FRET at the ICBM

- Q: Can FRET be done at the ICBM?
- A: Yes, FRET can be done at the ICBM.
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- Q: Which microscope system should I use for FRET?
- A: Any of the systems at the ICBM can be used for FRET. Which system is best for you depends on the exitation and emission spectra of your donor and acceptor pair and whether or not you are using live cells for your FRET experiments. You should take what hardware is available into consideration during the planning phase for your FRET experiments.
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- Q: Can I see an example of FRET done at the ICBM?
- A: Yes. Recently a student in our Biomolecular Imaging Program class G613, Advanced Biomolecular Imaging, characterized single molecule FRET using a CFP/YFP donor/acceptor pair on two of our systems, the Spinning Disc system and the Zeiss 510 LSM Meta system. Her results can be seen here. Exitation and emission spectra for CFP and YFP and the exitation and emission filters available on the Spinning Disc system and the Zeiss 510 Meta system can be seen below.
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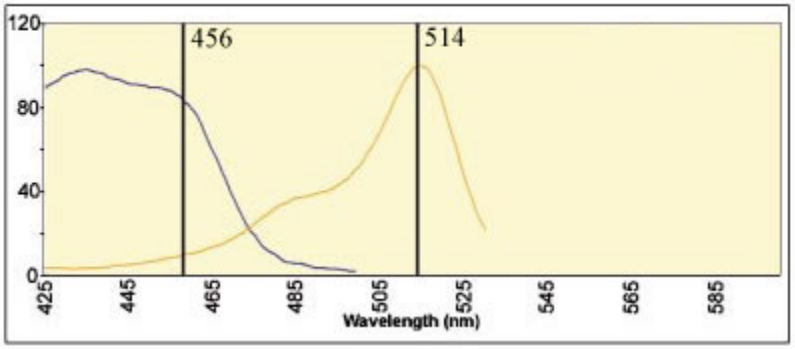
Fluorescence resonance energy transfer (FRET) is the radiationless transfer of energy from a donor fluorophore to an acceptor fluorophore. For FRET to occur, the emmision spectra of the donor fluorophore must overlap significantly with the exitation spectra of the acceptor fluorophore, the distance between the donor and acceptor must be less than 10 nm and they must be oriented in a way that favors the energy transfer.

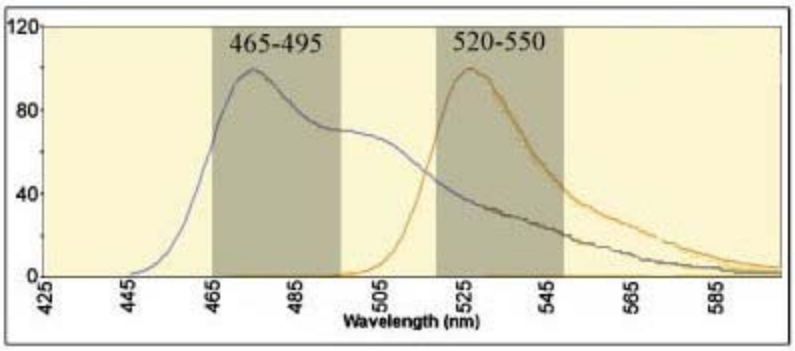
CFP and YFP are one commonly used donor and acceptor pair for FRET. Shown below are the exitation and emission spectra of CFP and YFP, overlaid with the laser lines and emission filters available for use with CFP and YFP at the ICBM. Appropriate laser lines and emission filters are available for the CFP/YFP FRET pair on the Spinning Disk system and the Zeiss 510 LSM Meta system. The Spinning Disk system is mounted on an inverted microscope and is suitable for live cell or fixed cell experiments. The Zeiss 510 LSM Meta system is mounted on an upright microscope and is better suited to fixed cell work.

