 3D Objects** Create 3D objects by connecting existing binary layers.

**All Objects** Shows all the objects in all the Z-stacks.

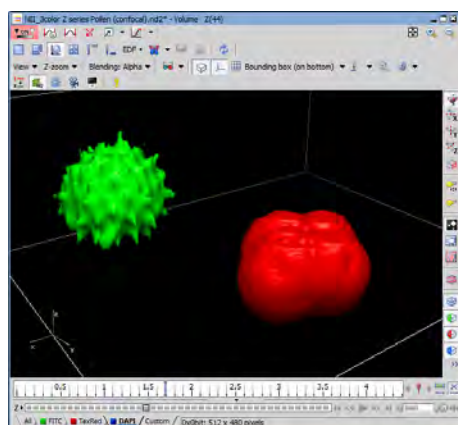
**Current Z-Stack** Shows only the objects of currently selected Z-stack.

**Delete Selected Object** Deletes selected object in the measurement table.

**Invert Selection** Inverts the selection of the objects in the image as well as in the table.

**3D Object Measurement to Excel** Allows you to export the measurement results to Excel.

Figure 8.19. Defined 3D objects



## 8.10. Measurement Features

The following list describes all the features that can be measured within *NIS-Elements AR* Measurements. Suitable type of measurement (object, field, manual) and the required image layer (binary, color/binary) are included in the description.

**AcqTime** Calling the Grab Sequence command, the AcqTime is set to zero at the beginning. Then, *NIS-Elements AR* assigns the AcqTime to every newly grabbed image file header, which denotes time elapsed from the beginning of the grabbing. If the Grab Sequence command is not called, then the AcqTime is the time elapsed since the start of *NIS-Elements AR*.

*Measurement Type:* object, field

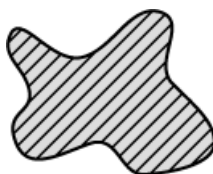
*Image Type:* binary

**Area** Area is a principal size criterion. In a non-calibrated system, it expresses the number of pixels; in a calibrated one, it expresses the real area.

*Measurement Type:* object, field, manual

*Image Type:* binary

Figure 8.20. Area





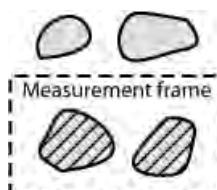
**AreaFraction** AreaFraction is the ratio of the segmented image area and the MeasuredArea. It has a strong stereological interpretation: in the case of isotropic uniform random sections it is equal to the volume fraction.

$\text{AreaFraction} = \text{Area} / \text{MeasuredArea}$

*Measurement Type:* field

*Image Type:* binary

Figure 8.21. Area fraction



**Blue** Arithmetic mean of pixel intensities of the blue component.

*Measurement Type:* manual

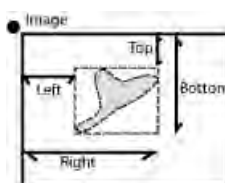
*Image Type:* color

**BoundsLeft, BoundsRight, BoundsTop, BoundsBottom** These are distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle. The units of calibration are used if the image is calibrated. Otherwise, the coordinate value is in pixels.

*Measurement Type:* object

*Image Type:* binary

Figure 8.22. Bounds

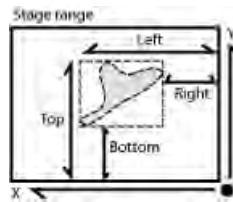


**BoundsAbsLeft, BoundsAbsRight, BoundsAbsTop, BoundsAbsBottom** These are absolute distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle within a motorized stage range. This feature can be measured only when a motorized stage is connected.

*Measurement Type:* object

*Image Type:* binary

Figure 8.23. BoundsAbs



**BoundsPxLeft, BoundsPxRight, BoundsPxTop, BoundsPxBottom** These are distances (X or Y coordinates) of the left, right, top, and bottom edge of the object bounding rectangle. Unlike the BoundsLeft,... distance, this value is always in pixels.

*Measurement Type:* object

*Image Type:* binary

**BrightVariation** It is the usual standard deviation of brightness values.

*Measurement Type:* object, field

*Image Type:* color/binary

**CentreX** CentreX is the x co-ordinate of the center of gravity. The units of calibration are used if the image is calibrated. Otherwise, the coordinate value is in pixels.

*Measurement Type:* object

*Image Type:* binary

**CentreY** CentreY is the y co-ordinate of the center of gravity. The units of calibration are used if the image is calibrated. Otherwise, the coordinate value is in pixels.

*Measurement Type:* object

*Image Type:* binary

**CentreXabs** CentreXabs is the absolute x co-ordinate of the center of gravity within a motorized stage range. This feature can be measured only when a motorized stage is connected.

*Measurement Type:* object

*Image Type:* binary

**CentreYabs** CentreYabs is the absolute y co-ordinate of the center of gravity within a motorized stage range. This feature can be measured only when a motorized stage is connected.

*Measurement Type:* object

*Image Type:* binary

**CentreXpx** CentreXpx is the x co-ordinate of the center of gravity. Unlike the CentreX coordinate, this value is always in pixels.

*Measurement Type:* object

*Image Type:* binary

**CentreYpx** CentreYpx is the y co-ordinate of the center of gravity. Unlike the CentreY coordinate, this value is always in pixels.

*Measurement Type:* object

*Image Type:* binary

**Circularity** Circularity equals to 1 only for circles; all other shapes are characterized by circularity smaller than 1. It is a derived shape measure, calculated from the area and perimeter. This feature is useful for examining shape characteristics.

$$\text{Circularity} = 4 * \pi * \text{Area} / \text{Perimeter}^2$$

*Measurement Type:* object

*Image Type:* binary

**Class** *NIS-Elements AR* recognizes 12 classes (1-12). If you have selected class feature (function Object Features or Field Features), system automatically asks you to specify the class via dialog box. In field or scan objects measurements, the application asks for a class before measurement is performed on the current image. In single object measurement, *NIS-Elements AR* asks for a class for every measured object. In the case you want to pass by, there is a possibility to run the `SetClass` function.

*Measurement Type:* object, field, manual

*Image Type:* binary

**Convexity** Indicates convexity of the object edges.

$$\text{Convexity} = \text{Area} / \text{Convex Hull Area}$$

*Measurement Type:* object

*Image Type:* binary

**DensityVariation** DensityVariation is derived from density values. It is a usual standard deviation of density values.

*Measurement Type:* object, field

*Image Type:* color/binary

**EdfSurface** EdfSurface is based on 3D model. It determines the surface area of an 3D object.

*Measurement Type:* object, field

*Image Type:* binary, EDF

**EdfRoughness** EdfRoughness is based on 3D model. It indicates how much the 3D object is rough.

$$\text{EdfRoughness} = \text{EdfSurface} / \text{Area}$$

*Measurement Type:* object, field

*Image Type:* binary, EDF

**Elongation** Elongation is determined as a ratio of MaxFerret and MinFerret features. This feature is useful for shape characteristics.

$$\text{Elongation} = \text{MaxFerret} / \text{MinFerret}$$

*Measurement Type:* object

---

*Image Type:* binary

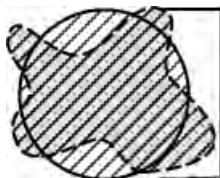
**EqDiameter** The equivalent diameter is a size feature derived from the area. It determines the diameter of a circle with the same area as the measured object:

$$\text{Eqdia} = \sqrt{4 * \text{Area} / \pi}$$

*Measurement Type:* object, manual

*Image Type:* binary

Figure 8.24. EqDiameter



**ExPurity** This feature specifies the amount of white in the measured color, if the measured color can be composed of white and a pure spectral color.

*Measurement Type:* object, field, manual

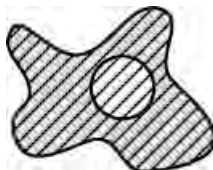
*Image Type:* color

**FillArea** In case an object does not contain holes then the FillArea is equivalent to the Area. If an object contains holes, FillArea remains the same while Area is reduced by the area of the holes.

*Measurement Type:* object

*Image Type:* binary

Figure 8.25. FillArea



**FillRatio** FillRatio is the ratio of Area and FillArea:

$$\text{FillRatio} = \text{Area} / \text{FillArea}$$

If an object does not contain holes the FillRatio is equal 1. If an object contains holes, FillRatio is less than 1. This feature can distinguish objects with and without holes.

*Measurement Type:* object

*Image Type:* binary

**IntensityVariation** IntensityVariation is derived from an intensity histogram. It is an usual standard deviation of intensity values. This feature describes the inner structure of an object or a field.

*Measurement Type:* object, field

*Image Type:* color

**Green** Arithmetic mean of pixel intensities of the Green component.

*Measurement Type:* manual

*Image Type:* color

**HueTypical** HueTypical is the hue value with maximum frequency in a hue value histogram. This feature describes the most frequent hue (color) in an object or field.

Figure 8.26. Calculation of Hue ( $H$ )

$$\begin{aligned}\alpha &= \frac{1}{2}(2R - G - B) \\ \beta &= \frac{\sqrt{3}}{2}(G - B) \\ H &= \text{atan2}(\beta, \alpha)\end{aligned}$$

*Measurement Type:* object, field

*Image Type:* color/binary

**HueVariation** HueVariation is the usual standard deviation of hue values. This feature describes hue (color) distribution of inner structure of an object or field.

Figure 8.27. Calculation of Hue ( $H$ )

$$\begin{aligned}\alpha &= \frac{1}{2}(2R - G - B) \\ \beta &= \frac{\sqrt{3}}{2}(G - B) \\ H &= \text{atan2}(\beta, \alpha)\end{aligned}$$

*Measurement Type:* object, field

*Image Type:* color/binary

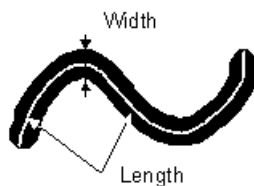
**Length** Length is a derived feature appropriate for elongated or thin structures. As based on the rod model, it is useful for calculating length of medial axis of thin rods.

$$\text{Length} = (\text{Perimeter} + \sqrt{\text{Perimeter}^2 - 16 \cdot \text{Area}})/4$$

*Measurement Type:* object, manual

*Image Type:* binary

Figure 8.28. Length



**LineLength** Line length is defined as the length of the object with elongated shape.

*Measurement Type:* object

*Image Type:* color/gray

**Luminance** Luminance is defined as a radiant power weighted by a spectral sensitivity that has characteristics of human vision.

*Measurement Type:* object, field, manual

*Image Type:* color

**MaxFeret** The MaxFeret is the maximal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle  $\alpha$  equals the projected length of object at angle  $\alpha$ ,  $\alpha$  (0,180); *NIS-Elements AR* calculates Feret's diameter for  $\alpha = 0, 10, 20, 30, \dots, 180$ .

*Measurement Type:* object, manual

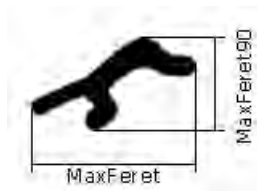
*Image Type:* binary

**MaxFeret90** The MaxFeret90 is a length projected across the MaxFeret diameter.

*Measurement Type:* object, manual

*Image Type:* binary

Figure 8.29. MaxFeret



**MaxIntensity** MaxIntensity value is derived from the intensity histogram. It is the maximal of intensity pixel values.

Figure 8.30. Calculation of Intensity (I)

$$I = \frac{1}{3}(R + G + B)$$

*Measurement Type:* manual

*Image Type:* color/gray

**MeanBlue** Arithmetic mean of pixel intensities of the Blue component.

*Measurement Type:* object, field, manual

*Image Type:* color/binary

**MeanBrightness** Arithmetic mean of brightness values of pixels.

*Measurement Type:* object, field, manual

*Image Type:* color/binary

**MeanDensity** Arithmetic mean of density values of pixels.

*Measurement Type:* object, field, manual

*Image Type:* color/binary

**MeanIntensity** MeanIntensity value is derived from the intensity histogram. It is the arithmetic mean of pixel intensities.

Figure 8.31. Calculation of Intensity (I)

$$I = \frac{1}{3}(R + G + B)$$

*Measurement Type:* object, field, manual

*Image Type:* color/binary

**MeanGreen** Arithmetic mean of pixel intensities of the Green component.

*Measurement Type:* object, field, manual

*Image Type:* color/binary

**MeanChord** MeanChord is the mean value of secants in the 0, 45, 90 and 135 degrees directions. It is a derived feature and is calculated from the Area and mean projection according to the following formula.

$$\text{MeanChord} = 4 * \text{Area} / (\text{Pr}_0 + \text{Pr}_{45} + \text{Pr}_{90} + \text{Pr}_{135})$$

*Measurement Type:* object, field

*Image Type:* binary

**MeanRed** Arithmetic mean of pixel intensities of the Red component.

*Measurement Type:* object, field, manual

---

*Image Type:* color/binary

**MeanSaturation** Arithmetic mean of saturation values of pixels.

*Figure 8.32. Chroma (C) definition*

$$M = \max(R, G, B)$$

$$m = \min(R, G, B)$$

$$C = M - m$$

*Figure 8.33. Calculation of Saturation (S)*

$$S_{HSI} = \begin{cases} 0, & \text{if } C = 0 \\ 1 - \frac{m}{T}, & \text{otherwise} \end{cases}$$

*Measurement Type:* object, field

*Image Type:* color/binary

**MeasuredArea** MeasuredArea value is the area of the measurement frame or of a mask within the measurement frame, if the mask status is on.

*Measurement Type:* object, field

*Image Type:* binary

**MinFeret** The MinFeret value is the minimal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle  $\alpha$  equals the projected length of object at angle  $\alpha$ ,  $\alpha$  (0,180); *NIS-Elements AR* calculates Feret's diameter for  $\alpha = 0, 10, 20, 30, \dots, 180$ .

*Measurement Type:* object, manual

*Image Type:* binary

**MinIntensity** MinIntensity value is derived from the intensity histogram. It is the minimum of intensity values of pixels.

*Figure 8.34. Calculation of Intensity (I)*

$$I = \frac{1}{3}(R + G + B)$$

*Measurement Type:* manual

*Image Type:* color/gray

**NumberObjects** NumberObjects value returns the number of objects in the measurement frame. Exclusion rules for counting objects are taken into account.

*Measurement Type:* field



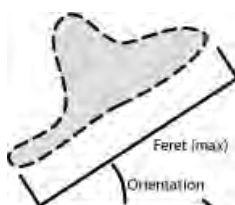
*Image Type:* binary

**Orientation** Orientation is the angle at which Feret's diameters have their maximum. The diameters are calculated with 5 degrees angle increment.

*Measurement Type:* object

*Image Type:* binary

Figure 8.35. Orientation



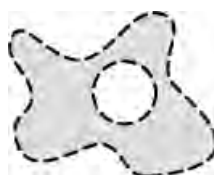
**Perimeter** Perimeter is the total boundary measure. It includes both the outer and inner boundary (if there are holes inside an object). The perimeter is calculated from four projections in the directions 0, 45, 90 and 135 degrees using Crofton's formula

$$\text{Perimeter} = \pi * (\text{Pr}_0 + \text{Pr}_{45} + \text{Pr}_{90} + \text{Pr}_{135}) / 4$$

*Measurement Type:* object, field, manual

*Image Type:* binary

Figure 8.36. Perimeter



**Red** Arithmetic mean of pixel intensities of the Red component.

*Measurement Type:* manual

*Image Type:* color

**Roughness** This feature indicates how much the object is rough. "1" means the object roughness is minimal (the object is circular). The feature may acquire values in range <0;1>.

$$\text{Roughness} = \text{Convex hull perimeter} / \text{Perimeter}$$

*Measurement Type:* object, manual

*Image Type:* binary

---

**RoughnessInf** This feature indicates how much the object is rough. “1” means the object roughness is minimal (the object is circular). The feature may acquire values in range <1;inf>.

$\text{RoughnessInf} = 1 / \text{Roughness}$

*Measurement Type:* object, manual

*Image Type:* binary

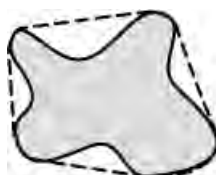
**ShapeFactor** This parameter is to define whether the object is rough or not.

$\text{Shape factor} = 4 \pi (\text{area}) / (\text{Convex hull perimeter})^2$

*Measurement Type:* object, manual

*Image Type:* binary

Figure 8.37. ShapeFactor

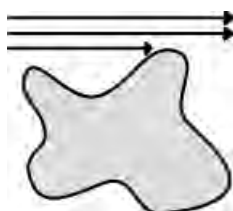


**StartX** StartX is the x co-ordinate of a pixel on the object boundary. When you scan the image from the origin as indicated (left->right), the first pixel of the object you hit is the one with StartX and StartY co-ordinates.

*Measurement Type:* object

*Image Type:* binary

Figure 8.38. StartX



**StartY** StartY is the y co-ordinate of a pixel on the object boundary.

*Measurement Type:* object

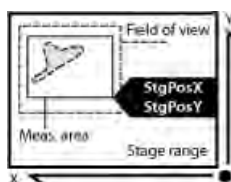
*Image Type:* binary

**StgPosX** StgPosX feature is the x co-ordinate of the absolute position of the measured field. It is available only for systems equipped with a stage.

*Measurement Type:* object, field

*Image Type:* binary

Figure 8.39. StgPos



**StgPosY** StgPosY feature is the y co-ordinate of the absolute position of the measured field. It is available only for systems equipped with a stage.

*Measurement Type:* object, field

*Image Type:* binary

**Straightness** Feature which shows overall straightness of the object trajectory calculated by formula: End Points Distance / Trajectory Length. Values range is 0.0 - 1.0.

*Measurement Type:* object, field

*Image Type:* binary

**SumBrightness** SumBrightness is defined as the sum of brightness in every pixel of the object.

*Measurement Type:* object, manual

*Image Type:* color/gray

**SumDensity** SumDensity is the sum of individual optical densities (O.D.) of each pixel in the area being measured. This feature describes, for instance, the amount of a substance in biological sections. Optical density is evaluated according to the following formula:

$$\text{O.D.} = -\log((\text{PixelIntensityValue} + 0.5 / \text{MaxIntensityValue}))$$

*Measurement Type:* object, field, manual

*Image Type:* color/binary

**SumIntensity** Sum Intensity is defined as the sum of intensity in every pixel of the object.

*Measurement Type:* object

*Image Type:* color/gray

**SurfVolumeRatio** SurfVolumeRatio is a feature with a strong stereological interpretation: if you measure on fields that are sampled systematically and independently of the content of the sections, then the feature is an unbiased estimator of the surface area of objects (inner structure) per volume of the whole sample.

$$\text{SurfVolumeRatio} = (4 / \pi) * \text{Perimeter} / \text{MeasuredArea}$$

*Measurement Type:* field

---

*Image Type:* binary

**Time** Time assigns the time to a field (object) after a measurement has been performed. See the `SetReferenceTime` function help.

