

## Workflow of the full 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 perform the following actions:

#### **1. Operate the equipment.**

- Turn the equipment on/off in a safe way.
- Mount and find the sample in a way that is safe for the sample and the equipment.
- Set appropriate multidimensional acquisition parameters for the scientific question.
  - xy/multiple positions
  - Tiling
  - z stacks
  - Multiple channel
  - Timelapse
- Explain how to set and maintain the focus to acquire reliable and reproducible data.
- Run an acquisition pipeline established by another LCI user.

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

- Assess the efficiency of imaging and the potential for bleedthrough for the fluorophores in the sample when using the microscope selected for the training.
- Recognize saturation/underexposure in acquired images, explain the consequence of both and explain how to eliminate these artifacts.
- Estimate the resolution needed to answer the scientific question and calculate which optical and digital resolutions fulfill these needs.

#### **3. Adapt the imaging parameters to ensure extraction of reliable data.**

- Set up the acquisition parameters (laser power, exposure time, gain, bit depth) to optimize acquisition speed and signal to noise ratio according to the scientific question.

#### **4. Deal with the data.**

- Transfer the data safely.

#### **5. Optional items depending on the experimental needs.**

- Adapt a JOBS pipeline
- Acquire and process DeepSIM images. Recognize artifacts and explain what can be done to avoid them.
- Set the parameters for the spectral detector.
- Set up a live imaging experiment.
- Set the acquisition parameters to image in TIRF/Hilo.
- Perform laser microdissection (MMI).
- Perform micropatterning and microfabrication (Primo).
- Deconvolution and post acquisition stitching (Aurora).