Deep Ultraviolet Excitation of Fluorescent Proteins for Multi-Color Imaging

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Studying a cell's organelles is important for understanding many biological processes. Currently, conventional fluorescence microscopes observe different organelles and proteins simultaneously with multicolor fluorescent protein (FP) labeling. However, the number of available colors in the simultaneous observation is limited because each FP requires a different excitation wavelength. Because most FPs share an excitation wavelength peak at 280nm while maintaining distinguishable emission wavelengths, deep ultraviolet (DUV) excitation may be a preferred alternative for cell imaging. We focus on the ability to simultaneously image multiple organelles in a cell sample by DUV excitation. We transfected FPs staining specific organelles in a cell sample. We focus on three fluorescence proteins: Green FP (GFP), Red FP (RFP), and Cyan FP (CFP), which emit light in different wavelength ranges for the Golgi body, mitochondrion, and nucleosome, respectively. Next, we developed a DUV-excitation microscope that measures fluorescence emission in the visible spectral range resulting from the excitation of FPs. The sample is irradiated with a DUV laser beam focused by an NA=1.35 objective lens and emits visible light that is collected and guided by lenses to a spectrometer. The spectrometer records the intensity of light received in the range between 300 and 700 nm. To get images, the sample is scanned in the X or Y direction over the focal spot with a step of 300 nm after each recording. By sampling fluorescence signals from a DUV laser focused over a cell, we obtained a hyperspectral FP image of the cell.

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DUV Excitation for Imaging Cells

Fluorescent Protein (FP) Imaging

- Study of cell's organelles important to understand biological processes
- FPs transfected in cells distinguish specific organelles

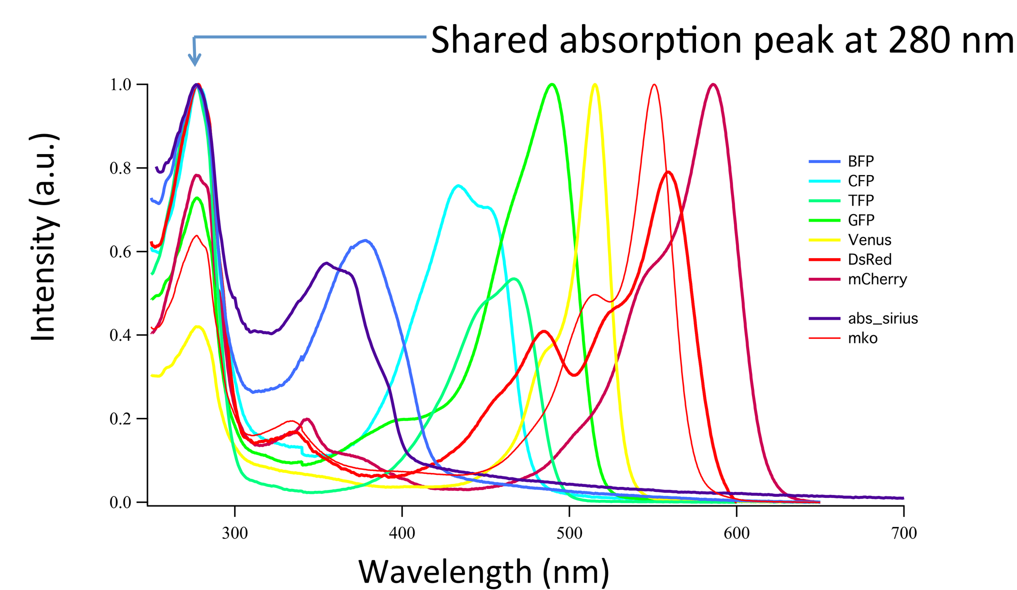
Visible Light Excitation

- Fluorescence microscope uses visible light excitation
- FPs have differing excitation wavelengths in visible light range