*Measurement Type:* object, field

*Image Type:* color/binary

**VolumeEqCylinder** This parameter is based on the rod model. Length is interpreted as height and Width as the base diameter of a cylinder. Bases are considered to be spherical.

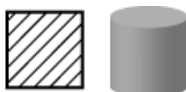
$$\text{VolumeEqCylinder} = (\pi d^2) * (l-d) / 4 + \pi d^3 / 6,$$

where  $l = \max(\text{MaxFerret}, \text{Length})$ ,  $d = \min(\text{MinFerret}, \text{Width})$ .

*Measurement Type:* object

*Image Type:* binary

Figure 8.40. VolumeEqCylinder



**VolumeEqSphere** Supposing a profile was created as an intersection of a ball and a section that contains the center of the ball. VolumeEqSphere is the volume of the ball.

$$\text{VolumeEqSphere} = \pi * \text{Eqdia}^3 / 6$$

*Measurement Type:* object

*Image Type:* binary

Figure 8.41. VolumeWqSphere



**WaveLen** Dominant wavelength. This feature is defined as a wavelength of the pure spectral color that is, together with white, the measured color composed of. The color in the purple must be composed of more than one pure spectral colors therefore they have no dominant wavelength.

*Measurement Type:* object, field, manual

*Image Type:* color

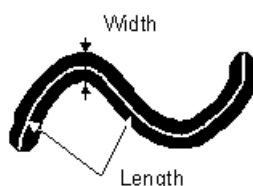
**Width** Width is a derived feature appropriate for elongated or thin structures. It is based on the rod model and is calculated according to the following formula:

$$\text{Width} = \text{Area} / \text{Length}$$

Measurement Type: object

Image Type: binary

Figure 8.42. Width



## 8.11. Measurement Features 3D

The following list describes all the features that can be measured within the *Measure > 3D Object Measurement* window.

**\_Alpha, \_Beta** Polar coordinates of the direction vector of a particular axis (Major, Minor, Minor2).

Measurement Type: object

Image Type: binary

**Axis Length (Major, Minor, Minor2)** Object size in the direction of the selected axis:

Axis Length =  $\max(|\text{dif}|)$  {over all P1, P2}

vector (dif = scalar \* AxisDirection = P1 - P2); P1 and P2 are the object points.

This feature measures length between the most distant edges of the two (outer) voxels / contrary to the Axis X, Y, Z [?] feature where voxel centers are measured.

### Note

*The Major axis length is not necessarily the longest one.*

Measurement Type: object

Image Type: binary

**Axis X, Y, Z** X, Y, Z are coordinates of the covariance matrix vectors, the covariance matrix being calculated from voxel coordinates. The prefixes Major, Minor and Minor2 relate the axes and their values. Major axis, Minor axis and the secondary Minor axis (Minor2). The method applied is called PCA (Principal Component Analysis).

Measurement Type: object

Image Type: binary

**Elongation** Elongation is calculated as a ratio of axes lengths (AxisLength [?]). This feature characterizes object shape well.

---

Elongation M1M2 = Major/Minor

Elongation M2M3 = Minor/Minor2

Elongation M1M3 = Major/Minor2

*Measurement Type:* object

*Image Type:* binary

**EqDiameter** Equivalent diameter is a feature derived from the volume. It determines the diameter of a sphere with the same volume as the measured object:

$$\text{EqDiameter} = (6 * \text{Volume} / \pi)^{(1/3)}$$

*Measurement Type:* object

*Image Type:* binary

**MeanIntensity** Arithmetic mean of intensities inside the object.

$$\text{MeanInt} = (\text{SumIntensity} / \text{Volume} / \text{Voxel Volume})$$

*Measurement Type:* object, field, manual

*Image Type:* color/binary

**MinDistance** Distance between the centroid of the object and the centroid of the nearest object.

*Measurement Type:* object

*Image Type:* binary

**RefLength** Distance from the object centroid to the reference object. Any of the 3D object can be set as reference from context menu in the *Measure > 3D Object Measurement* window.

*Measurement Type:* object

*Image Type:* binary

**SumIntensity** Sum of voxel intensities inside the object.

*Measurement Type:* object

*Image Type:* binary + color

**Surface** Surface of the object.

*Measurement Type:* object

*Image Type:* binary

**Volume** Volume of the object.

$$\text{Volume} = (\text{number of voxels} * \text{voxel volume})$$

*Measurement Type:* object

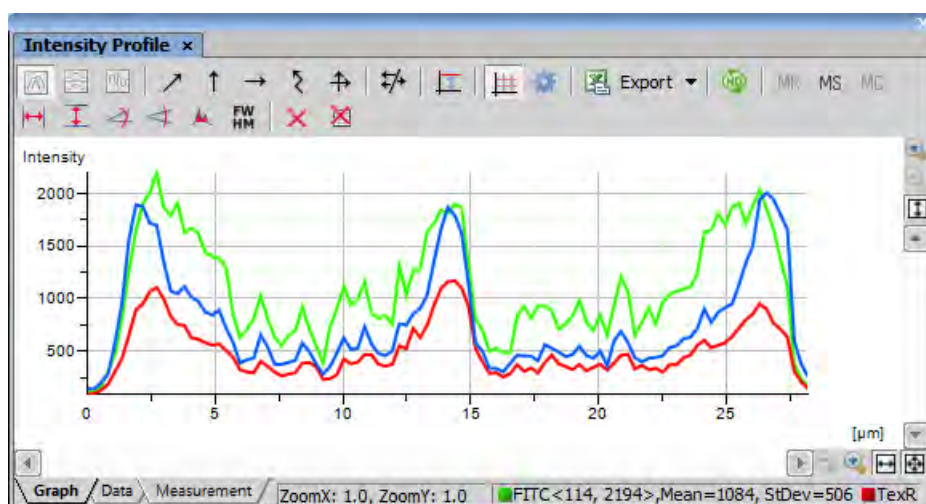
*Image Type:* binary

**x, Y, Z** Coordinates of the binary centroid.

*Measurement Type:* object

*Image Type:* binary

## 8.12. Measurement on Graph



The following interactive measurements can be performed within graphs.

**Vertical, Horizontal** Pick this tool to measure distances within the graph.

**Angle** This tool enables selecting an angle and its both line lengths. The resulting number represents a ratio between the projection of the y arrow on y axis and projection of the x arrow on x axis. The resulting number is a dimensionless value. This tool does not allow adjusting the x line length.

**Free Angle** This tool measures the same features as the previous angle tool but allows to adjust all of the angle's anchor points.

**Area of Graph** This tool enables you to draw a rectangle in the graph. Area will be measured of intersection of the rectangle with the area below the graph curve.

**FWHM** This tool measures the *Full Width at Half Maximum* value on the given graph range.

**Clear Measurement Objects** Deletes all the measured objects in the graph.

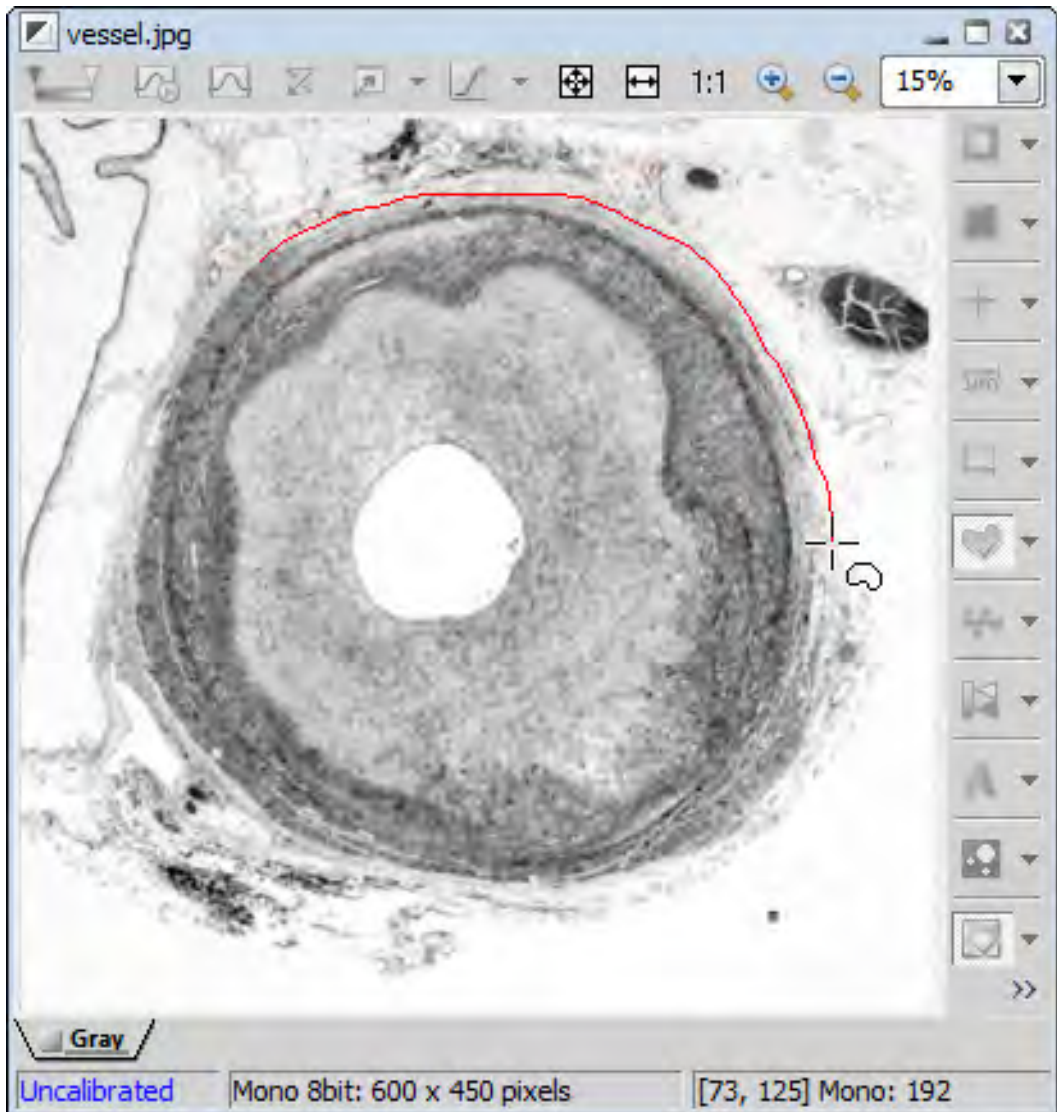
**Reset Data** Clears the table with the measurement results.

## 8.13. Pixel Classifier

A typical task for Pixel Classifier may be counting the area of a cross-section of a blood vessel. Since there are at least three different types of tissue whose area should be calculated while background should not be counted at all.

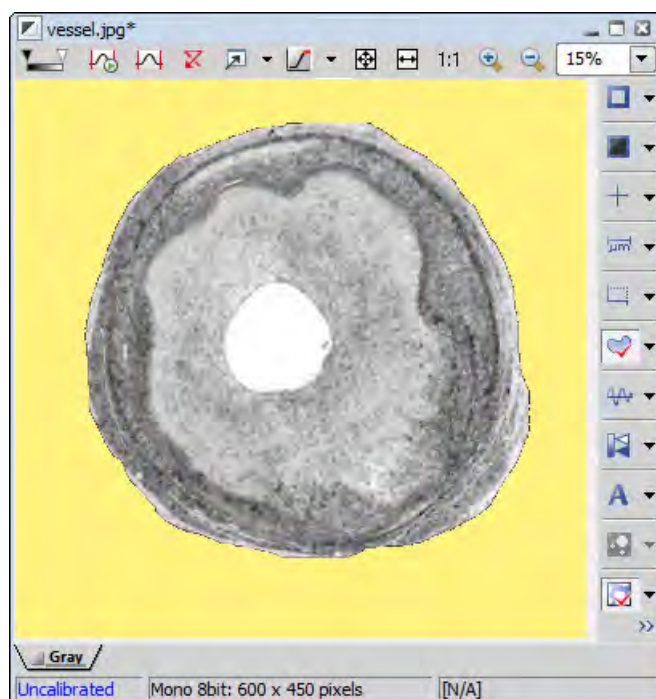
1. Eliminate the background. You can:



- define a region of interest. See 7.6. Regions of Interest - ROIs [131] for more information.
- use the method of accepted region. Run the *Edit > Region > Region Settings* command first. Choose color of the background in the *Region Settings* dialog that appears. You can fill in the value 0, which is easy to threshold. Next run the *Edit > Region > Accepted Region* command. Draw the boundary around the area to keep.



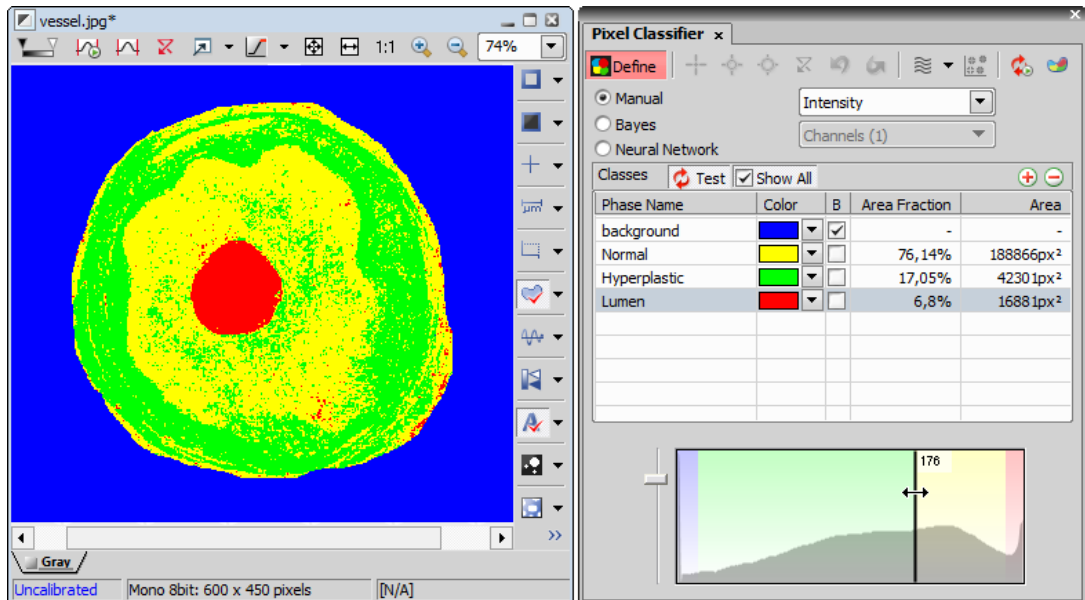
Finish drawing with a right click. The image is cropped according to the boundary. Area of image which remained outside the border is deleted and filled with the set background color.





2. Run *View > Analysis Controls > Pixel Classifier* to display the classifier panel. Press the *Define* button and edit all phases you want to detect separately. Use the  and  buttons and commands in the contextual menu to *Add*, *Rename* or *Delete* any class.

If you choose the *Manual* method of classifying, you can manually choose the intensity ranges of all phases.

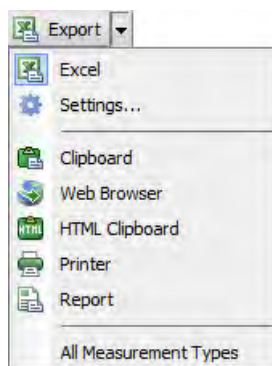


Alternatively, you can sample areas and train the other algorithms to find phase boundaries.

- Once all phases have been defined properly, the settings can be re-used to classify another images.

## 8.14. Exporting Results

Some image analysis data and measurement results can be transferred out of *NIS-Elements AR* so they could be used further. There is a standard *Export* pull-down menu in some of the control panels (intensity profile, histogram, measurement, etc...) which enables the export.



The set of commands of the menu differs according to the type of data (tables or graphs) which can be exported. Click the arrow button to display the pull-down menu. When you select the destination, the

pull down menu hides and the icon of the selected export type appears on the *Export* button. The export action is performed after the button is pressed once more.

### **Export Destinations**

It is possible to export tabular data or a graph:

**To MS Excel** Tabular data can be exported to MS Excel. A new XLS sheet opens and the table is copied to it automatically.

**To File** Tabular data can be exported to an MS Excel sheet (xls) or a text file (tab delimited txt).


**To Clipboard** Data tables and graph images can be exported (copied) to MS Windows clipboard. Then the data or the image can be inserted into any appropriate application (text editor, spreadsheet processor, graphics editor) typically by the *Paste* command.

**To Report** Tabular data and graph images can be exported to *NIS-Elements AR* report. If a report is already opened for editing, the data/graph will be appended to it. Otherwise, a new report will be created and the exported data inserted to it.

**To Web Browser, To HTML Clipboard** Some tabular data can be exported as a HTML table. It can be displayed in a default Internet browser (the *Web Browser* option), or the HTML code can be copied to Windows clipboard - ready to be inserted to any HTML/text editor.

**To Printer** Tabular data can be printed directly. This option opens a standard *Print* dialog window where it is possible to select a printer and print the data.

### **Data Export Options**

Run the *Options* command and switch to the *Data export* tab or press the *Settings*  button within the export pull-down menu. There are two tabs available:

#### **Global Settings Options**

**Export into Microsoft Excel application** Select this option if you want to export the data to MS Excel application. You can choose if the data will be exported into currently opened file or a new file will be created. Or you can set to export the data to a specific file. If this file does not exist, it will be created. Choose the path and name of the file to be created/opened.

Set the *Start at column* values (column and row indexes). These values mark the first cell in the MS Excel application which will hold the exported data.

**Export text files into folder** Select this item if you prefer to export the data to a text file. Press the ... button and specify the target folder and the file name.

**Data delimiter** Pick up the type (tabulator, space, semicolon) of data delimiter which will be used when exporting data to MS Excel or clipboard.

**Append exported data after previous exports** If you check this item the existing data will not be overwritten by the new data.

