NYU Langone MEDICAL CENTER

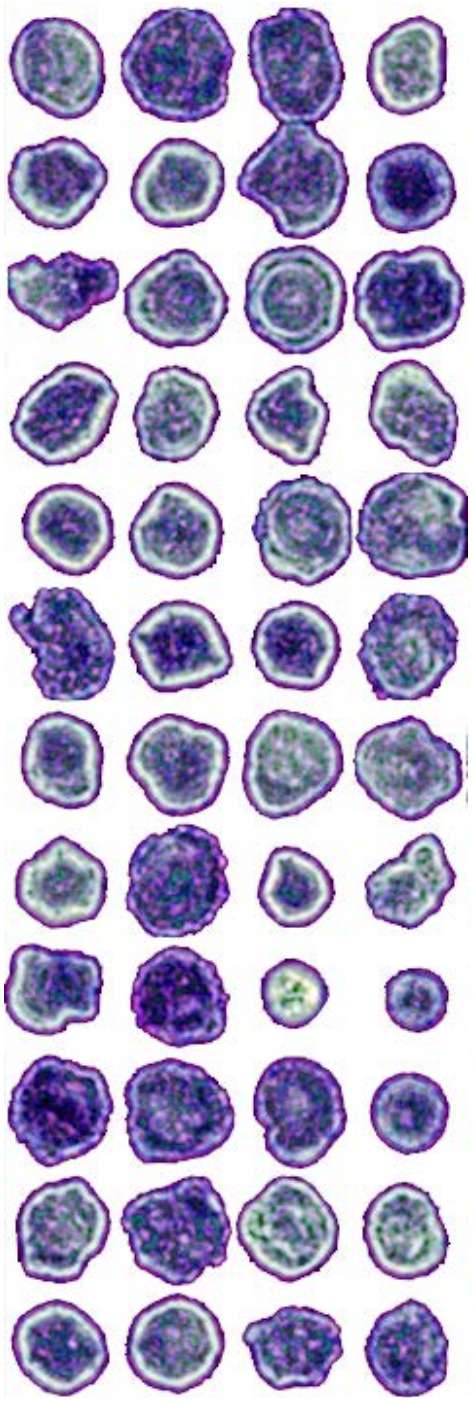
TIRFm

(**T**otal **I**nternal **R**eflectance **F**luorescence **M**icroscopy)

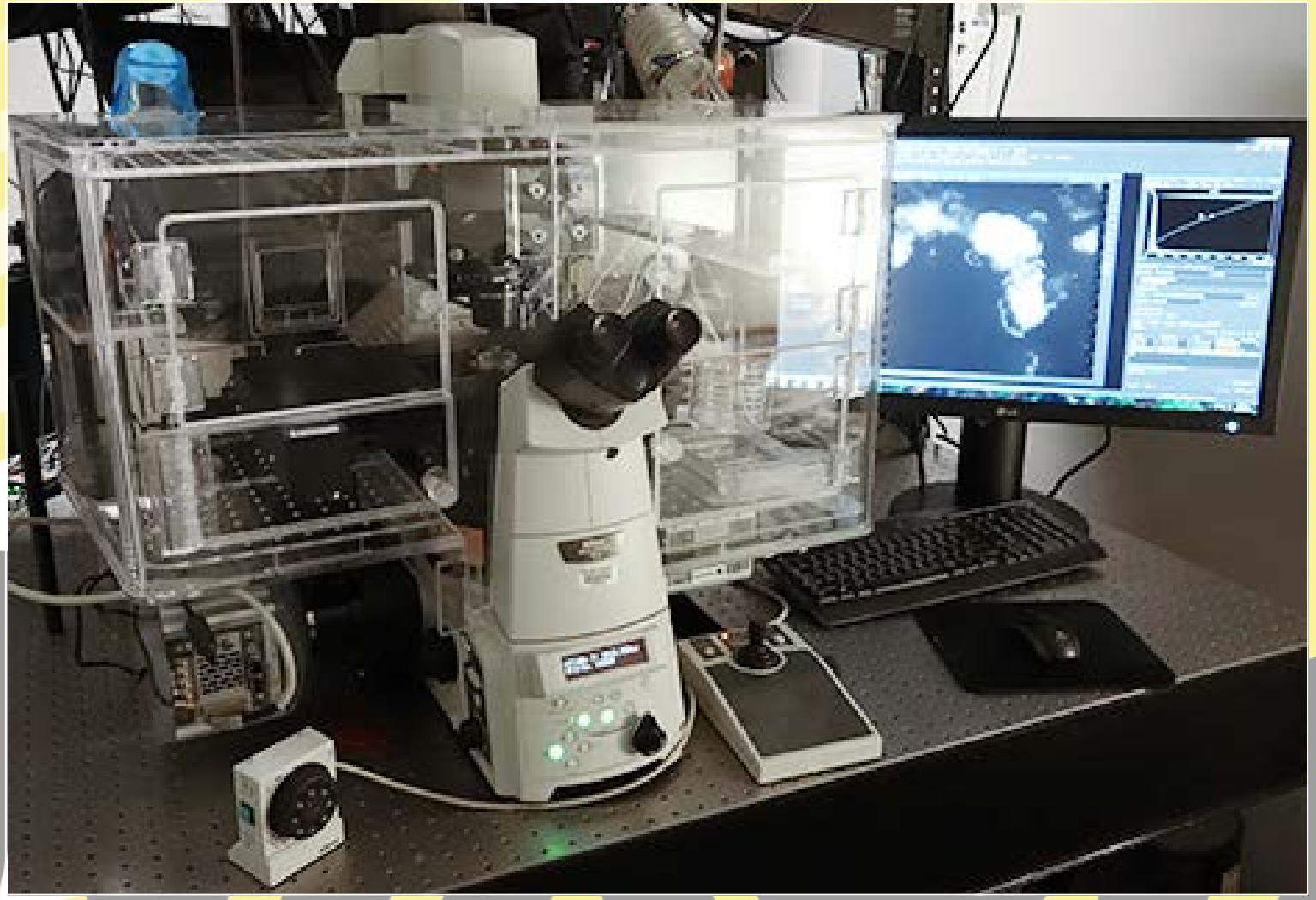
New at the OCS Microscopy Core

Lunch Talk March 2015

Michael Cammer



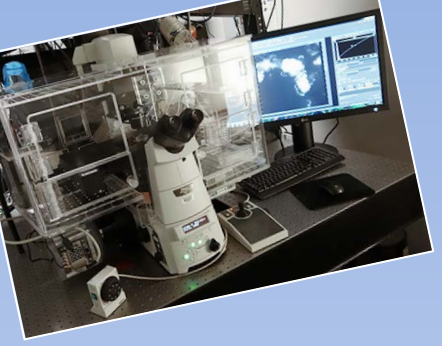
Nikon Eclipse Ti microscope



History

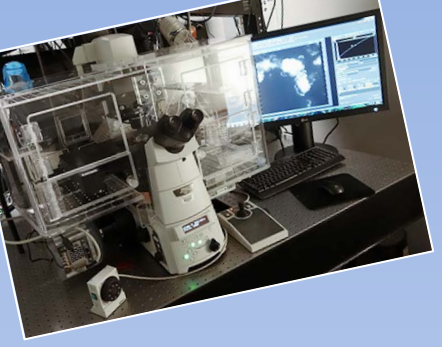
2010: Purchased with lasers for TIRF by Dr. Michael Dustin

2014: Transferred to Microscopy Core and upgraded by OCS for epifluorescence (LED light sources, new computer, additional lenses & sCMOS camera)



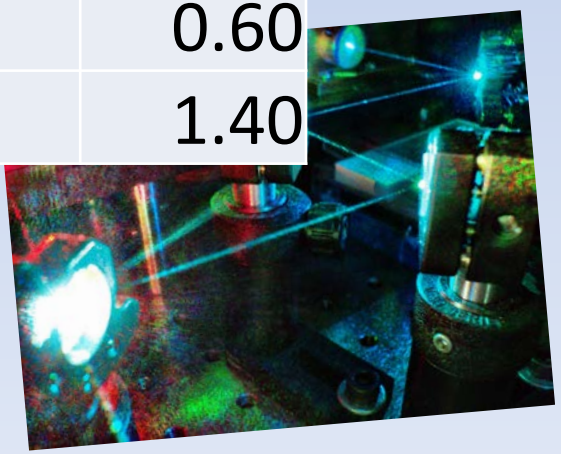
Technical Details

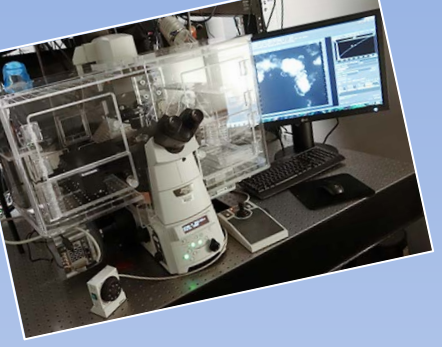
- Nikon Eclipse Ti inverted microscope
- Environment chamber with heat unit
- Motorized stage for tiling and multiple fields imaging
- Autofocus stability
- NIS-Elements software



Technical Details

Lens Type	N.A.
Plan Fluor **40x** DIC M N2	0.75
Apo TIRF **100x** Oil DIC N2	1.49
Plan Fluor **10x** Ph1 DLL	0.30
Plan Apo **20x** DIC M N2	0.75
S Plan Fluor ELWD **40x** Ph2 ADM	0.60
Plan Apo λ **60x** Oil Ph3 DM	1.40





Technical Details

Standard Epifluorescence with Andor Zyla sCMOS Camera

Excitation wavelengths

nm
395
440
508
555
640

→ 550
→ 575

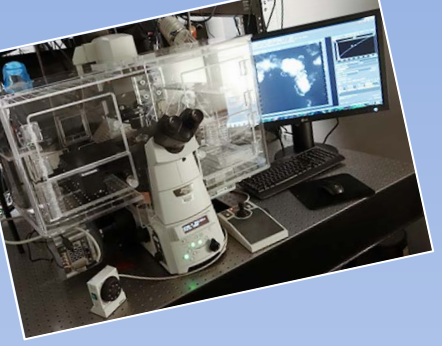
Dichroics

CFP/YFP/Dsred

Dapi/FITC/ TxRed/Cy5

Emission wavelengths

nm
435/26
475/20
515/30
540/21
595/40
632/60
700/75



Technical Details

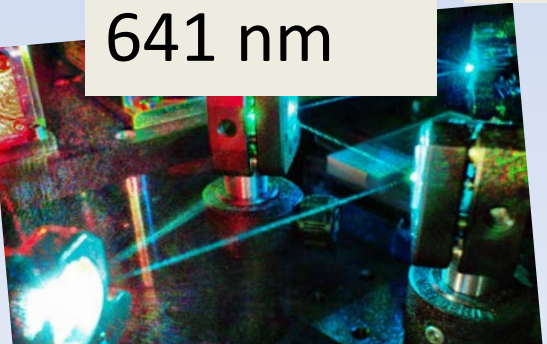
TIRF with Andor DU897 Camera

Excitation Lasers
405 nm
488 nm
561 nm
641 nm

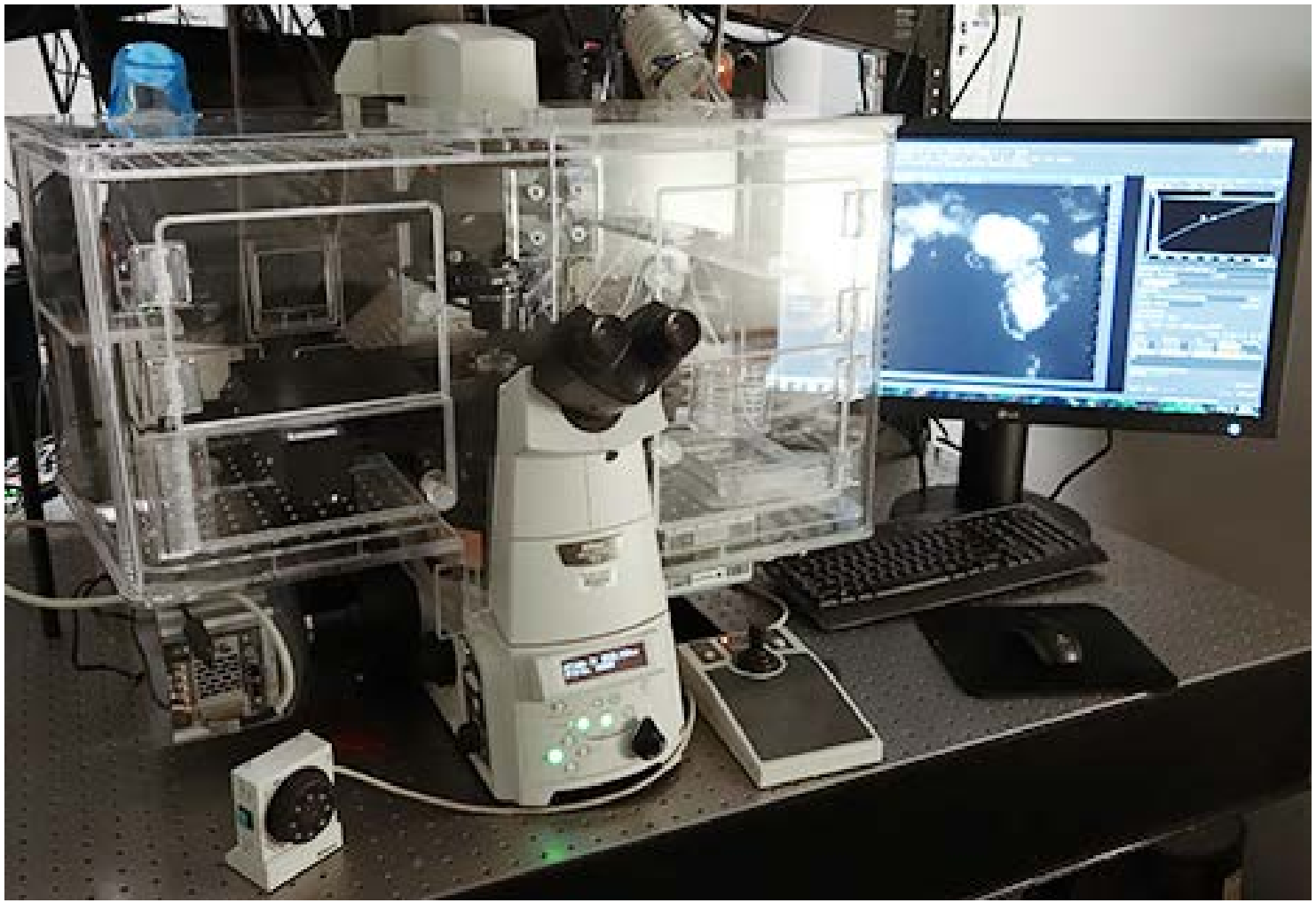
Chroma 97327 C-TIRF zet405/488/561/635x quad-band clean-up/excitation filter zt405/488/561/640rpc