→ no simultaneous excitation

Deep Ultraviolet (DUV) Excitation

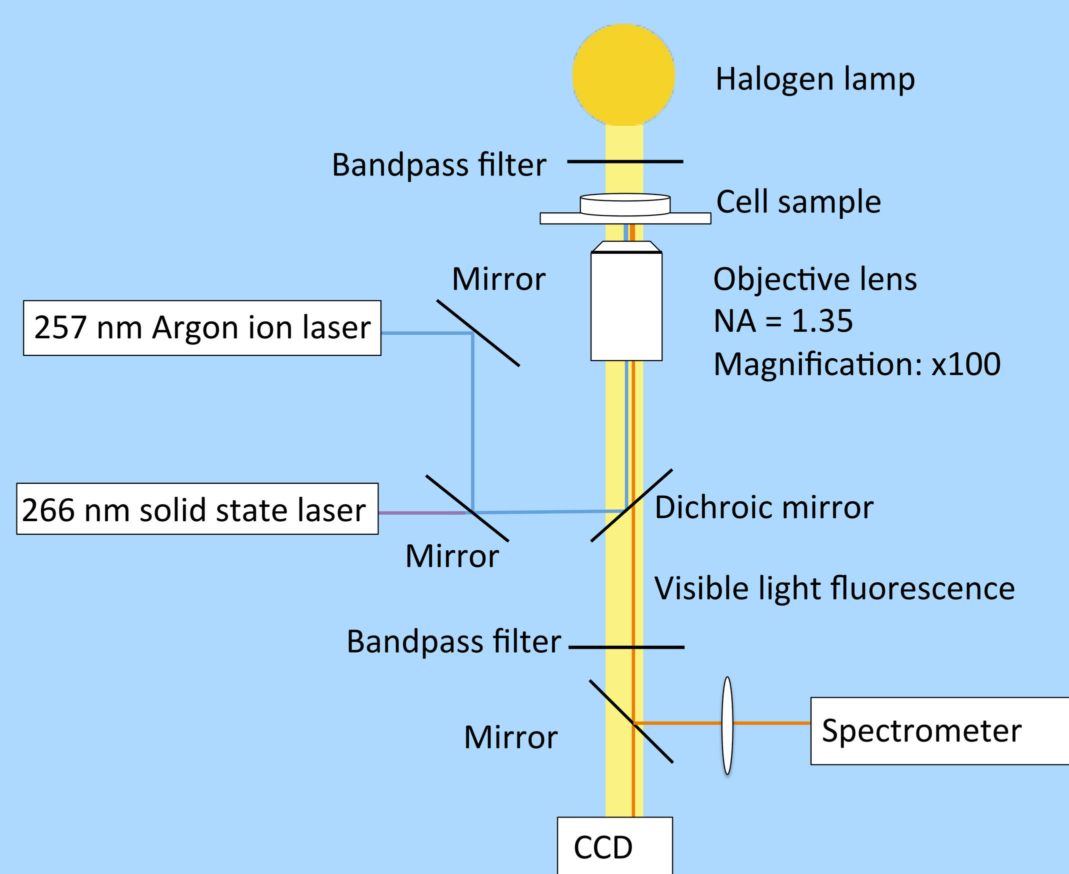
- DUV radiation wavelength: < 350 nm
- Many FPs share absorption peak in DUV range
- Possible simultaneous excitation