**Export also column headers if not appending** Include the titles of columns in the files which are being created or overwritten. If the *Append exported data after previous exports* is selected, no headers will be included in the export.


---

**Active target application after export** If you check this item the exported file is opened in the default application (MS Excel or the default text editor) automatically.

**Insert empty lines** Check this item to allow insertion of empty lines in the exported data.

### **Data export Options**

You can modify the amount of data included in each export type. The control panels which contain the Export to file functionality are listed in the top pull-down menu. Select the control panel of which the export shall be modified.


**Export Name** A control panel such as  *View > Analysis Controls > Annotations and Measurements* stores information about the measurement in multiple tables according to the measurement type (area, length, etc.). In this column, you can select the table you would like to modify.

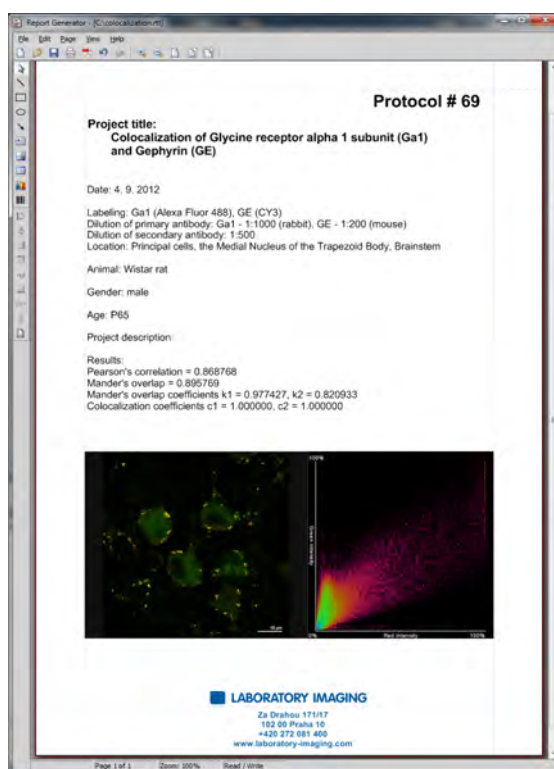
**Sheet name** In this column you can define an arbitrary name which will be used to name a sheet or a txt file to which the data will be exported.

**Options for:** This section of the window enables you to select the items to be included/excluded from the selected table during export.

## 9. Creating Reports

### 9.1. Report Generator

Report Generator allows the user to create a print-ready output containing measured data, images, graphs etc. Once you start it by the  **File > Report > New Blank Report** command, a graphical editor appears.



Creating of a report is very similar to creating any document in a common text editor in many ways, so you can insert text fields, simple shapes, images, tables or graphs. As well as in the text editors, you can create report templates containing static objects (which do not change) and dynamic objects to be filled with data upon report creation. This comes especially handy in connection with the 14.7. Database [230] module where database fields are loaded to the report(s) automatically.

#### **Context Menu Commands**

Once you insert an object, its appearance, behavior, and position can be changed via the context menu which appears on right-click:

- If there are more than one objects selected, the *Group* command makes them to behave as a single object.
- The *Align or Distribute* commands enable moving objects within the page or align them with another object(s).
- The *Resize object* commands enables to resize two objects to the same size.
- The *Bring to front* command changes the order of overlapping objects and brings the current object to front.
- The *Send to back* command moves the object to the bottom layer.
- The *Lock* command locks the object disabling any further changes to it until it is unlocked.
- The *Properties* command opens a window where object properties such as color, background color, border size and color, object size, font, aspect ratio, alignment, and shape can be adjusted.

## 9.2. Report Objects

When you double-click any of the report objects, a window appears where the properties can be adjusted. Some properties are common to all types of objects, and some are special.

### **Common Properties**

- The precise position can be determined by defining the XY coordinates of the top-left corner of the object.
- Line and fill colors can be selected.
- Line widths can be adjusted within the range of 0,25 to 6 pts.
- Precise width and height of the objects can be adjusted.
- Rotation of some objects can be adjusted.

#### Tip

Vector objects (report objects, annotations, interactive measurement objects, ROIs) can be copied by “drag and drop” while holding the **Shift** key down.

1. Select the object(s).
2. Press **Shift** and drag the object somewhere.
3. A copy of the object is placed to where you release the mouse button.

### **Special Object Properties**

- **Ellipse** Minor and major axes lengths can be modified.

 **Arrow** The arrow tip shape and size can be modified.

### **Text Box**

- Format of the text can be adjusted like in a standard text editor.
- The current date and time can be inserted from a pull-down menu.

### **Image**

- A new picture can be loaded to the image frame via the *Load Picture* button.
- The current image scale is displayed in the properties window. It shows the *current scale/original image dimensions* ratio (the image must be calibrated to use this feature).
- A scale bar can be displayed below the image. The position and width of the scale bar can be defined.

### **Table**

- Inner and outer borders can be hidden or displayed.
- Number of rows/columns can be modified.

### **Graph**

- The range of displayed values can be limited by defining the min/max values.
- Histogram labels can be edited.


### **Insert bar code** (*requires:Local Option*)

It is possible to insert bar-codes to the report.

## **Aligning Objects**

### **Align Objects**

You can align two objects to the same horizontal or vertical level.

1. Select more objects (e.g. by holding down **CTRL** and clicking with the left mouse button).
2. Right-click one of the selected objects and select the *Align or Distribute > Align ...* command from the context menu.
3. The objects will be aligned as indicated on the command icon.
4. If the *Relative to Page* () option is turned *ON*, the objects will be aligned to the edges or the center of the page.

### **Distribute Objects**

Objects of similar size can be distributed uniformly in the horizontal/vertical direction.

1. Select three or more objects.

- 
2. Right click one of the objects and select the *Align or Distribute > Distribute...* command from the context menu.
  3. Distances between the objects will be adjusted to equal.
  4. If the *Relative to Page* option is turned ON, the outer objects will be moved to the edges of the page.

### **Match Object Sizes**

Sizes of objects can be unified too.

1. Select two or more objects.
2. Right-click the “master” object to the size of which you want to resize the other objects.
3. Select one of the *Resize Objects* sub-menu commands.

### **Dynamic Data**

Results of automatic-interactive measurement, graphs, or the current image can be inserted to reports.

1. Insert an object which can contain dynamic data (text, picture, table, or graph).
2. Right-click the object and select *Insert Dynamic Data/Insert Dynamic Picture* from the context menu.
3. A window appears.
4. Select one of the available sources, and click *Next* .
5. Finish the source definition and click *OK* . The data appear on the report page.

### **List of Available Dynamic Data Sources:**

- *Data inserted by user (available for: image, text, table )* - the system will ask you to type a text or browse for an image to insert during the report creation. When creating a dynamic object of this type, a query text which will be used to prompt you for the data can be defined.
- *System data (available for: text, table )* - enables you to insert some general data such as date, the name of the user account currently logged in, the page number, or the page count.
- *Macro (available for: text, table )* - enables you to insert expressions, values, or results of a macro.
- *Measurement (available for: text, table, graph )* - enables you to insert results of automatic/interactive measurement.
- *Database (available for: image, text, table )* - this data source is displayed only when creating a database report template (see below). It enables you to insert a link to any of the database records.


The real purpose of the dynamic data turns up when creating reports using report templates.



## 9.3. Report Templates

A report template is a layout defining the appearance of future reports, which is ready to be filled with data. The data can be inserted by the user “on demand” or automatically (dynamic data).

### **To Create a Report Template**



1. Run the  *File > Report > New Blank Report* command. An empty report appears.
2. In the Report Generator, select *File > Change to Template*.
3. Edit the report template in the same way as a common report.
4. Insert the dynamic data where it is appropriate - measurement results to text boxes or tables, the current image to the image field, etc.).
5. Save the report template (\*.rtt) via the *File > Save* command.

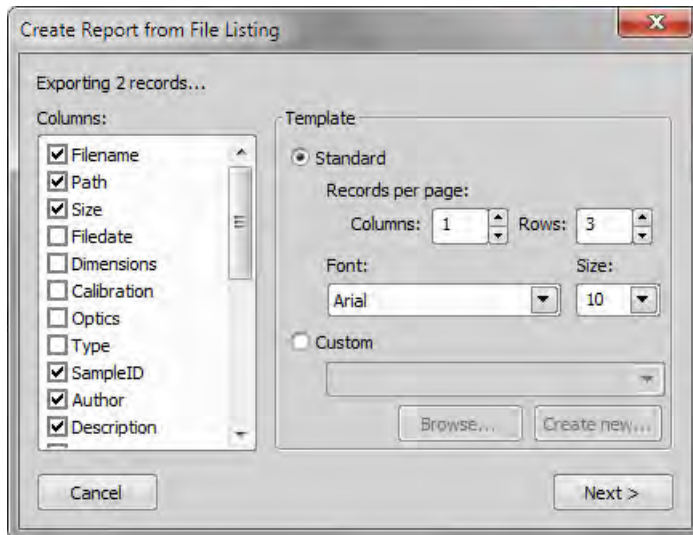
### **To Create a Report FROM Template**

1. If the template is opened inside the Report Generator, run the *File > Create Report* command. Otherwise, use the *File > Report > New Report from Template > Browse* command inside the main application window to open a template from harddisk.
2. A report opens and the dynamic data is inserted automatically.
3. Save the report, print it, or export the page(s) to PDF using the commands from the *File* menu.

## 9.4. Creating Reports from Database

Pictures of a database together with the associated table data, or common images with the associated *Image Info* can be exported straight to a report.

1. Switch *NIS-Elements AR* to *Organizer* by the  *View > Organizer Layout* command.
2. Select one or more images. These images will be inserted to the report.
3. Click the *Report*  button on the main tool bar. The following window appears:



4. In the *Columns* section, you can select fields, which will be included in the report. If you are exporting images from a database, the database table fields will be listed. If you are exporting images from a directory, image description items will appear.
5. The *Template* portion of the dialog specifies the layout details. If you select the *Standard* template, images will be organized in rows and columns. It is possible to set the number of columns and rows. If you select the *Custom* report template, a user report template (\*.rtt) can be opened and used for the report creation. If such report does not exist yet, you can create it using the *Create New* button.

#### **If You Pressed the Create New Button**

1. A wizard appears. Select number of columns and rows of the new report template. Click *Next*.
2. Select the paper size, page orientation, and define margins. Click *OK*.
3. An untitled report template opens containing a grid of images spaced according to the *columns/rows* settings.
4. Edit the top-left cell of the image grid. You can change the text/image boxes position, size, and mapping. During report creation, all the other cells of the grid will be filled automatically according to the settings of the first one.
5. A custom header or automatic page numbering can be added to the report.

## 10. HCA

### 10.1. Introduction to HCA

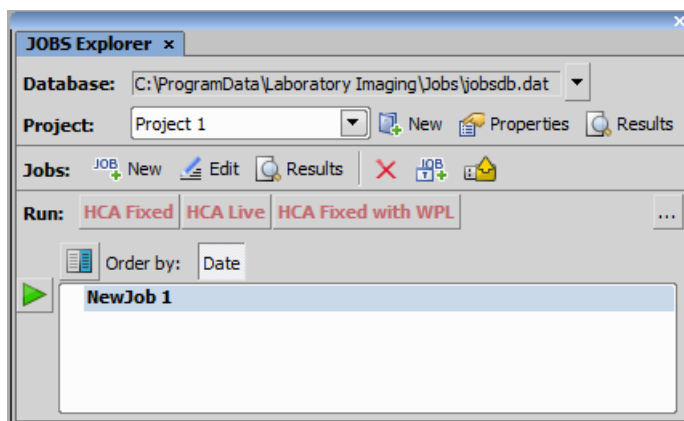
The HCA (High-content analysis) module enables the user to perform full scale HCA experiments by combining the capturing and the analysis phases to a single “HCA job”. There are job templates designed to analyze live or fixed samples with well-plate loader support.

Images and results are organized in a database - the database containing just links to image files placed in a separate folder. Before you start, you need to open or create a jobs database.

#### 10.1.1. JOBS Explorer

*Jobs Explorer* is the heart of the 14.10. HCA [230] and 14.14. Jobs [231] modules. Run the *View > Analysis Controls > JOBS Explorer* to display it.

Figure 10.1. *JOBS Explorer with the HCA module*

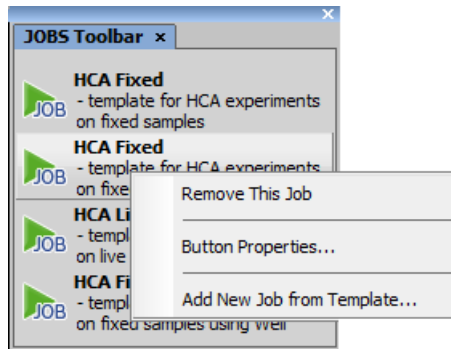


Please see 11.2. JOBS Explorer [201].

#### 10.1.2. JOBS Toolbar

The JOBS Toolbar (displayed by the *View > Analysis Controls > JOBS Toolbar* command) has some common features with the 11.2. JOBS Explorer [201]. It enables organizing, editing and running single Jobs, Job Wizards or Test Runs.

A job can be added to the toolbar from within the *View > Analysis Controls > JOBS Explorer* window: right click on the job name and select *Show on Toolbar* or just drag and drop Jobs from the Job Explorer.



## 10.2. Running a HCA job

### 1) Open JOBS Explorer

Run View > Analysis Controls > JOBS Explorer.

### 2) Connect to a job database

A database for storing the job data must be opened or created before running a job. Use the database pull-down menu within *JOBS Explorer*.

### 3) Run the HCA Job template

Click one of the buttons in the *Run:* section of *JOBS Explorer*. The following jobs are prepared there with the HCA module installed:

**HCA Fixed** Analysis of fixed samples prepared on well plates.

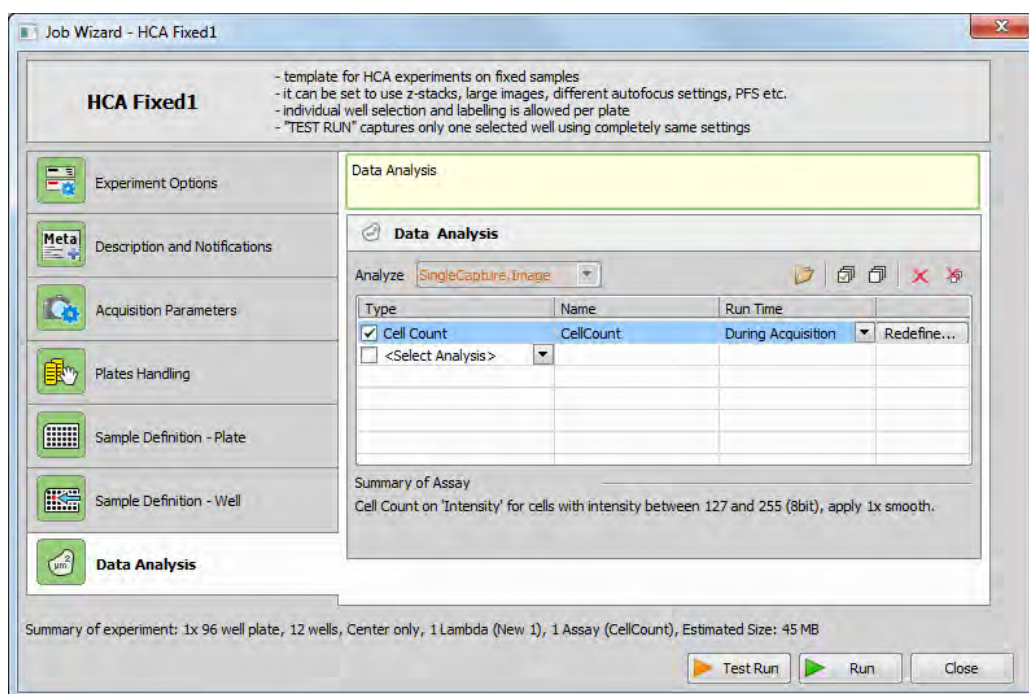
**HCA Fixed with WPL** “WPL” meaning *Well Plate Loader*.

**HCA Live** Analysis of live (motile or growing) samples.

### 4) Define parameters of the JOB

The following window appears. You should browse all the tabs (tasks) and adjust settings of the job correctly. Once you visit a tab and the settings are acceptable - in the scope of the whole job - the background of the task icon turns green.

Figure 10.3. HCA Fixed Job wizard enabling to set up parameters of the Job step by step.



Start by setting your first tab called *Job Parameters* . All the subsequent steps of your job will be affected by these settings (some will even disappear).

Continue setting up task after task until all are highlighted green and ready for the job run.

## 5) Test the job on a single well

You can click the **Test Run** button to run the current job on a selected well.

## 6) Run the job

When everything is set up correctly, click the **Run** button. The HCA Progress window opens. See for details.

Some tasks may appear during run time waiting for user interaction. These tasks have to be set up and confirmed clicking **Next/Run** .

## 7) Browse results

Results are displayed automatically in a separate window once the job is finished.


## 10.3. HCA Analysis



*NIS-Elements* provides the following analysis modules available within the

- 7.8.2. Cell Count Analysis [138]
- 7.8.5. General Analysis [140]
- 7.8.6. Image Intensity Analysis [141]
- 7.8.7. Live/Dead Analysis [141]

You can include one or more of these analysis modules to be a part of your HCA job or you can just use them to process the resulting images after running the acquisition part of the job.

### **Analysis performed on job results**

1. Go to the Job Results window e.g. by double-clicking the job run record.
2. In the result view (10.4. Viewing Results [192]), click the  *Run Analysis* button.
3. The Run Analysis dialog window opens.

Click the  *Add New Analysis* button and choose the type of your analysis in the pull down menu. Continue setting up your analysis as described in the following chapters. After (re)defining your analysis click the *Run* button. Your analysis is then processed and the resulting variables are added to the *Show Quantities*  panel.