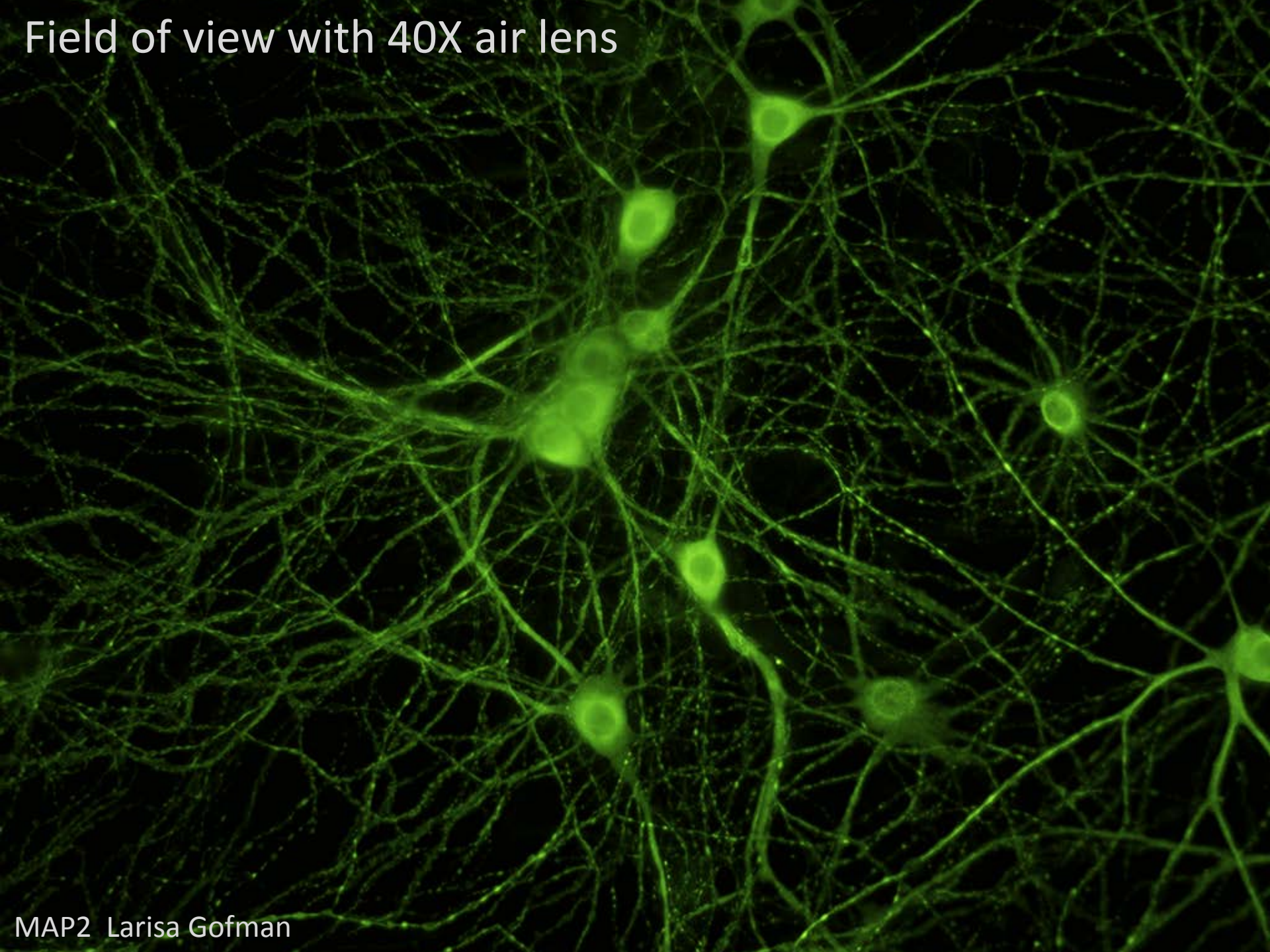
Filters in external emission wheel:

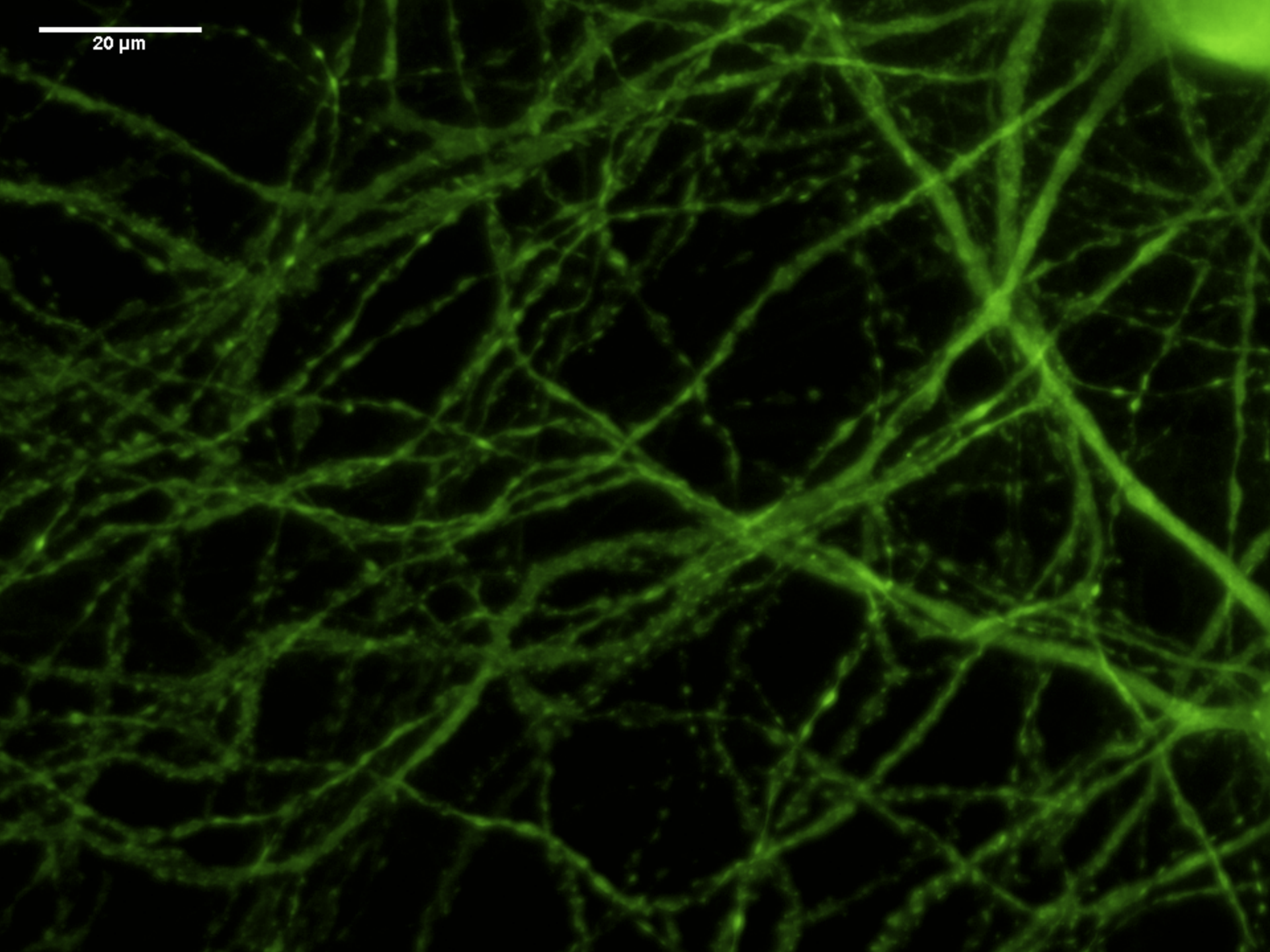
ET450/40M
ET525/50M
ET600/50M
ET700/75M



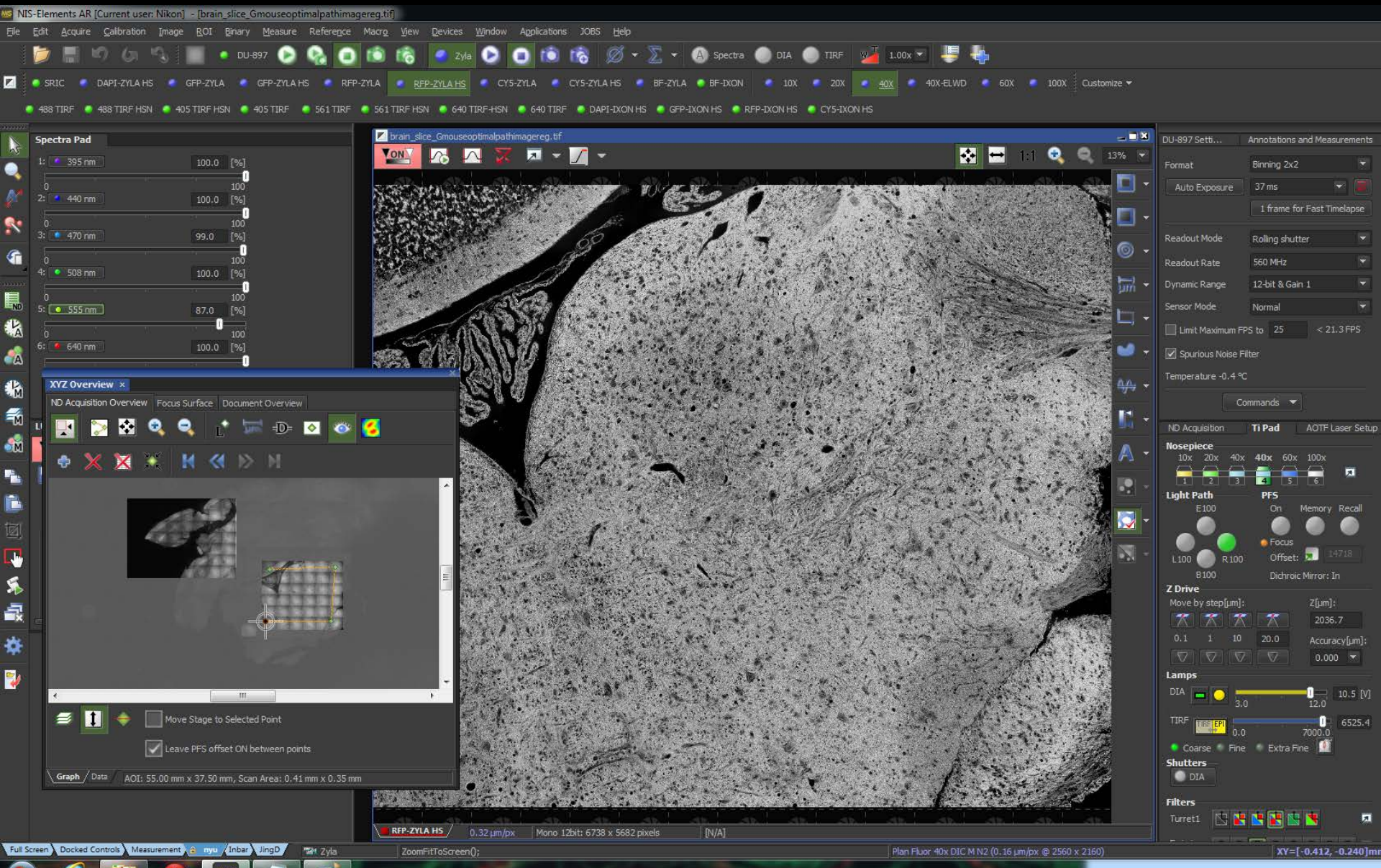
Before we discuss TIRF, highlights of the Nikon microscope in standard modes.




