qbio
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IMAGING BIOLOGICAL SYSTEMS

Practical : building a confocal

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OBJECTIVES

1. Basic design of a confocal microscope : optics
2. Selections of appropriate fluorescence filters
3. Fluorescence correlation spectroscopy : some reminders
4. Measurements
5. Option 1 : How to get an image using the confocal ?
6. Option 2 : How to build a multicolour microscope ?

EQUIPEMENTS

1. Lasers : THORLABS LDM405, LDM635, LDM850
2. Objectives : Zeiss Plan Apochromat 100X, NA 1.4
3. Detector : Perkin Elmer SPCM AQR-14
4. Fiber for detection : Thorlabs M18L01
5. Pinole : 100µm diameter
6. Lenses : achromat 40mm, 100mm, 125mm, 400mm
7. Dicroics mirrors : Semrock FF555 and FF660
8. Filters : Semrock FF01-708/75, FF01-673/11, FF01-628/32

NB : you want to use Atto655 as fluorophore

MEASUREMENTS

1. Determine the concentration of a concentrated(>µM) solution of Atto655 without using its fluorescence properties

2. Determine the concentration of a diluted (~nM) solution of Atto655 using FCS. You can use the concentrated solution as a standard

3. Determine the size of fluorescent beads in solution using FCS. For reference, the diffusion coefficient of the Atto655 dye is 300µm²/s

FOR THE NEXT SESSION

1. Prepare a design of the confocal microscope with all its components properly placed
2. Propose a protocol to measure the concentration of the two solutions (measurement 1 & 2)
3. Propose a protocol to measure the size of the fluorescent beads (measurement 3)