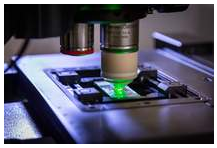
PI Preclinical Imaging

Multiphoton Microscopy Online Training: Part 1 - Overview and Theory



The multiphoton microscope can perform 2-photon fluorescence microscopy, second harmonic and third harmonic generation imaging.

This module is the first in the training series to access and use Preclinical Imaging's Multiphoton Microscope (MPM) within Comparative Medicine.

The order of training in the MPM series is:

1. Multiphoton Microscope scientific background and theory (this training)
2. in-person equipment training
3. online MPM System Operations training

The order of steps 2 and 3 can be interchanged, but step 1 must be completed prior to either step 2 or 3.

Comparative Medicine's multiphoton microscope is equipped with a tunable wavelength Chameleon laser (690 nm - 1100 nm) and a tunable Compact Chameleon OPO (1080 nm

- 1600 nm). The microscope system is a Zeiss LSM 7 MP microscope and is equipped with 2 BiG detectors.

Additionally, this online training can be referenced as a refresher-course and serve as a reference for any future use of the equipment.

Introduction

What kind of images can we take on the MPM?

Brief Equipment Overview

Do you need to use the OPO?

The Electromagnetic Spectrum

Optical Microscopy overview

Fluorescence microscopy basics

the MPM in vivo advantage

putting it all together: THG, SHG, 2PF example

Further Resources

Introduction

PI **Preclinical Imaging**

What can the Multiphoton Microscope do?

Of all the preclinical imaging equipment available in Comparative Medicine, the Multiphoton Microscope (MPM) allows for the fastest and highest resolution imaging for in vivo studies.

Our multiphoton microscope uses a tunable wavelength laser that outputs longer wavelengths than those typically found in a confocal microscope. This allows for:

- **Less power** required to image - which means less tissue damage to your animals!
- **Higher resolution** imaging (down to 0.4 microns resolution!)
- **Deeper tissue imaging** compared to confocal microscopes (making MPM the method of choice for single-cell level in vivo studies).
- A **wider probe range** available for fluorescence applications
- **Faster imaging**. We can image cellular dynamics as they are happening, including observing neurons firing and mitotic events.
- **Reduced photobleaching** of fluorophores when performing 2-photon fluorescence compared to confocal fluorescence

In the next section we will delve into how these features of multiphoton microscopy are possible.

- **Caveat:** Compared to other *non-optical* in vivo imaging methods **(**like MRI, CT, ultrasound) multiphoton microscopy methods have a **low penetration depth.**

- Here, imaging depth is being sacrificed for the sake of imaging resolution and imaging speed.
- Imaging depth limits for MPM methods range from 0.4 mm - 2 mm, depending on the specific imaging application. These values are deeper than those that can be achieved on a confocal microscope, which gives comparable imaging resolutions.
- To compare the spatial resolution limits, imaging depth limits, and imaging speeds of various PCI instruments, please visit the following graph: https://centernet.fredhutch.org/content/dam/centernet/u/shared-resources/comparativemedcine/preclinical-imaging/Internal_Modalitie_At_FHSRCM.png

What kinds of microscopy can the CM multiphoton microscope do? Select all that apply

- [ ] Confocal fluorescence microscopy
- [ ] Third Harmonic Generation (THG) microscopy
- [ ] Two-photon fluorescence microscopy
- [ ] Second Harmonic Generation (SHG) microscopy
- [ ] Scanning Electron Microscopy

SUBMIT

Complete the content above before moving on.

What kind of images can we take on the MPM?

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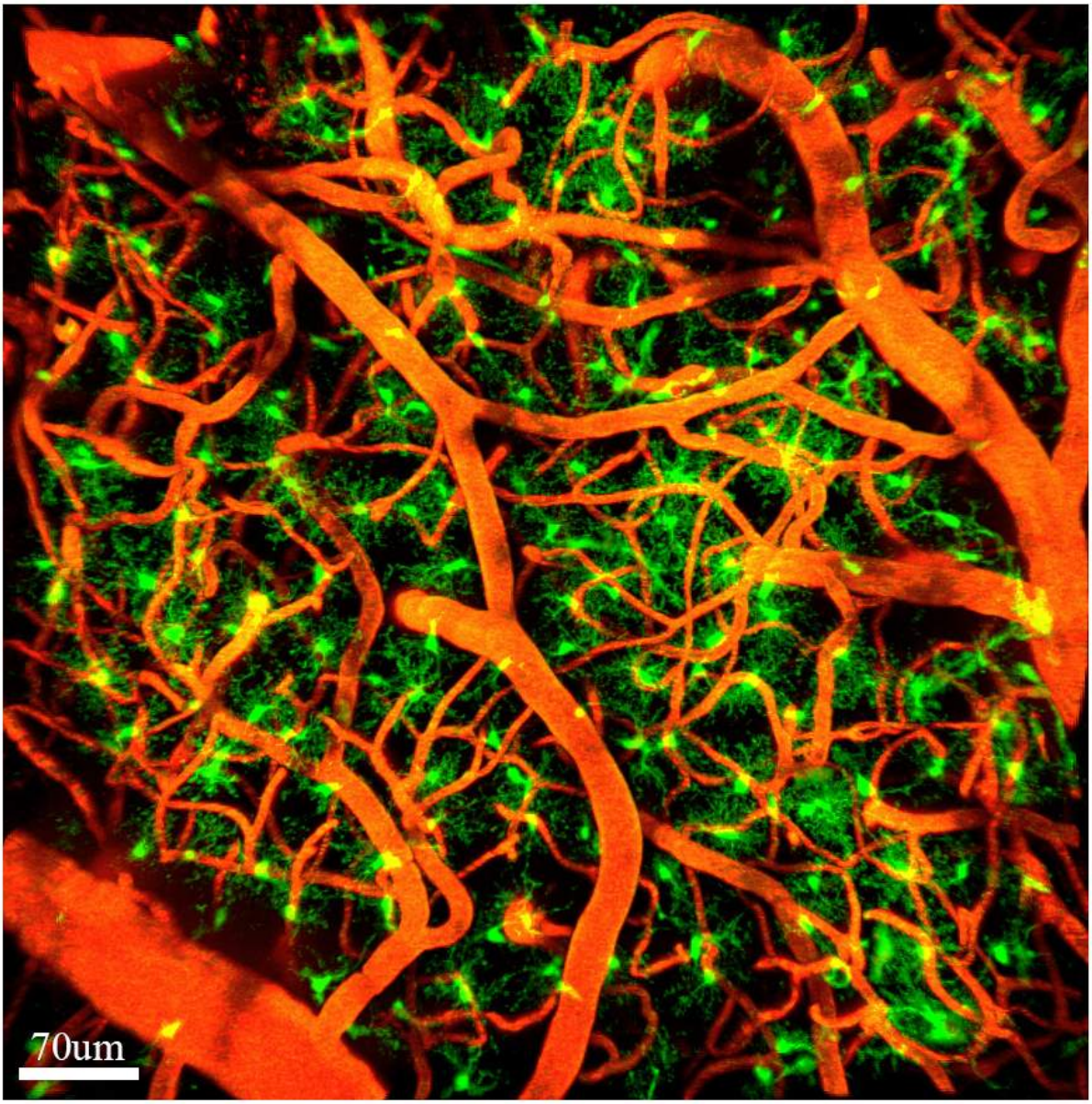
What kind of images can we acquire on the MPM?

There are three main types of microscopy the MPM can perform: 2-photon fluorescence microscopy (2PF) , Second Harmonic Generation (SHG) microscopy, and Third Harmonic Generation (THG) microscopy.

2-Photon Fluorescence Microscopy



Red: vasculature labeled with Tx Red; Green: Astrocytes expressing green fluorescent protein (GFP)

Dai, J., Cimino, P.J., Gouin, K.H. et al. Astrocytic laminin-211 drives disseminated breast tumor cell dormancy in brain. Nat Cancer 3, 25–42 (2022). https://doi.org/10.1038/s43018-021-00297-3

2-photon fluorescence (2PF) is the most common application of our multiphoton microscope.

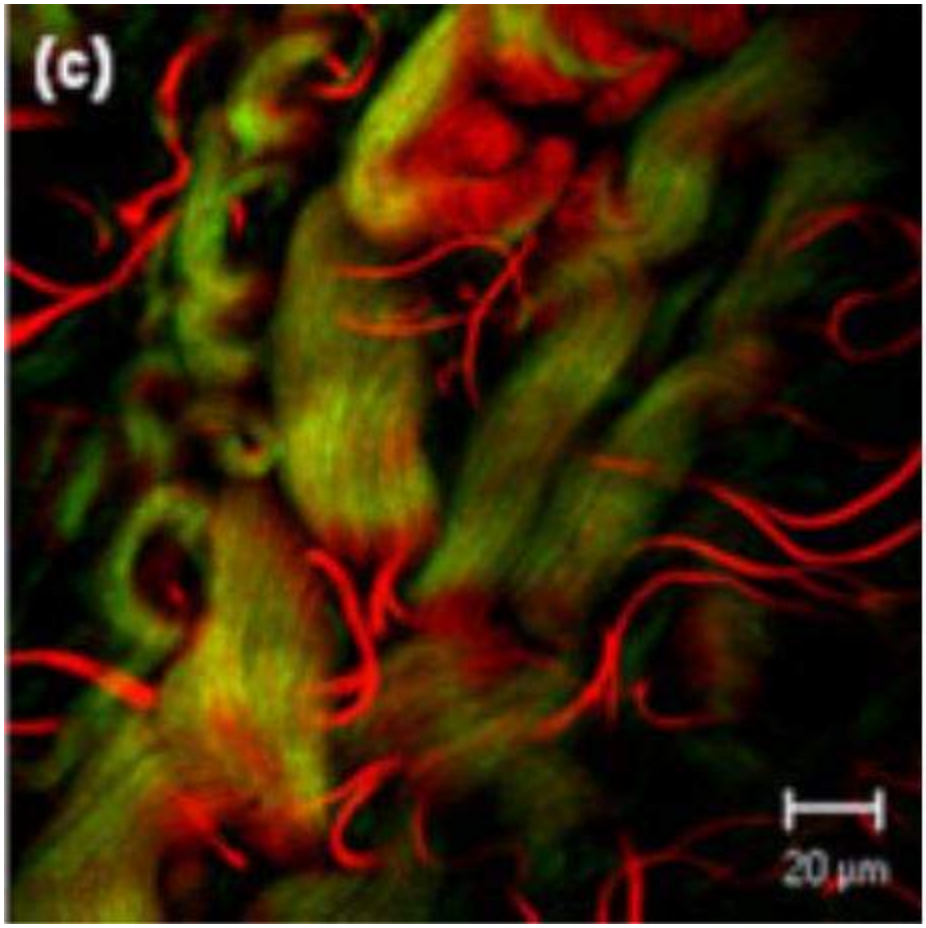
Fluorophores are expressed in animal tissue within specific proteins, cell types, or specific membranes or vessels. With a careful study design, multiple fluorophores can be imaged at the

same time to study how various cells or proteins interact with each other in physiological phenomena.