Field of view with 40X air lens
MAP2 Larisa Gofman

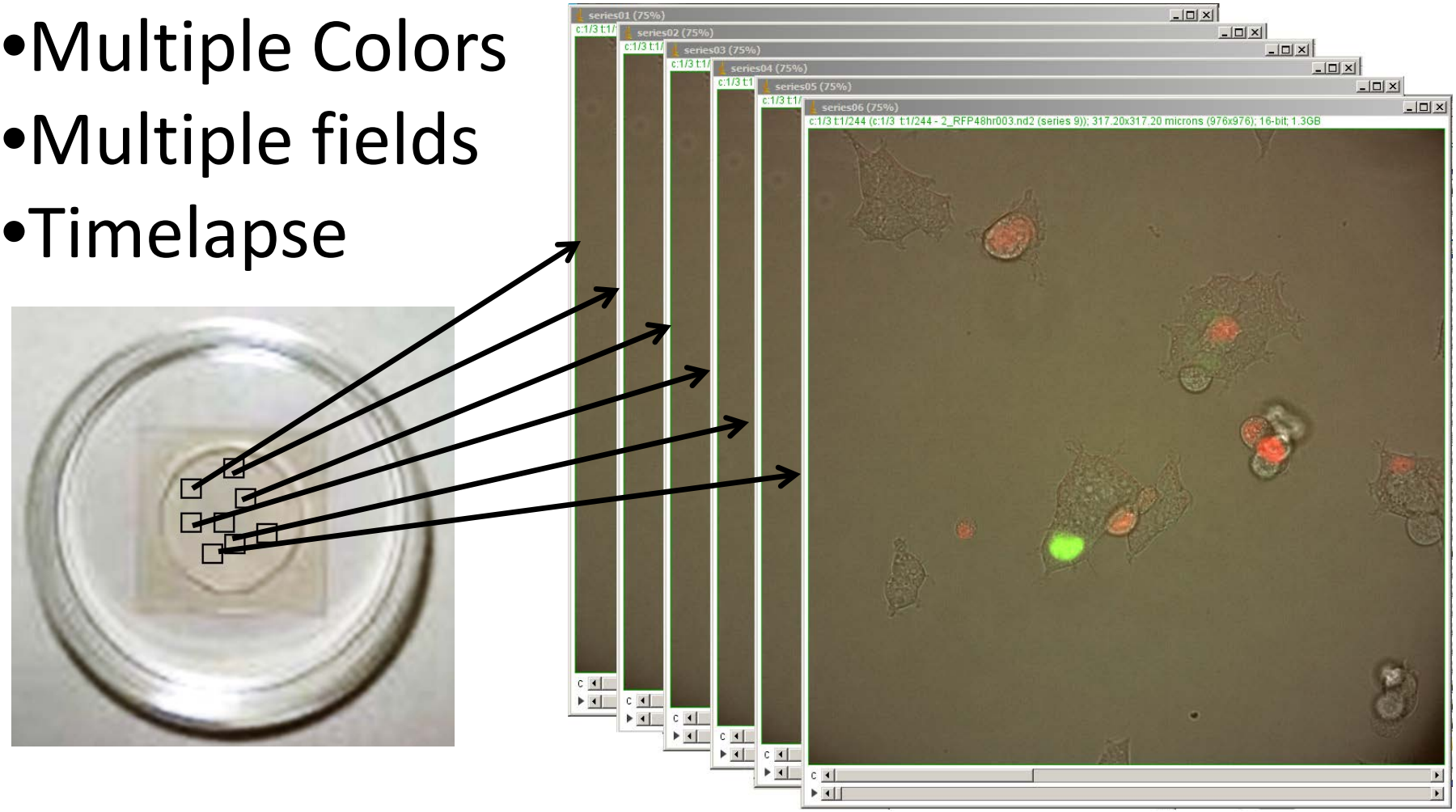

20 µm

Large Tissue Scanning



Multiple Field Timelapse

- Multiple Colors
- Multiple fields
- Timelapse



Multiple Field Timelapse

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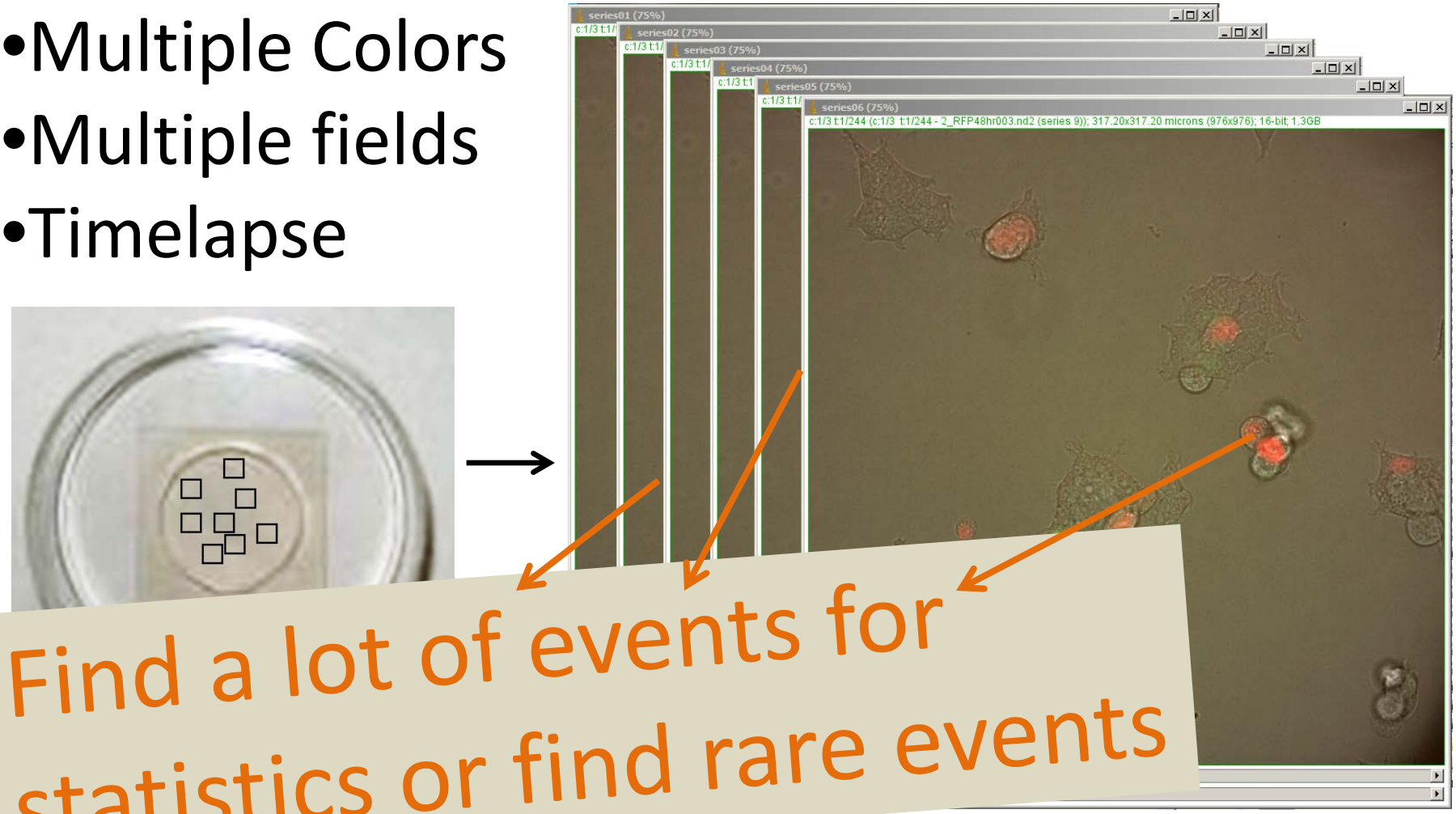
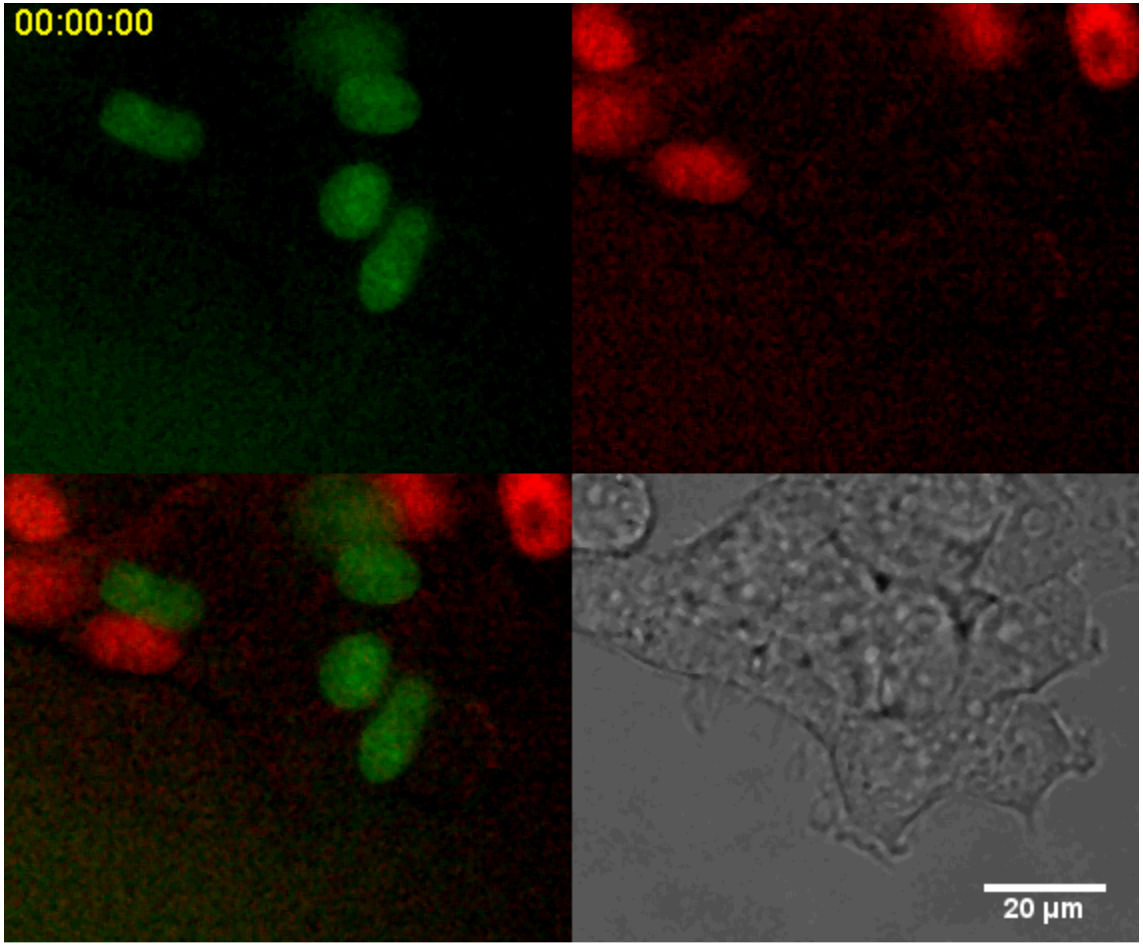
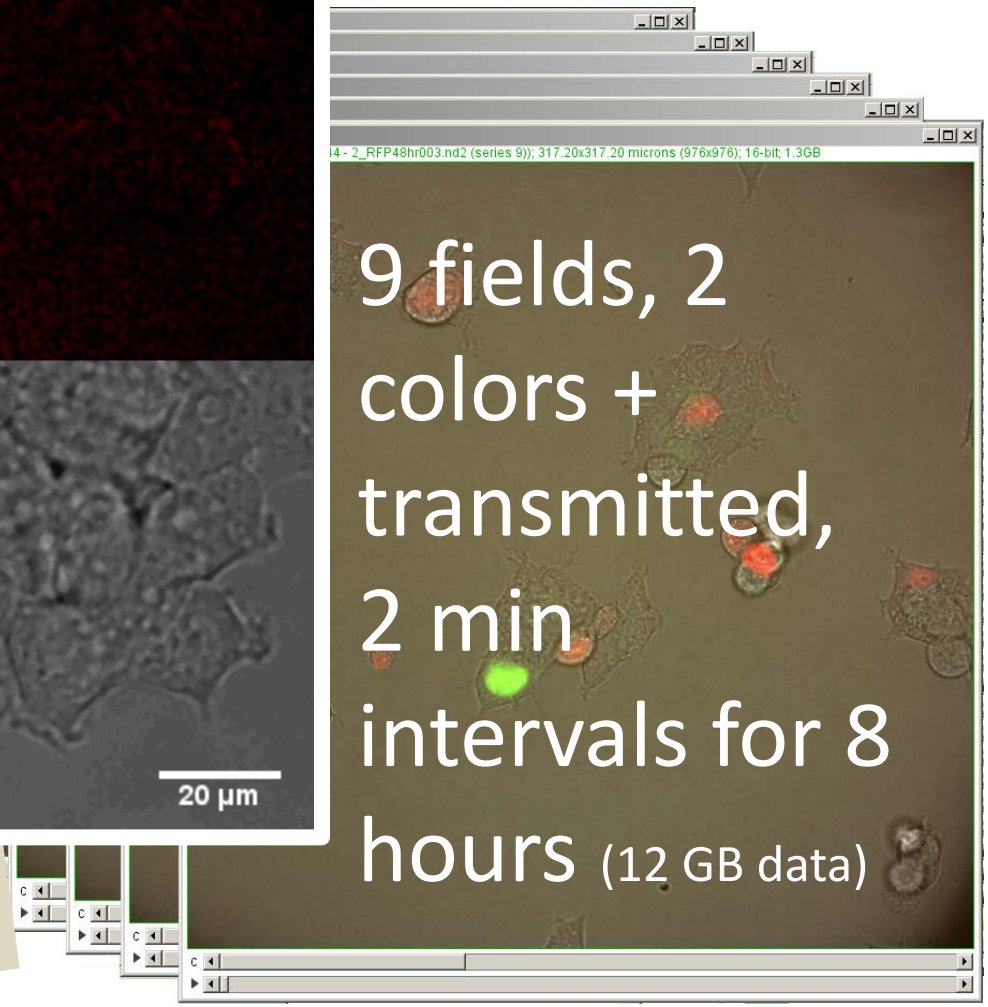


Find a lot of events for statistics or find rare events

Multiple Field Timelapse





Find rare events

Evgenia Korol in Mamta Tahiliani lab

Live Cell Imaging Techniques Workshop

Friday, April 24th, 2015

1:00 p.m.—2:00 p.m.

Seminar @Skirball 2nd floor, Conference Room

2:00 p.m.—5:00 p.m.

Hands-On Demonstrations (Microscopy Core)

The CellASIC™ ONIX Microfluidic Platform. The control system is connected to the microfluidic plate via a low-profile manifold, which enables setup on any inverted microscope.

The CellASIC™ ONIX Microfluidic Plates deliver unprecedented control for live cell imaging.

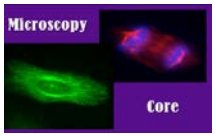
❖ The microfluidic platform is designed to enable perfusion based microenvironment control for long term, high quality live cell microscopy.

❖ Continuous perfusion of culture medium to the cells recreates the physiologic mass transport condition for optimized cell health, giving a suitable growth environment for long-term experiments from 4-72 hours on the microscope stage.

❖ The system enables single or multi-cell tracking while automated perfusion controls washout, drug changes, and dynamic solution profiles.

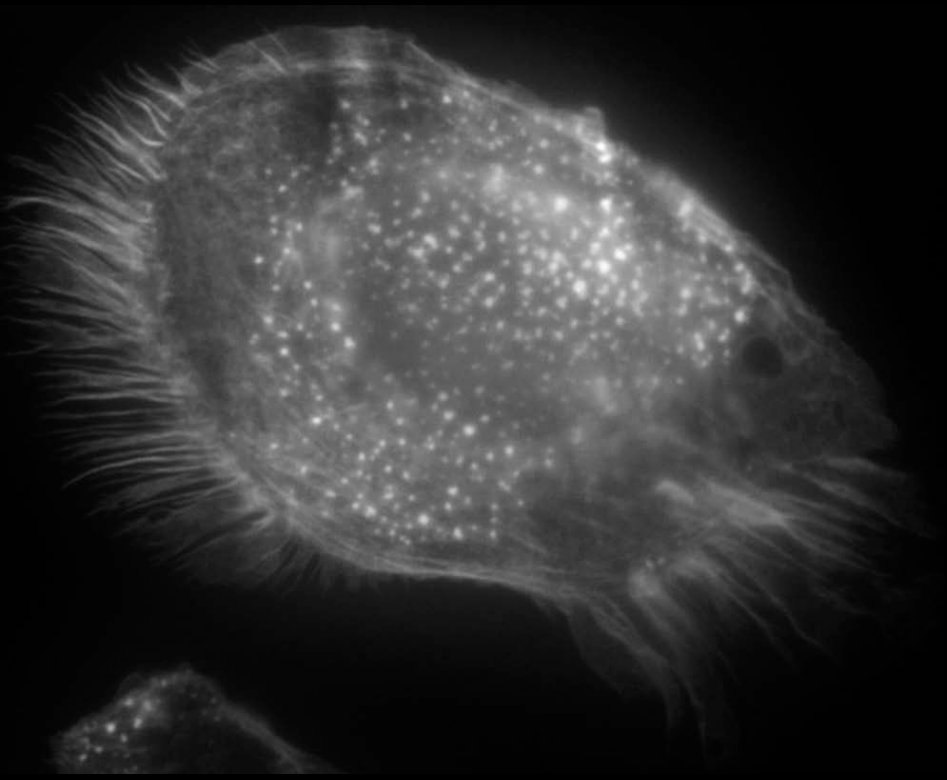
❖ Temperature and CO_2 control is maintained by an on-chip microincubator.