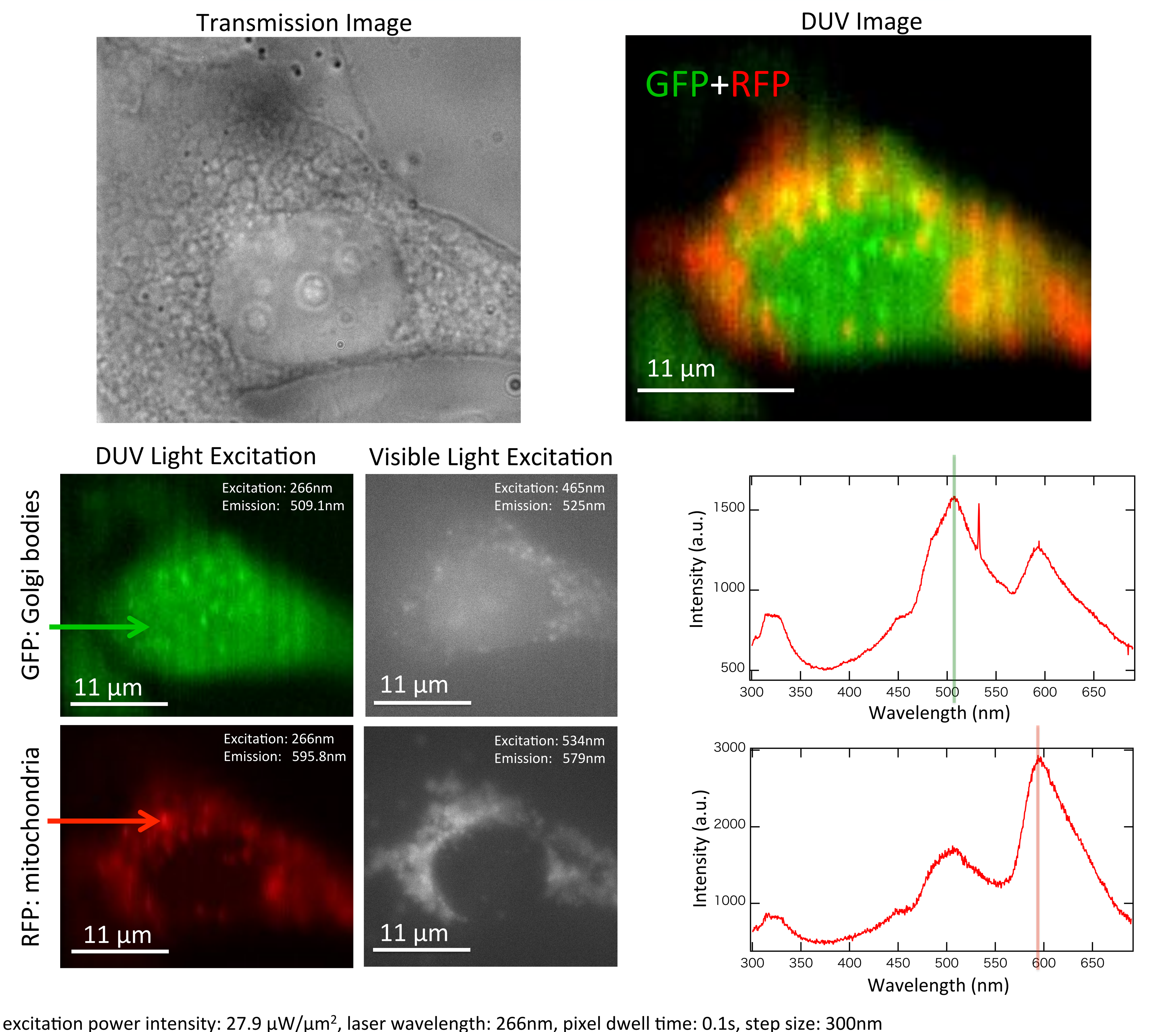
Goal:

- To obtain DUV images of multiple FPs simultaneously
- To study optical properties of FPs in order to distinguish them