#### Tip

*Hide your preview by clicking the  *Show preview* button to see more of your analysis results.*

## 10.4. Viewing Results

### 10.4.1. Result View


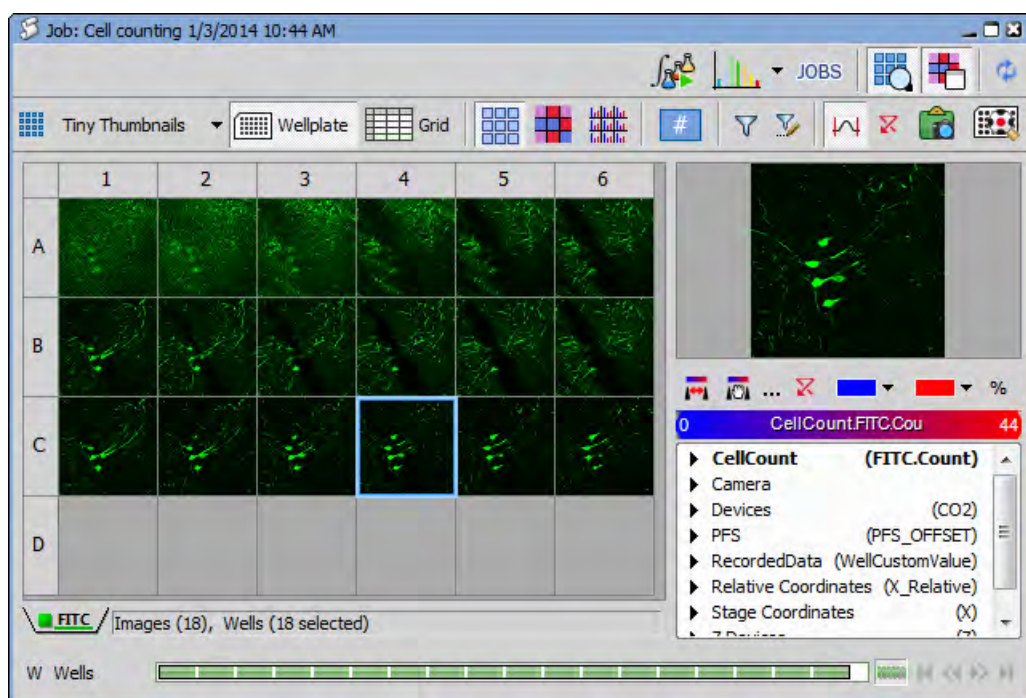
Once the job is finished, results are saved automatically and the Job results window opens. To open some other saved job results, select the particular job/job-run in the 11.2. JOBS Explorer [201] and click  *Results*. The Job result window shows captured images and all associated captured data of a single job run.

Figure 10.4. Job Results window



This picture shows a result of a well-plate experiment displaying preview of the captured images in each well. If multiple images are captured in a single well, they are automatically stitched into a single large image.

### **Basic dialog areas**

Basic navigation is done using the ND navigation bars in the bottom part of the dialog window. Right side shows a preview of the selected well/frame with heat-map controls and acquired data browser.

The Job results window enables to view the results from “various angles”. There are three basic views:

- Thumbnail view
- Wellplate view (if wellplate was used during acquisition)
- Grid view

In *Thumbnail* and *Plate* view you can view Images, Heat-map of selected data values (Quantities, Acquisition metadata and Analysis results) and labels (in case of wellplates). *Grid* view displays all results in a tabular form.

### **ND2 Interconnection**

Double-clicking an image in the result view opens the underlying ND2 file with that particular image frame displayed. If the job consists of multiple ND files, just a single image window stays open. To display



---


each ND2 file in a separate window, use `alt + double click`. Both options are available also on the context menu.


### **Reusing a Job Definition**


ND2 files created in Jobs are saved together with their Job Definition. To display or reuse this definition, simply right-click on the opened image and choose *Reuse Job Definition*.


### **Top Toolbar options**

 **Huge Thumbnails,**  **Large Thumbnails, etc.** Thumbnail view displays all acquired images in the order of acquisition.

 **Wellplate** Real layout of the plate is displayed.

 **Grid** Images along with metadata are displayed in a grid (see below). Filtering and grouping is supported (you can sort and group by any column). Statistics can be turned on.

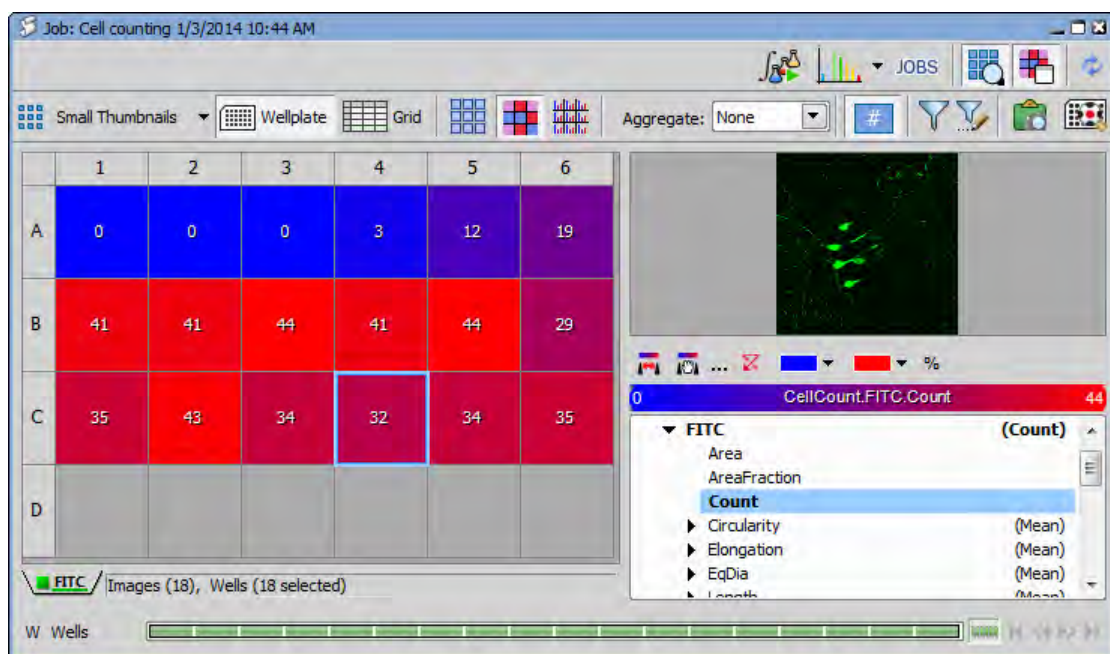
 **Show Images** Displays all acquired results as images.

 **Show Heatmap** Independently on the current view (thumbnails, well plate, grid), you can select a heat-map which represents one of the meta-data values (e.g. analysis results, gas concentration, temperature, etc.). In the heat-map view you see the selected colored squares instead of images. The color is based on the value and can be tuned. If multiple data are acquired in one well/frame (e.g. when multi points were captured in each well of the well plate), it is possible to *aggregate* these data (calculate min, max, mean, sum, SD, etc.) in order to get a single value per well. If one of the aggregating features is selected from the combo box, entire multi point bar is selected and the results show the aggregated values. If one multi point is selected in the multi point control bar, *Aggregate* combo box is automatically switched to *None*, displaying only non-aggregated results of the currently selected multi point.

An example of a heatmap is displayed in the following picture. Displayed as thumbnails, each image is overlaid by a color and a value both of which carry information about the meta-data selected in the bottom-right corner of the window. By default the color range of the selected feature uses minimal and maximal values found in the entire job run. The color scale range and colors can be modified by the user.



Figure 10.5. Job Result: Thumbnail View (Heatmap)



#### Note

Select the meta-data to be displayed in the heat-map in the Quantities panel (bottom-right area of the window).



**Show Histogram** Data of each image are represented by a histogram.



**Time Trend** Time trend represented by arrows for each image can be displayed using this button.



**Show Feature Values** Displays a numeric value of the selected feature over each well.



**Use Filter** Activate this button to filter the results. The filter can be defined by clicking the **Define Filter** button.



**Define Filter** A filter may be applied to images displayed in the result view. Click the **Define Filter** button to display a table with the filter definition. Toggle the **Use Filter** button to turn the filter ON and OFF.




**Export View** Exports the well plate overview.




**Show Selected Well/Jump to Wellplate** Switches between single well view and well plate view.


---


 **View Graph** Click the black arrow next to this button to choose a graph view from the list. Then click the button to open the selected graph view. For more information about viewing results in different graph modes, please see: 10.4.2. Graph View [196].

 **Run Analysis** This button runs an analysis on images captured using a job definition.


 **View Definition** (*requires:Local Option*)

Click this button to display the job definition window.


 **Grid Settings** Shows a table with all acquired metadata. Each feature can be displayed or hidden in the *Grid View* using the check boxes.



 **Show Preview** Displays a small image preview window.

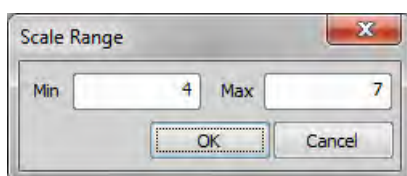
 **Show Quantities** Display or hide the preview and meta-data of the currently selected image.


 **Refresh Contents** Hit this button to refresh the currently displayed results.


### Quantities tool bar

 **Auto Scale Current View** This button assigns the left color to the lowest value and the right color to the highest value present in the current data results view.

 **Manual Color Scale** Applies the custom minimal/maximal scale range. Click the  button and enter the minimum and maximum values to be used instead of the default ones.




Click  to apply the manual scale.

 **Reset scale** Any manual scale changes are turned off and the default values are shown.

% Feature values are represented by their percentage. 100% is assigned to the maximal and 0% to the minimal value.

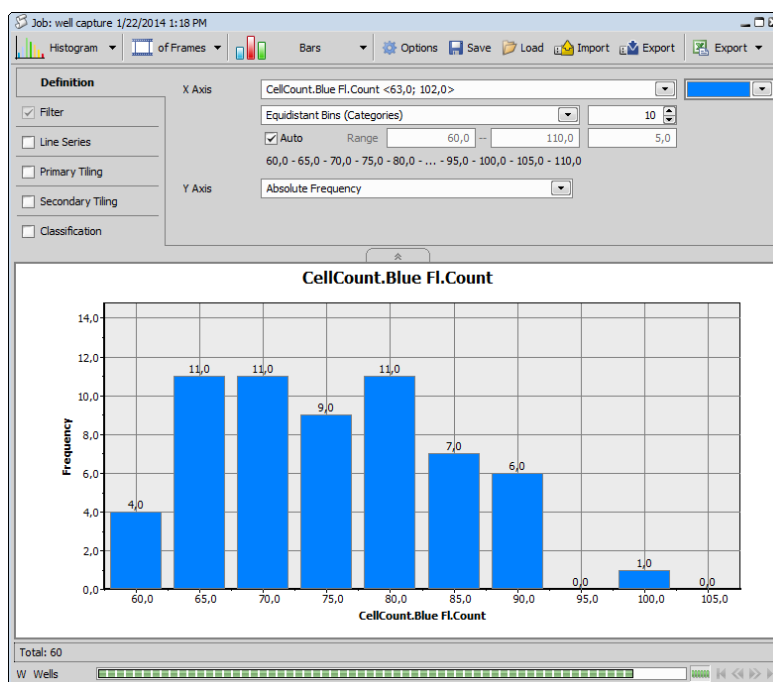
## 10.4.2. Graph View

Clicking on the  *View Graph* icon in the result view opens the graph view.

## Note

To display different graphs of your results, click the graph button to expand a combo box containing more options.

Figure 10.7. Histogram graph view



## Top toolbar options



**Graph selection** The first button is used for selecting the graph type.



**of Frames/of Objects/of Tracks** Select whether your graph results are related to image *Frames*, binary *Objects* or *Tracks*.



**Graph sub-type** Specifies the graph sub-type. Following types are available: *Histogram*, *Scatterplot*, *Barchart*, *XY Line*, *Timechart*.



**Options** Opens the Graph Options dialog window. Color and line features of the graph can be adjusted in the *Appearance* tab. X Axis and Y Axis Left modify the axes.





**Save** Opens the *Save Graph Settings* window enabling to save the current graph settings into the database.



**Load** Opens the *Load Graph Settings* window used for loading graphs settings from the database.

 **Import** Imports the graph settings from a .graph file.

 **Export** Exports the current graph settings into a .graph file.

 **Export** This button enables to export the resulting graph images into raster files or Clipboard. Data can be exported into MS Excel or copied to Clipboard as well. Clicking the black arrow next to this button reveals a menu which specifies further export settings (  **Settings**) and which graph features will be exported.

 **Zoom in/out** Zoom in/out inside the graph area.

Tabs on the left represent steps of setting-up your graph visualization. Each tab has a check box, which determines whether the tab parameters are influencing the resulting graph or not.

### **Definition tab**

In this tab, the user defines main properties of each graph type. Settings are not the same for each graph type. Generally features shown on the X and Y axis are selected and their range, bins trend lines and other variables can be adjusted.

### **Filter tab**







*Filter* tab enables filtering results based on selected features. These can be selected from the combo box in the *Column* column. After choosing the feature for filtering, select the *Comparison* type. Click the combo box in the *Value* column to reveal a histogram suitable for quick value estimation or enter the numeric value directly into the edit box. If you add multiple filtering features, you can move them up/down in the list using these   arrows. You can also delete each filter clicking on this  icon.

Figure 10.8. Filter tab

Definition	AND/OR	Column	Comparison	Value			
<input checked="" type="checkbox"/> <b>Filter</b>	<input checked="" type="checkbox"/>	CellCount.Blue Fl.Circula...	Is Greater or Equal	0,8595			
	<input type="checkbox"/>	<select>					

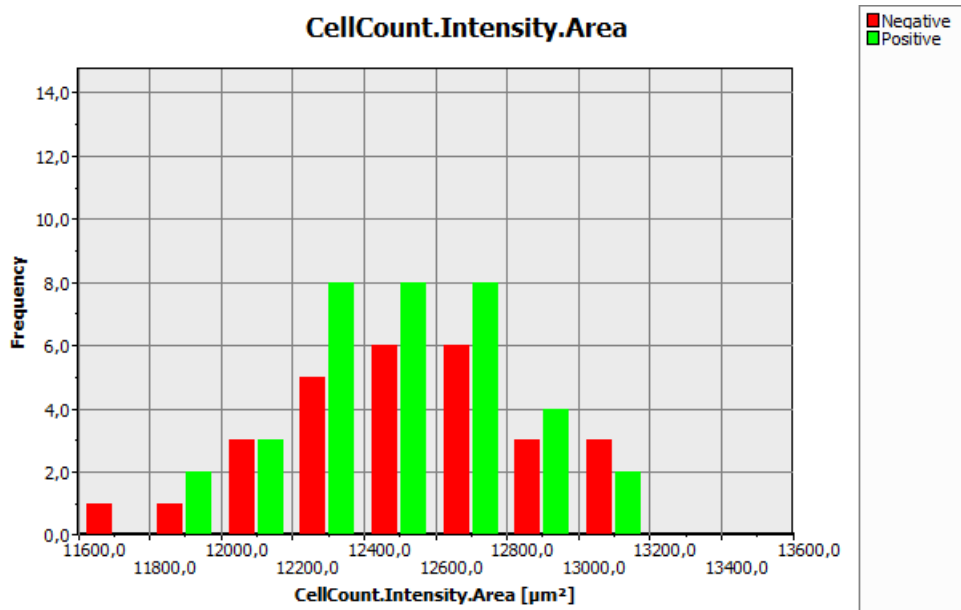
#### Note

Only filters with the check box marked as checked are used for result filtering.

### **Line Series tab**

Series enable to view different groups of data in the same graph. Each data series can be displayed in a different color. Select one feature from the combo box (label, quantity, class or any measurement result) and adjust its starting and ending color. Bin types, their ranges and limits, legend and other parameters can be adjusted.

Figure 10.9. Line Series graph



### Primary/Secondary Tiling tabs

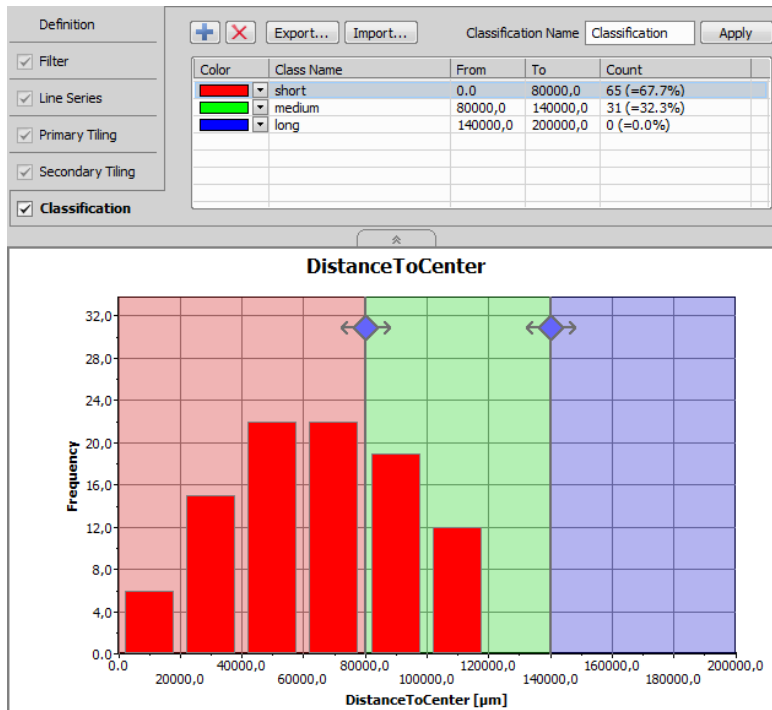
Both define another two groupings enabling the user to see more instances of the same graph populated with different group data. *Primary Tiling* defines the groups to be displayed in graph tiles horizontally whereas *Secondary Tiling* defines them to be displayed vertically. Grouping is defined as in the Line Series tab except the colors.

Figure 10.10. Primary Tiling tab

Definition	CellCount.Blue Fl.Count <63,0; 102,0>
<input checked="" type="checkbox"/> Filter	User Defined Bins
<input type="checkbox"/> Line Series	50,0;55,0;60,0;65,0;70,0;75,0;80,0;85,0;90,0;95,0;100,0;105,0;110,0
<input checked="" type="checkbox"/> Primary Tiling	
<input checked="" type="checkbox"/> Secondary Tiling	
<input type="checkbox"/> Classification	<input checked="" type="checkbox"/> Limit Graphs per Row to 3

### Classification

This tab is used to create classes over the resulting graphs. These classes are easily defined by selecting the class color, entering its name and specifying its range (by editing *from* and *to* columns or by moving the rhomb symbol directly inside the graph).



# 11. JOBS

## 11.1. Introduction to the JOBS Module

JOBS is an environment aiming at complex acquisition and data analysis with easy to use and comprehensive user interface.