CELLASIC
Bringing Microfluidics to Life

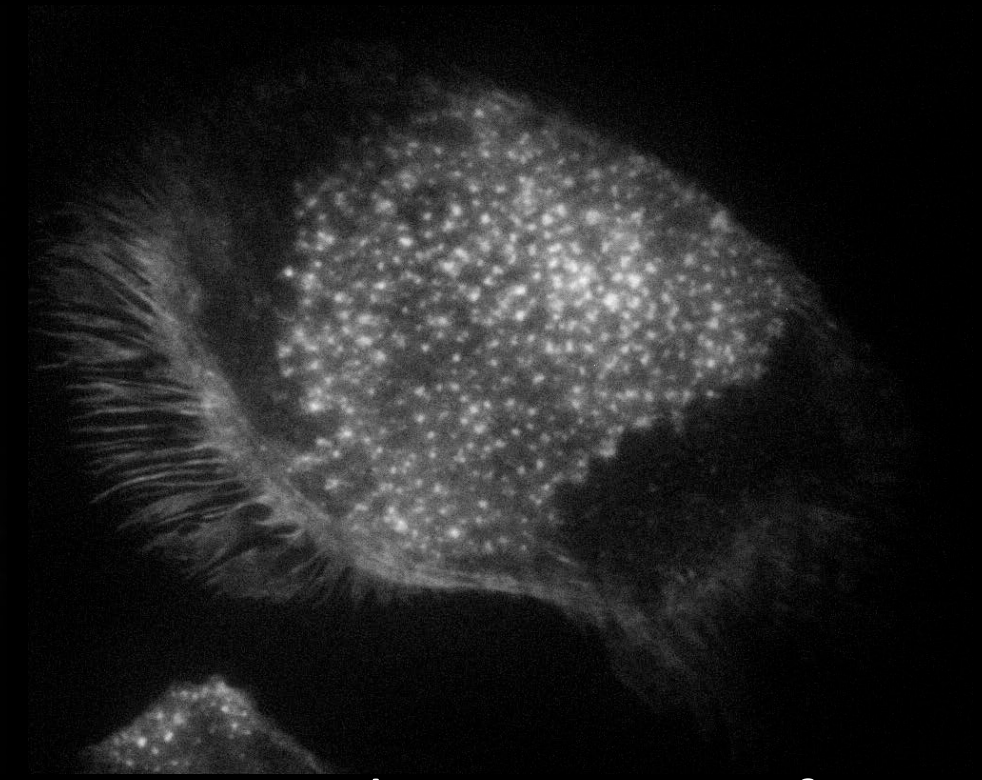


TIRF (Total Internal Reflectance Fluorescence)

Standard Epifluorescence



TIRF



Higher contrast of molecules at substrate

50 to 200 nm Z Axis “Resolution”

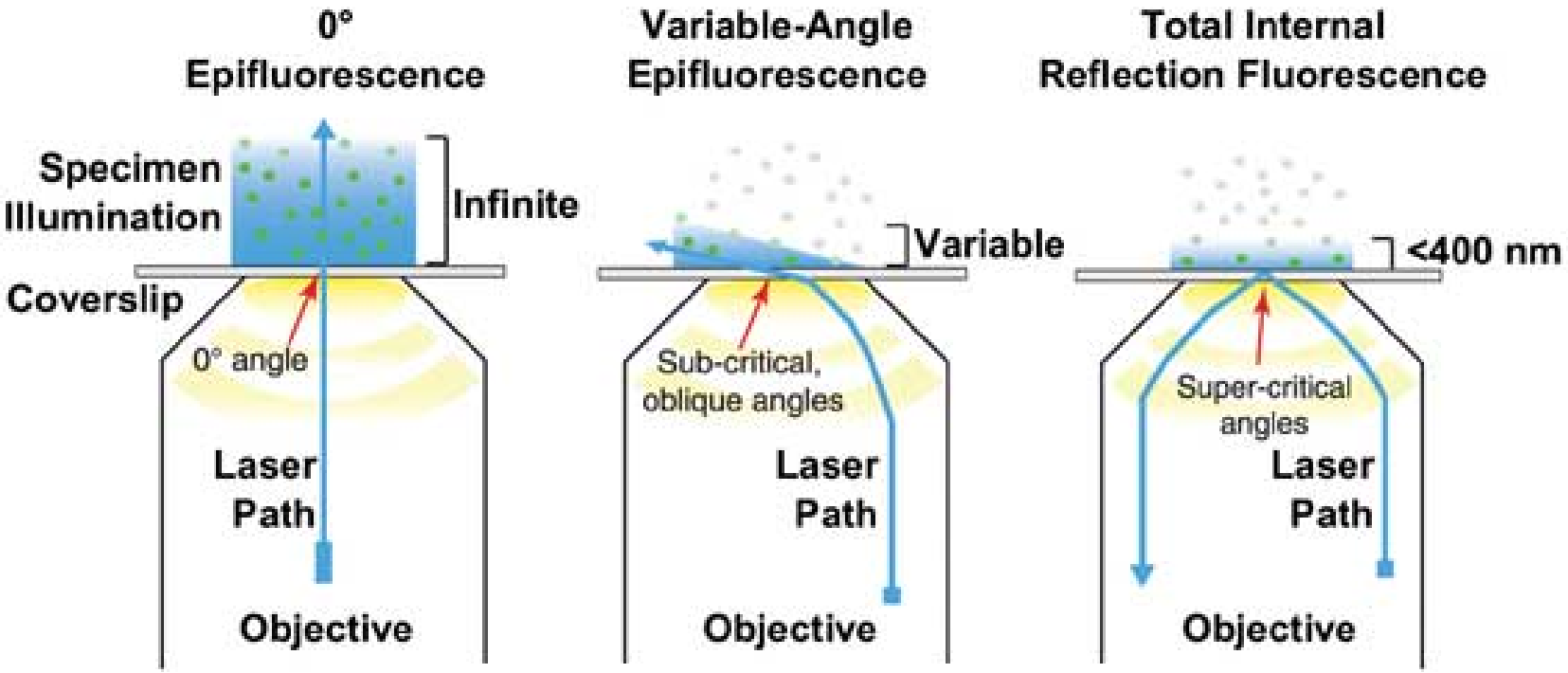
- Technically, the spatial resolution in the Z axis isn’t improved.
- The energy activating the fluorescent molecules is limited to a depth of 200 nm maximum.
- Effectively, the result is imaging molecules only within 50 to 200 nm of the substrate, or *effective resolution* of 50 – 200 nm in the Z axis.

How do we do this?

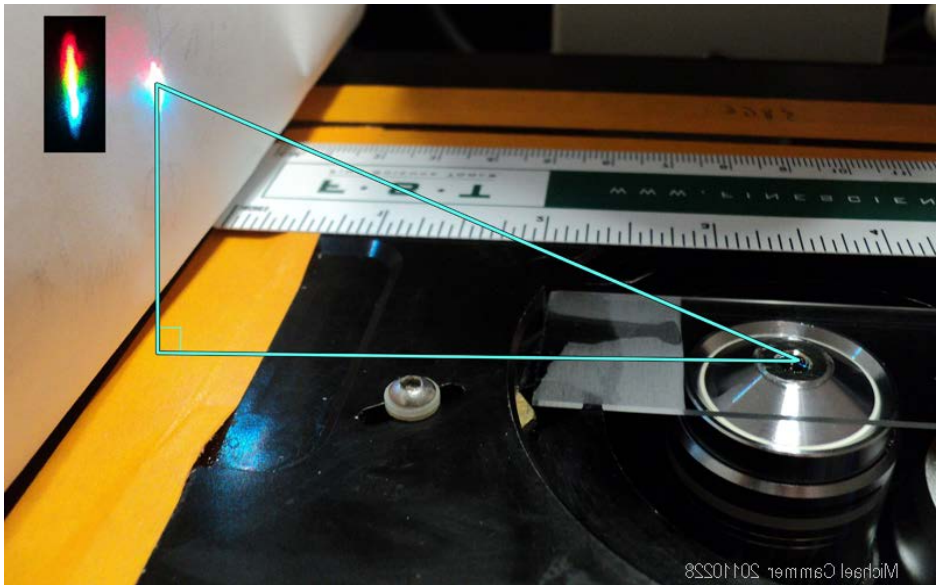
Total Internal Reflectance Fluorescence Microscopy is based on an evanescent field that is produced at the critical angle between two interfaces of different refractive indexes.

Three Worlds" 1955 by M.C. Escher

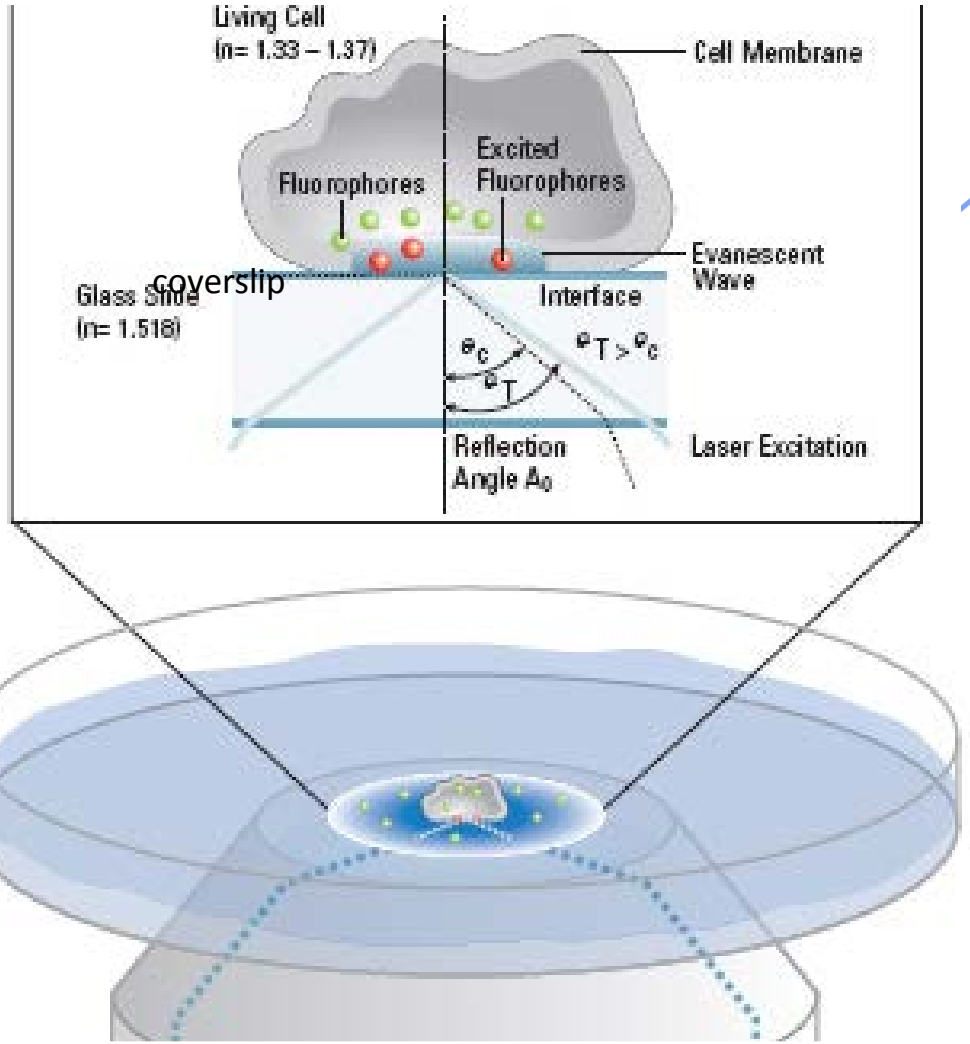
http://www.photonics.com/Article.aspx?AID=48690



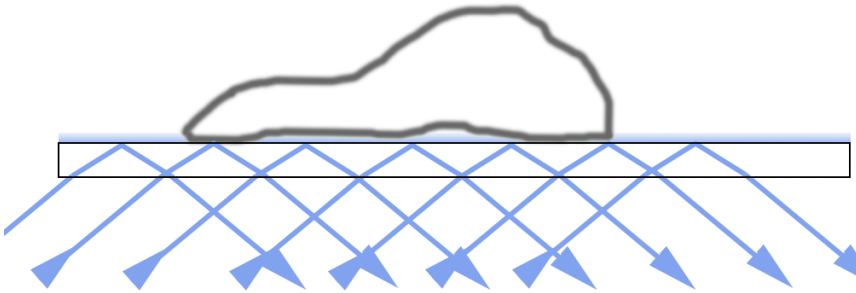
Approaching Critical Angle



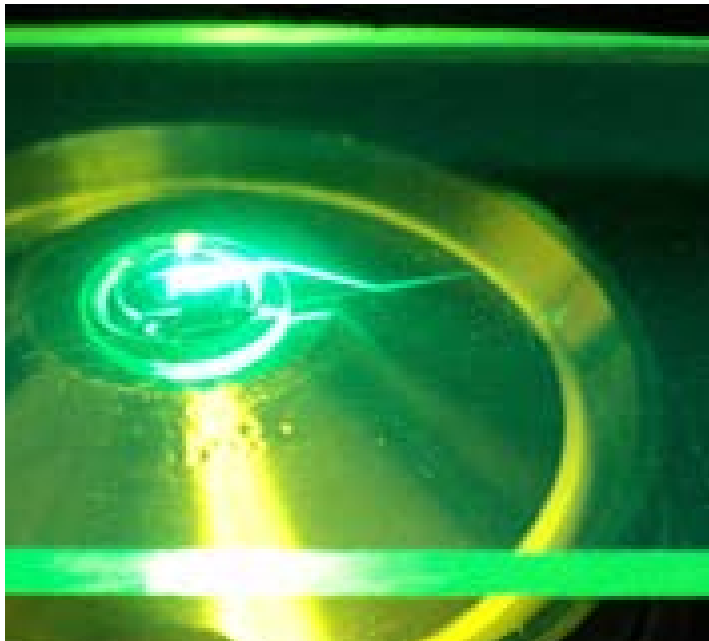
How It Works



Schematic from Duke microscopy website

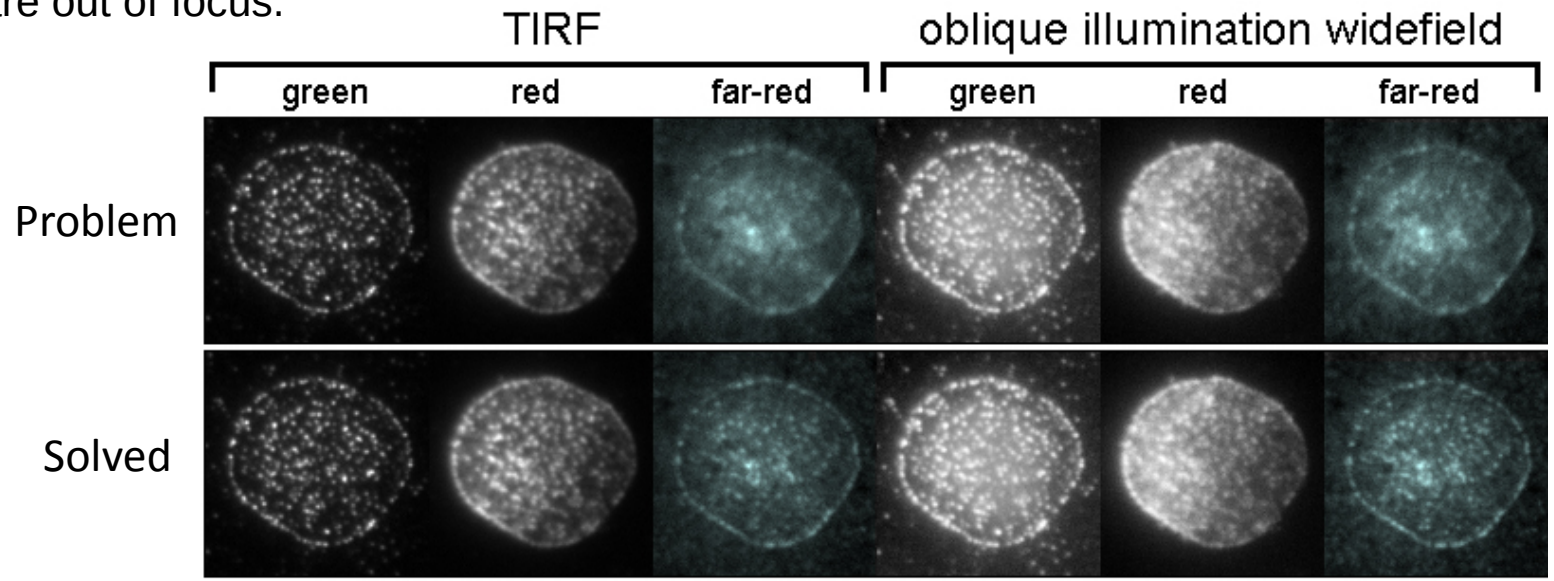


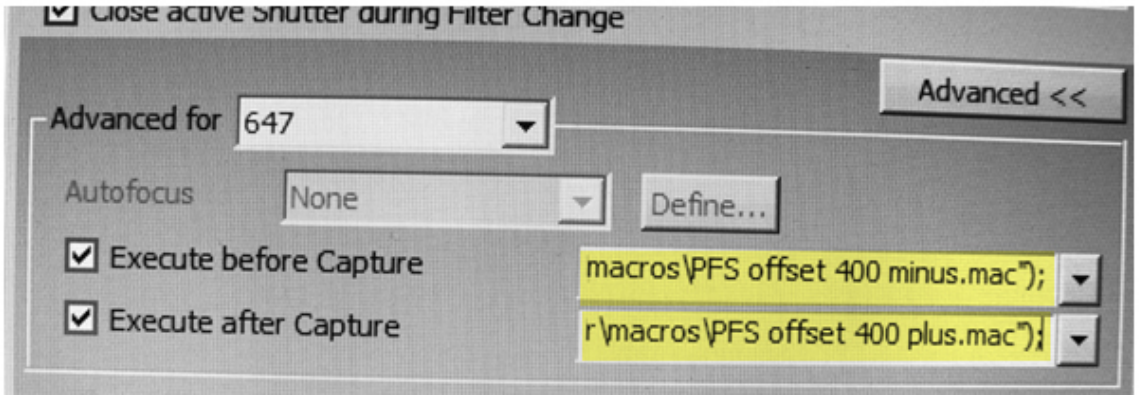
Internal Reflection



Chromatic Aberration

Different colors of light focus on different focal planes. This is a problem in microscopy where you want to take pictures of violet through near infra-red fluorescent emitters. When you focus on the green fluorescence, with all microscopes to some extent the violet and far-red images are out of focus.