There are a vast array of possible fluorophores available for these types of applications. Fluorophores can be expressed to the areas of interest using genetic techniques, virus activation, injecting dye directly into particular membranes or vessels, and a host of other methods as well.

In the left image, 2PF enables high resolution imaging of brain blood vessels (stained with Texas Red fluorescent dye) and astrocyte cells (expressing GFP fluorescent dye) in a live mouse brain.

Second Harmonic Generation microscopy



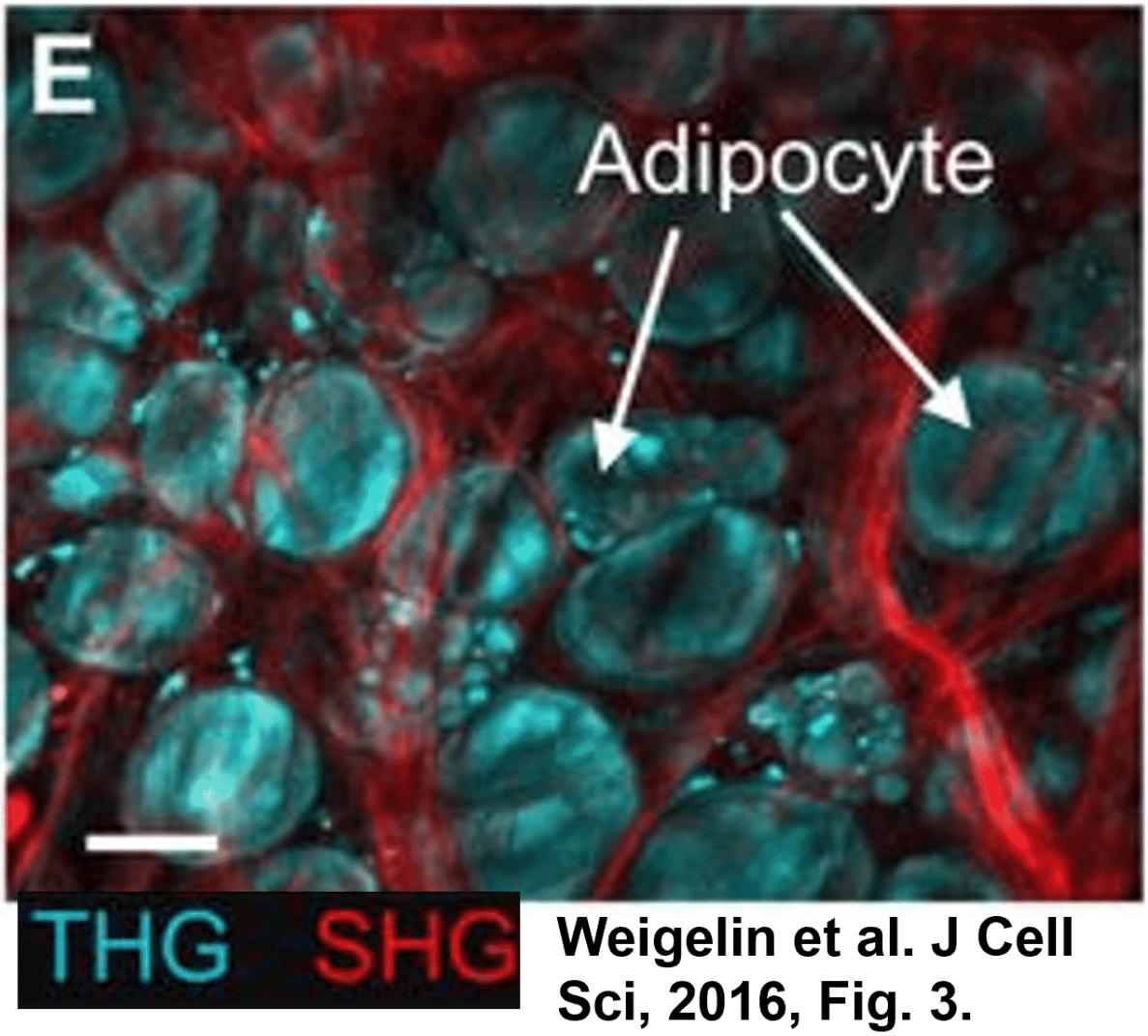
Red: SHG of collagen fiber bundles ; Green: 2-photon fluorescence off elastic fibers and collagen bundles
Image from: Jiang et al. Scanning Vol. 33, 53-56 (2011). https://onlinelibrary.wiley.com/doi/epdf/10.1002/sca.20219

Second Harmonic Generation (SHG) microscopy occurs when laser light around 850 nm or 1300 nm interacts with an ordered non-centrosymmetric structure, such as types I & III collagen and certain kinds of muscle fiber. The light is frequency doubled - hence the term second harmonic.

SHG can be considered a kind of autofluorescence. Because of this, SHG is inherently a label and dye-free imaging technique. The biggest benefit of SHG imaging is the fact that it doesn't require *any* additional labels or dyes to be expressed or injected into an animal!

The image to the left shows overlaid SHG imaging of collagen fibers (red channel) in colonic submucosa, and 2PF signal from collagen bundles and elastic fibers (green channel).

Third Harmonic Generation microscopy



Overlayed THG and SHG signal from mouse skin showing cellular membranes and ECM structures. THG signal comes from lipid-rich adipocyte cells, SHG signal comes from collagen in the skin.

Neither technique uses any additional dyes or labels.

Scale bar: 50 microns.

Image from : Weigelin et al. J Cell Sci, 2016.

https://journals.biologists.com/jcs/article/129/2/245/55706/Third-harmonic-generation-microscopy-of-cells-and

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Third Harmonic Generation (THG) is similar to SHG in that it also looks at endogenous features based solely off the symmetry properties of certain structures without any need for additional labels or dyes.

However, in the case of THG the laser light is frequency *tripled* - hence the term *third* harmonic generation. Because *three* photons of light are required for this process, THG process are much more sensitive to power changes and the effects of light scattering in tissue which affects the excitation light's polarization. This can make THG imaging more complex to achieve when compared with 2PF or SHG, however its applications are much broader than what can be achieved with SHG alone.

In tissues, THG excitation occurs predominantly at interfaces that are formed between aqueous interstitial fluids and lipid-rich structures.

Examples of these types of THG compatible interfaces are:

- **cellular membranes**[1]
    - e.g., THG with 1200 nm excitation - see references below for published examples
- **lipid droplets**[2]
    - e.g. THG with 1180 nm excitation
- **Interfaces between water and large protein aggregates**, such as collagen bundles or muscle fibers[3]
    - e.g. 1270 nm for dual SHG and THG excitation

There are many instances where SHG, THG, and 2PF can be combined for simultaneous tri-modal imaging - offering a wealth of biological information.

To perform Second Harmonic Generation (SHG) imaging, you need to express a special type of fluorescent probe in your animal to get signal.

- ◯ True

False

SUBMIT

Fill in the blank for the below sentence:

THG imaging occurs predominantly at interfaces formed between aqueous interstitial fluids and _____.

Type your answer here

SUBMIT

Please match each MPM imaging technique with some of its common inn vivo imaging applications

2-Photon Fluorescence Microscopy	Fluorescent dyes attached to various proteins, cell types

Microscopy	various proteins, cell types, ...
Second Harmonic Generation (SHG)	Collagen (types I and III), myocin, striated muscle fibers
Third Harmonic Generation (THG)	cellular membranes, lipid droplets, collagen bundles or muscle fibers

SUBMIT

THG Publications and References

1) Aptel, F., Olivier, N., Deniset-Besseau, A., Legeais, J.-M., Plamann, K., Schanne-Klein, M.-C. and Beaurepaire, E. (2010). Multimodal nonlinear imaging of the human cornea. Invest. Ophthalmol. Vis. Sci. 51, 2459-2465. https://doi.org/doi:10.1167/iovs.09-4586

2) Débarre, D., Supatto, W., Pena, A.-M., Fabre, A., Tordjmann, T., Combettes, L., Schanne-Klein, M.-C. and Beaurepaire, E. (2006). Imaging lipid bodies in cells and tissues using third-harmonic generation microscopy. Nat. Methods 3, 47-53. https://doi.org/doi:10.1038/nmeth813

3) Rehberg, M., Krombach, F., Pohl, U. and Dietzel, S. (2011). Label-free 3D visualization of cellular and tissue structures in intact muscle with second and third harmonic generation microscopy. PLoS ONE 6, e28237. https://doi.org/doi:10.1371/journal.pone.0028237

Complete the content above before moving on.