Optical Setup for Obtaining Images

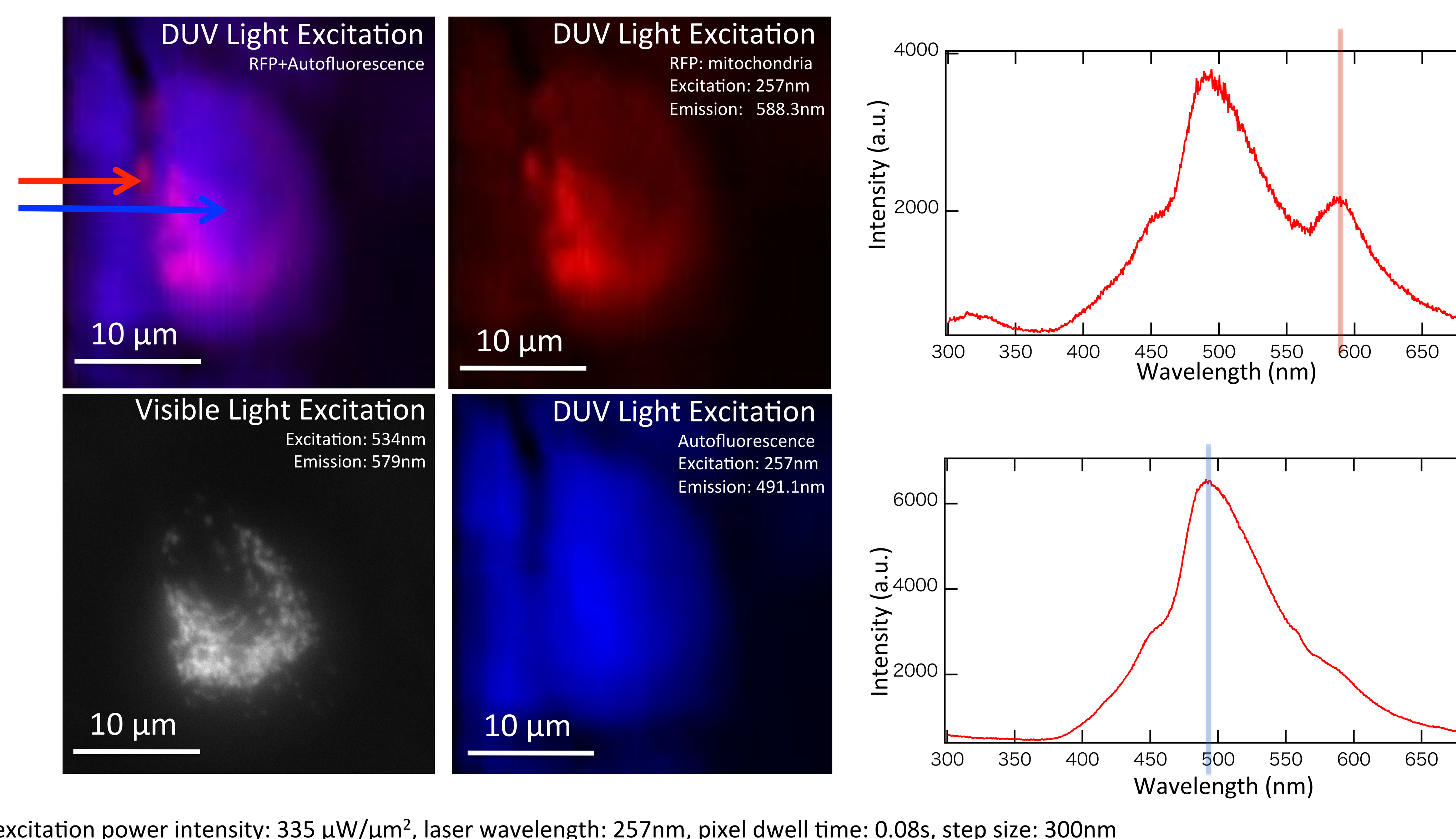


Simultaneous 2-Color Imaging: GFP, RFP



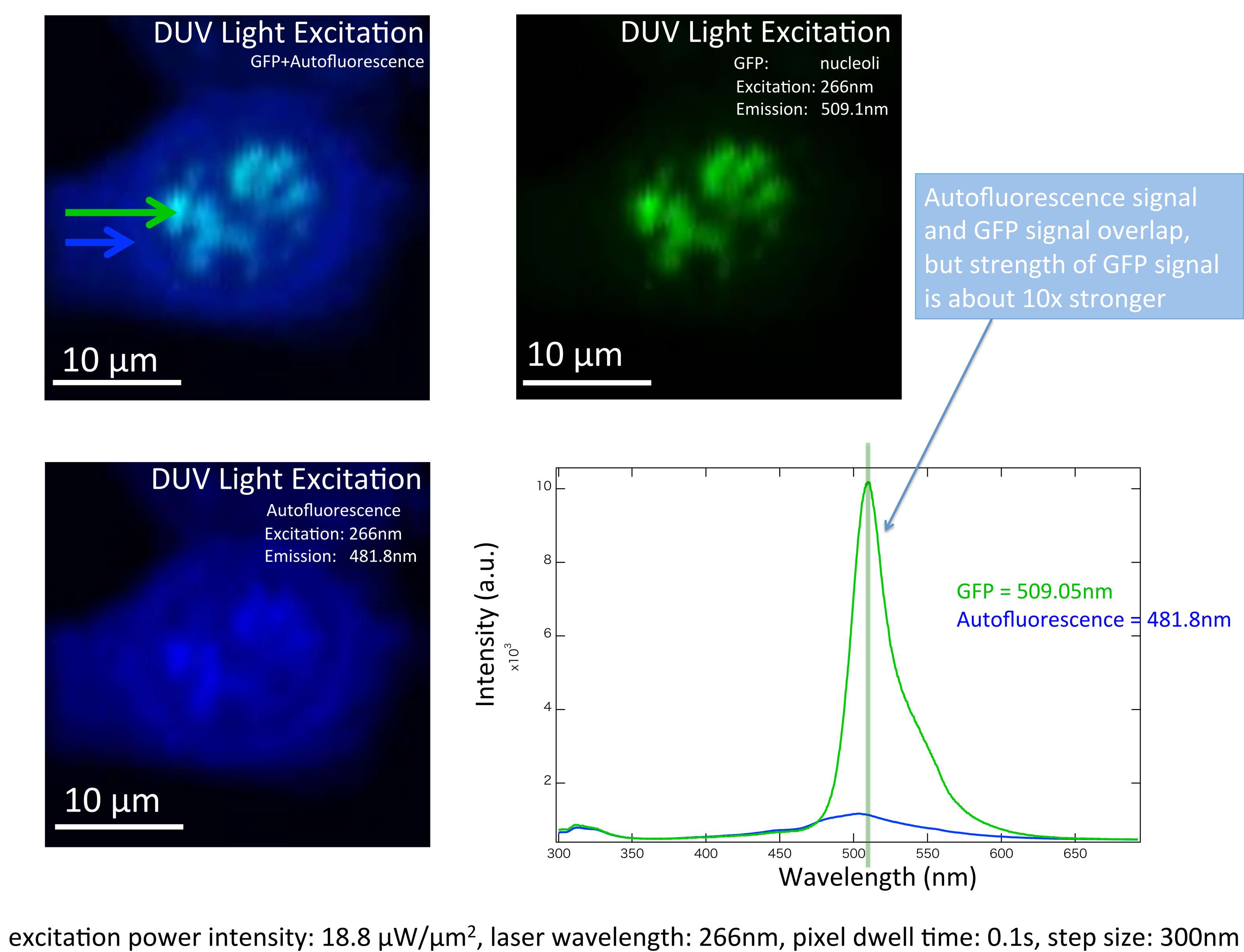
excitation power intensity: 27.9 µW/µm², laser wavelength: 266nm, pixel dwell time: 0.1s, step size: 300nm

Distinguishing Autofluorescence and RFP Signal by Peak Position



excitation power intensity: 335 µW/µm², laser wavelength: 257nm, pixel dwell time: 0.08s, step size: 300nm

Distinguishing Autofluorescence and GFP Signal By Fluorescence Intensity



excitation power intensity: 18.8 µW/µm², laser wavelength: 266nm, pixel dwell time: 0.1s, step size: 300nm

3-Color Imaging Challenges

- CFP's signal weak
- Overlaps with autofluorescence
- Choice of FP limited by signal strength

Conclusion

- Multiple FPs imaged simultaneously using DUV excitation
- **RFP** and **GFP** can be clearly detected at **590 nm**, **510 nm**
- CFP and autofluorescence signals overlap

Next Steps

- Develop algorithm to unmix FP and autofluorescence signals

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