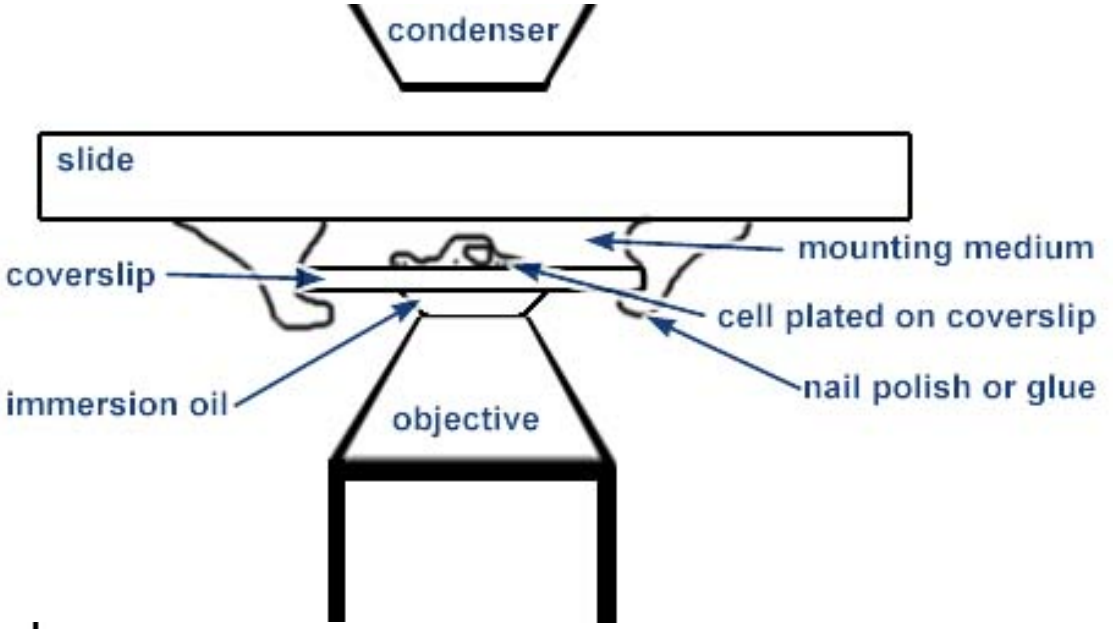


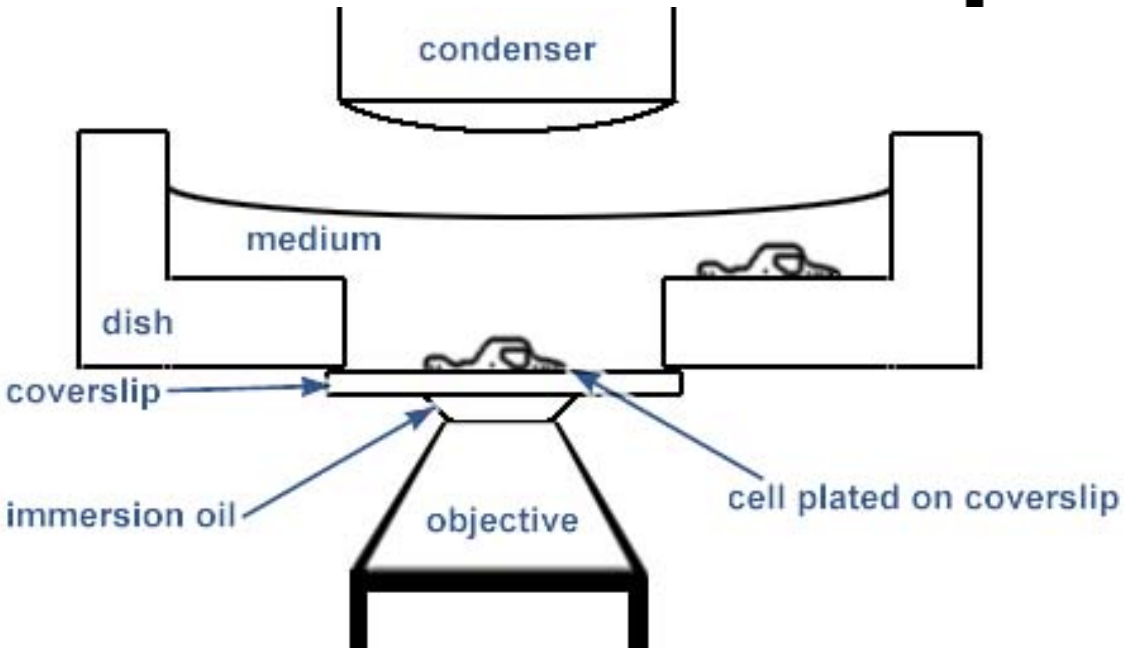


Practicalities

 # 1.5 coverslip

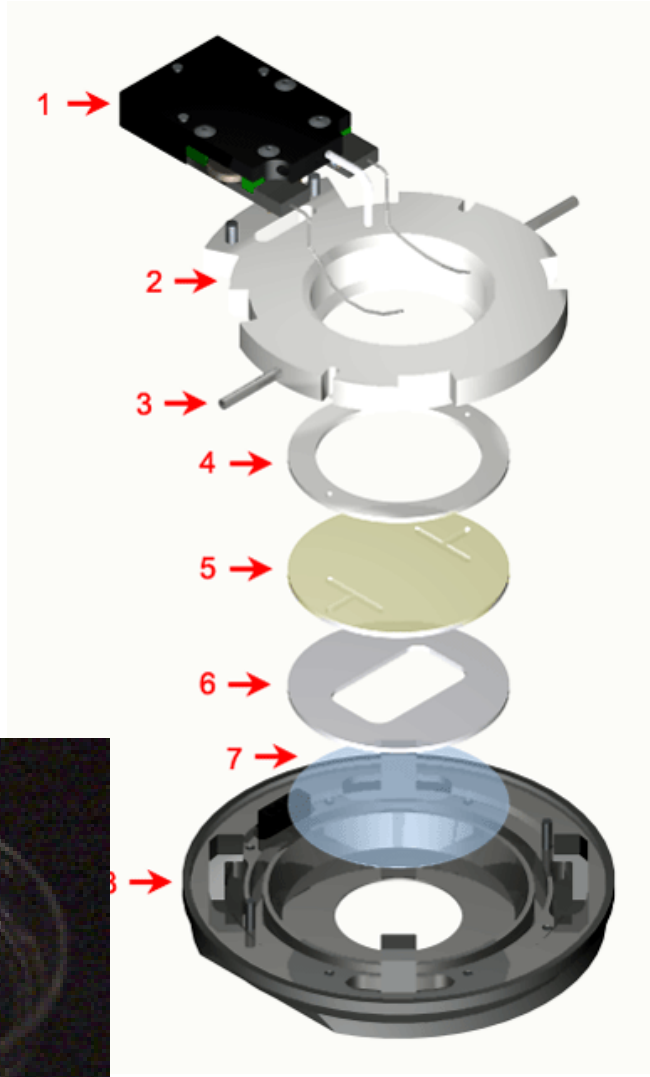
Not #0, not #1…

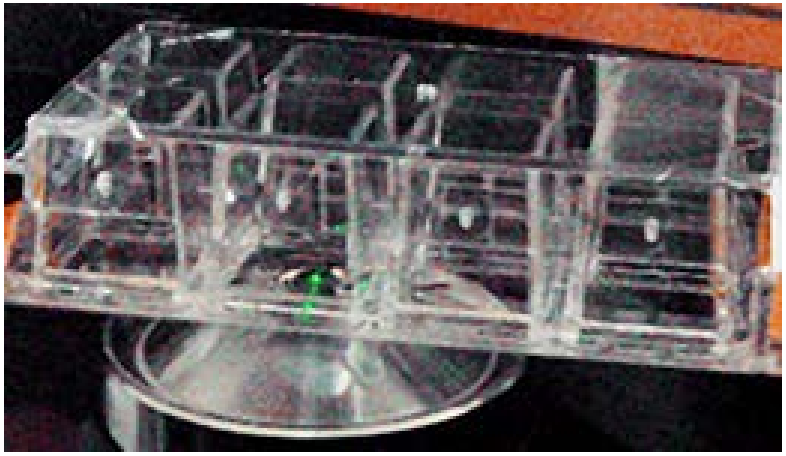


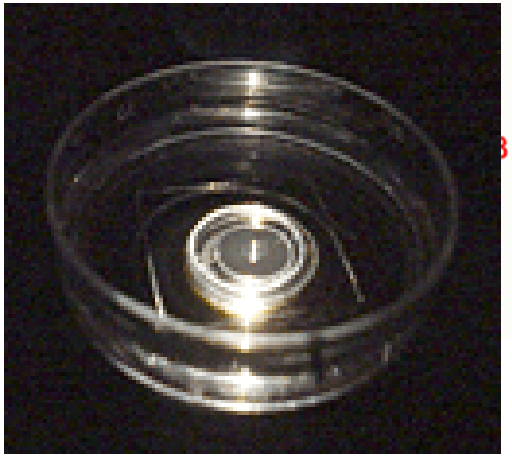


http://microscopynotes.com/coverslips/index.html

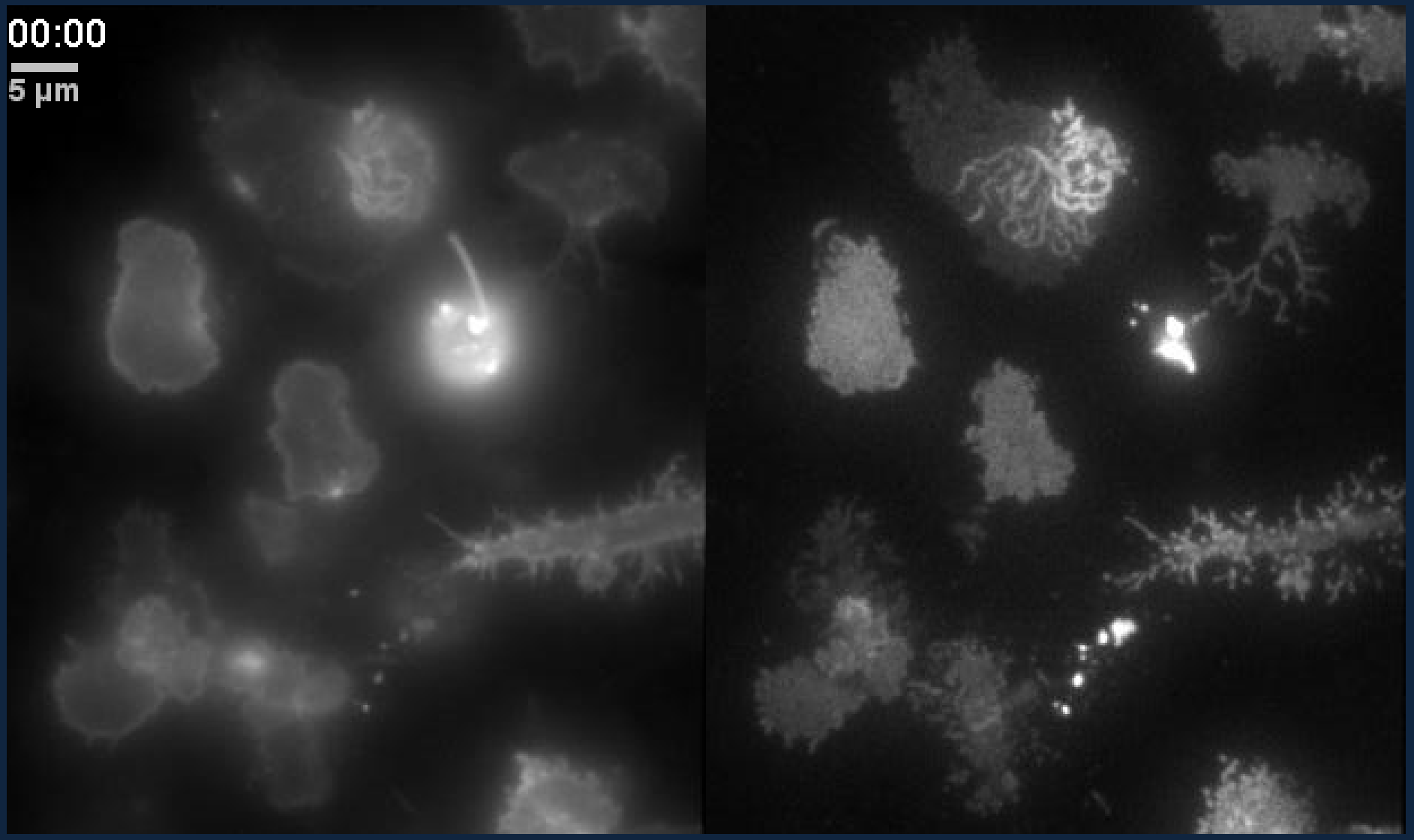
Example #1.5 Coverslip Bottom Chambers



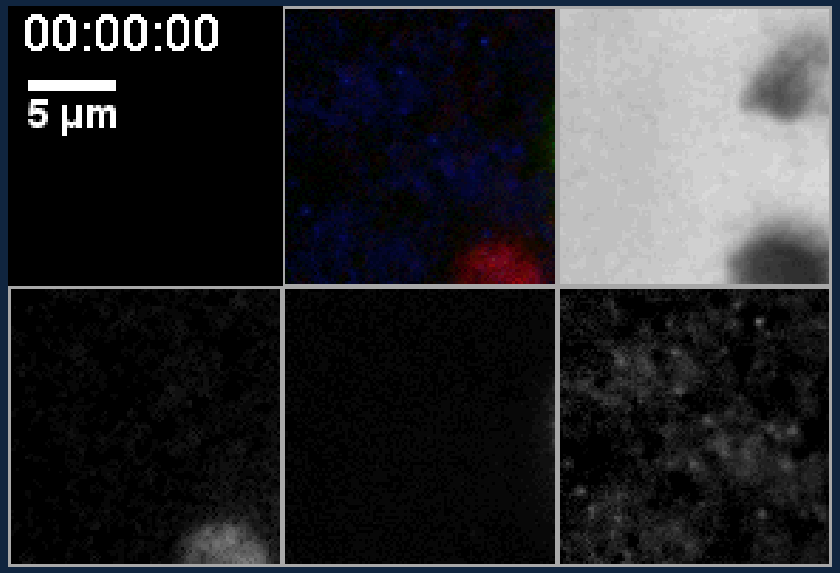


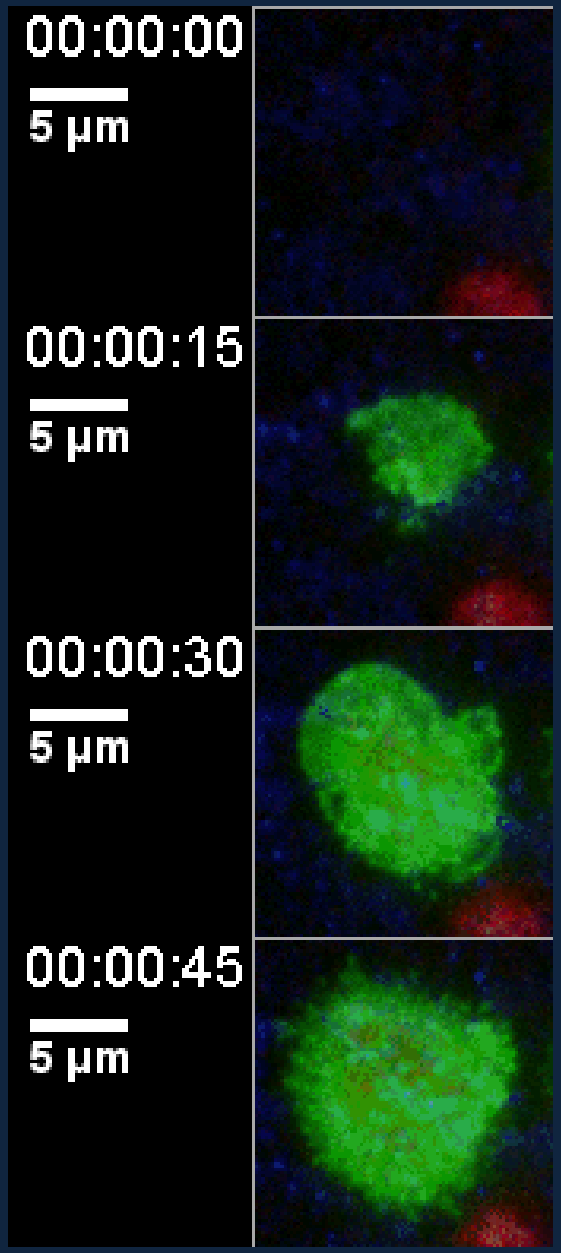


http://microscopynotes.com/coverslips/index.html
http://www.bioptechs.com/Products/FCS2/fcs2.html


00:00
5 µm

Sequential Colors





To go faster, need to use fewer imaging modes and fewer colors.

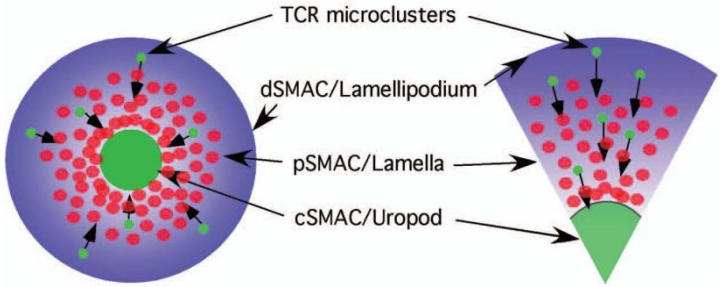
Easy to sequence different conditions; run fast single color then slower multiple channels.