### **Features highlights:**

- Well-plate, dish and slide support
- Conditional acquisition
- Set of ready-to-use templates
- Wizard for Operators
- Visual Programming Style
- Integrated Analysis
- Asset Management via Database
- Data Mining
- Remote Access and Sharing
- *NIS-Elements* platform

Jobs are automated procedures for complex image acquisition and data analysis. Typically it is a conditional acquisition on well plates, dishes or slides for long times. It may include Confocal and wide-field camera acquisition combined with photoactivation or bleaching.

The strength of Jobs is their flexibility. They rely on building blocks that can be freely assembled into arbitrary procedures in order to fulfill any particular needs. As all this is done visually using Drag&Drop it is called Visual Programming. This approach combines advantages of programming – flexibility – and limits the drawbacks: having to learn how to write a programming source code.

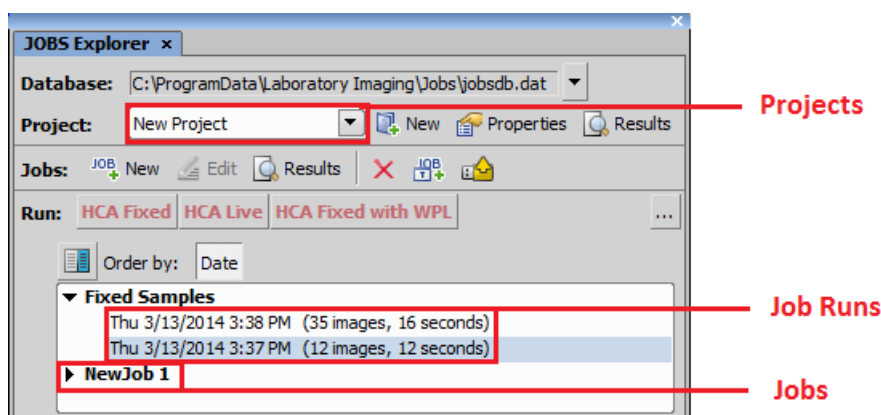
When such procedures – Jobs – are executed, images and analysis data are produced. The images are stored in ND2 files. Metadata and analysis results are stored in the database together with links to the ND2 files. The complete acquisition history is stored in the database as well as the whole organization and structure of Jobs and Projects.

## 11.2. JOBS Explorer

### 11.2.1. Introduction to JOBS Explorer

*Jobs Explorer* is the heart of the 14.10. HCA [230] and 14.14. Jobs [231] modules. Run the *View > Analysis Controls > JOBS Explorer* command to display it. All jobs, job runs and all acquired data are viewed and managed throughout this window.

Figure 11.1. JOBS Explorer with the 14.14. Jobs [231] module



**Database** Name of the currently connected job database is displayed in this row. The pull-down menu provides tools for managing job databases.

**Project** Jobs are organized into *Projects* - virtual containers which help you organize your jobs. Create your own project or use the default one. You can have projects named e.g. “cell proliferation”, “translocation” etc. or name it by its author e.g. “Catherine”, “John”, or for example use dates as project names.

However, since you can display results of the whole project in a single result view, it may be a good idea to keep similar jobs together. For instance it may be useful to have a 5hour and a 10 hour proliferation jobs together in one project, but it may produce some complications to put 6-well-plate data together with a 96-well-plates.

**Jobs**  *Results* open Results of the selected Job.

**Run** This area aggregates predefined job templates which are executed by clicking on their button.

**List of job runs** Jobs Explorer shows the contents of one project at a time as a list of jobs. Each job can be unfolded to disclose all instances of when it was run. Single jobs (highlighted in bold) represent the definition which can be viewed and modified in the job editor, wizard or run time. The run instances can be opened to reveal a view on acquired images and data.

*Jobs Explorer* shows the contents of one project at a time as a list of jobs. Each job can be unfolded to display all instances of when it was run - so called *Job Runs*. Single jobs (in bold type) represent the



definition which can be viewed and modified in the job editor, wizard or run time. The run instances can be opened to reveal a view on acquired images and data.

## 11.3. Basic Job Rules

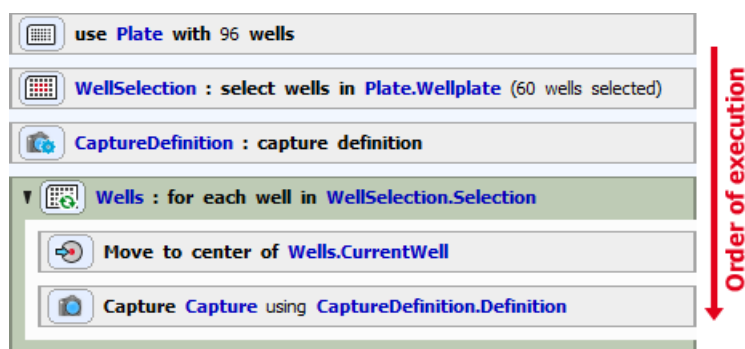
### Introduction

Jobs are automated procedures for image acquisition and analysis. When a job is executed, images and analysis data are produced. The images are stored in ND2 files while metadata and analysis results are stored in a database including links to the corresponding ND2 files. A complete acquisition history is stored in the database as well.

### Order of execution

Jobs are composed of *tasks*. The order of tasks in a job is essential because they are executed in that order beginning at the top. There are two kinds of tasks: simple tasks and tasks which have blocks of other tasks nested inside them (e.g. loops and conditions). If there were only simple tasks, the job would be mere chain of operations, very easy to assemble. On the other hand, it would be too difficult to create a simple time-lapse with 100 repetitions - having to copy the *Capture* task 100 times in a row. In order to avoid this copying, JOBS provide the concept of loops. Loops can embed other tasks. If a loop is run, it repeatedly executes the whole inner block of tasks until the defined number of repetitions is achieved. Another task which embeds other tasks is the *Condition*. Conditions are used for branching (deciding within the job flow). Embedded tasks in a condition are executed only if the specified condition is satisfied. Such a condition in a job which scans well plate can go like this: “capture a multi-point sequence on a well only if there is a fluorescence signal detected, otherwise proceed to the next well”.



Figure 11.2. A simple job capturing an image on each well center of the well plate selection

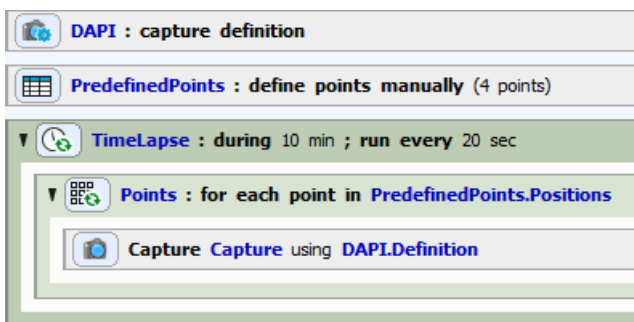




### Rules

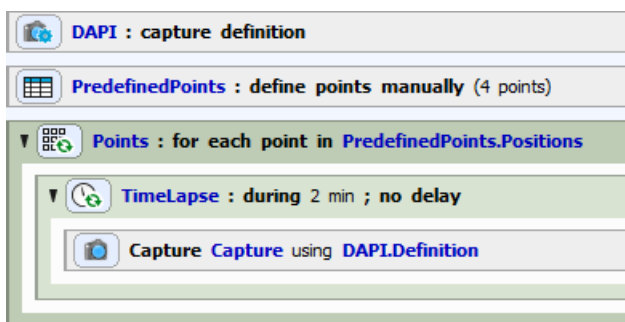
- Tasks are executed in order (from top to bottom).
- If a task contains child tasks, these child tasks are executed in order (conditions) or repeatedly (loops).
- Tasks inside a condition are executed only if this condition is fulfilled.

## Task position

Position of each task in the job definition is substantial. In the following example,  Time Lapse and  Loop over Points tasks are swapped, bringing completely different results.



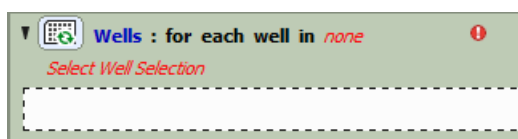
Herein, points on the sample are created manually. *Time Lapse* loop is then started for 10 minutes, executing embedded tasks every 20 seconds. Therefore a  Loop over Points (containing  Capture) is executed. Hence, an image is captured for each predefined point one after another. This capturing takes place every 20 seconds during the 10 minute *Time Lapse* interval.




If the *Time Lapse* task is placed inside the *Loop over Points* task, the job works differently. Images are captured (for the duration defined in the *Time Lapse* task - 2 min in our example) on the first point, then for 2 minutes on the second point, etc. When all 2 min time lapses of all points we are looping over are captured, the job is finished.

## Task dependency

Some of the tasks require other tasks to be present in the job - they depend on them. For instance, a well-loop which goes over wells requires a selection of wells on a well plate in order to loop over it (otherwise the job wouldn't know which wells to loop through). Moreover, the well selection task requires a well plate definition in order to know the dimensions of the well plate, how many wells are present and their exact position.

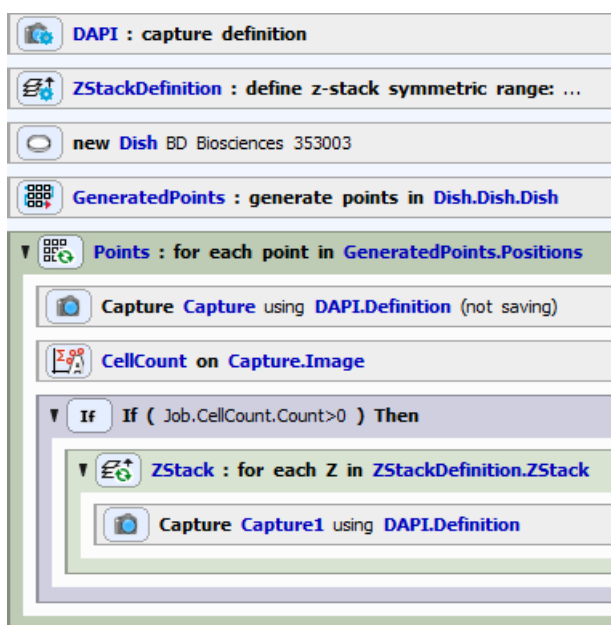








#### Note



If a task is not set-up properly (e.g.: missing dependent task, wrong task order in the definition, wrong task setting,...) a red exclamation mark  appears in the caption of the concerned task. The job cannot be executed until all such errors are resolved.

### Definition-Execution type tasks

Some operations consist of two separate tasks - one for *definition* and one for *execution*. This is often useful in situations where a single definition is to be executed in different places of the job. If you wish to use such a task, always place the definition task before the execution task.



In this example, DAPI optical configuration is used in the  Capture Definition task and  Define Z-Stack parameters are defined. Points are generated on a dish and a loop over these points is created. On every point an image is captured (not saved) and used for the  Cell Count Analysis. Information about the cell quantity at each point of the dish is used in a condition. If *Cell Count* is bigger than 0, all the tasks inside this **If** Condition IF task are executed (  Define Z-Stack containing  Capture). Hence, image on each plane of the Z-Stack is captured (and saved). Notice that  Capture uses the same definition in both places. If the user decides to switch the Optical Configuration used (e.g. from

DAPI to FITC), he can simply change it in the  Capture Definition and all  Capture tasks linked with this definition will use the new setting.

## Rules

- The *Definition* task (usually containing the word Define, Definition,...) has to be placed above its *Execution* task.

## Loops

We can consider a well plate as a set of many wells. To sequentially pass through all the wells in Jobs we use Loops. Each processes (tasks) which we want to execute on the well level have to be placed inside this loop.

Note





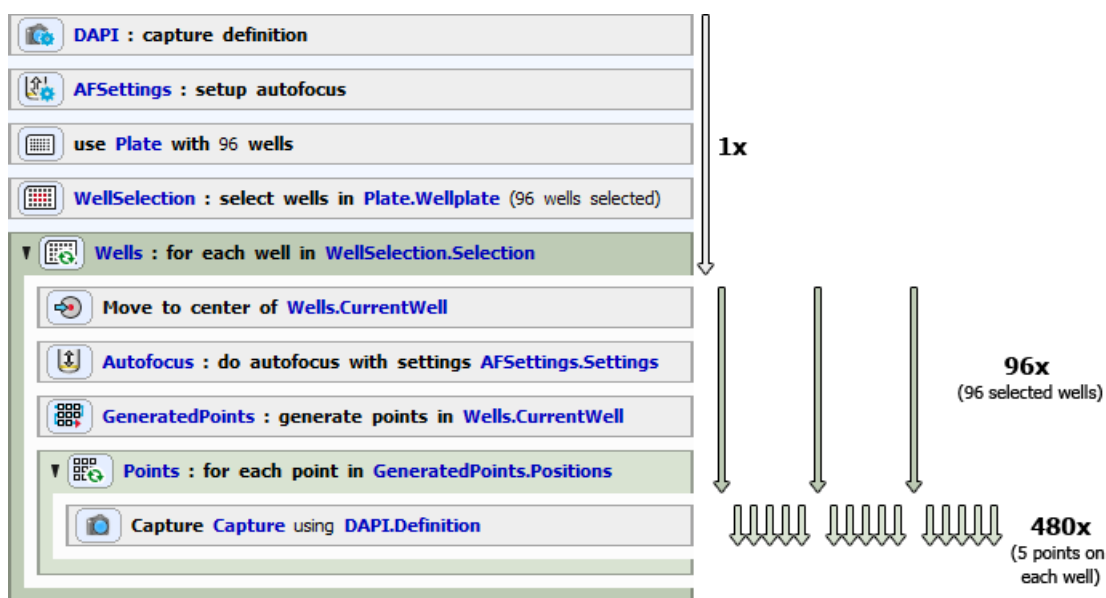
 Loop Over Wells does not move between the wells on a well plate by itself. It is therefore impossible to expect pictures of single wells by placing just  Capture inside the  Loop Over Wells. To do so, you have to place the  Move to Well Center task inside the loop right before capture. This task tells the loop to move to the appropriate well.

Figure 11.7. Loop over Wells and Loop over Points



In this job, stage moves to the center of the first well of the well selection, auto focus is performed and random points are generated (first level loop). Then the job encounters the embedded second level loop. Still on the same well these random points are looped through and image is captured for each point. First level loop was now completed for the first well. Stage moves to the second well and the inside of the loops is executed repeatedly until all the wells of the well selection are run through.

## **Rules**

- Tasks contained in a loop task are executed repeatedly and from top to bottom.

## **Adjusting the Task Settings**

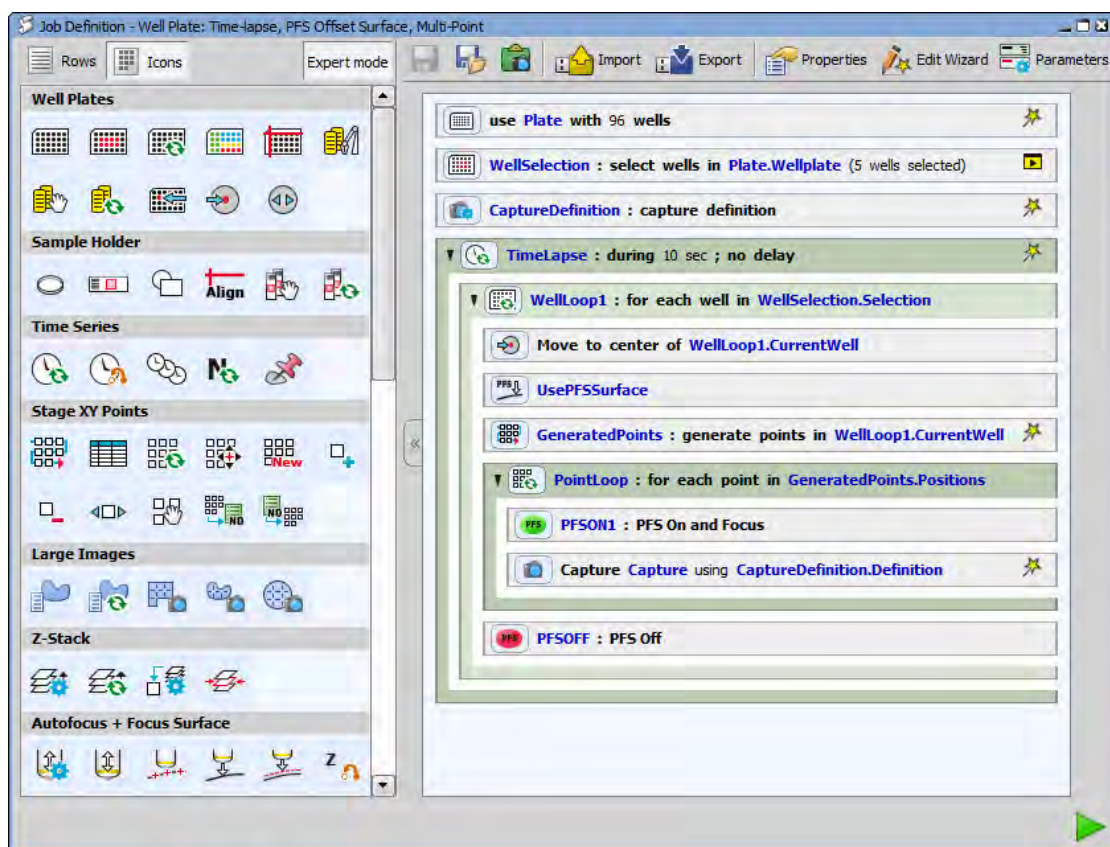
Tasks have different parameters which need to be set. These parameters are usually set at job-design time by double-clicking the caption of the task. However, crucial tasks can be selected and displayed within a wizard out of the main job body, so that the person running the job is not distracted by the whole job structure. Similarly, the same tasks can be displayed at runtime which enables the user to interact with the job or make decisions. Complex jobs can be parametrized to be more user friendly (see online help).

## **Effective job building**

To avoid errors when building a job, all of the rules mentioned above have to be followed. The best practice is to think about the results we want to acquire and then go backwards. Which devices are necessary for our procedure? Is the procedure done only once or shall it repeat? Do I need to change sample holders (slides, well plates) during the job? Are there any decision-making points? What images exactly do we want to capture? Which device operations need to be done before an image is captured? Also think about the user interaction. Will the job be fully automatic or do you want to adjust the parameters during the job run? Try to answer all of these questions to make the job as simple and effective as possible. After that, you can start creating the job.

