

How to record a lifetime sample (Quick Reference)

Recording the Reference

1. Start LI-FLIM software
2. Place reference material with known mono-exponential lifetime under the microscope
3. Switch the light beam to the eye port of the microscope
4. Set the LED current to 100 mA or laser to 10 mW
5. Switch the LED/laser on by clicking the LED DC/MOD. DC checkbox
6. Focus using the eyepiece
7. Click "Idle" to switch off the LED/laser
8. Switch the light beam to the camera port of the microscope
9. Set the MCP voltage to minimum (400 V) and the Exposure time to 100 ms
10. Click "FLIM" to switch on the LED/laser and the image intensifier in modulation mode
11. Draw a region of interest in the middle of the camera image
12. Enable the Statistics tab and find the average ADU value
13. Determine the brightest phase (= highest average ADU) by sliding the phase control from 0 to 359 (if you do not see a difference between phases incrementally increase the MCP voltage by 10 V)
14. To make use of the whole dynamic range of the camera, increase the MCP voltage until the maximum ADU in the statistics reaches 55.000-65.000 (anything above 65.000 means overexposure and thus possible damage to the camera). You might also want to adjust the Exposure time
15. Click "Reference" to record a reference phase image stack
16. Click "Save Reference" to store the stack on disk (obsolete if Autosave is enabled)
17. Remove the reference material
18. Switch the light beam to the eye port of the microscope

Recording the Sample

1. Unless noted here do not change any parameter of the system between reference and sample!
2. Place specimen under the microscope
3. Switch the LED/laser on by clicking the LED DC/MOD. DC checkbox
4. Focus using the eyepiece
5. Click "Idle" to switch off the LED/laser
6. Select "Edit comment" on the Info tab and give a description of your sample (very useful afterwards)
7. Set the MCP voltage to minimum (400 V) to prevent overexposure
8. Switch the light beam to the camera port of the microscope
9. Click "Focus" to switch on the LED and the image intensifier
10. Increase the MCP voltage until you see the image appear on screen.
11. Click "FLIM" and determine the brightest phase and optimal MCP and Exposure time settings (see steps 1.13 and 1.14). Best results are obtained with approximately the same MCP voltage (± 15 V) as used for the reference. Exposure time may be increased
12. Refocus the microscope using the camera image if necessary
13. Click the "Sample" button to record the sample phase stack and get a lifetime image
14. Don't forget to save your data!