Time Intervals Seconds

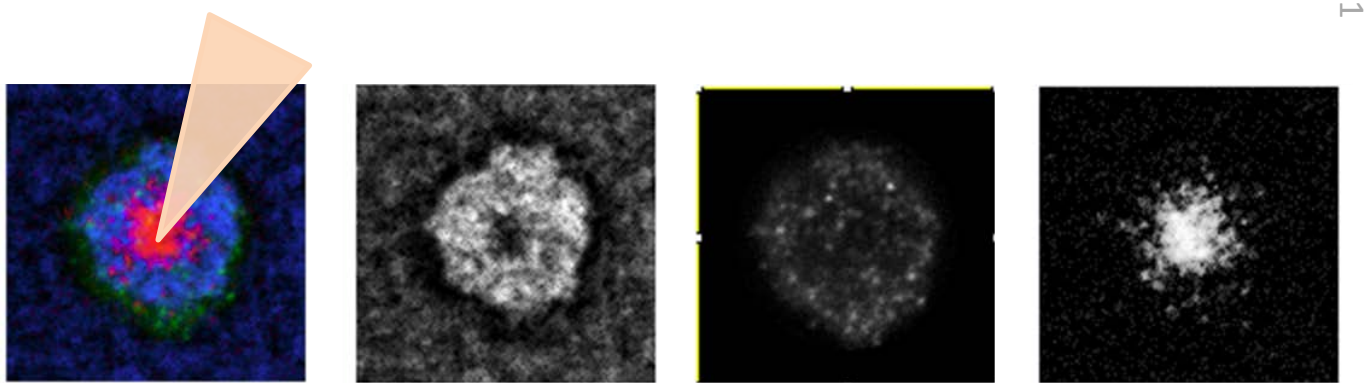
14.95
15.11
14.99
14.91
15.03
15.06
14.95
15.00
14.98
15.05
14.91
15.02
15.00

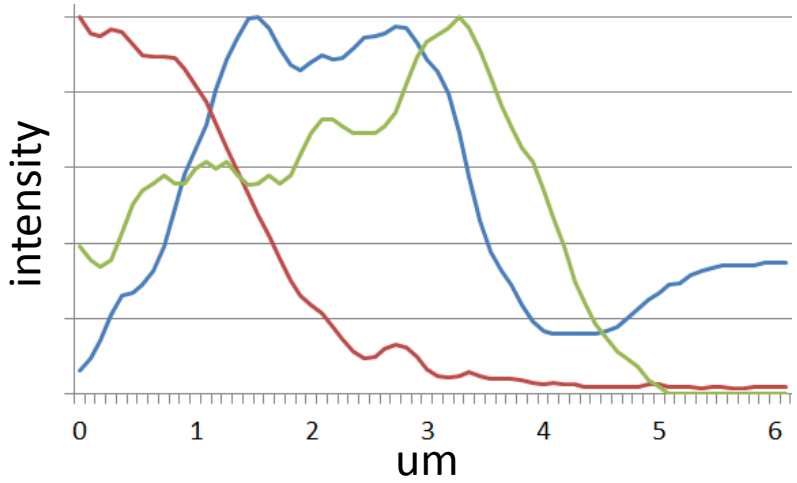
Example Quantification: Radial Intensity Plots Show Locations of Molecules Per Cell Compartments

En face view of the synapse with cSMAC, pSMAC and dSMAC and en face view of a kinapse.



From Dustin, 2011

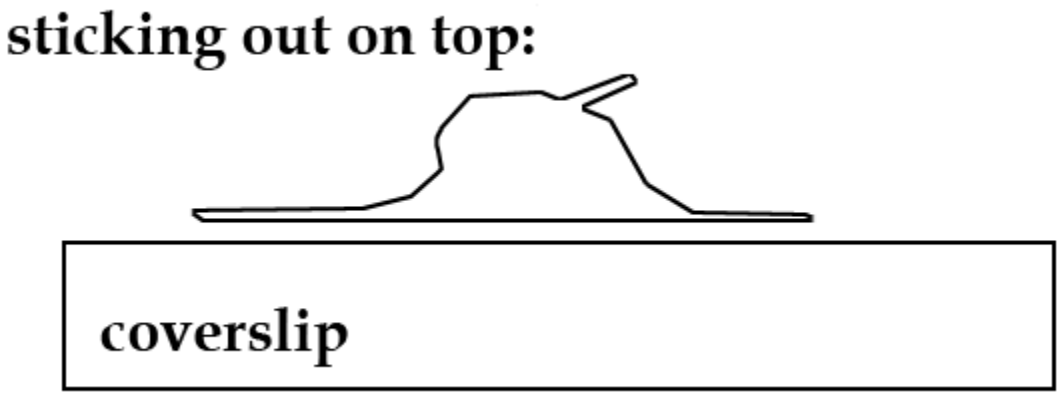




Radial intensity plot from cell center (left) to periphery (right)

Primary Cilia

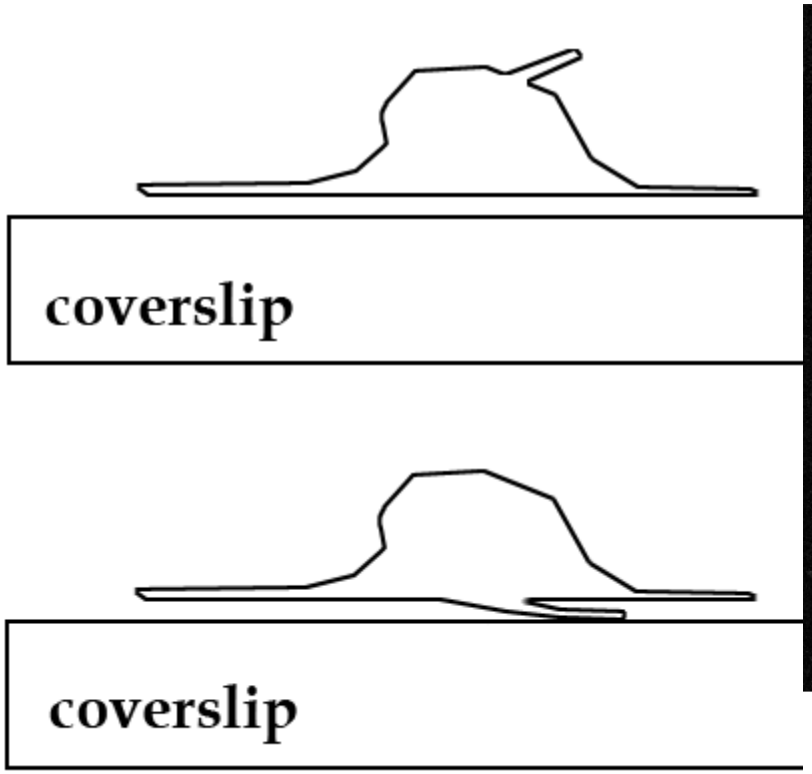
Primary cilium in fibroblasts (marker acetylated α tubulin)

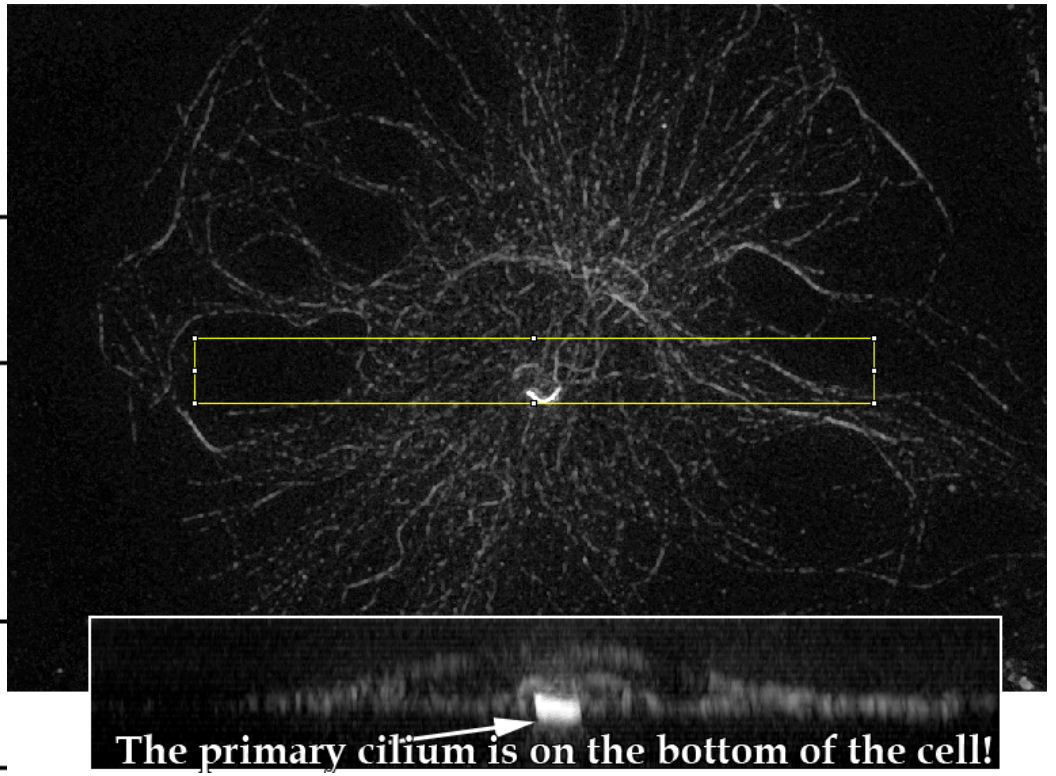


unpublished Linda Schneider (P. Satir & S.T. Christensen labs)
& Michael Cammer - 2006