## **11.4. Job Definition Window**

Once an empty job is created or an existing job is opened, the job definition window appears.



This window is the main place for the Job program flow and for changing task parameters. Apart from the main toolbar there are two main areas available:

**The leftmost task palette** All tasks available are displayed here in the form of icons (or icon+description). *Expert mode* button switches between the simple (basic tasks) and advance view mode (all tasks). Tasks are organized into logical groups. Some tasks depend on the presence of some devices. They may not appear in the palette until that device is added into the device manager ( *Devices > Manage devices*). To insert a task to the job, drag its icon into the main area which forms the job definition. Width and thus the number of the icons(tasks) in every row in the task list can be set to any number of icons by sliding the right border of the task list.

**The main program area containing the job definition** This pane holds the tasks that form the program. Each task has an icon and text that describes what the task will do when executed. Every task has different parameters which are revealed by double-clicking on its caption. Tasks can be marked as to be shown in the Wizard or at runtime. All tasks which were dragged here will be run one after another until the job is finished.

## Operation

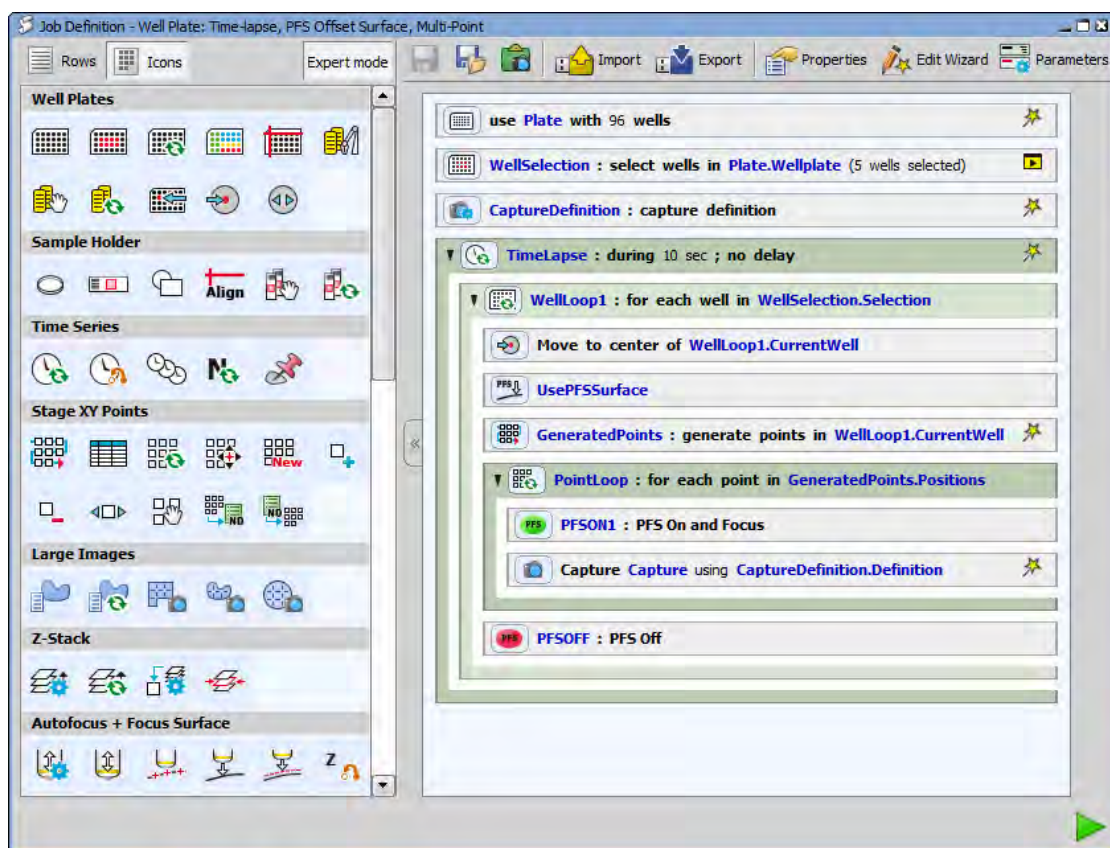
Most of the editing operations are done by moving the Tasks around. By simple dragging and dropping the tasks from the left to the right you create your complete Job sequence in a matter of minutes.

- To insert a task into the job program, drag the icon to the main program area which contains the job definition.
- To move a single task or multiple tasks to different place, hold the task in the main area and drag it to another place in the program.
- To delete a task from the program area, simply drag it out of the job main program area.
- To set parameters of each task, double-click its caption.

## Warning

Some tasks depend on other and their order of execution needs to be correct. Please see 11.3. Basic Job Rules [203] to avoid error warnings when setting up your Job.




Figure 11.9. Job Definition window






---


## Window Options

 **Rows**,  **Icons**,  **Hidden** These buttons change the appearance of the task panel. It is possible to switch between the *Rows* + description view mode and *Icons* view mode. In the *Icons* view mode it is possible to change the width of the task panel by dragging the right border line of the scroll bar. The task panel can also be fully hidden by clicking on the *Hide* button in the middle of the 11.4. Job Definition Window [207].


**Expert mode** Expert mode in JOBS displays all available tasks. By default, this mode is off and the Job Definition window shows only the basic tasks.


 **Save Changes** Automatically saves any changes in the *Job Definition*.

 **Save As** Saves the *Job Definition* with desired name into a Project that could be then selected from the 11.2. JOBS Explorer [201].

 **Print Job Definition to Clipboard** Copies a screen shot of the current Job including key parameters into the clipboard.

 **Import** Imports existing Job .BIN files into the *Job Definition* window.

 **Export** Exports current Job to a single .BIN file.

 **Properties** This button opens the *Job Properties* dialog window where following features related to the current job can be set.


In the *Job description* edit box a description of each job can be made and saved together with the Job. This description is displayed in the caption of the *Job Wizard* and *Job Execution Progress* window.


*Progress window* section enables to Show/Hide Progress Window during the Job run or to preview image in the Progress window after the first capture or on the job start.

*When Job definition changes* section defines what is done when changes in the job definition are made. These changes can be saved to the current Job definition, saved as a new definition or the user is asked to specify the location where to save them. *Treat adjustments in Wizard as Job change* should be checked if it is requested to apply the changes made in wizard directly to the job definition.

*Wizard* section specifies when the Run button is shown in wizard - *always* or *only if all tasks are visited* (highlighted green).

*Advanced features* section groups advanced features such as enabling the *Test Run*, *Parameters* or *Programmable Actions in Wizard*.

 **Edit Wizard** This button opens the Job Wizard edit dialog window.

 **Parameters** Each job can be parametrized on the task level. This button opens the Job Parameters dialog window where you can define the name of your parameter, type, values it takes and whether it requires another parameter from the list to be set to a certain value. You can also mark each parameter to be shown in wizard/runtime.

---

### Note

*This button is visible only if Parameters are enabled inside Job Properties.*





**Run Job** Executes the current Job.




# 12. Macros

## 12.1. Creating Macros

A macro - an executable sequence of commands - can make the work very effective. *NIS-Elements AR* provides a C-like programming language utilizing its internal set of functions. The sequence of functions can be created either by recording the performed actions, by writing the functions within the macro editor, or by modifying the command history (the history is recorded automatically during the work). The macro can be saved to an external (\*.mac) file for later use.

### Recording a Macro

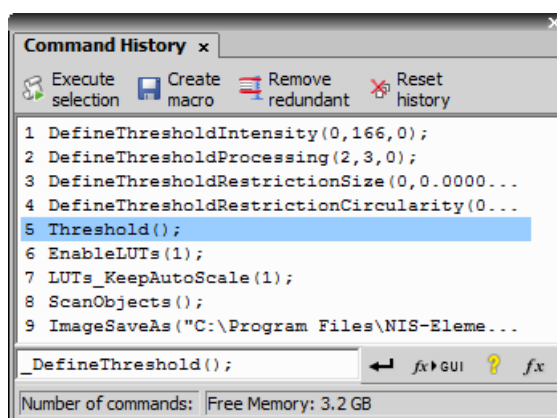
The fastest way to create a macro is to record it.

- Start the *NIS-Elements AR* macro recorder by selecting the *Macro > Record* command.
- Perform the series of actions you would like to record.
- Finish the recording by the same command again (its name changes to “Stop Recording”)
- It is recommended to check the macro in the macro editor before saving. Run the  *Macro > Edit* command to display it.
- Save the macro to a file via the *Macro > Save As* command.

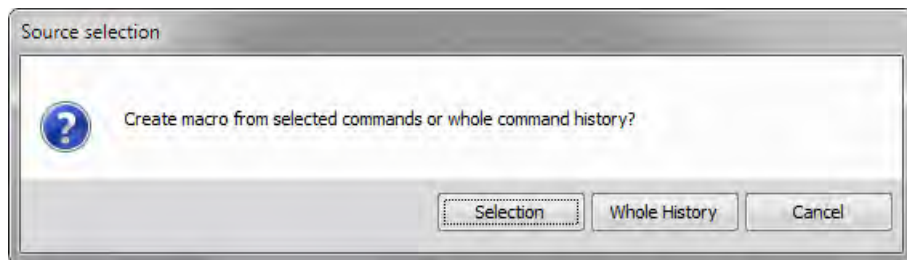
### Creating a Macro from History

You can create a macro using the list of recently performed commands.

- 1) Run the *Macro > History* command to display the *Command History* control panel:



- 2) Push the *Create Macro* button.
- 3) Select what portion of the command history will be used in the macro:



**Selection** This option will use only commands which you have previously selected by mouse within the *Command History* window. Group selection (holding **Shift** or **Ctrl**) is available.


**Whole History** All commands listed in the *Command History* window will be used for the new macro.

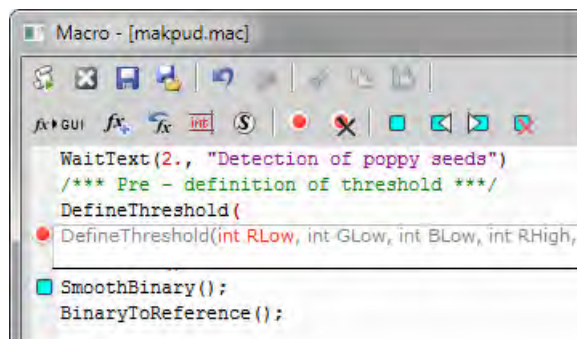
- 4) After you selected one of the options, the macro editor appears containing the new macro source code. Use the editor to fine-tune it.

#### Note





*The Remove Redundant command is automatically performed before the macro is opened in the editor.*

### **Writing/Editing a Macro**

If you possess programmers skills, a macro can be written by hand in the built-in macro editor. Run the  *Macro > Edit* command to display it.






The macro editor provides:

- Undo/Redo functionality.
- Command insertion from the list of available commands.
- Interactive command names list. Press `Ctrl+Space` to display a simplified list of all commands while typing the macro.
- Syntax hints (parameter types and names) appear as you type a command name.
- Bookmarks  can be placed to the code so you can easily roll to the important parts of the macro.
- Breakpoints  can be placed to the code. A breakpoint forces the macro to stop the execution at a certain point so you can check the state of variables and therefore inspect the macro functionality thoroughly.
- Syntax highlighting.
- Help on commands with detailed description.
- Use the *Save Macro*  and the *Save Macro As*  commands in the macro editor to save the created macro.

NIS-E automatically remembers the folder from where the macros were run the last time and will open this folder when user clicks on Open/Save command in Macro menu.

## 12.2. Running a Macro

*NIS-Elements AR* provides several ways to run a macro.

- You can run the current macro loaded to *NIS-Elements AR* by choosing the  *Macro > Run* command or by pressing *F4*.
- A *hotkey* combination (*Ctrl+Alt+1,2,3 ... 9*) can be assigned to it in the  *Macro > Options* window.
- You can run a macro at the beginning of the *NIS-Elements AR* session, by assigning the *StartUp* flag to the macro in the  *Macro > Options* window.
- You can run a macro saved to a disk by using the *Macro > Run Macro From File* command.
- A tool bar button can be assigned to run a macro. See 3.6. Modifying Tool Bars [32].

### **Breaking the Macro**

The macro execution can whenever be stopped by pressing the `Ctrl+Break` key shortcut.

## 12.3. Macro Language Syntax

Specifies the *NIS-Elements AR* Macro Language features.

---

## **Variable types**

The following data types are implemented:

**char** text

**char8** <-128, 127>

**byte** <0, 255>

**int** <-32768, 32767>

**word** <0, 65535>

**long** <-2 147 483 648, 2 147 483 647>

**dword** <0, 4 294 967 295>

**double** <1.7E +/- 308 (15 digits)>

## **Structures and Unions**

Structures and unions are not supported.

## **Arrays**

One and two dimensional arrays are supported.

## **Local and Global Variables**

You should declare local variables at the beginning of macro or function only. You should declare global variables only at the beginning of macro. You can run two nested macros declaring the same global variables, but they must be of the same type. You can declare global variables by prefixing the declaration *global*. Eg.:

```
global int Number_Rows;  
global char buffer[200];
```

The “global” keyword in front of the variable definition assigns the variable to the global scope. Such variable is then accessible from all function scopes within the macro interpreter.

## **Statements Supported**

**for** The *for* statement lets you repeat a statement a specified number of times.

Syntax

```
for([init-expr]; [cond-expr]; [loop-expr])  
    statement
```

First, the initialization (init-expr) is evaluated. Then, while the conditional expression (cond-expr) evaluates to a non zero value, Statement is executed and the loop expression (loop-expr) is evaluated. When cond-expr becomes 0, control passes to the statement the following the for loop.

**while** The *while* statement lets you repeat a statement until a specified expression becomes false.

## Syntax

```
while(expression) statement
```

First, the expression is evaluated. If expression is initially false, the body of the while statement is never executed, and control passes from the while statement to the next statement in the program. If expression is true (nonzero), the body of the statement is executed and the process is repeated.

**if, else** Conditionally executes a statement or group of statements, depending on the value of an expression.

## Syntax

```
if(expression) statement1
    [else statement2]
```

The *if* keyword executes statement1 if expression is true (nonzero); if *else* is present and expression is false (zero), it executes statement2. After executing statement1 or statement2, control passes to the next statement.

**goto** Transfers control of the program execution.

## Syntax

```
goto name;
    . . .
    name: statement
```

You cannot use goto to jump inside the block from outside. E.g. following is not allowed:

```
goto label;
    if(k>5)
    {
    label:
        DilateBinary(3, 5);
        FillHoles();
    }
```

You cannot use goto to jump out from the block more than 2 block levels down. E.g. the following is not allowed:

```
for(i=0; i<64; i=i+1)
{
    for(j=0; j<64; j=j+1)
    {
        if(a[i] > b[i])
        {
            value = i;
        }
    }
}
```

---

```

        goto end; // crossing 3 right brackets
    }
}
end

```

**break** Terminates the execution of the nearest enclosing statement.

Syntax

```
break;
```

The *break* keyword terminates the execution of the smallest enclosing *for* or *while* statement in which it appears. Control passes to the statement that follows the terminated statement.

**continue** Passes control to the next iteration of the statement in which it appears.

Syntax

```
continue;
```

The *continue* keyword passes control to the next iteration of the *for* or *while* statement in which it appears. Within a while statement, the next iteration starts by reevaluating the expression of the while statement. Within a for statement, the first expression of the for statement is evaluated. Then the compiler reevaluates the conditional expression and, depending on the result, either terminates or iterates the statement body.

### **Statements Not Supported**

**do, switch, case, default, typedef** These statements are not supported.

### **Directives**

Following directives are supported by the system.

**define** The *#define* directive assigns a meaningful name to a constant in a program.

//Syntax

```
#define identifier token-string
```

The directive substitutes token-string for all subsequent occurrences of the identifier in the source file. Token-string can be a value or a string (only for 32-bit version of *NIS-Elements AR*).

//Example

```
#define ERROR_SPRINTF 0
#define MAINDIR "c:\Images"
```



```

int main()
{
char buf[256];
int retval;

retval = sprintf(buf, "%s", "MAINDIR");
if(retval == ERROR_SPRINTF)
Beep();
else WaitText(0., buf);
return TRUE;
} \

```

**include** Specifies the name of the file to be included.

//Syntax

```
#include filename
```

The **#include** directive includes the contents a file with a specified filename in the source program at the point where the directive appears.

Example

// if you do not specify the full path, NIS-Elements assumes, that \ it is a relative path to a main directory

```

#include "macros\my_macro.h"
#include "c:\NIS-Elements\macros\my_macro1.h"

```

**import** The **#import** directive is used to incorporate information from an external library.

//Syntax

```

#import("DLLname");
#import function_declaration

```

*NIS-Elements* can call functions from external DLL's. You should import the DLL, where the functions resides and then make a declaration of the functions. You should not import the following system DLL's: kernel32.dll, user32.dll, gdi32.dll, com32.dll, comdlg32.dll. This feature is available only for 32 bit version of *NIS-Elements*.

//Example

```

#import("luc_13.dll");
#import int RTF_ReplaceVariables(LPSTR destfile, LPSTR sourfile);

```

---

```
#import int RTF_FindQuestion(LPSTR sourfile, LPSTR question, long \
*length, LPSTR defvar);
#import int RTF_ReplaceQuestion(LPSTR destfile, LPSTR sourfile, LPSTR \
replacement);
```

## **Operators**

The following operators are supported by the system. If an expression contains more than one operator, the order of operations is given by the priority of operators. Following operators have higher priority than the others: / \* %. You should use brackets to define evaluation order other than implemented in *NIS-Elements*, which is: from right to the left.

### **arithmetic operators**

- + Addition
- Subtraction
- \* Multiplication
- / Division

### **assignment operators**

The assignment operator assigns the value of the right operand to the left operand.

- = Addition

### **bitwise operators**

The bitwise operators compare each bit of the first operand to the corresponding bit of the second operand. The following bitwise operators are supported.

- & Bitwise AND
- | Bitwise OR
- ~ One's complement

### **pointer operators**

- & Address of ?
- \* Indirection

### **relational operators**

- < Less than
- <= Less than or equal to
- > Greater than

**>=** Greater than or equal

**==** Equal

**!=** Not equal

### **logical operators**

The logical operators perform logical operations on expressions. The following logical operators are supported:

**&&** Logical AND

**||** Logical OR

**!** Logical NOT

### **Expression evaluator**

Expression evaluator support the precedence of operators / \* %. It evaluates the expression strictly from right to left, so you need to use brackets.

