

Prerequisites for FLIM experiments

The document summarizes the prerequisites for a successful introduction of new users for the FLIM microscopes at the LIC. The following knowledge about your sample is necessary and the samples preparation should follow the recommendations below, when you want to perform Fluorescence Lifetime Imaging Microscopy (FLIM) in general and specifically on the LEICA SP8-U-FLIM microscope.

Fluorophore knowledge:

You have to know exactly at least the fluorophores or fluorescent proteins, you have used in your sample. If these are very special ones, where the excitation and emission spectra cannot be found easily on the web, try to find them before you are coming to the introduction session or your experimental session. If there is literature available of FLIM measurements performed using these fluorophores, please bring it with you as a PDF or sent it in advance to the LIC.

Samples needed for FLIM measurements:

Every sample from A) to C) should be at least available as a doublet (2 independent slides).

- A) Unlabeled sample (for checking the sample autofluorescence)
- B) Labelled samples – only labelled with a **single** fluorophore
Separate samples for each of the fluorophores you want to use in your final multi-labeled sample are needed.
- C) Final sample with all the fluorophores you want to image together.

Sample preparation:

- A) Coverglasses
Use only coverglasses of defined thickness (named 1.5H 170 μm \pm 10 or 5 μm). These coverglasses can be bought from many suppliers also glass bottom petri dishes or multi-well plates. We have a separate information sheet about coverglasses for microscopy.
- B) Embedding media
You have to know all of the following information about the embedding media (exact name, brand, article number), if possible also refractive index. If you do not find the information, please contact us. We can also measure the refractive index in the LIC with a refractometer. We also have a separate information sheet about embedding media. Please contact us, if you have questions or would like to have test samples of embedding media. The LIC has a number of commercial embedding media in stock.
- C) In General, non-fixed samples are superior for most FLIM experiments. For fixed FLIM samples, use non-hardening embedding media like Glycerin or Vectashield as

hardening embedding media like Mowiol or the Prolong family of media from Thermo Fisher quite often will strongly change the lifetime properties of the fluorophores.

This document will be revised continuously, when new requirements or recommendations become obvious.