# TUTORIAL WORKSHOP – FUNDAMENTALS OF FLUORESCENCE IMAGING

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This tutorial will consist of two lectures, with a break for refreshments and discussion between them, and further time for discussion afterwards

## Lecture 1 – Basics of fluorescence and fluorescence microscopy.

- Fluorescence – how it works, Stokes Law, Kasha's Law, lifetime.
- Properties of fluorochromes (extinction coefficient, quantum yield), photobleaching.
- Widefield (Ploem) fluorescence microscopes, light sources, filters.
- Confocal microscopes.
- Multiphoton excitation, second harmonic generation.

## Lecture 2 – Getting the picture – acquiring an optimal image

*Optical considerations*

- Point spread function - Rayleigh resolution, confocal resolution
- Aberrations – spherical and chromatic.
- Apparent depth

*Digital imaging*

- Pixels and voxels, bit depth
- Sampling considerations in x, y z & t, Nyquist criterion
- Signal to noise ratio
- What is a colour image? Real colour, 3-channel images, false colour