Brief Equipment Overview

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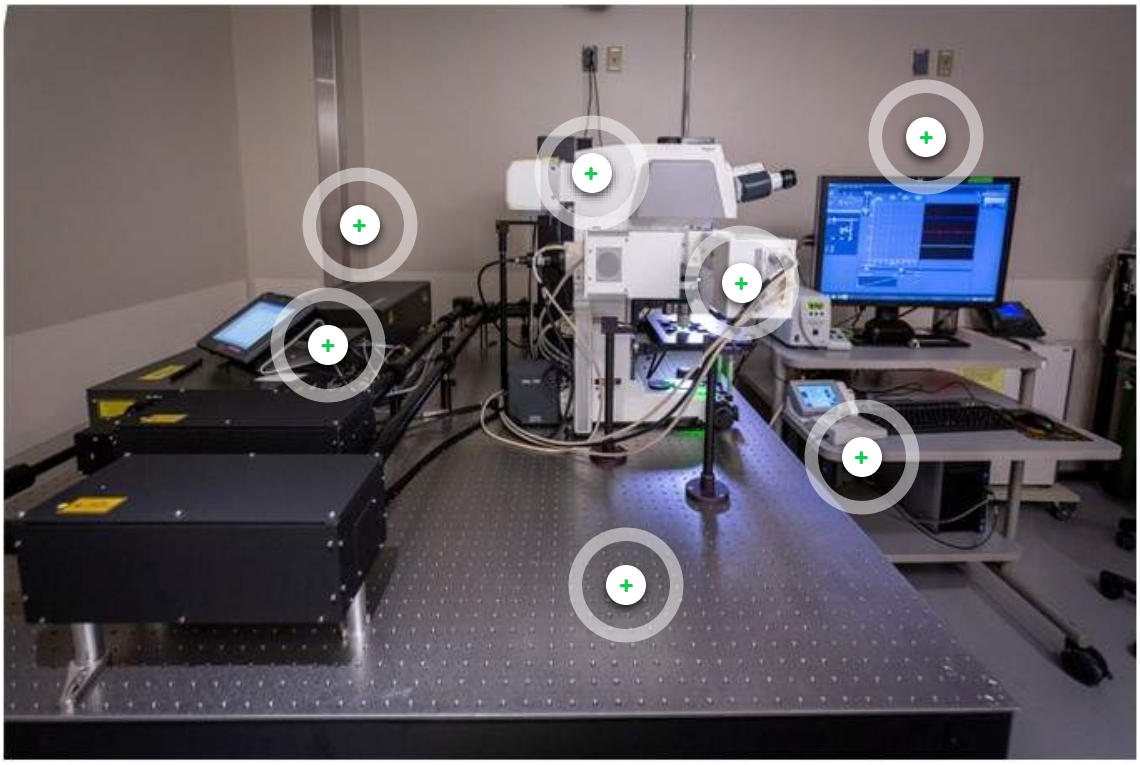
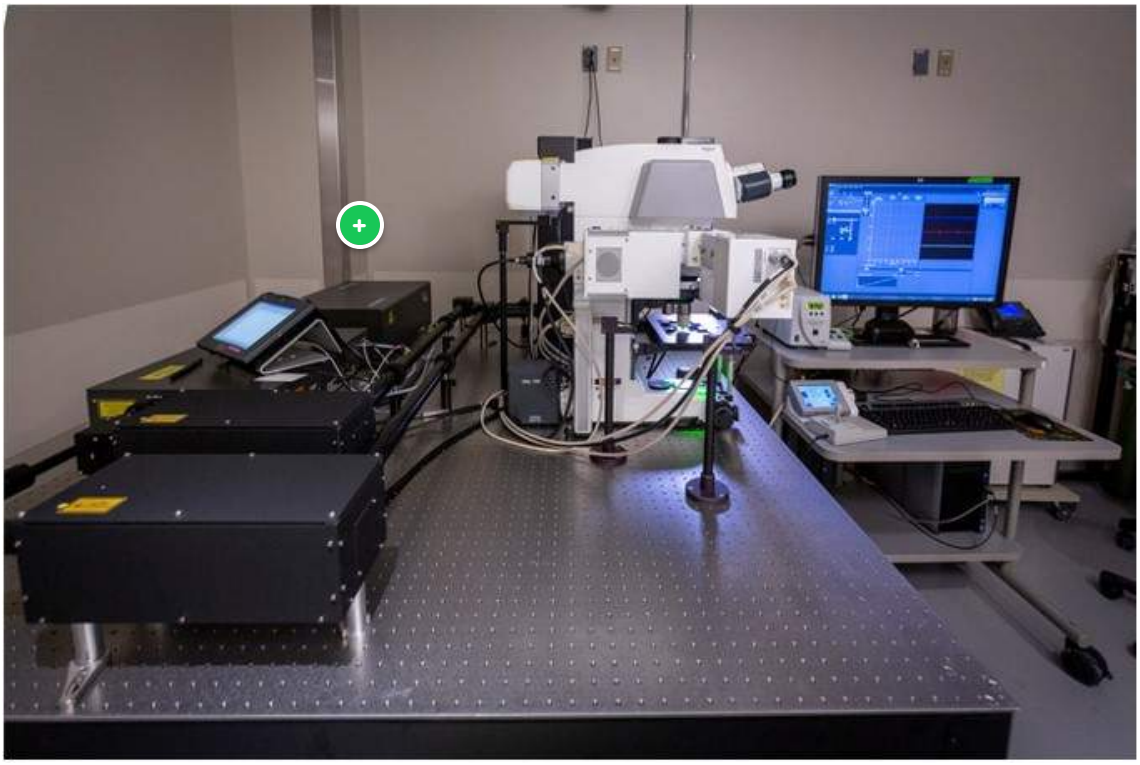
Multiphoton Microscope Equipment

To understand the scientific background behind MPM, it is helpful to be somewhat familiar with the equipment and components of the microscope system.

Click through each feature of the MPM equipment to understand some greater detail about each of the components.

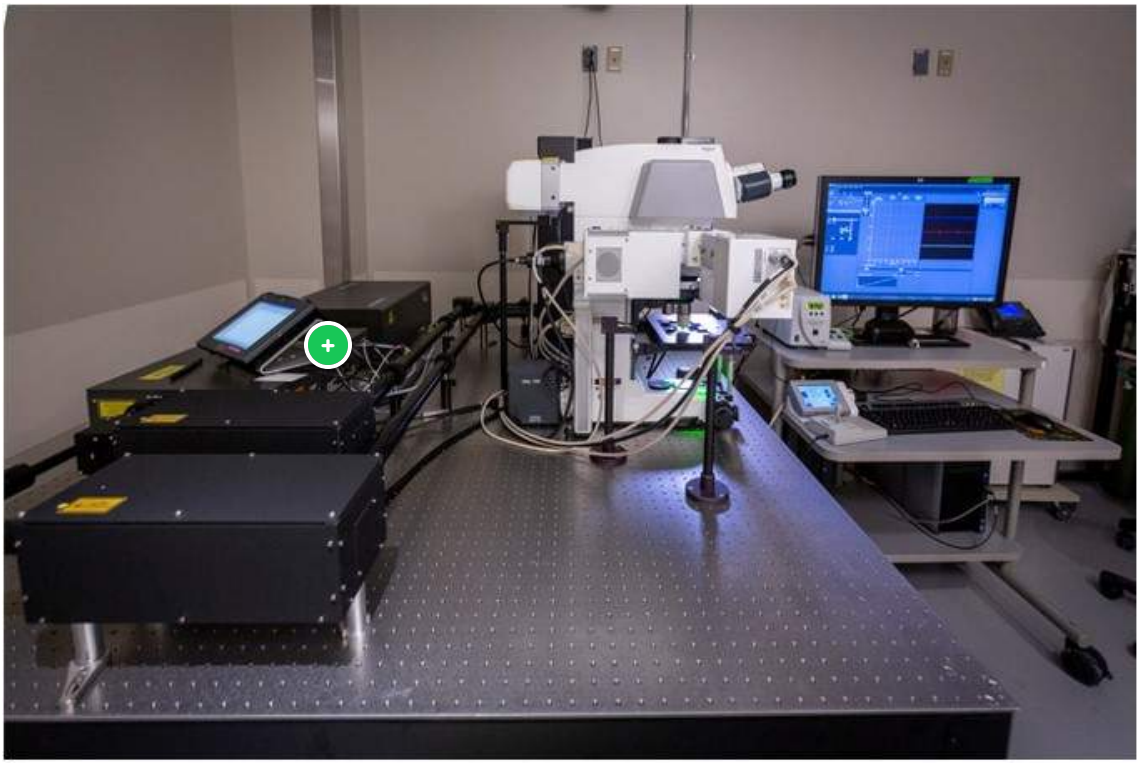




Chameleon Laser Output

Tunable laser spans 680 nm - 1080 nm

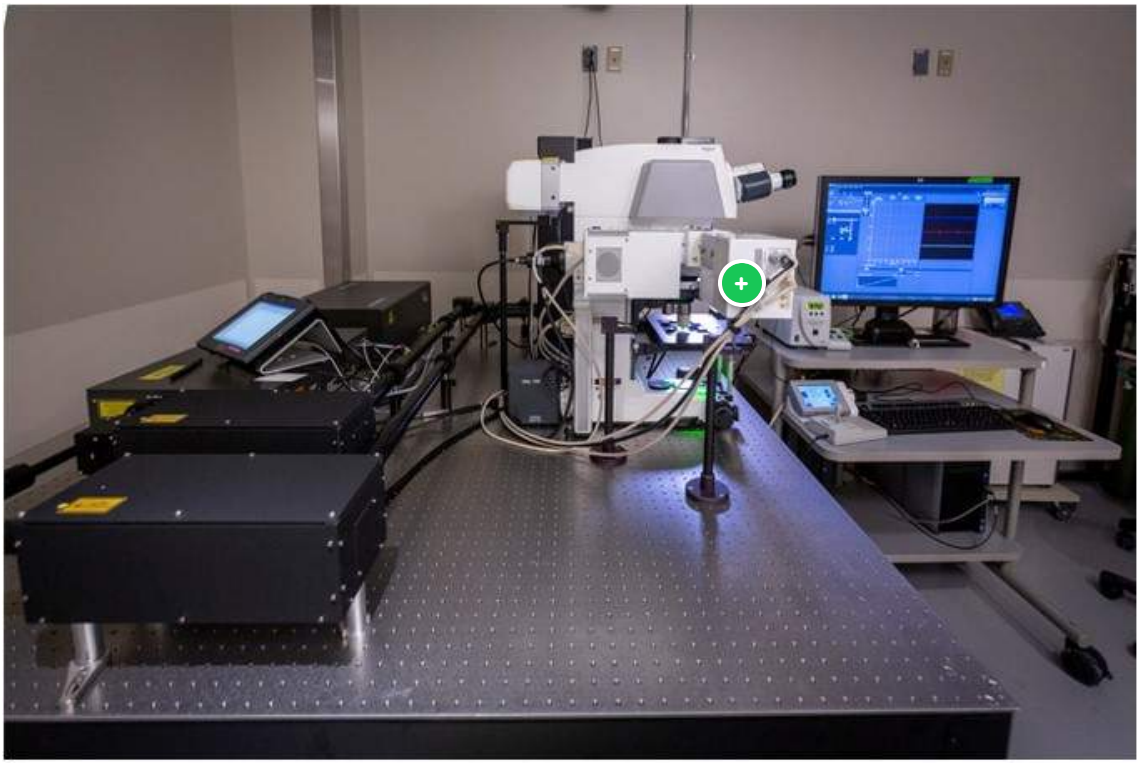
Can output up to 4 Watts of power so be mindful of laser safety when it is on!



OPO tablet and box

The Optical Parametric Oscillator (OPO) can take an input wavelength from the Chameleon laser and convert it into a different laser wavelength, at a longer range than the Chameleon's range.

