qbio
quantitative
biology

QBIO MASTER PROGRAM

quantitative biology in practice

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. Laser : THORLABS LDM635
2. Objective : Zeiss Plan Apochromat 100X, NA 1.4
3. Detector : Perkin Elmer SPCM AQR-14
4. Fiber for detection : Thorlabs M18L01
5. Pinole : 100µm diameter

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
3. Propose a protocol to measure the size of the fluorescent beads