## Learning objectives for the LCI training

During the LCI training, we provide teaching as well as scientific advice.

# A. Scientific advice

We discuss with you how to adapt your sample preparation and experiment goals to minimize sample to sample variability and ensure extraction of reliable data by minimizing refraction in the sample and optimizing the signal to background ratio.

#### B. Training

At the end of the training, you should be able to independently perform the following actions:

#### 1. Operate the equipment to ensure extraction of reliable data for your scientific question.

□ Turn the equipment on/off in a safe way that leaves the equipment and the whole area clean.

 $\Box$  Mount and find your sample in a way that is safe for the sample and the equipment.

□ Identify and eliminate refraction index mismatch and chromatic aberration artifacts.

□ Set the acquisition parameters to optimize acquisition speed and signal to noise ratio for your scientific question.

- $\Box$  Laser power
- □ Exposure time
- 🗌 Gain
- □ Bit depth
- □ Look-Up Tables

□ Set the optical and digital resolutions to avoid undersampling artifacts and be able to reliably answer your scientific question.

- □ Objective
- □ Pixel size
- □ Extra magnification lens
- □ Binning

□ Set appropriate multidimensional parameters for your scientific question.

- $\Box$  Multiple xy positions
- □ Overview/tiling
- □ z stacks
- □ Multiple channels
- □ Timelapse

 $\Box$  Set and maintain the focus to acquire reliable and reproducible data.

□ Transfer the data safely.

 $\Box$  Write the Material and Method section for your imaging experiment to enable reproducibility by other researchers.

# 2. Identify and eliminate typical microscopy artifacts: bleedthrough and saturation.

 $\Box$  Assess the efficiency of imaging and the potential for bleedthrough for the fluorophores in your sample when using the microscope selected for the training.

 $\Box$  Identify saturation/underexposure in acquired images, explain the consequence of both and explain how to eliminate these artifacts.

## 3. Optional items depending on the experimental needs.

 $\Box$  Run an acquisition pipeline established by another LCI user.

 $\Box$  Set the parameters for the spectral detector.

□ Adapt a JOBS pipeline

 $\Box$  Acquire and process DeepSIM images. Recognize artifacts and explain what can be done to avoid them.

 $\Box$  Set the incubator for a live imaging experiment.

□ Set the acquisition parameters to image in TIRF/Hilo.

- □ Perform laser microdissection (MMI).
- □ Perform micropatterning and microfabrication (Primo).
- □ Stitch tiled images (Aurora).
- □ Deconvolve the images and explain how to recognize and eliminate deconvolution artifacts.