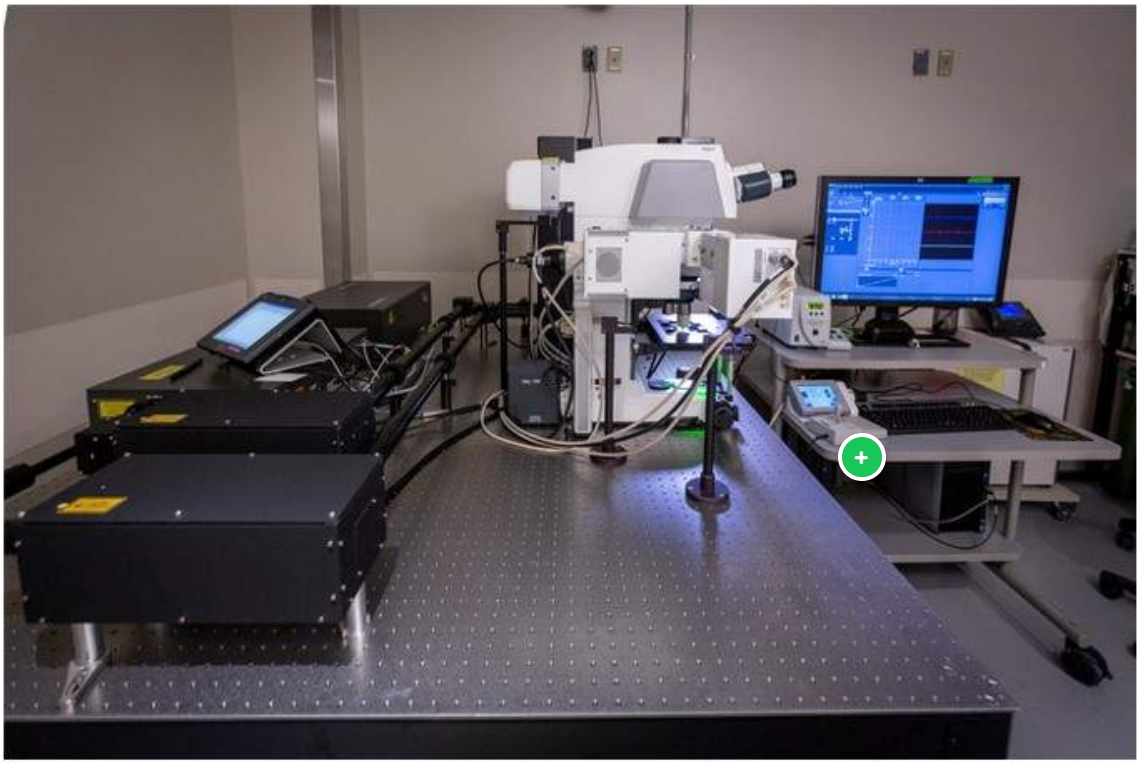
OPO output: 1080 nm - 1600 nm



Dectectors

Two BiG non-descanned detectors collect our light signal.

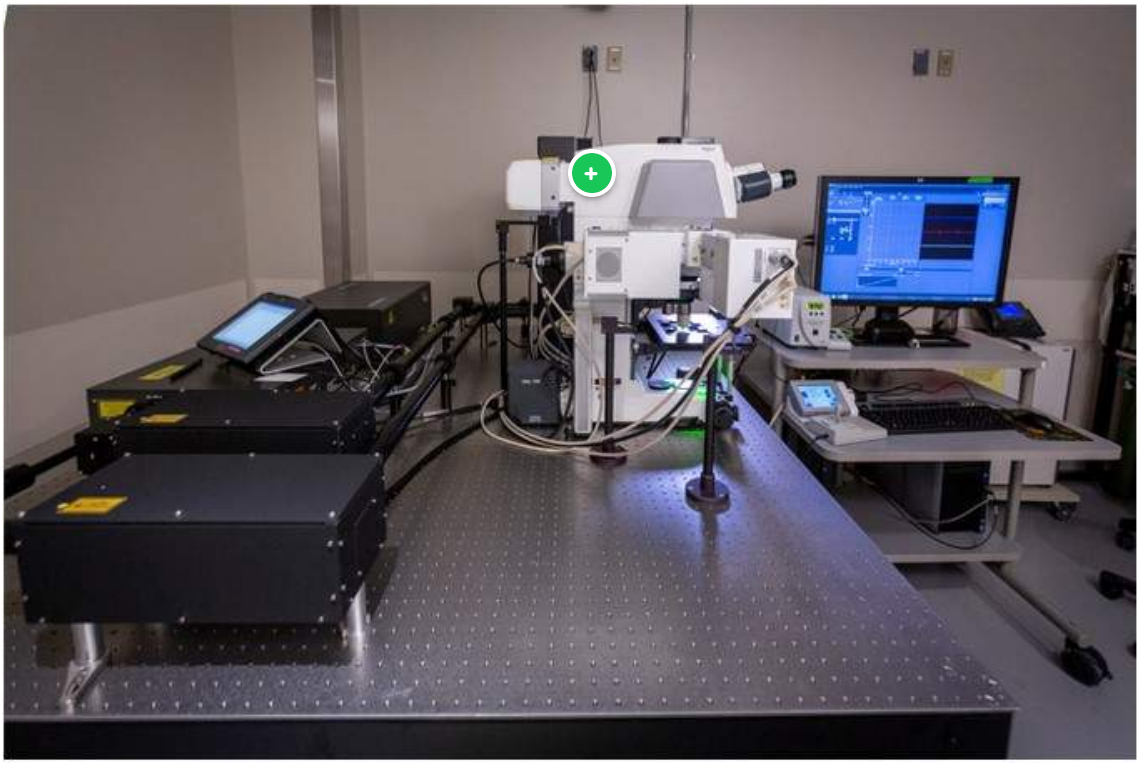
These cameras can detect very faint, dim light from weakly emitting signal sources. However, in order to do this, this means that **the BiG detectors are EXTREMELY light sensitive!**
The room lights should **never** be on when the laser is actively scanning. Room light will oversaturate and ***break*** the detectors!



Stage motor controls

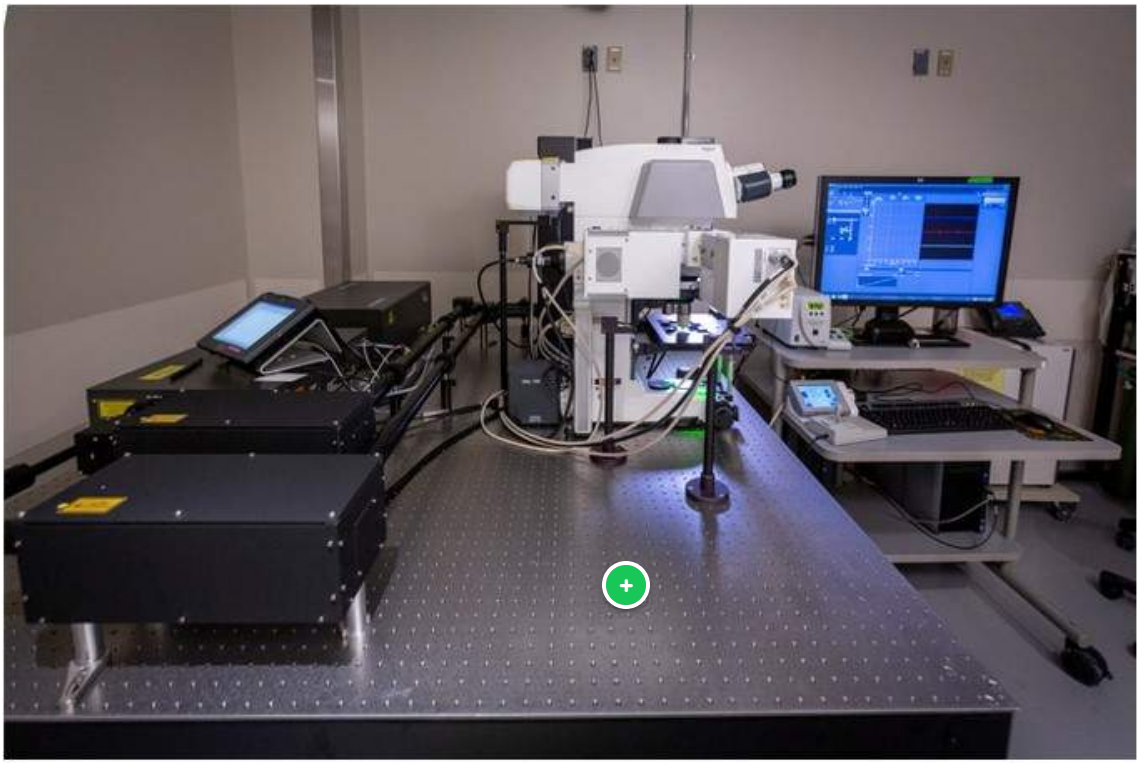
Joystick moves the stage in the X and Y directiions.

The objective controller interface can move the objective up and down (in the Z direction) and track the X, Y, and Z positions of where the sample is.



Microscope

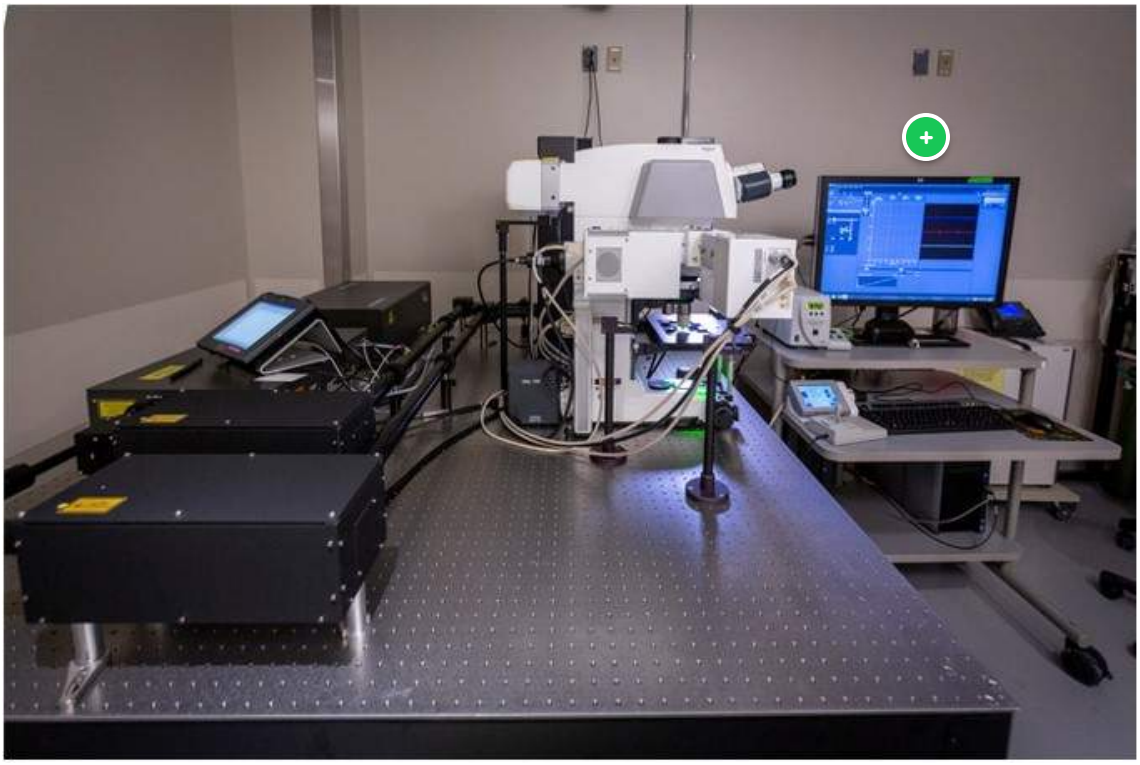
Zeiss LSM 7 MP, which is equipped with:
- a 20x water-immersion objective (1.0 NA)
-a 5x air objective (NA 0.25)
- Prior XY stage
- Brightfield lamp
- retroreflector
- filter sets



Floated Optics Table

The floated optics table is hooked up to pressurized air, which keeps the table even and flat, minimizes the effect of vibrations (stomps, bumps, etc) while in the midst of imaging a sample.

