Build your setup

1- Build an epifluorescence / TIRF microscope

Teacher : Antoine Le Gall

Students will learn how to build a widefield fluorescence microscope from scratch! The setup will be designed such that students will be able to switch from Epifluorescence to Total Internal Reflection Fluorescence (TIRF) mode.

To achieve this, students will first design the setup: based on the specifications of the available laser, microscope objective, and camera, they will choose the right optics to properly illuminate the sample and collect light from the correct part of the sample. Then, based on their design, students will assemble the microscope from scratch by setting the objective, camera, sample stage, fluorescence filters, etc… on an empty frame, and align the excitation laser to shine the sample. Then they will characterize the setup and perform experiments on what they have built!

Sample : nuclear pores labeled with red organic fluorescent dyes will be used to see the contrast gain of the acquired images when illuminated in TIRF compared to epifluorescence.

2- Build a confocal microscope

Teacher : Emmanuel Margeat
We will build a simple confocal microscope, with one (or two) channels, and use it for FCS experiments.

We will learn how to align the lasers into the optical path of the microscope, expand the beam with a telescope, and check the collimation. We will have to place the pinoles and detectors at the right position and align them. We will then use Fluorescence Correlation Microscopy to determine the concentration and the diffusion coefficient of fluorescent species
Sample: Fluorophores at various concentration, fluorescent beads.

3- Build an AFM

Teacher : Luca Costa

The idea is to design and assemble an atomic force microscope.
Students will make use of piezoelectic elements for the scanner and for the excitation of the cantilever in amplitude modulation mode (AM-AFM). They will design and build up an interferometric Fabry-Perot system to measure the cantilever deflection. For this, they will assemble a 3-stage inertial motors setup to

place an optical fiber at a few microns of distance from the cantilever backside. Moreover, they will use a photodiode and a transimpedance amplifier to convert the Fabry-Perot optical interference into a measurable voltage signal. Additionally, they will use three motorized rotary stages to displace the AFM tip in close vicinity of the sample. Students will use a Stanford Research Lock-In Amplifier to evaluate the cantilever oscillation amplitude and phase and use them to run AM-AFM experiments.
Microscopy and Force Spectroscopy data acquisition will be performed using an SPM Nanonis (SPECS) control unit. Hardware (motors, stages, piezos, etc, etc) will be controlled making use of the Nanonis tools or using Labview/custom made/existing commercial softwares.

Sample: Silicon calibration gratings (measurement in air) or eventually supported lipid bilayers (measurement in DPBS) if the AFM will be very performant.

4- Build an Optical Tweezer

Teacher : Jean-Bernard Fiche

Students will construct an optical tweezer by aligning an infrared (IR) laser into a high numerical aperture (NA) objective to generate an optical trap. This trap will be used to hold and manipulate microscopic objects such as polystyrene beads, red blood cells, or bacteria. Students will learn how to align the laser beam through the optical path, optimize the focus at the sample plane, and verify stable trapping. They will then calibrate the trap stiffness by analyzing the Brownian motion of a trapped bead, thereby quantifying the optical forces exerted by the trap.

Sample: Polystyrene beads for calibration; red blood cells or bacteria for trapping demonstrations.