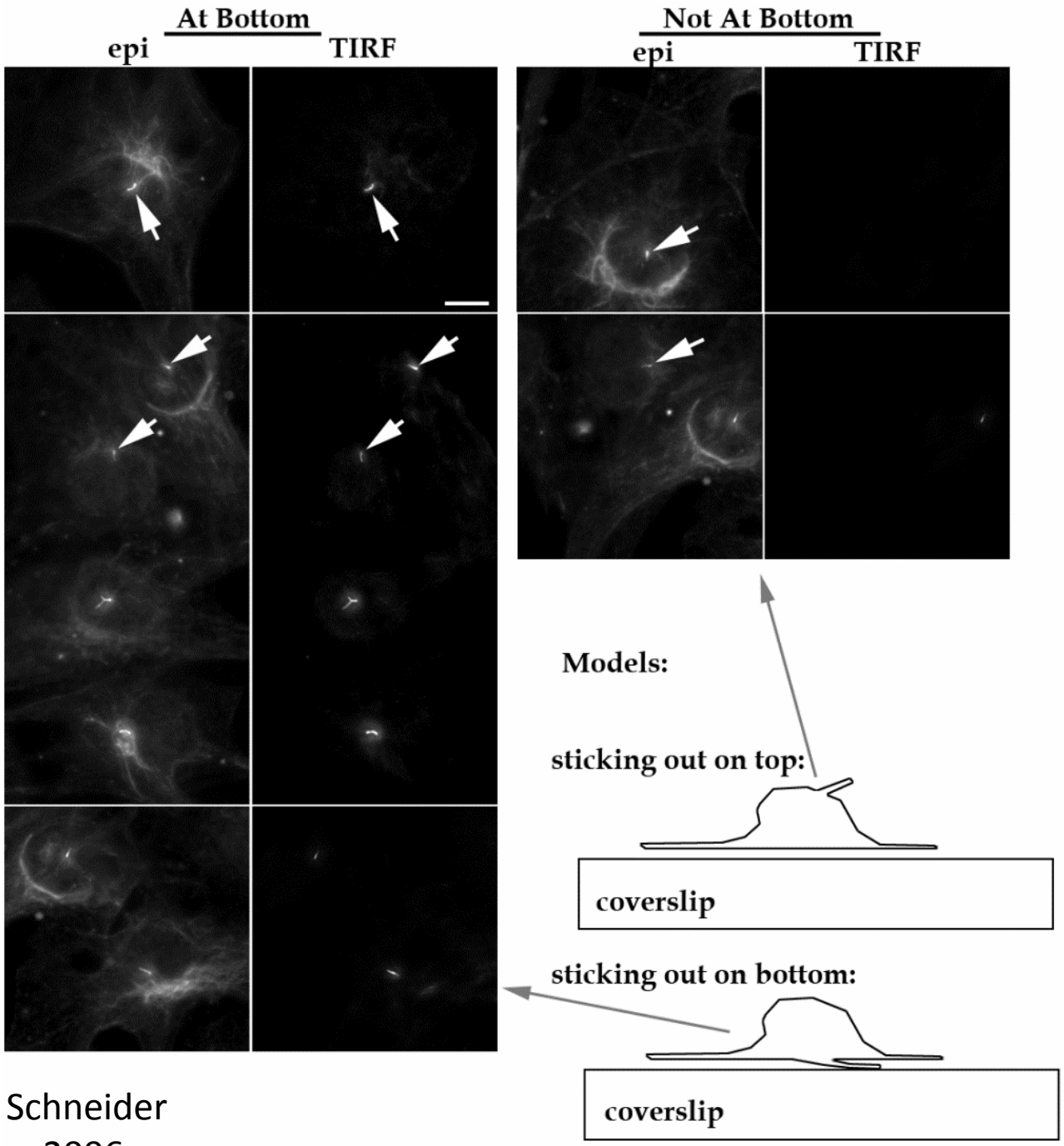
Primary Cilia

Primary cilium in fibroblasts (marker acetylated α tubulin)



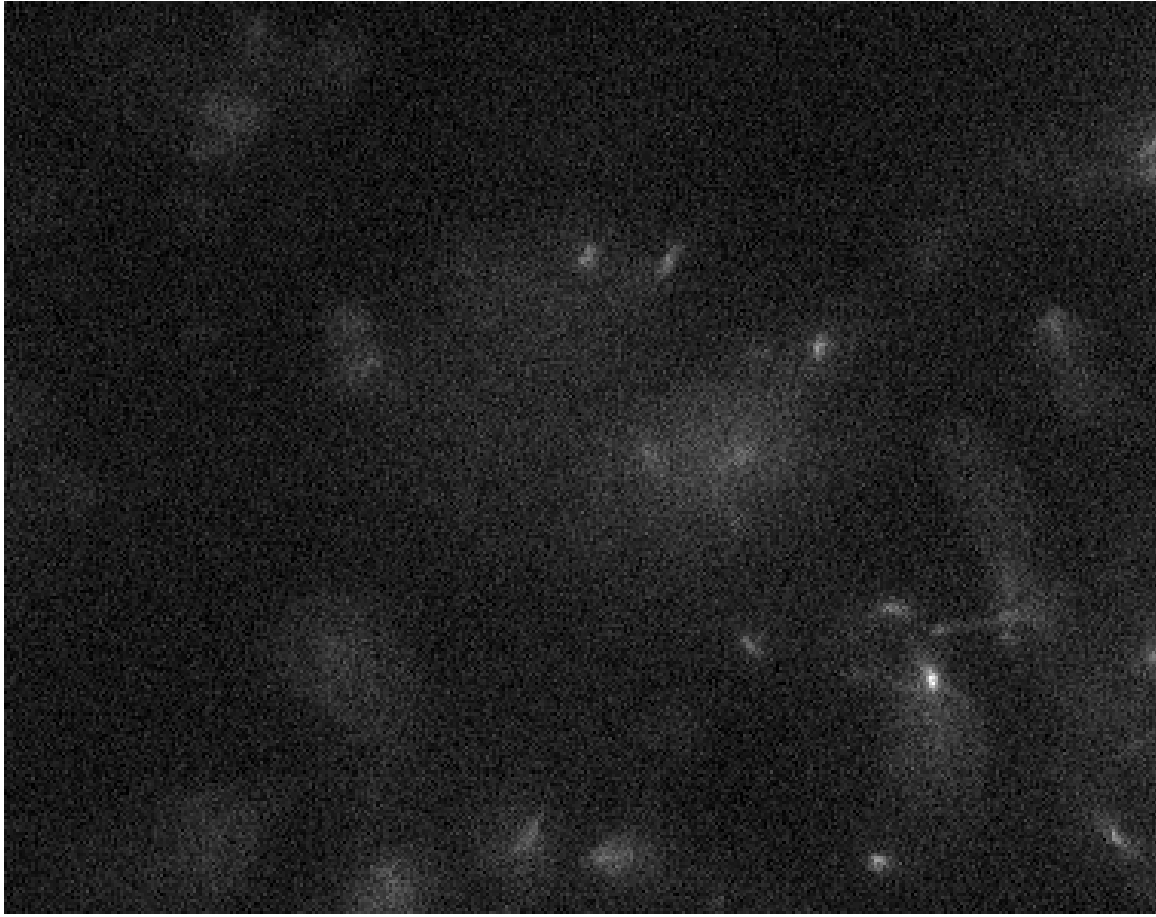


Allows for high contrast imaging of MT or associated proteins (motors, receptors, etc.) in intact primary cilia