Please avoid leaning on the table, especially while scanning, as it can negatively affect the optical alignment of the OPO and other microscope components.



Computer assembly

Computer is the control center for imaging and operating the microscope.

It is connected to the internet so data can be transferred according to standard homelink data drive transfer methods .
USB drives are not allowed.

Using information given in the above graphic, sort the laser wavelength output ranges by what component of the MPM system they are outputted from:

Chameleon laser output wavelengths

680 nm - 1080 nm

OPO output wavelengths

1080 nm - 1600 nm

Please fill in the blank for the following two questions:

The BiG detectors are extremely _____ sensitive!

Type your answer here

SUBMIT

Because of this, I should NEVER acquire an image or set the laser to "Live" mode when the _____ are ON.

Type your answer here

SUBMIT

Complete the content above before moving on.

Do you need to use the OPO?

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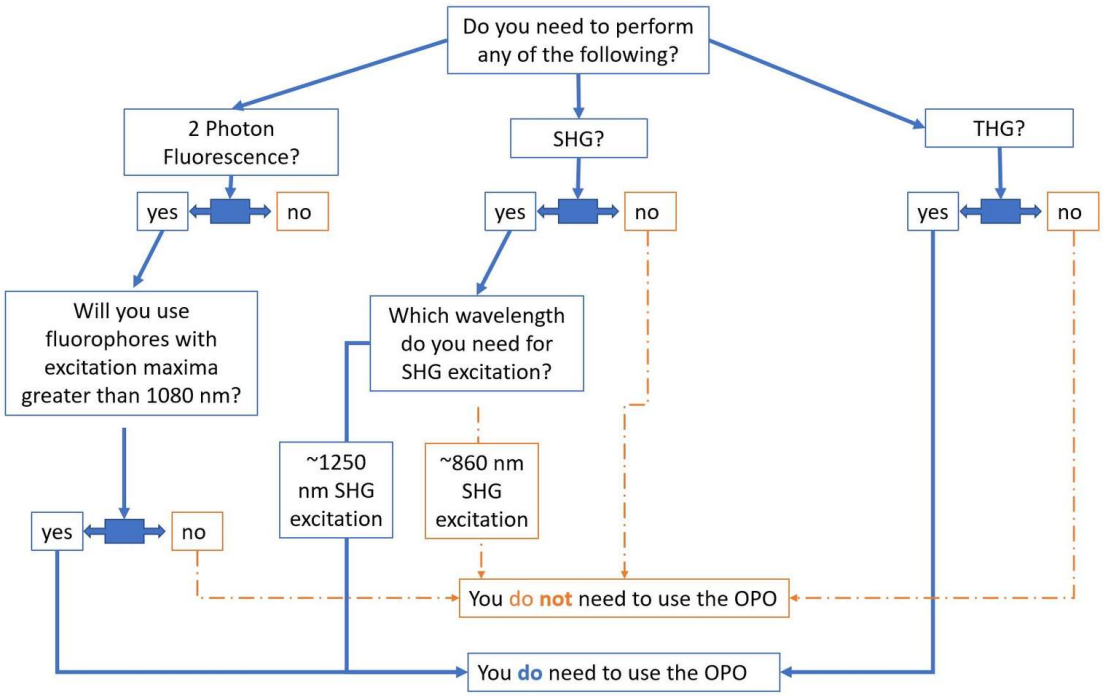
Do you need to learn to use the OPO?

Now that we are familiar with what the MPM equipment can do, and some of the different types of microscopy it can perform, before your in-person training you should determine if you will need to use the OPO for longer wavelength imaging.

The Optical Parametric Oscillator (OPO) is an add-on instrument that can output even longer laser wavelengths than what the Chameleon laser is capable. This is useful for certain kinds of 2PF, SHG, and necessary for all THG applications.

Please consult the below chart to determine if you need to use the OPO:



IF you are unsure what the excitation maxima of your fluorophore is, please visit the following website:

https://www.aatbio.com/fluorescence-excitation-emission-spectrum-graph-viewer - AAT Bioquest, Fluorescence Spectrum Viewer

1. On the left hand tool bar click "Add Fluorophore"
2. Search for the name of your fluorophore and select the correct one.
3. In the right hand tool bar under Physical properties, you should see a number for exitation (e.g. '489'). This is the excitation wavelength maximum (in nanometers).
4. Double this number (e.g. 489 * 2= 978). This resulting value is what you should reference when answering the above question.

If you DO need to use the OPO, please let the PCI staff member who does your in-person training know this.

Additionally when you complete the second online MPM training, there will be an additional instruction section on the OPO's operation.

If you **DO NOT** need to use the OPO, please let the PCI staff member who does your in-person training know this.

When you do the online instrument training you can skip the section on OPO operations.

However, if in the future you do need it you can retake this training and review that section.

CONTINUE

The Electromagnetic Spectrum

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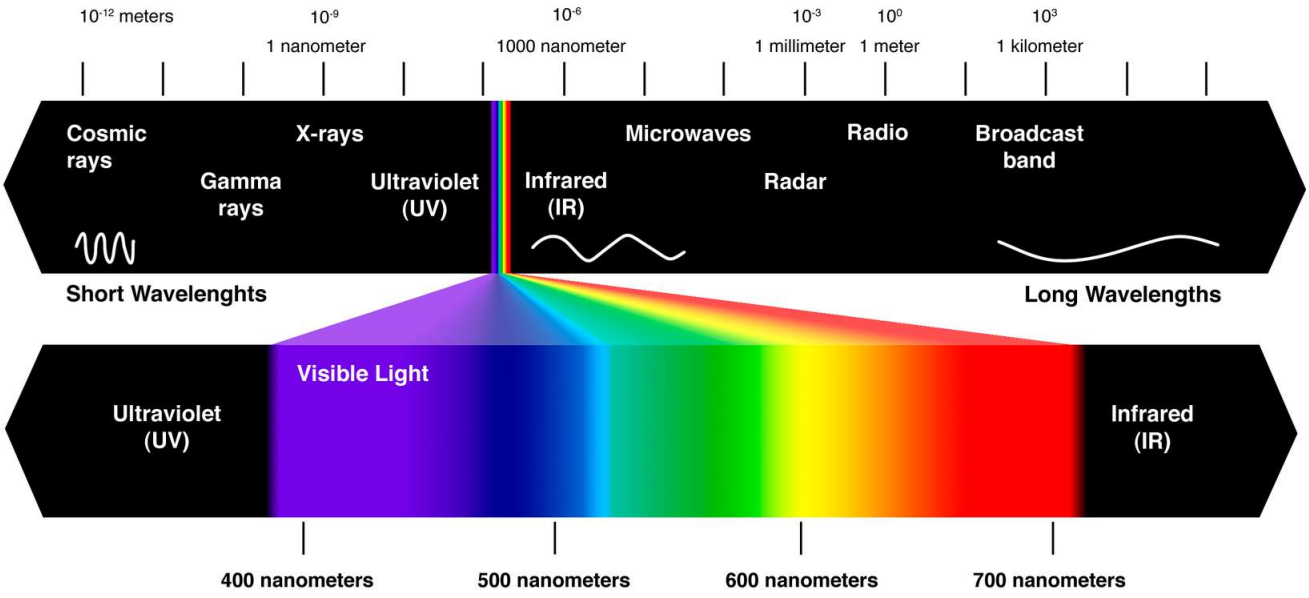
The Electromagnetic Spectrum of Light

Optical imaging methods rely on the detection of light, or photons, to form an image. Most optical imaging methods use photons within and around the visible spectrum of light, from 350 nm wavelength (ultra-violet) to 1300 nm (near-infrared).



Full Electromagnetic Spectrum. Optical imaging methods like fluorescence typically only use light at wavelengths between 350 nm (ultraviolet) to 1200 nm (near infrared).

Light's wavelength and energy are inversely proportional

The energy of a photon determines its wavelength.

Light's energy and wavelength values are inversely proportional.

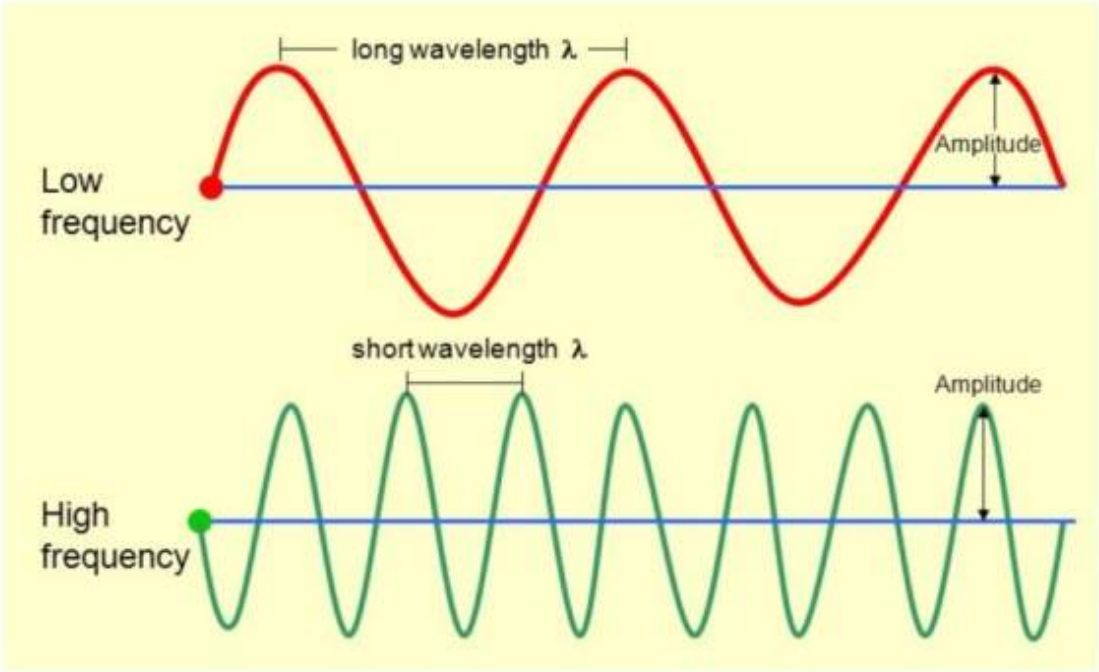


Diagram illustrating that the relationship between frequency and wavelength is inversely proportional.
image credit: https://www.vedantu.com/physics/frequency-and-wavelength

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This inverse relationship based on Einstein's equations:

$$\text{Energy} \propto \text{frequency}$$

OR

Energy ∝ 1/wavelength

So if a wavelength's value is higher in number, this means the light particle's energy (and thus, frequency) is lower.