### **C-like functions**

The system can interpret your own C - like functions. An entry point to program is the main() function in your macro. If main() is not presented, for backward compatibility, the body of the macro is also considered as "main function". The new macros should use main() as an entry point.

The general C-like functions (also called interpreted functions, as opposed to basic, system functions from *NIS-Elements* or *LUC32\_1.DLL*) have the following syntax.

Syntax

```
int MyFunction(int a, LPSTR str, double d)
{
    int retval;
    . . .
    return retval;
}
```

**Return Value** The return value can be any basic data types (char, int, word, dword, int, double or pointer).

**Parameters** Parameters can be any basic data types (char int, word, dword, int, double or pointer).

Note

*Int and double parameters are automatically converted to string type, when they are assigned to text variable, or are one of the parameters of a function.*

Example

```
int main()
{
    char buf[256];
```

```

my_function1(buf);
WaitText(0., buf);
return TRUE;
}

int my_function1(char *buf)
{
strcpy(buf, "This function has a pointer to char array as a \
parameter");
return TRUE;
}

```

## 12.4. Controlling Cameras by Macro

Every camera can be controlled from within a macro by changing its properties. There is a `CameraGet_*` and `CameraSet_*` macro function available for each camera property (e.g. `CameraGet_ExposureTime()`). The number of functions (beginning with `CameraSet_` and `CameraGet_`) and their actual names depend on the type of camera(s) you have currently connected to *NIS-Elements AR*.

**CameraGet\_** The *CameraGet\_...* functions retrieve current values of the properties determined by the function name postfix. So the `CameraGet_Exposure(int Mode, double *Exposure);` function retrieves the current value of Exposure of the specified Mode.

**CameraSet** The *CameraSet\_...* functions allows you to adjust camera properties. So the `CameraSet_Exposure(int Mode, double *Exposure);` function sets a new Exposure value for the specified Mode.

### How To Set Camera Properties

Instead of describing each property function, it is better to show you a universal way to handle a camera by macro:

- 1) Display the *View > Acquisition Controls > \*Camera\* Settings* control panel and find the property you would like to control.
- 2) Make a change of the property from within the control panel. For example, change the exposure time.
- 3) Display the *View > Macro Controls > Command History* window and see the last function which has been called and its parameters (e.g. `CameraSet_ExposureTime(1, 500);`). This is the function you will control the exposure time with.
- 4) Experiment with the camera control panel in order to determine the right parameter values of the functions to be called within your macro.

## 12.5. Interactive Advanced Macro (API)

(requires: *Interactive Advanced Macro (API)*)

If the API module is installed, it dramatically extends the macro programming capabilities. It adds the following functionality to the main application:

**User interaction functions** Contains functions, which enable interaction with user like: type value, text, combo boxes, list boxes and many other Windows standard controls with easy and intuitive code handling.

**Support of importing functions from external DLLs** Functions from external DLLs can be imported at the start of the macro and used during the macro execution.

### Directives (only for 32bit operating systems)

**\_\_underC** The `__underC` directive assigns a function to be interpreted by the UnderC engine instead of the standard interpreter.

//Syntax


```
__underC int inter_sharpen(int cols, int rows) { }
```

**#importUC** The `#importUC` directive imports an API function to the UnderC engine so that it can be used from within there.


//Syntax



```
#importUC DisplayCurrentPicture;
```

## 12.6. Macro Preferences

This window enables you to configure key shortcuts to macros and set a macro to run automatically on startup. Run the  *Edit > Options* command and switch to the *Macro* tab.

**Macros** Lists of macros that can be executed using hot keys or automatically at the beginning of the *NIS-Elements AR* session.

**Start Up** To launch a macro automatically after starting the *NIS-Elements AR* program, select it and press the  button. StartUp field of the selected macro in the list box is filled.

**Hot Key** To assign a hot key to a macro, select the macro in the macro list box and press the  button. Select one of the pre-defined key combination and press  button.

**Full Path** Shows the full pathname of the selected macro.

---

**Add** Adds a macro to the macro list box. Select Macro dialog box appears and you can search the disk for the macro.

**Remove** Removes a selected macro from the macro list box.

**Edit** Enables to edits a selected macro from the macro list box.

**Filename substitution** These lines display the current values of substitution strings available in macro commands.

**#** Letter representing a drive e.g. "C"

**##** A customizable path e.g. "C:\Images"

**###** Path to a directory according to the macro function used. It leads to the folder where *NIS-Elements* EXE file is placed and to a subdirectory - "Images" for functions concerning image files or "macros" for functions concerning macros. E.g.:

```
ImageOpen ("###\agnor.tif");           //leads to the IMAGES subdir  
RunMacro ("###\macro_001.mac");        //leads to the MACROS subdir
```

**####, #####** The current filename including/excluding the path. These substitutions can be used with the **Sequences** or **SequencesEx** macro commands.

**Information Shown in Caption** Optionally, the name of the current macro and an arbitrary user text can be displayed in the *NIS-Elements AR* caption.


**Defaults for this Page** Restores the default setting for Macro Options.

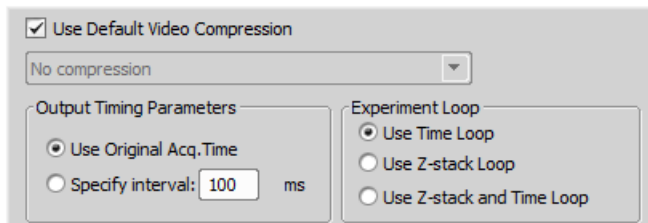
## 13. Movies


### 13.1. Capturing AVI Movie

1. Display the AVI Acquisition control panel by the *Acquire > AVI Acquisition* command.
2. Adjust the advanced settings, especially define the file name and the destination folder.
3. Click the *Record* button. The live image appears and recording starts according to the settings.
4. Stop recording by the *Stop* button, or wait until the time defined in the *Duration* field passes.
5. When stopped, the AVI file remains opened in a new image window.

### 13.2. Save ND2 as AVI

You can easily create an AVI movie from an nd2 file. Run the  *File > Save As* command and select the AVI format in the *Save As Type* pull-down menu. The *Save As* window expands as follows:




Adjust the AVI file settings and press *Save* to finish. Please see the  *File > Save As* command description for more details.

#### Note

*If you have the QuickTime Player installed on your PC, a QuickTime movie (\*.mov) can be created as well.*

See also 13.4. About Video Compression [227].

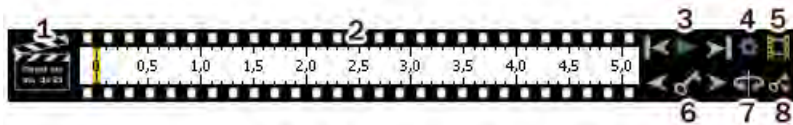
### 13.3. Movie Maker

Models displayed in *3D Volume View* or *EDF 3D Surface View* (requires: *Extended Depth of Focus*) can become actors of your movies once the *NIS-Elements AR* movie-maker is started. The *Movie Maker* control panel appears after you press the  button located on the image tool bar (Volume View or

Surface View must be active). The user is supposed to create a set of key-frames. The rest of the movie will be created by interpolation between these key frames. This gives the user absolute freedom in how the movie will look like.


### **Movie Maker Controls**

When you start the movie maker, the following bar appears at the bottom of the image view.




1. The *Clapper* button. By this button, the director's mode can be turned ON/OFF. See Directors Mode [227].
2. Time line. By clicking to the time line you can browse the movie frames. When a key frame is added, a bold line appears in the time line to indicate its position.
3. *First Frame*, *Play*, *Last Frame* buttons.
4. *Settings* - by this button, you can define the movie length and the frame rate.
5. *Create Movie*
6. The key frame buttons. The existing key frames may be browsed by pressing the *Previous/Next Key Frame* buttons. The *Add/Update Key Frame* button inserts a key frame to the current position on the time line.
7. *Presets* - a predefined rotation can be loaded. When you press the button and select the rotation type, the existing key frames settings will be overwritten.
8. *Import/Export Key Frames* - this enables you to save and load the key frame settings. A file with the \*.KEY extension will be created during the export.

### **How to Create a Movie**

1. Run the Movie Maker by the  button.
2. Adjust the movie settings by the *Settings* button a window appears where the movie duration and frame rate can be defined.
3. You can use one of the predefined presets or import some previously defined key frames by the *Export/Import* button.
4. Either enable or disable the Director's mode by the clapper button. See Directors Mode [227].
5. Go to the beginning of the time line and define the initial zoom, position, rotation or component appearance of the object. This step is not necessary, but very much recommended. Press the *Add/Update key frame* button to fix the initial appearance.



6. Browse the time line, modify the object position each time, and set key frames by clicking the *Add/Update key frame* button. Press the *Play Movie* button for a preview.
7. When all the key frames are properly set, press the *Create Movie* button. The movie is created and the AVI file will open in a new image window.
8. Save the new file by the standard  *File > Save As* command.

### **Directors Mode**

In the directors mode it is not needed to press the *Add key frame* button every time the position of the object is changed. The director's mode detects all changes made to the object position and adds a key frame to the time line whenever such change is detected. A typical work-flow would look like this:

1. Turn ON the directors mode by the *Clapper* button. The time line gets highlighted with red color.
2. Click to the time line where a certain position of the object shall occur.
3. Move the object by mouse or adjust it in some way (zoom, crop, etc.) - at this moment, the key frame will be added to the time line automatically.
4. Click to the time line where another position shall occur, and so on...

The director's mode shall be used when you know precisely how the movie shall look.

## 13.4. About Video Compression

It is common to encode (compress) movies in order to save some disk capacity. This can be done using various types of codecs. A video codec is a software tool which can code (compress) and decode (de-compress) video files. Remember that the codec you use to save the video will be required for playback as well (when playing the video on other computers).

Look at the list of codecs coming with *NIS-Elements AR* by default. If other codecs are installed on the computer, they become also available to *NIS-Elements AR*.

### Caution

*32bit operating systems provide better choice of codecs because a 64bit codec must be used on a 64bit operating system, but there are not many of those in the meantime.*

### **NIS-Elements AR compression options**

We performed a test by converting a 1 GB / 4000 frames ND2 file to AVI using different codecs. The results are displayed in the list:

**No compression** Original quality, file size: 787 MB.

**DV Video Encoder** This codec produces only videos with 640 x 480 resolution. Videos with other resolutions are stretched to fit, therefore we do not recommend to use this codec.

**MJPEG Compressor** Very good quality, 240 MB.

---

**Cinepack Codec by Radius** Average quality, file size: 70 MB.

**Intel IYUV codec** Very good quality, file size: 390 MB. Provides the best quality / compression rate.

**Microsoft RLE** Very poor quality, file size: 90 MB.

**Microsoft Video 1** Very poor quality, file size: 6 MB.

## 14. Additional Modules

### 14.1. 3D Measurement

(requires:Local Option)

If you have this module enabled in HASP, some extra commands and features for analysis of multiple binary layers over Z become available in the *Binary* menu.

See also: 8.9. 3D Measurement [160].

### 14.2. 4D/6D Acquisition

The 4D/6D Acquisition module enables users to combine several acquisition types into one ND experiment. A multi-dimensional data set comes out from such an experiment.

### 14.3. AQI 2D Real Time Deconvolution

One of the deconvolution modules available with *NIS-Elements*.

### 14.4. AQI 3D Blind Deconvolution

One of the deconvolution modules available with *NIS-Elements*.

### 14.5. Bio Analysis

---

#### Note

*This module requires a 64bit operating system.*

This module enables to perform bio-analytical tasks. The Bio Analysis module measures and analyses time-lapse and multipoint ND images (Z-stack data sets are not supported). Several analysis modules are available - see 7.8. Analysis Modules [135].

### 14.6. Calcium and FRET

Installing the *Calcium and FRET* module adds several tools to the system:

- A control panel suited for capturing FRET images.

- Automated FRET calibration algorithm.
- The *Create FRET Image* window for “passive” creation of FRET images from files.
- Calcium ion concentration measurement ability.

Please see the electronic help for more details.

## 14.7. Database

If the database module was installed, the system can create and control MS Access databases (\*.MDB). A database can help you to efficiently organize your image archives and manage additional information related to the images. The images themselves are stored on hard disk, only links to them and the additional data are stored in the database file.

Please see 6.9. Database [107] and 2.1.9. Installing the Database Module on 64-bit Systems [11].

## 14.8. Extended Depth of Focus

The EDF module allows you to combine an existing Z-stack of images into one focused image by picking the focused regions from each frame and the pieces together.

## 14.9. Filters Particle Analysis

*(requires:Local Option)*

This module is aimed at analysis of filters according to the ISO 16232 standard.

Please see the electronic help for more details.

## 14.10. HCA

HCA is a simple-to-use high content analysis tool which combines the following procedures into one:

- Acquisition experiment setup including well plates handling.
- Running the experiment - the HCA job.
- HCA Job management, sorting into projects, each job run is saved to a database.
- Results management with data analysis.

## 14.11. HDR

The High Dynamic Range module brings the functionality of creating HDR images within *NIS-Elements AR*.

## 14.12. Illumination Sequence

(requires: *Local Option*)

This module provides an easy graphical user interface for setting and running advanced triggered-acquisition experiments.

## 14.13. Interactive Advanced Macro (API)

This module dramatically extends the macro programming capabilities, it:

- adds a set of user-interaction macro functions
- allows importing functions from external DLLs

See 12.5. Interactive Advanced Macro (API) [222].

## 14.14. JOBS

- JOBS is an environment aiming at complex acquisition and data analysis with easy to use and comprehensive user interface.
- JOBS is a “visual programming” tool enabling non-programmers to design their own automated acquisition / analysis experiments.

Please see the 11. *JOBS* [201] chapter for thorough description of JOBS.

## 14.15. LIM 2D/3D Deconvolution

The imperfection of a microscope optical path causes convolution of the original image signal which makes the resulting image blurry. Deconvolution algorithms reduce the impact of convolution on the image. Several deconvolution modules can be used with *NIS-Elements*.

## 14.16. LIM Legacy Deconvolution

One of the deconvolution modules available with *NIS-Elements*.

---

## 14.17. Local Option

Local Option is a pseudo-additional module. When Local Option is installed (see Step 2 [6]), *NIS-Elements AR* will provide some advanced features which did not pass the quality assurance procedure yet. We recommend to wait until they are released officially.

## 14.18. Metalo - Cast Iron Analysis

After the module installation, a new item called *Metallography* appears in the *Applications* menu. Select the *Cast Iron* command to display the cast iron measurement layout.

## 14.19. Metalo - Grain Size Analysis

After the module installation, a new item called *Metallography* appears in the *Applications* menu. Select the *Grain Size* command to display the grain size measurement layout.

## 14.20. ND Advanced Acquisition

(requires:Local Option)

This module enables users to perform complex timelapse/multipoint ND acquisitions and also adds some other features. Please ask your local dealer of *NIS-Elements* for details.

## 14.21. Single Particle Tracking

Single Particle Tracking (SPT) is used to track single molecules and for analysing parameters of these tracks.

## 14.22. Time Measurement

The time measurement tool enables to record average pixel intensities within Regions Of Interest (ROIs) during a time interval. Please see 8.8. Time Measurement [155].

## 14.23. Tracking

Motion characteristics of objects such as living cells can be measured automatically or manually using the *Tracking* module. Tracking is based on ROIs.

# General Index

## Symbols

3D Measurement (Additional Modules), 229  
 3D Measurement (Measurement), 160  
 3D Object Measurement Window, 161  
 4D/6D Acquisition (Additional Modules), 229

## A

A simple job capturing an image on each well center of the well plate selection, 203  
 A T/Z/multi-channel image containing 11 channels and the time line hidden, 87  
 A T/Z/multi-channel image with some loops selected., 87  
 About ND Acquisition (Image Acquisition), 59  
 About Organizer (Displaying Images), 103  
 About Video Compression (Movies), 227  
 Add relevant color channels to the analysis, 140  
 Adding Buttons to the Left Tool Bar, 32  
 Additional Module/Device Installation (Installation and Settings), 9  
 Additional Modules, 229  
 Adjust the Camera Settings, 38  
 Adjusting Program Preferences (Installation and Settings), 19  
 Adjusting the Task Settings, 207  
 Advanced Mode, 104  
 Advanced Options, 72  
 Align Objects, 185  
 Aligning Objects, 185  
 An HDR multi-exposure image, 88  
 Analysis Explorer (Image Analysis), 136  
 Analysis Explorer Basics (Image Analysis), 136  
 Analysis Modules (Image Analysis), 136  
 Analysis performed on job results, 192  
 Another instance running, 13  
 Appearance Options (User Interface), 35  
 Apply the new policies, 13  
 Applying Restrictions (Measurement), 149  
 AQI 2D Real Time Deconvolution (Additional Modules), 229  
 AQI 3D Blind Deconvolution (Additional Modules), 229  
 Area, 162

Area fraction, 163  
 arithmetic operators, 220  
 Arranging User Interface (User Interface), 29  
 Arrays, 216  
 Assigning Colors to Channels (Displaying Images), 85  
 Assigning Objective to a Nosepiece Position (Cameras & Devices), 42  
 assignment operators, 220  
 Auto Scale Settings, 96  
 Auto-calibration window, 44  
 Auto-Detect Tool (Image Analysis), 134  
 Automated Measurement (Measurement), 153  
 Automatic Calibration, 44  
 Available Logical Devices, 46  
 Available ROI tools, 132  
 AWB, 97

## B

Basic dialog areas, 193  
 Basic Job Rules (JOBS), 203  
 Basic Mode, 104  
 Basic Workflows (Cameras & Devices), 37  
 Binary Layer (Image Analysis), 126  
 Binary Layer Color and Transparency, 126  
 Binary Operations, 121  
 Bio Analysis (Additional Modules), 229  
 bitwise operators, 220  
 Bounds, 163  
 BoundsAbs, 164  
 Breaking the Macro, 215  
 Browse results, 191

## C

C-like functions, 221  
 Calcium and FRET (Additional Modules), 229  
 Calculation of Hue (H), 167  
 Calculation of Intensity (I), 169, 170  
 Calculation of Saturation (S), 170  
 Calibrate Using Objective, 45  
 Calibrating an Uncalibrated Image, 143  
 Calibration (Measurement), 143  
 Camera ROI (Image Acquisition), 57  
 Camera ROI Definition, 58  
 Camera Selection on Startup (Cameras & Devices), 37  
 Camera Settings (Cameras & Devices), 50