A student in our Biomolecular Imaging Program class G613, Advanced Biomolecular Imaging, characterized single molecule FRET on the Spinning Disc system and the Zeiss 510 LSM Meta system. Her results can be seen here. The student FRET project utilized a previously well characterized single molecule probe tagged with CFP and YFP (Mochizuki et al 2001). Both sensitized emission FRET experiments and acceptor photobleaching FRET experiments were done on each system.

ICBM Zeiss LSM 510 Meta

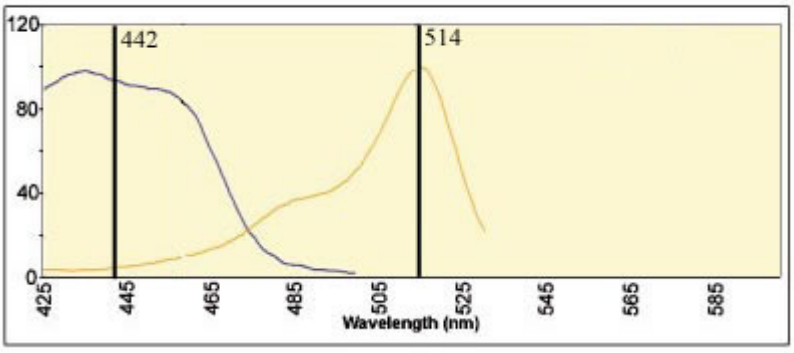


Exitation spectra for CFP and YFP with the appropriate laser lines available on the Zeiss LSM 510 Meta overlaid.

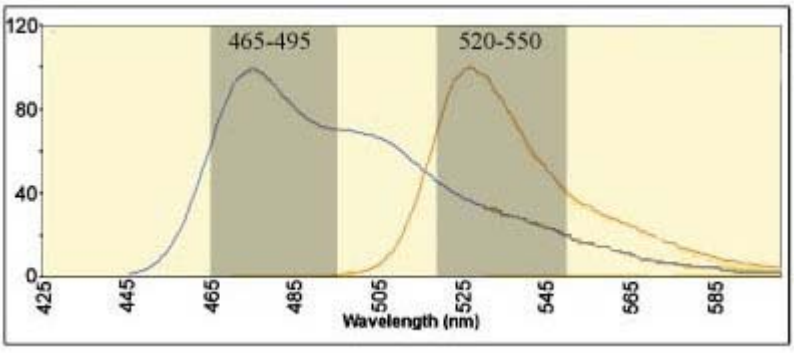


Emission spectra for CFP and YFP with the appropriate emission filters available on the Zeiss LSM Meta overlaid.

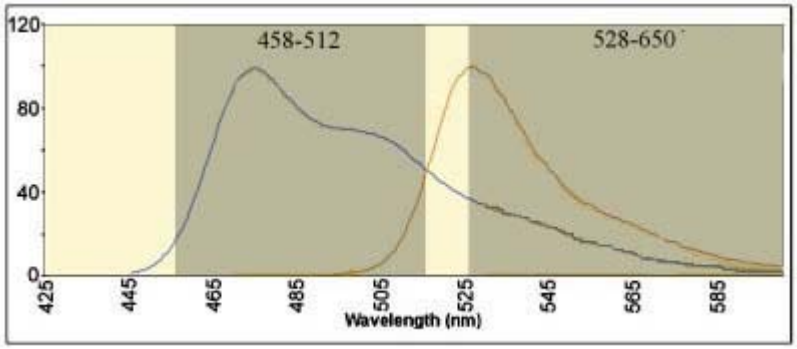
ICBM Spinning Disk System



Exitation spectra for CFP and YFP with the appropriate laser lines available on the Spinning Disk system overlaid.



Emission spectra for CFP and YFP with the appropriate filter sets available on the Spinning Disk system for FRET overlaid.



Note that there are two sets of CFP and YFP emission filters on the Spinning Disk system. Use the narrow band pass filters for FRET experiments to minimize crosstalk.