

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 potential sample preparation improvements to minimize sample to sample variability and ensure extraction of reliable data. We help you define your experimental goals in terms of which metrics need to be extracted from the image.

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 according to the recommended procedure that leaves the equipment and the whole area clean.
- ☐ Mount and find your sample according to the recommended procedure that is safe for the sample and the equipment.
- ☐ Choose appropriate acquisition parameters to optimize acquisition speed and signal to noise ratio to be able to reliably answer your scientific question.
 - ☐ Illumination power
 - ☐ Exposure time
 - ☐ Gain
 - ☐ Bit depth
- ☐ Display the image in a way that allows viewers to see the details relevant to the scientific question.
- ☐ Choose appropriate optical and digital resolutions to minimize undersampling artifacts and be able to reliably answer your scientific question.
 - ☐ Objective
 - ☐ Pixel size
 - ☐ Extra magnification lens
 - ☐ Binning
- ☐ Choose appropriate multidimensional parameters to be able to reliably answer your scientific question.
 - ☐ Multiple xy positions
 - ☐ Overview/tiling
 - ☐ z stacks
 - ☐ Multiple channels
 - ☐ Timelapse
- ☐ Define a focus strategy to first set then maintain the focus to ensure reliable and reproducible data acquisition.
- ☐ Transfer the data according to the recommended safe procedure.

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

2. Identify and eliminate typical microscopy artifacts: bleedthrough, saturation and optical aberrations.

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

☐ Identify saturation/underexposure in acquired images, explain the consequence of both and explain which options are available to eliminate these artifacts.

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

3. Optional items depending on the experimental needs.

☐ Set the incubator for a live imaging experiment.

☐ Adjust the Koehler illumination to optimize transmitted light imaging.

☐ Run an acquisition pipeline established by another LCI user.

☐ Set the parameters for unmixing.

☐ Adapt a JOBS pipeline

☐ Acquire and process DeepSIM images. Recognize artifacts and explain what can be done to eliminate them.

☐ Align the NSPARC detector and set the acquisition parameters to eliminate the Pile up effect.

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

☐ Perform laser microdissection.

☐ Perform micropatterning and microfabrication.

☐ Stitch tiled images post-acquisition.

☐ Deconvolve images and explain how to recognize and eliminate deconvolution artifacts.