---

Cameras & Devices, 37  
 Capture the Image Sequence, 80  
 Capturing AVI Movie (Movies), 225  
 Capturing to Ring Buffer (Image Acquisition), 79  
 Cell Count Analysis (Image Analysis), 138  
 Cell Motility (Image Analysis), 138  
 Cell Proliferation (Image Analysis), 140  
 Changing Appearance of the Threshold Layer, 122  
 Channel Tabs, 28  
 Channel tabs of an RGB image, 28  
 Channels Setup, 71  
 Chroma (C) definition, 170  
 Classification, 199  
 Closing Another Instance of NIS-Elements (Installation and Settings), 13  
 Closing Images (Displaying Images), 112  
 Combine binary layers (optional), 141  
 Combined ND Acquisition (Image Acquisition), 74  
 Command Line Installation Options (Installation and Settings), 9  
 Command Line Startup Options, 1  
 Command line switches syntax, 1  
 Common ND Experiment Options, 60  
 Common Properties, 184  
 Connect to a job database, 190  
 Connected Incubation Phase, 62  
 Connecting a Device to NIS-Elements (Cameras & Devices), 45  
 Connectivity (Image Analysis), 128  
 Context Menu Commands, 183  
 Control Bar (Displaying Images), 87  
 Controlling Cameras by Macro (Macros), 222  
 Controlling Illumination Devices (Cameras & Devices), 53  
 Copying Channels by Drag and Drop (Displaying Images), 86  
 Creating a Macro from History, 213  
 Creating a Shared Layout (Installation and Settings), 12  
 Creating and editing ROIs (Image Analysis), 131  
 Creating Macros (Macros), 213  
 Creating New Optical Configuration, 38  
 Creating new user, 15  
 Creating Reports, 183  
 Creating Reports from Database (Creating Reports), 187  
 Custom Color Displaying Mode, 100

## D

Data Export Options, 181  
 Data export Options, 182  
 Database (Additional Modules), 230  
 Database (Displaying Images), 107  
 Database Backup (Displaying Images), 109  
 Database Tables (Displaying Images), 108  
 Database View within Organizer (Displaying Images), 109  
 Define analysis of each channel, 140  
 Define calculations, 141  
 Define Events, 159  
 Define Graticule Properties, 145  
 Define parameters of the JOB, 190  
 Define ROI, 141  
 Defined 3D objects, 162  
 Defining 3D Threshold, 161  
 Definition tab, 198  
 Definition-Execution type tasks, 205  
 Device Updates (Installation and Settings), 10  
 Dilation, 129  
 Directives, 218  
 Directives (only for 32bit operating systems), 223  
 Directors Mode, 227  
 Disable layout modification, 13  
 Displaying Image Layers (Displaying Images), 84  
 Displaying Images, 81  
 Distribute Objects, 185  
 Docking Panes (User Interface), 24  
 Drawing Style, 118  
 Drawing tools (Image Analysis), 126  
 Dynamic Data, 186

## E

Effective job building, 207  
 EqDiameter, 166  
 Erasing Single objects, 127  
 Erosion, 129  
 Event and Selection Controls, 89  
 Events (Displaying Images), 89  
 Example, 125  
 Example of the warning message, 45  
 Example Procedure, 147  
 Experiment Timing (requires:Local Option) (Image Acquisition), 77  
 Experiment Timing window, 77  
 Export (Image Analysis), 117



Export Destinations, 181  
 Exporting Results (Measurement), 180  
 Expression evaluator, 221  
 Extended Depth of Focus (Additional Modules), 230

## F

Features Available in the Database View, 110  
 Features highlights:, 201  
 FillArea, 166  
 Filter tab, 198  
 Filters Particle Analysis (Additional Modules), 230  
 Fixed Grabber Startup, 2  
 Fixes (Installation and Settings), 11  
 Functions applied subsequently, 130

## G

General (Installation and Settings), 20  
 General Analysis (Image Analysis), 140  
 General Index, 233  
 General Options tab, 157  
 Global Settings Options, 181  
 Graph Memorizing (Image Analysis), 118  
 Graph View (HCA), 196  
 Graticules Density, 145  
 Gray Scale Displaying Mode, 102  
 Grouped Displaying Mode, 101  
 Grouping of Images, 107  
 Groups tab, 17  
 Groups Tab Options (Installation and Settings), 17

## H

Handling Control Panels, 25  
 Hardware key, 10  
 HCA, 189  
 HCA (Additional Modules), 230  
 HCA Analysis (HCA), 192  
 HCA Fixed Job wizard enabling to set up parameters of the Job step by step., 191  
 HDR (Additional Modules), 231  
 Hiding Toolbar Buttons, 32  
 Histogram (Image Analysis), 116  
 Histogram graph view, 197  
 Histogram Options (Image Analysis), 118  
 Histogram Options Window, 118  
 Histogram Scaling (Image Analysis), 117

Home Position, Range, 69  
 Homotopic Transformations, 128  
 How to capture a single image, 55  
 How to Create a Movie, 226  
 How To Set Camera Properties, 222  
 How To Set General Analysis (Image Analysis), 140  
 How to setup a shading correction, 57  
 How to use the histogram to threshold an image, 121  
 How to use the picker tool to threshold an image, 120  
 HSI Mode (Image Analysis), 122

## I

If You Pressed the Create New Button, 188  
 Illumination Sequence (Additional Modules), 231  
 Image acquired and corrected, 57  
 Image acquired without the shading correction, 56  
 Image Acquisition, 55  
 Image Analysis, 115  
 Image Filter (Displaying Images), 104  
 Image Intensity Analysis (Image Analysis), 141  
 Image Layers (Displaying Images), 83  
 Image Layers Involved in Measurement, 154  
 Image Types (Displaying Images), 84  
 Image Window, 27  
 Image Window (User Interface), 26  
 Inhomogeneous illumination, 56  
 Input/Output Example, 74  
 Inputs/Outputs Control (Image Acquisition), 74  
 Insert analysis info, 140  
 Install Local Options, 6  
 Installation, 5  
 Installation and Settings, 5  
 Installation and Updates (Installation and Settings), 5  
 Installing the Database Module on 64-bit Systems (Installation and Settings), 11  
 Intensity Mode (Image Analysis), 122  
 Interaction with ROIs (Image Analysis), 133  
 Interactive Advanced Macro (API) (Additional Modules), 231  
 Interactive Advanced Macro (API) (Macros), 223  
 Introduction, 203  
 Introduction to General Analysis (Image Analysis), 140  
 Introduction to HCA (HCA), 189

---

Introduction to Image Acquisition (Image Acquisition), 55  
Introduction to Image Layers (Displaying Images), 83  
Introduction to JOBS Explorer (JOBS), 202  
Introduction to Moving ROIs (Image Analysis), 135  
Introduction to the JOBS Module (JOBS), 201

## J

Job Definition window, 209  
Job Definition Window (JOBS), 207  
Job Result: Thumbnail View (Heatmap), 195  
Job Results window, 193  
JOBS, 201  
Jobs (Additional Modules), 231  
JOBS Explorer (HCA), 189  
JOBS Explorer (JOBS), 202  
JOBS Explorer with the HCA module, 189  
JOBS Explorer with the module, 202  
JOBS Toolbar (HCA), 189

## L

Large Image (Covering) Multi-Point (Image Acquisition), 66  
Large Images (Displaying Images), 93  
Large-image Acquisition (Image Acquisition), 72  
Layout Manager (User Interface), 31  
Layout Manager Tools, 31  
Layout tabs, 30  
Layouts (User Interface), 30  
Legend:, 64, 65  
Length, 168  
LIM 2D/3D Deconvolution (Additional Modules), 231  
LIM Legacy Deconvolution (Additional Modules), 231  
Line Series graph, 199  
Line Series tab, 198  
Line-length Calibration, 144  
List of Available Dynamic Data Sources:, 186  
List of Layouts, 31  
Live/Dead Analysis (Image Analysis), 142  
Local and Global Variables, 216  
Local Option (Additional Modules), 232  
Local Options installation, 6  
Locating XY Positions Between Images (Displaying Images), 94

logical operators, 221  
Login As, 14  
Loop over Wells and Loop over Points, 206  
Loops, 206  
LUTs (Look-Up Tables) (Displaying Images), 94  
LUTs on Monochromatic Images (Displaying Images), 97  
LUTs on Multichannel Images (Displaying Images), 99  
LUTs on RGB Images (Displaying Images), 95  
LUTs on Spectral Images (Displaying Images), 99  
LUTs Tools, 95  
LUTs window on two-channel image, 99

## M

Macro Language Syntax (Macros), 215  
Macro Preferences (Macros), 223  
Macros, 213  
Main Menu (User Interface), 23  
Main View, Max/Min Intensity Projection View, 91  
Main Window Components (User Interface), 23  
Managing Objectives (Cameras & Devices), 41  
Managing Optical Configurations, 40  
Managing Recipes (Image Analysis), 137  
Manual Calibration, 42  
Manual Capture, 72  
Manual Measurement (Measurement), 146  
Match Object Sizes, 186  
Mathematical Morphology Basics (Image Analysis), 127  
Mathematical Morphology Examples (Image Analysis), 129  
MaxFerret, 168  
MCH Mode (Image Analysis), 122  
Measure the Image, 145  
Measurement, 143  
Measurement Features (Measurement), 162  
Measurement Features 3D (Measurement), 175  
Measurement on Graph (Measurement), 177  
Measurement Options (Measurement), 155  
Metallo - Cast Iron Analysis (Additional Modules), 232  
Metallo - Grain Size Analysis (Additional Modules), 232  
Modifying Menus (User Interface), 34  
Modifying the Layout Settings, 31  
Modifying the Main Menu, 34

Modifying Tool Bars (User Interface), 32  
 Motorized Stage Initialization, 48  
 Movie Maker (Movies), 225  
 Movie Maker Controls, 226  
 Movies, 225  
 Multi-channel Acquisition (Image Acquisition), 71  
 Multi-channel Images, 84  
 Multi-point Acquisition (Image Acquisition), 65  
 Multiple Binary Layers, 127  
 Multiple Cameras, 37

## N

Navigation in ND2 Files (Displaying Images), 87  
 ND Advanced Acquisition (Additional Modules), 232  
 ND Experiment Progress (Image Acquisition), 77  
 ND Sequence Options, 61  
 ND Views (Displaying Images), 90  
 ND2 (multi-dimensional) files / datasets, 84  
 ND2 files, 86  
 ND2 Files Processing (Image Analysis), 115  
 ND2 Information (Displaying Images), 92  
 ND2 Interconnection, 193  
 New Connection (Displaying Images), 108  
 New Database (Displaying Images), 107  
 NIS-Elements AR compression options, 227  
 NIS-Elements authentication (Installation and Settings), 12  
 NIS-Elements Installation Steps (Installation and Settings), 5  
 NIS-Elements Preferences (Installation and Settings), 19

## O

Object Count (Measurement), 148  
 Object Count Procedure Example (Measurement), 152  
 Object/Field Measurement, 154  
 Objective Calibration (Cameras & Devices), 42  
 Objectives (Cameras & Devices), 40  
 Open and Close, 129  
 Open JOBS Explorer, 190  
 Opening Files in Progressive Mode (Displaying Images), 93  
 Opening Image Files (Displaying Images), 81  
 Operating with Images, 110  
 Operation, 209

Operations with Images within Organizer (Displaying Images), 105  
 Operations with Optical Configurations, 40  
 Operators, 220  
 Optical Configurations (Cameras & Devices), 38  
 Options, 139, 161  
 Options (Measurement), 157  
 Options for the Open Next Command (Displaying Images), 82  
 Order of execution, 203  
 Organizer (Displaying Images), 103  
 Orientation, 171  
 Other functions applied subsequently, 131  
 Overrun Indication (Image Analysis), 118

## P

Perimeter, 171  
 Pixel Classifier (Measurement), 177  
 Pixel Size Calibration, 144  
 Playing Controls, 88  
 Playing options, 88  
 Point by Point (Manual) Multi-Point (Image Acquisition), 66  
 pointer operators, 220  
 Predefined Camera ROI, 59  
 Preprocessing (Image Analysis), 115  
 Primary Tiling tab, 199  
 Primary/Secondary Tiling tabs, 199  
 Privileges tab, 18  
 Privileges Tab Options (Installation and Settings), 18  
 Processing On: Intensity/RGB/Channels (Image Analysis), 115

## Q

Quantities tool bar, 196  
 Quick Guide, 5  
 Quick Guide (Measurement), 156

## R

Random Multi-Point (Image Acquisition), 67  
 Recorded Data (Image Acquisition), 76  
 Recording a Macro, 213  
 Regions of Interest - ROIs (Image Analysis), 131  
 relational operators, 220  
 Renaming of devices, 46

Repetition issues, 129  
 Report Generator (Creating Reports), 183  
 Report Objects (Creating Reports), 184  
 Report Templates (Creating Reports), 187  
 Reset of password, 16  
 Resizing the Organizer Panes (Displaying Images), 107  
 Restricting the Area of Measurement, 154  
 Restrictions, 121  
 Restrictions from the Image, 150  
 Result View (HCA), 192  
 Results (Measurement), 151  
 Reusing a Job Definition, 194  
 RGB Images, 84  
 RGB Mode (Image Analysis), 119  
 RGB/Mono/Multichannel Image Processing (Image Analysis), 116  
 ROIs in Time - Moving ROIs (Image Analysis), 135  
 Rough Measurement (Measurement), 145  
 Rules, 203, 206, 207  
 Run the analysis, 141  
 Run the HCA Job template, 190  
 Run the job, 191  
 Running a HCA job (HCA), 190  
 Running a Macro (Macros), 215  
 Running a Macro Upon Layout Change (User Interface), 35

## S

Sample Database Installation (Installation and Settings), 10  
 Save ND2 as AVI (Movies), 225  
 Save Next Options (Displaying Images), 111  
 Save to File (Image Acquisition), 76  
 Saving Image Files (Displaying Images), 110  
 Saving Images with UAC (Displaying Images), 111  
 Scan Area, 73  
 Select Camera Driver, 37  
 Select Graticule Type, 145  
 Selecting a Camera, 38  
 Set layout visibility, 13  
 Setting Software Limits to Stage Movement, 49  
 Setting Up the Camera, 37  
 Shading Correction (Image Acquisition), 56  
 ShapeFactor, 172  
 Shifting Image Channels (Displaying Images), 87  
 Simple ROI Editor (Image Analysis), 134

Simple ROI Editor toolbar, 134  
 Single function applied to the original, 130  
 Single Particle Tracking (Additional Modules), 232  
 Slices View, 92  
 Software Copy Protection (Installation and Settings), 10  
 Sorting of Images, 106  
 Source Data (Image Analysis), 117  
 Special Object Properties, 184  
 Special Options, 61, 70, 71  
 Special Options (Image Acquisition), 67  
 Splitting Large Images (Displaying Images), 93  
 Start menu, 9  
 Startup Switches, 1  
 StartX, 172  
 Statements Not Supported, 218  
 Statements Supported, 216  
 Statistics and Data Presentation, 154  
 Status Bar, 28  
 Status Bar (User Interface), 24  
 Status bar of the image window, 29  
 Step by Step, 5, 123  
 StgPos, 173  
 Stitching, 73  
 Structures and Unions, 216  
 Structuring Element = Kernel = Matrix (Image Analysis), 128  
 Superresolution Calibration, 45  
 Supported Image Formats (Displaying Images), 113  
 Switching Between Loaded Images (Displaying Images), 81  
 Synchronizer (Displaying Images), 102

## T

Task dependency, 204  
 Task position, 204  
 TD channel, 100  
 Test the job on a single well, 191  
 The application status bar, 24  
 The Database View, 110  
 The Docked Control Panel Caption, 25  
 The Installation DVD-ROM Content (Installation and Settings), 5  
 The NIS-Elements Main Window, 23  
 The organizer layout., 104  
 Threshold Adjustments, 121  
 Thresholding (Image Analysis), 119

Thresholding Example (Image Analysis), 123  
Thresholding Large Images, 122  
Thresholding the Image (Measurement), 149  
Thumbnail Displaying Options, 106  
Tiled View, 92  
Time Measurement (Additional Modules), 232  
Time Measurement (Measurement), 155  
Time measurement window, 156  
Time-lapse Acquisition (Image Acquisition), 61  
Timing Explanation (Image Acquisition), 63  
Tips, 88, 94  
Tips on Moving ROI, 135  
To Change a Single Z Coordinate: (Image Acquisition), 67  
To Create a New Layout, 30  
To Create a Report FROM Template, 187  
To Create a Report Template, 187  
To define a moving ROI:, 135  
To Display a Docking Pane, 24  
To Reload Previous Layout Settings, 30  
Tool Bar, 152  
Tool Bars (User Interface), 24  
Tools for Handling the Live Image, 55  
Top and Bottom, Step, 69  
Top Toolbar options, 194  
Top toolbar options, 197  
Tracking (Additional Modules), 232  
True Color Displaying Mode, 100  
Turn Camera ROI ON/OFF, 58  
Turn the Buffer ON, 80  
Types of ROI (Image Analysis), 132

## U

Units (Measurement), 144  
Usage (Image Analysis), 136  
User Interface, 23  
User name and password properties, 15  
User Permissions (Displaying Images), 108  
User Rights (Installation and Settings), 11  
User Rights Options (Installation and Settings), 14  
Users Tab Options (Installation and Settings), 14  
Using ROIs for analysis (Image Analysis), 134  
Using two independent Z Drive devices, 49

## V

Variable types, 216  
Viewing Results (HCA), 192

Volume View, 91  
VolumeEqCylinder, 174  
VolumeWqSphere, 174

## W

Well Plate (Rectangular) Multi-Point (Image Acquisition), 66  
What are Logical Devices? (Cameras & Devices), 46  
Width, 175  
Window Options, 210  
Working with the Binary Layer, 126  
Working with the Measurement and Annotation Objects, 147  
Wound Healing (Image Analysis), 142  
Writing/Editing a Macro, 214

## X

X axis, 158  
XY Stages and Z Drives Tips (Cameras & Devices), 48

## Y

Y axis left/right, 159

## Z

Z Intensity Correction (Image Acquisition), 75  
Z-series Acquisition (Image Acquisition), 69