For example, the wavelength 550 nm shows as the color green. 550 nm is lower in energy than 400 nm (which corresponds to the color blue). Based on the above equations, 550 nm has a lower frequency value, and thus is of a lower energy than 400 nm light.

Yellow light is higher in energy than infrared light

- ◯ True
- ◯ False

SUBMIT

Yellow light has lower frequency value than infrared light does

True

False

SUBMIT

Complete the content above before moving on.

Optical Microscopy overview

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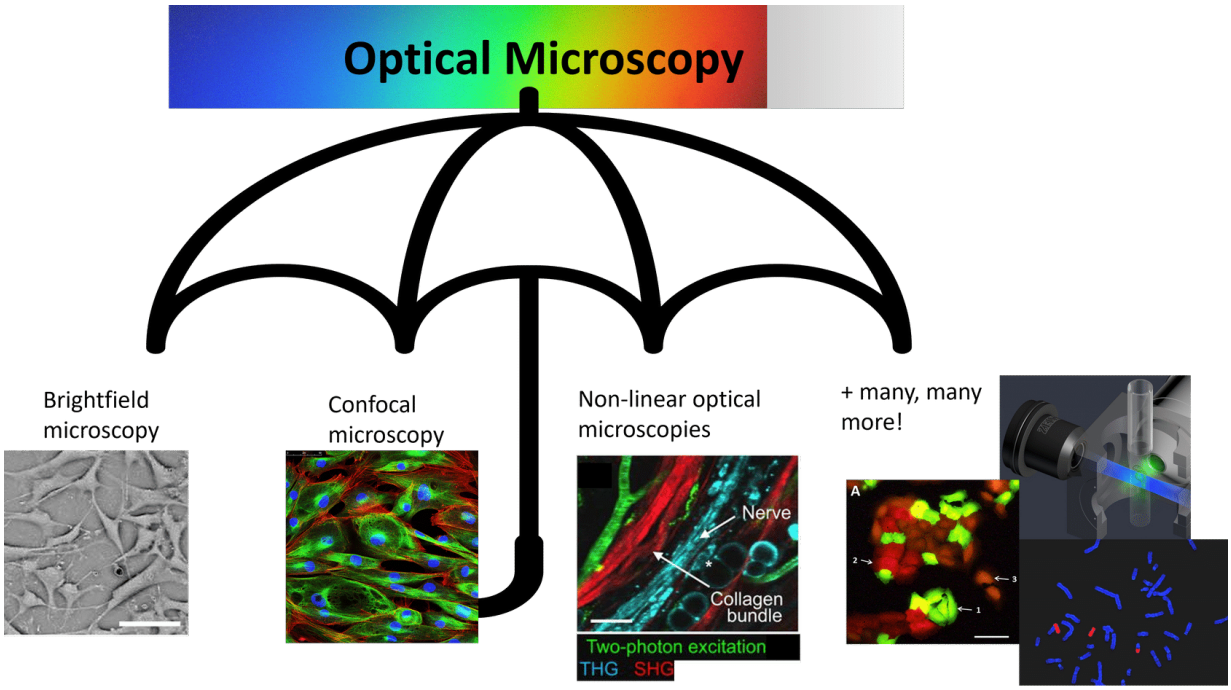
What is optical imaging? What is non-linear optical imaging?

There are many types of imaging and microscopy that rely on light to generate their images. These optical imaging methods include bright field, confocal fluorescence, bioluminescence, 2-photon fluorescence, SHG, and THG, in addition to many more applications.

Non-linear optical (NLO) microscopy relies upon longer wavelengths and higher powers of light, which can only be generated using lasers. There are many kinds of non-linear optical imaging microscopies. These include our multiphoton methods of 2PF, SHG, and THG.



Overview of some different types of optical microscopies. Note - we only can do *specific kinds* of NLO methods with our MPM!
Image credit: Elena Carlson, Preclinical Imaging Comp Med Fred Hutch Shared Resources

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Understanding the basics of NLO Microscopy for operating the Comparative Medicine Multiphoton Microscope

It is very important to understand some level of the scientific background behind how NLO microscopy works. This knowledge will help equip you to better design your studies, get better research outcomes, and to know what other information can be gathered from data you acquire on the Comparative Medicine MPM.

We will try to cover some NLO microscopy basics as are relevant to the MPM system. We will not discuss the underlying laser physics (but if interested, see the last module for further resources).

We will go step-by-step through light microscopy principles. Some of this won't be *directly* relevant to the CM MPM, but these principles are nonetheless essential for understanding NLO

microscopy applications with the CM MPM.

Complete the content above before moving on.

Fluorescence microscopy basics

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Microscopy and fluorescence fundamentals

Especially if microscopy is new to you, and if you are unfamiliar with fluorescence and why you may want to choose one probe versus another, watch the below video.

YOUTUBE

James Hayden - 2-Photon Microscopy: How Deep Can You Go? Part 1

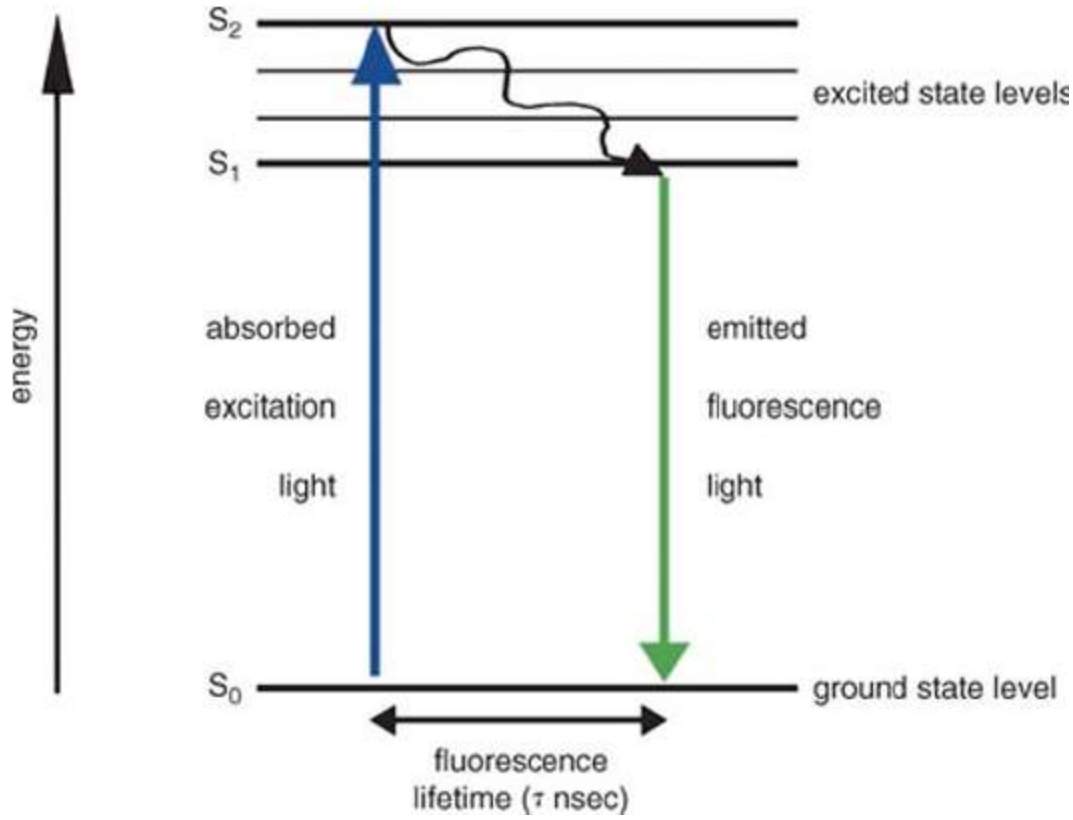
Widefield, fluorescence microscopy lets you see everything inyour biological sample, but a lot of it is out of focus, especiallyat higher magnifications. Con...

VIEW ON YOUTUBE >

Using some of the information we learned in the above video, we are going to go through some knowledge checks and a couple of examples to check that we understand fluorescence fundamentals.

Recall from the video that we need to **first excite** a fluorophore in order to **cause the emission** of a fluorescent photon. The energy lost in this process is responsible for the Stokes shift, and this is what causes a fluorophore's emission wavelength to be lower in energy than its excitation wavelength.

A good rule of thumb is that a dye's **excitation spectrum** tells you what **excitation laser wavelengths** to use. Its **emission spectrum** tells you what **detection filters** to use.



Jablonski diagram of a fluorescent process.

Please reference the above Jablonski Energy diagram.

What is/are the physical process(es) responsible for the shift in a fluorophore's excitation wavelength vs its emission wavelength?

