Useful Links for Two-Photon Microscopy: Fluorescently Probed Samples and Reviews

Spectral Viewers (mostly single photon data):

- These are great for planning fluorophore combinations to ensure that the single photon (1P) emission from excited samples will not overlap.
- 2P spectral excitation data for select fluorescent proteins (i.e., GFP, tdTomato) can be visualized depending on the viewer.

1. Spectra viewer by the Max Plank Institute for Brain Research
   - https://public.brain.mpg.de/shiny/apps/SpectraViewer/ (link to viewer)
   - https://brain.mpg.de/326043/spectra-viewer (About page)
   - Easily the most comprehensive spectral viewer for 1P, 2P and even 3P data.

2. FPdatabase (Fluorescent Protein Database)
   - https://www.fpbase.org/spectra/
   - Excellent and easy to use 1P spectral data viewer for fluorescent proteins and dyes. Includes 2P excitation data of many commonly used fluorescent proteins.

3. ThermoFisher Spectra-Viewer
   - https://www.thermofisher.com/order/spectra-viewer
   - Helpful tip and instructions for how best to utilize the viewer: https://www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook/technical-notes-and-product-highlights/using-the-fluorescence-spectraviewer.html
   - Great for 1P spectral data viewing. Highly diverse list of fluorophores. Great for visualizing custom ex/em filters relative to selected fluorophores.

Other helpful links of 2P excitation data.

1. https://www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook/technical-notes-and-product-highlights/fluorescent-probes-for-two-photon-microscopy.html
   - Helpful introduction to fluorescent probes for 2P microscopy with links to more data.

2. https://www.drbio.cornell.edu/cross_sections.html
   - 2P excitation cross sections for various fluorescent dyes and proteins.
   - Created by the lab of Dr. Warren Zipfel at Cornell University.

Published Articles of 2P excitation spectra:

1. Bestvater, F et al. "Two-photon fluorescence absorption and emission spectra of dyes relevant for cell imaging." Journal of microscopy vol. 208,Pt 2 (2002): 108-15.
    - DOI: 10.1046/j.1365-2818.2002.01074.x
    - 2P excitation spectral curves for a variety of fluorescent dyes/antibody conjugates
        - Quinacrine, Hoechst 33342, DAPI, DCF2, AMC, Rhodamine123, PI, SNARF-1, Bodipy FL, Bodipy-TRoad, Acridine Orange, Alexa Fluor 488, 546 and 594, Cy2, Cy3, RedX, lissamine rhodamine, PhenGreen-FL (+/- Fe), TexasRed, DTAF, FITC, ER-Tracker white/blue, MitoTracker Red, LysoTracker Yellow and Red.

2. Mütze, Jörg et al. "Excitation spectra and brightness optimization of two-photon excited probes." *Biophysical journal* vol. 102,4 (2012): 934-44.
    - DOI: 10.1016/j.bpj.2011.12.056
    - 2P excitation spectral curves for a variety of fluorescent dyes/antibody conjugates
        - Alexa Fluor 314, 350, 430, 488, 546, 555, 594, 610, 633, 647, 610, Fig 3, rhodamine dyes: tetramethylrhodamine, 5-carboxy-tetramethylrhodamine, Q-rhodamine, rhodamine 101, 110, 575 and sulforhodamine 101. Calcium ion indicators: eGFP (for comparison), GCaMP2, GCaMP3, CalciumGreen, CalciumOrange, CalciumCrimson, XRhod1, Rhod4, Rhod2, Fluo8, Fluo3, Fluo4, OGB1, OGB1 APO, OGB5N, OGB5N Apo. Fluorescin, Bodipy492/515, BodipyTR, Resorufin.

3. Drobizhev, Mikhail et al. "Two-photon absorption properties of fluorescent proteins." *Nature methods* vol. 8,5 (2011): 393-9.
    - DOI: 10.1038/nmeth.1596
    - 1P and 2P excitation spectral curves for select fluorescent proteins
        - EBFP2.0, EGFP, mBlueberry1, mAmetrine, Citrine, DsRed2, ECFP, mOrange, TagRFP. tdTomato, DsRed2, mBanana, mRFP, mCherry, mStrawberry. tdKatushka2.

4. Drobizhev, Mikhail et al. "Two-photon absorption properties of fluorescent proteins." *Nature methods* vol. 8,5 (2011): 393-9.
    - DOI: 10.1038/nmeth.1596
    - 1P and 2P excitation spectral curves for select fluorescent proteins
        - EBFP2.0, EGFP, mBlueberry1, mAmetrine, Citrine, DsRed2, ECFP, mOrange, TagRFP. tdTomato, DsRed2, mBanana, mRFP, mCherry, mStrawberry. tdKatushka2.

5. Ricard, Clément et al. "Two-photon probes for in vivo multicolor microscopy of the structure and signals of brain cells." *Brain structure & function* vol. 223,7 (2018): 3011-3043.
    - DOI: 10.1007/s00429-018-1678-1

    - 2P excitation data for 280 fluorescent molecules (dyes and proteins).
        - Data collected *in vivo*.

6. Velasco, MG et al (2015) Absolute two-photon excitation spectra of red and far-red fluorescent probes Optical Letters 40/21: 4915-4918

7. Drobizhev, M et al (2014) Multiphoton photochemistry of red fluorescent proteins in solution and live cells. Jour. Phys. Chem B. 118/31: 9167-9179.

8. Drobizhev, M et al (2009) Absolute two-photon absorption spectra and two-photon brightness of orange and red fluorescent proteins. Jour. Phys. Chem B. 113/4: 855-859.

9. Speiss, E et al (2005) Two-photon excitation and emission of the green fluorescent protein variants ECFP, EGFP and EYFP Journal of Microscopy 217/3: 200-204

10. Dickinson, ME et al (2003) Multiphoton excitation spectra in biological samples Jour. Biomed. Optics 8/3: 329–338

Some Great Review Articles:

1. Klein, JK & Smith, PG (2014) Figure 1, page 32 in: Deep dive in multi-photon imaging Imaging & Microscopy Oct 2014 16/4: 31-33 GIT-Verlag. https://www.spectra-physics.com/mam/celum/celum_assets/sp/resources/DeepDiveIntoMultiphotonImaging.pdf

2. Clément, R et al (2018) Two-photon probes for in vivo multicolor microscopy of the structure and signals of brain cells Brain Struct Funct. 223/7: 3011–3043.

3. Mostany R, Miquelajauregui A, Shtrahman M, Portera-Cailliau C. Two-photon excitation microscopy and its applications in neuroscience. Methods Mol Biol. 2015;1251:25-42. doi: 10.1007/978-1-4939-2080-8_2. PMID: 25391792.

4. Rubart M. Two-photon microscopy of cells and tissue. Circ Res. 2004 Dec 10;95(12):1154-66. doi: 10.1161/01.RES.0000150593.30324.42. PMID: 15591237.

5. Perry SW, Burke RM, Brown EB. Two-photon and second harmonic microscopy in clinical and translational cancer research. Ann Biomed Eng. 2012 Feb;40(2):277-91. doi: 10.1007/s10439-012-0512-9. Epub 2012 Jan 19. PMID: 22258888; PMCID: PMC3342697.

6. Liu, J. Two-photon microscopy in pre-clinical and clinical cancer research. Front. Optoelectron. 8, 141–151 (2015). https://doi.org/10.1007/s12200-014-0415-5