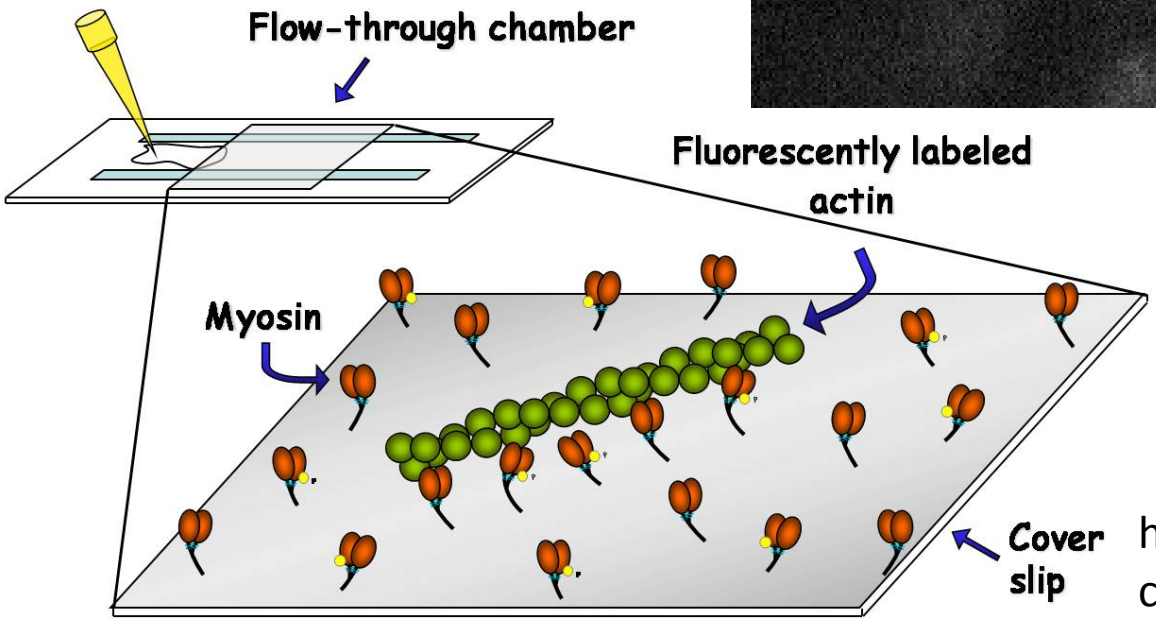


unpublished Linda Schneider & Michael Cammer - 2006

From standard epifluorescence to TIRF in an f-actin *in vitro* assay

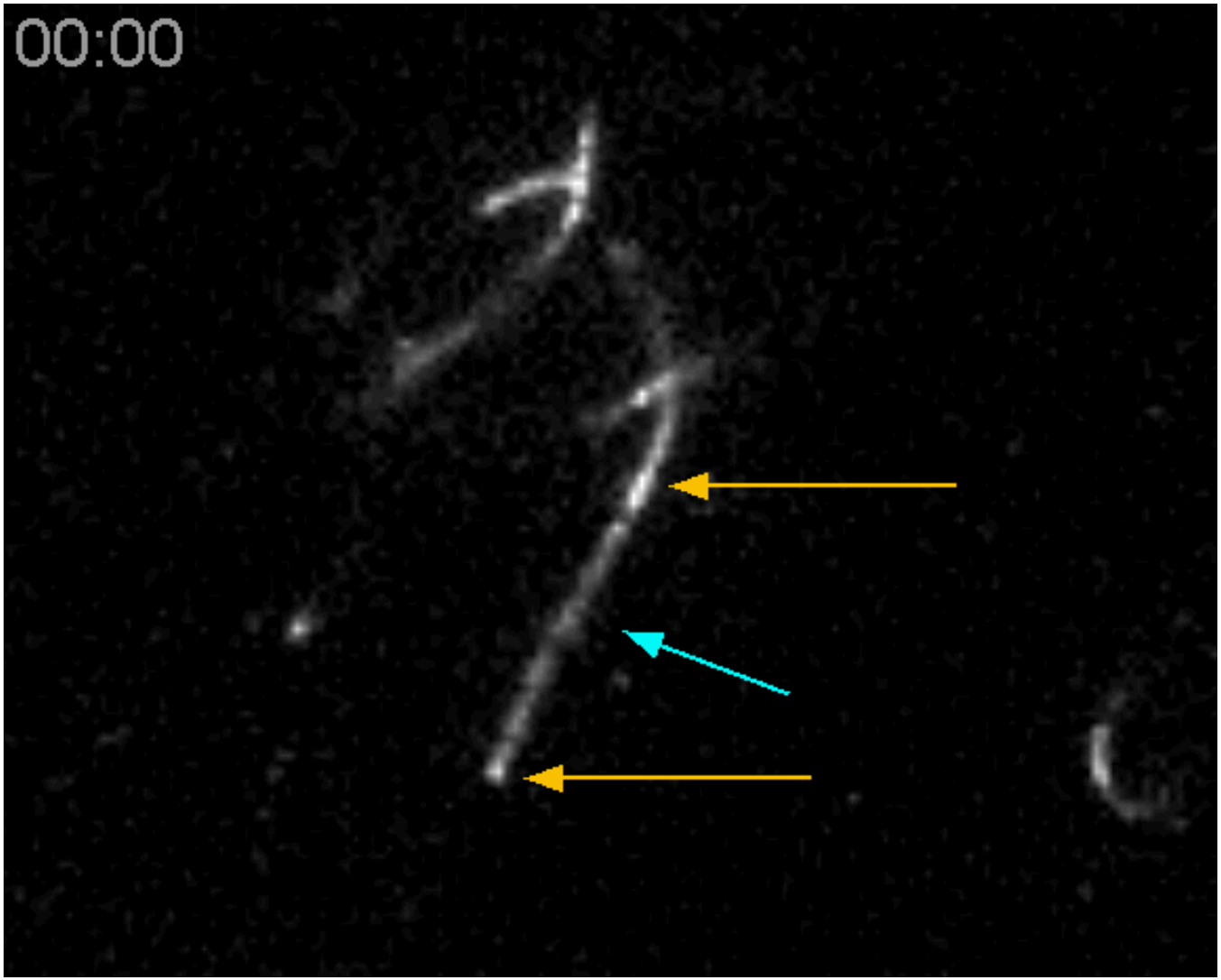


Rob Eddy
unpublished

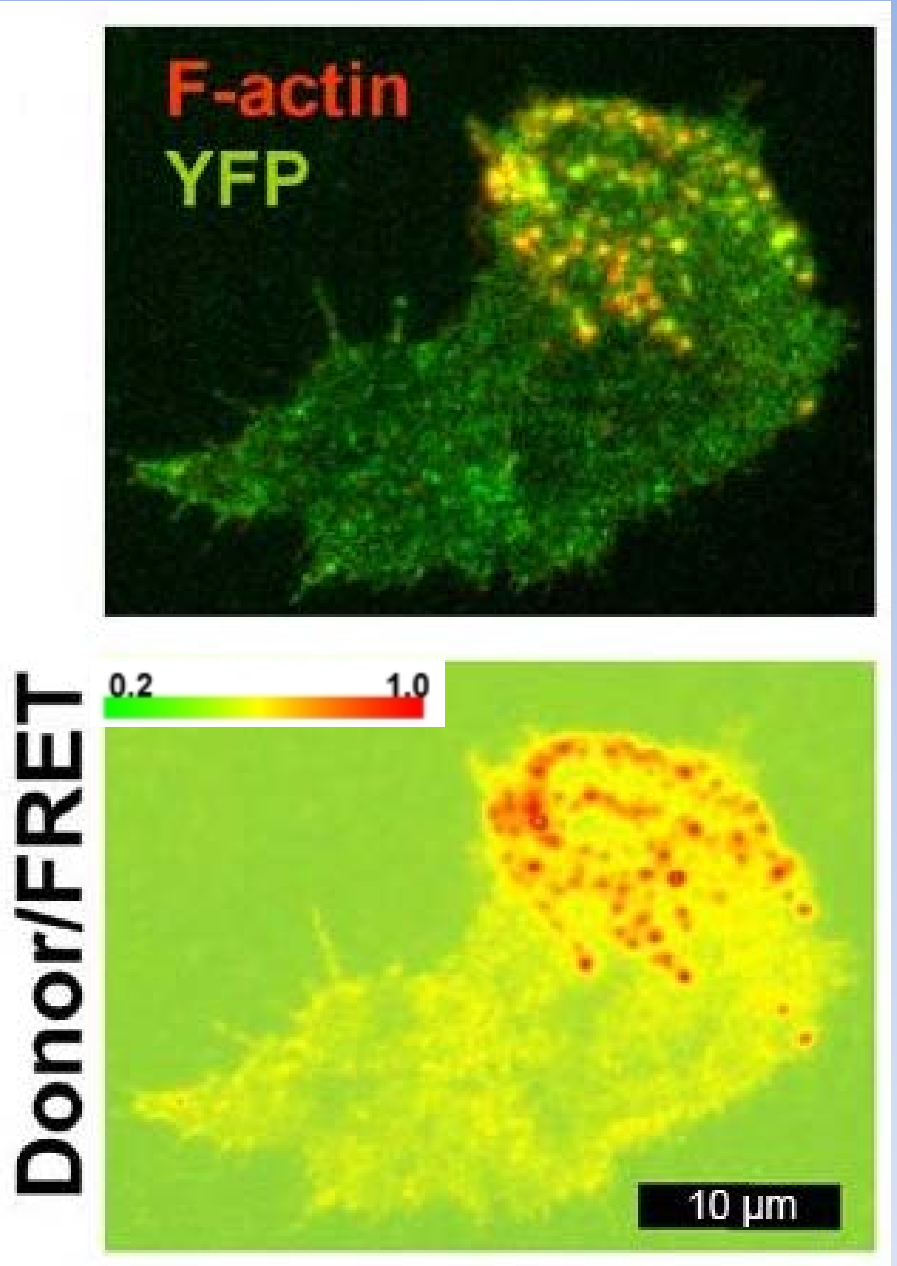


http://lauzonlabmcgill.com/wp-content/uploads/2014/06/Picture3.jpg

Anchor points closer to substrate are brighter



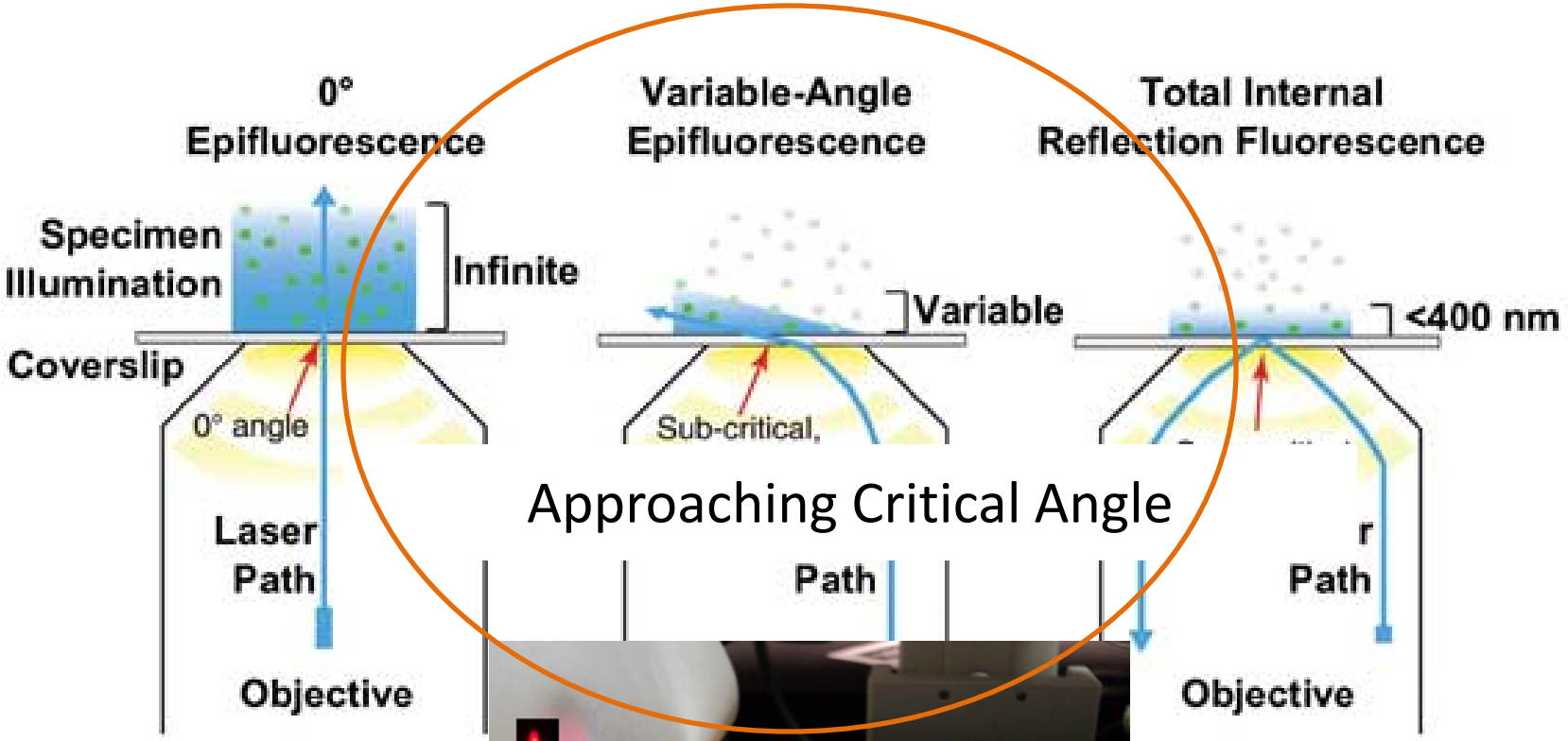
TIRF FRET

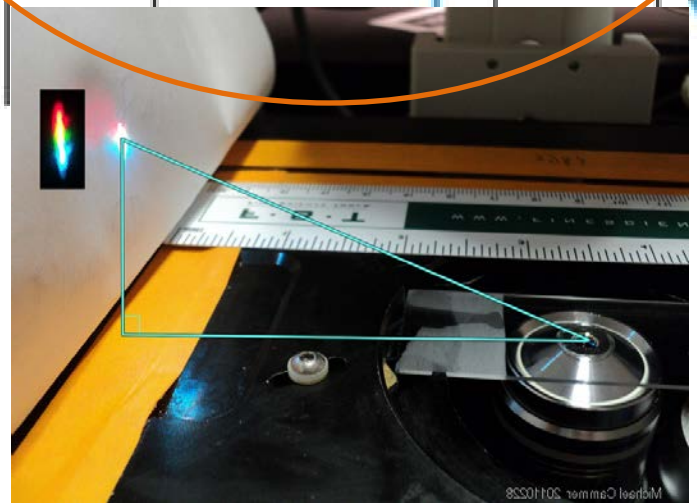


Active WASp is localized in podosomes and its activity is required for podosome maintenance. WASp is active in podosomes. RAW/LR5 cells transfected with a WASp biosensor, fixed and stained with Alexa Fluor 568-phalloidin and imaged by TIRF microscopy.

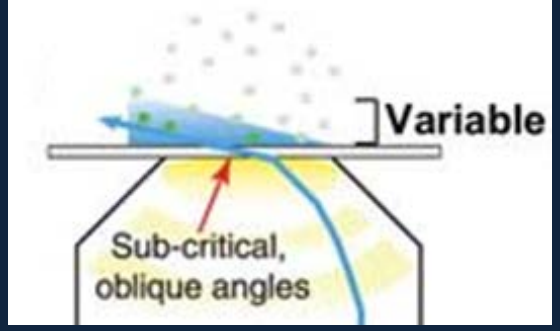
Dovas A, Gevrey JC, Grossi A, Park H, Abou-Kheir W, Cox D. Regulation of podosome dynamics by WASp phosphorylation: implication in matrix degradation and chemotaxis in macrophages. *J Cell Sci.* 2009 Nov 1;122(Pt 21):3873-82. doi: 10.1242/jcs.051755.

Hi Lo may be useful for thick samples

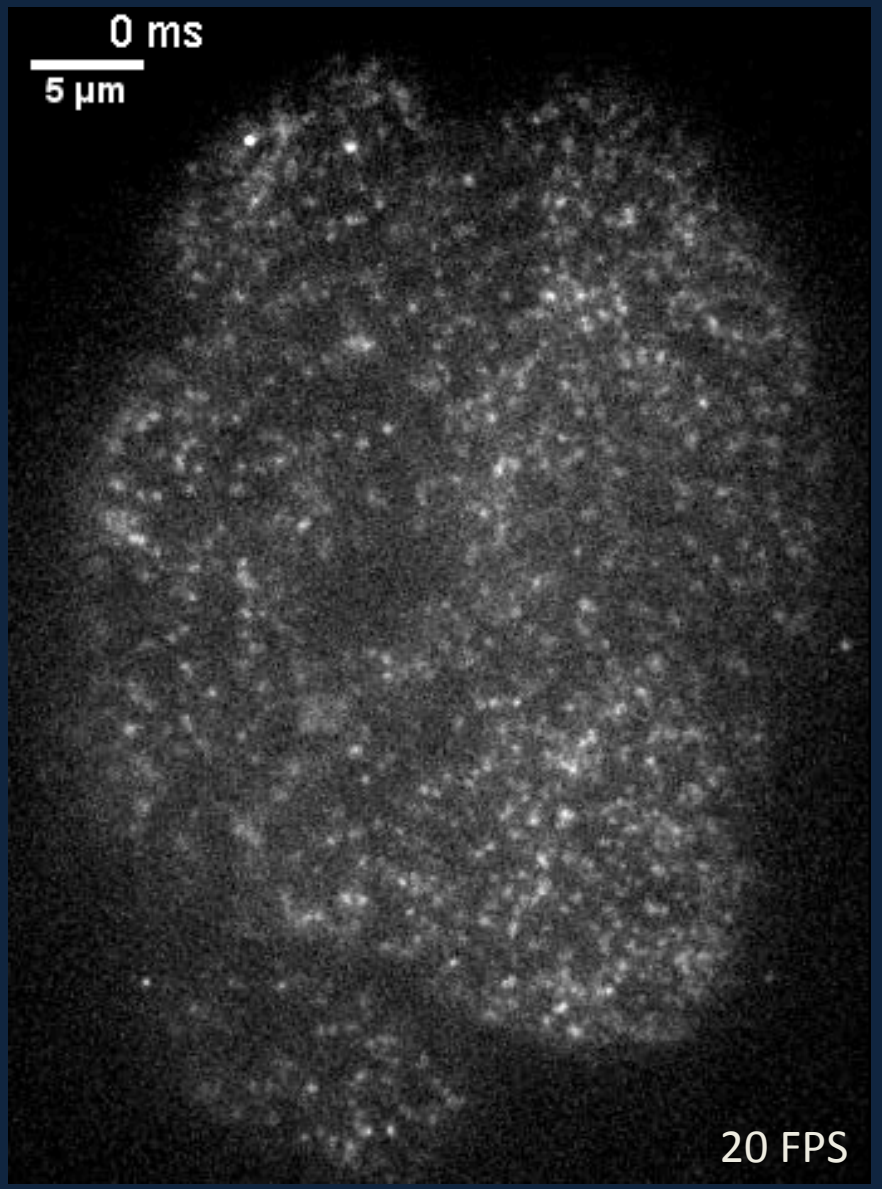




http://www.photonics.com/Article.aspx?AID=48690

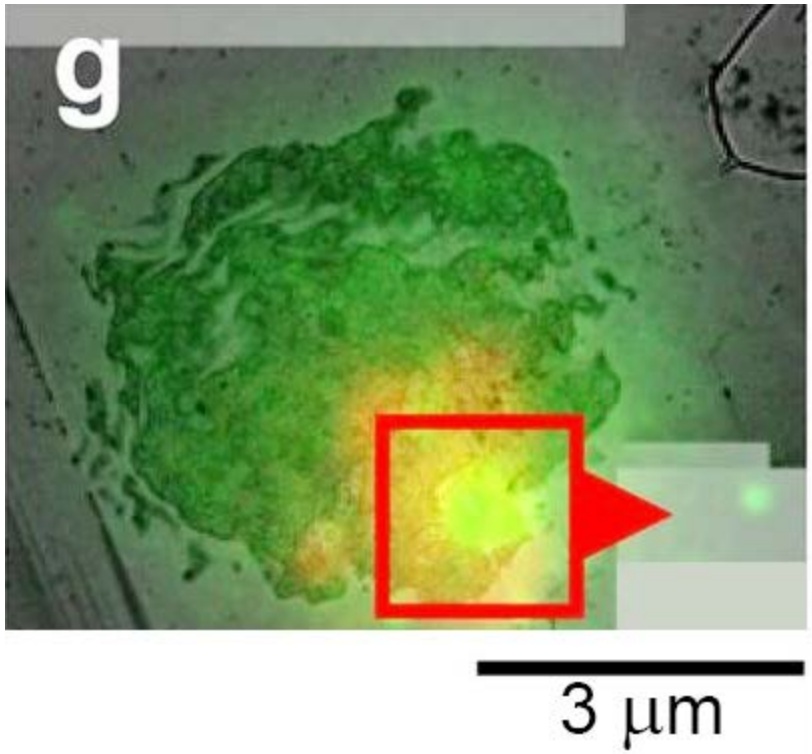


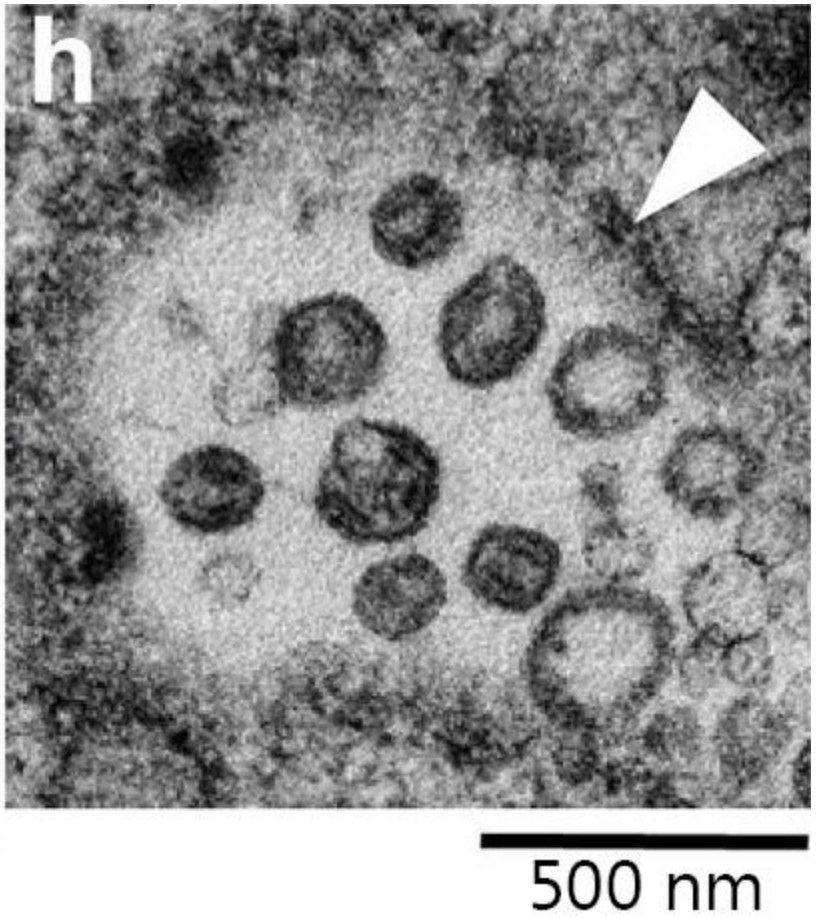
Early C. elegans embryo imaged with near-TIRF illumination to overcome the problem of the 200nm-thick eggshell that makes it difficult to use the true TIRF optics. The molecule here is Par-6-GFP a polarity protein. This method allows measuring the exchange rate and mobility of the single molecules at the membrane.



Yuliya Zilberman in Nance lab

Correlative TIRF and TEM





g. T cell with centrally accumulated GAG-GFP resuming motility and releasing GAG-GFP-containing microvesicles. **h.** Higher magnification image of boxed region in **g.** showing internal juxta-membrane density in GAG-containing microvesicles. Arrowhead, plasma membrane.

Modified from Choudhuri K, Llodrá J, Roth EW, Tsai J, Gordo S, Wucherpfennig KW, Kam LC, Stokes DL, Dustin ML. Polarized release of T-cell-receptor-enriched microvesicles at the immunological synapse. *Nature*. 2014 Mar 6;507(7490):118-23. doi: 10.1038/nature12951.

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(draft as of 20150330_1344)

Michael Cammer