- [ ] Conservation of energy
- [ ] Stokes shift

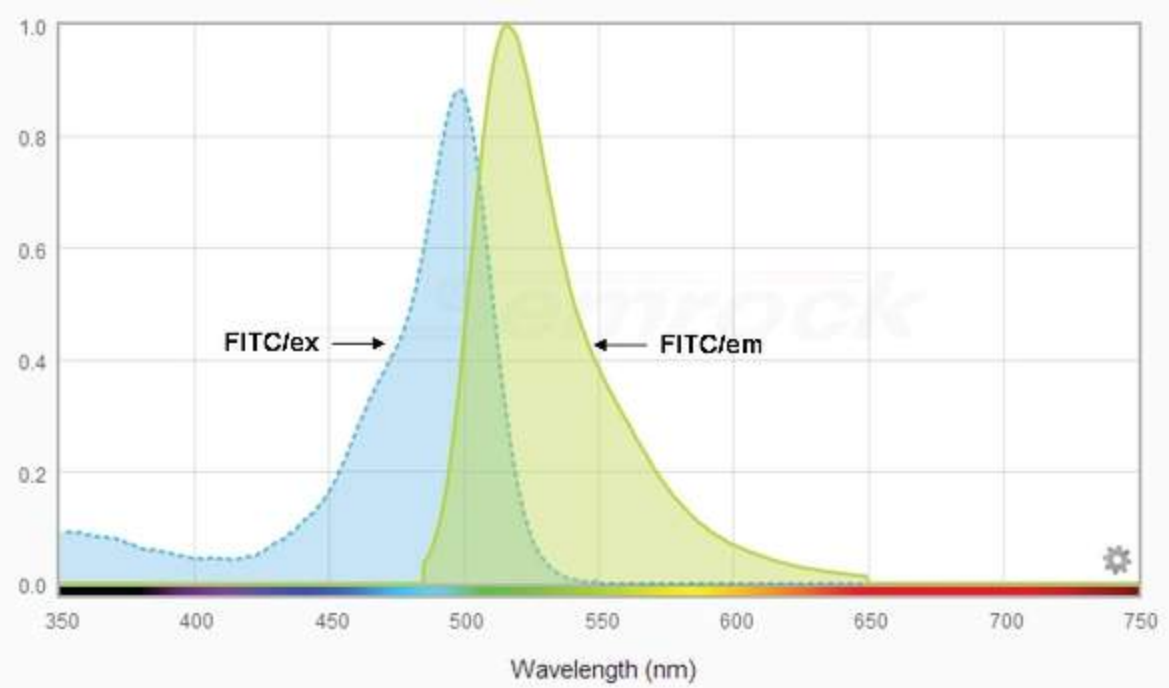
SUBMIT

What are some reasons that we need to use any detection filters in fluorescence imaging?

- [ ] All fluorescent dyes are too bright and could damage the camera, so we need to dim the light before it reaches the detector
- [ ] We need to filter out the laser excitation light, otherwise it will raise the background noise of our images and will make the actual fluorescent signal too difficult to detect

SUBMIT

Scenario 1: Determining confocal fluorescence imaging parameters for FITC fluorescent dye



Excitation (blue) and emission (green) spectrum for fluorescein isothiocyanate (FITC) dye.
FITC's excitation maximum: 491 nm ; emission maximum: 516 nm

Please reference the above image for the following knowledge check:

You want to image FITC-Dextran stained onto some tissue, using a confocal microscope (**not** the CM MPM).

What type of fluorescence excitation process will the FITC dye undergo on a confocal fluorescence microscope?

- ◯ One - photon excitation process

- ◯ Two-photon excitation process

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Using the above image which shows the excitation and emission spectrum of FITC-Dextran, what excitation laser source will be the best choice to get the most signal out of the dye in the tissue?

- ◯ 405 nm
- ◯ 488 nm
- ◯ 532 nm
- ◯ 640 nm

SUBMIT

Using the same figure above, what **filter** should you use to detect the most signal from FITC?

- ◯ 480/40 nm Bandpass filter (detects light from 460 nm to 500 nm)
- ◯ 525/40 nm Bandpass filter (detects light from 505 nm to 545 nm)

SUBMIT

Complete the content above before moving on.

the MPM in vivo advantage

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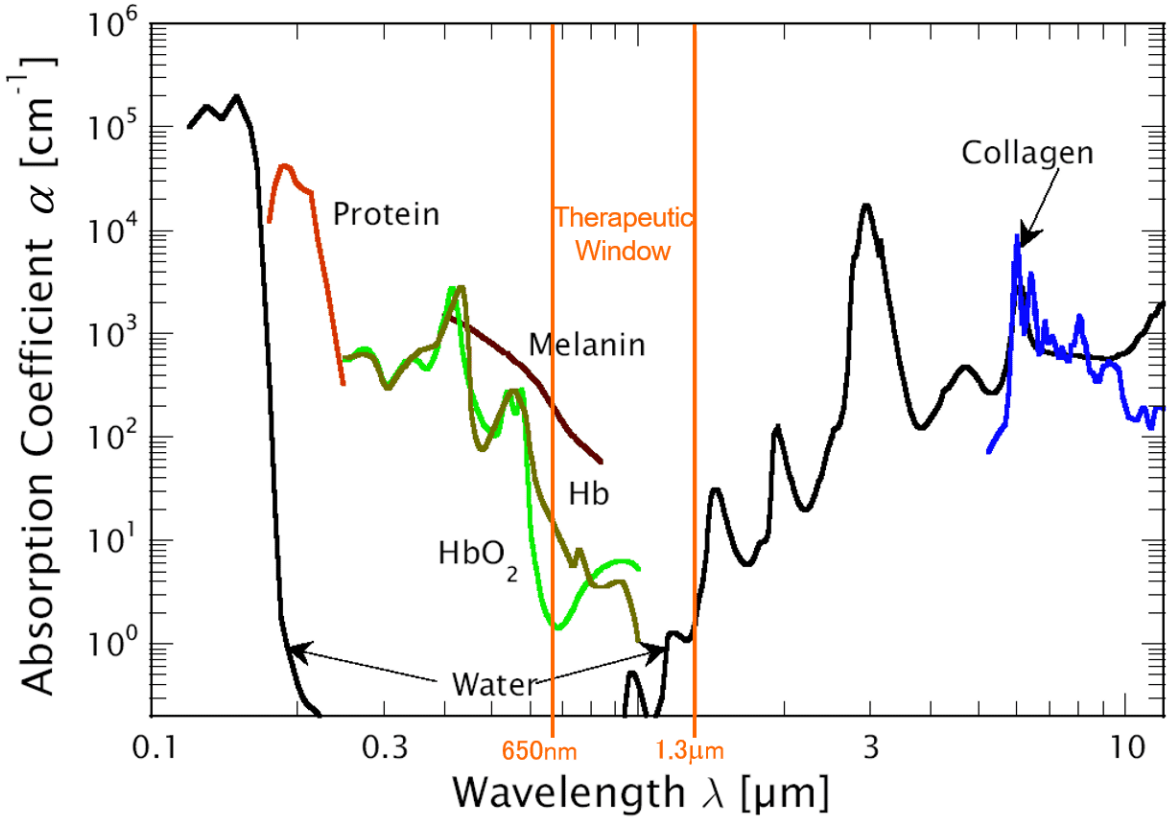
Why do MPM methods offer in vivo imaging advantages?



Absorption Spectrum of Human Tissue

plot credit: Warren S. Warren, Princeton

text credit: Dr. Rick Trebino; Non-linear Ultrafast Optics lectures (found at https://frog.gatech.edu/lectures.html)

Here are absorption spectra of various components of human tissue from the UV to the IR.

You can see that visible light doesn't penetrate very far into human tissue, which, of course, you already knew. After all, we can't see through each other. So if you'd like to see into the body, that's bad news.

Specifically, an absorption coefficient of 100/cm means that light of the relevant wavelength penetrates only about a hundredth of a cm into tissue.

Of course, x-rays aren't absorbed as strongly and penetrate much deeper and so are quite useful. But x-rays can be dangerous, and they don't see everything your doctor might want to see.

On the other hand, from this plot, we can see that there's some hope for wavelengths near 1 micron (1000 nm)

And advanced techniques using lasers are being developed using such wavelengths.

Rick Trebino, Non-linear Ultrafast Optics

With this in mind, 2-photon fluorescence is great for our purposes, as the "therapeutic window" shown in the above image covers wavelengths that our Chameleon laser + OPO works perfectly within and that work with most commercial fluorophores and dyes!

Additionally, for mouse imaging and in vivo applications - these longer wavelengths also travel farther into tissue, so we can see more of the tissue we are interested in studying. Longer wavelengths of light scatter *less* than shorter wavelengths of light do (this is also the reason the sky is blue, by the way..).

This is just one reason that MPM works so well for in vivo imaging. Please watch the second video below to understand better how and why this works.

YOUTUBE

Jamie Hayden - 2-Photon Microscopy: How Deep Can You Go? part 2

Widefield, fluorescence microscopy lets you see everything inyour biological sample, but a lot of it is out of focus, especiallyat higher magnifications. Con...

VIEW ON YOUTUBE >

Fill in the blank:

Compared with a 1-photon fluorescence process, two-photon fluorescence uses two photons with wavelengths approximately _____ the frequency of just 1 -photon to excite the same fluorophore.

Type your answer here

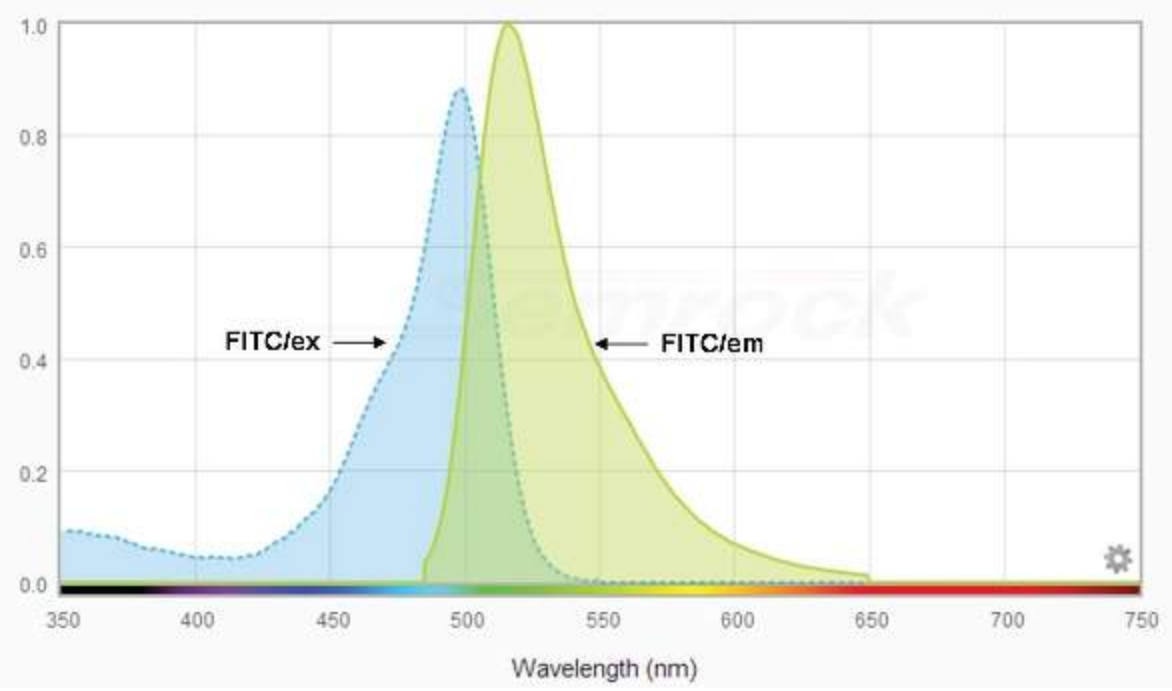
SUBMIT

Revisiting our scenario

Recall our earlier example where we wanted to image FITC dye on a confocal microscope (in other words, with 1-photon fluorescence).

Now, we want to image this same dye using our 2-photon microscope.

Below is the 1-photon excitation and emission spectra of FITC, the same image as was given in the previous example.



Excitation (blue) and emission (green) spectrum for fluorescein isothiocyanate (FITC) dye.
FITC's 1-photon excitation maximum: 491 nm emission maximum: 516 nm

Around approximately what excitation wavelength should we use to excite the dye via a *2-photon* excitation process?

- ○ 491 nm
- ○ 516 nm
- ○ 982 nm
- ○ 1032 nm

SUBMIT

How will the FITC dye's *emission* wavelength change now that we are using a 2-photon fluorescence process, rather than a 1-photon fluorescence process?

- ○ The dye's emission wavelength will decrease

- The dye's emission wavelength will be about the same
- The dye's emission wavelength will increase

SUBMIT

Complete the content above before moving on.

putting it all together: THG, SHG, 2PF example

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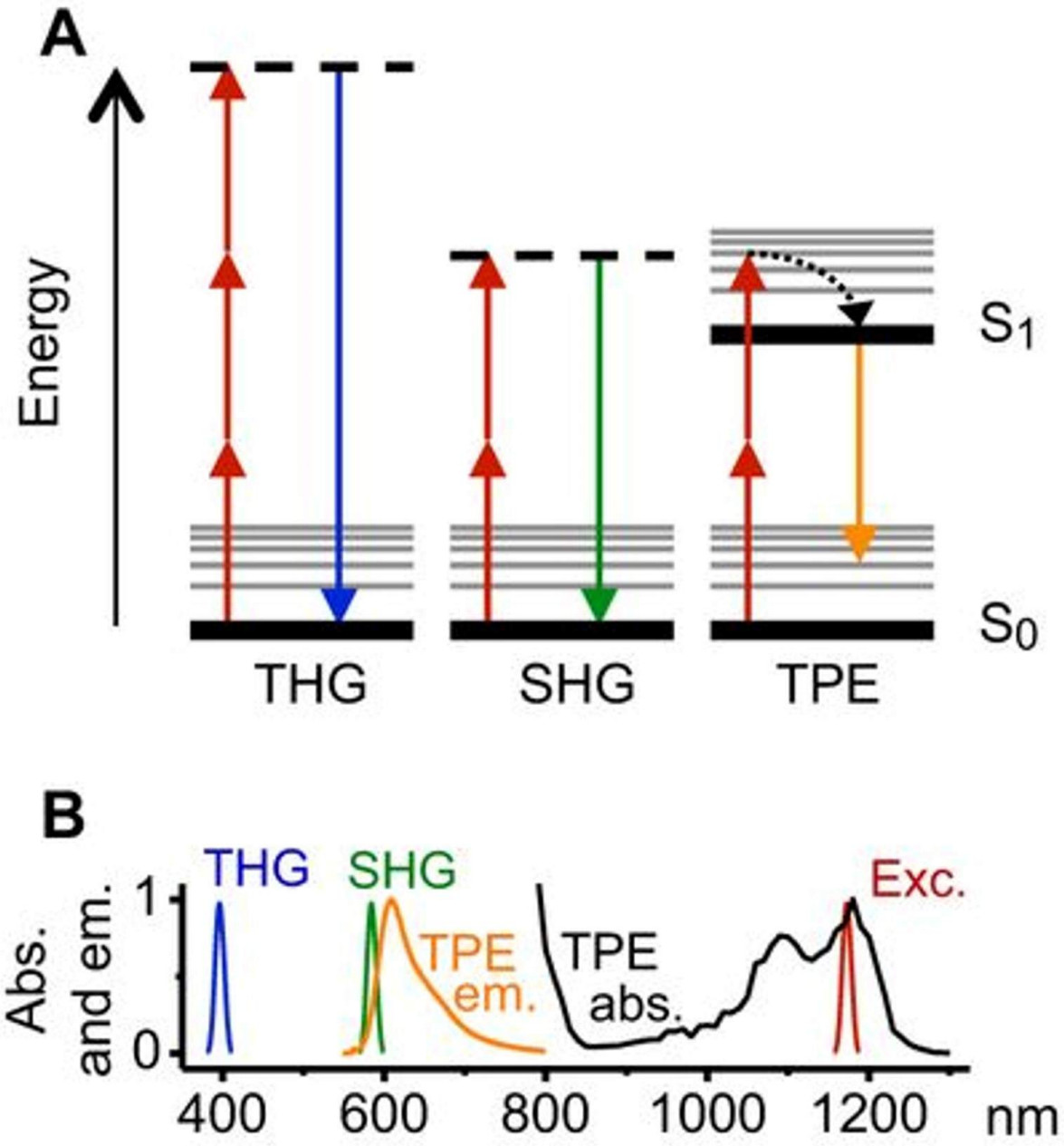
Example of using all three non-linear optical methods simultaneously together at 1200 nm wavelength excitation



Principles of THG signal generation. Excitation (Exc.) is shown in red and other colors refer to emission. (A) Jablonsky diagram of THG, SHG and two-photon fluorescence excitation (TPE). Excitation with the same wavelength results in signals of a distinct emission wavelength. Dashed lines, virtual states. S_0 and S_1, ground-level and first-excited electron state, respectively, with vibrational levels (thin lines). (B) Absorption (abs.) and emission (em.) spectra of THG, SHG and two-photon excitation (mCherry is shown here), excited with 1180-nm light and scaled to maximum absorption and emission.

Figure and text adapted from: Bettina Weigelin,Gert-Jan Bakker,Peter Friedl, Third harmonic generation microscopy of cells and tissue organization, J Cell Sci, 2016, Fig. 1. https://journals.biologists.com/jcs/article/129/2/245/55706/Third-harmonic-generation-microscopy-of-cells-and

Under the right excitation conditions and with the correct selection of fluorophore, simultaneous imaging like this can be done, but experimental planning is key!

CONTINUE

Further Resources

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Further resources

1. For a much more thorough, physical explanations of **non-linear optical microscopy and lasers**, we *highly* recommend looking further into the published lectures of Prof. Rick Trebino (Georgia Institute of Technology). https://frog.gatech.edu/talks.html

    a. For comparing and proving the properties of 1-photon versus 2-photon fluorescence microscopy, see his basic Optics course, lecture 27 on Ultrafast Optics.

    b. How lasers work: Ultrasfast optics, Lecture 2: Pulse Generation

2. AAT Bioquest has a helpful **Fluorescence Spectrum Viewer**, useful for experiment planning and which sometimes has other pertinent information such as fluorophore quantum yield and extinction coefficients. https://www.aatbio.com/fluorescence-excitation-emission-spectrum-graph-viewer

3. Fluorescence and Phosphorescence fundamentals: https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Electronic_Spectroscopy/Fluorescence_and_Phosphorescence

4. Jablonski energy diagrams: https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_%28Physical_and_Theoretical_Chemistry%29/Spectroscopy/Electronic_Spectroscopy/Jablonski_diagram

Click below if you would like to download a PDF printout of this training to keep on-hand for future reference

PDF

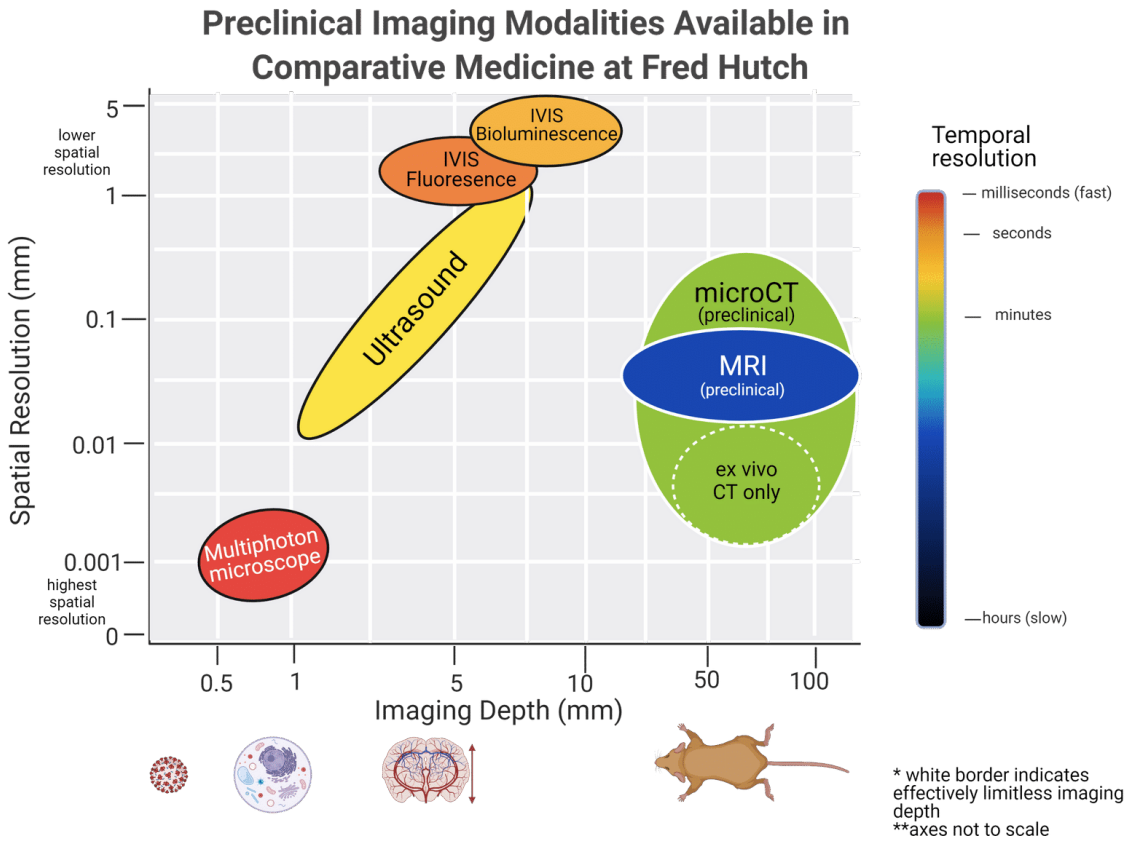
multiphoton-microscopy-online-training-part-1-overview-and-theory-RRp6dgmg.pdf

7 MB

Have questions or want further info?

Preclinical Imaging offers an array of imaging services, including one-on-one consultations and analysis advice!

See below charts for further information regarding the services provided by Preclinical Imaging and feel free to send an inquiry to us at preclinicalimaging@fredhutch.org.



Different imaging modalities available in Preclinical Imaging.

Thank you for completing this part of the MPM training!

You will need to complete an in-person component of this training before being able to access the Multiphoton Microscope. Please contact preclinicalimaging@fredhutch.org with any questions or feedback regarding this training.

CONTINUE