

# OPTICAL SECTIONING FRET/FLIM WITH A SPINNING DISK

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## 1. FLIM

Fluorescence Lifetime Imaging Microscopy (FLIM) is gaining importance as a specific microspectroscopic technique to visualise and quantitatively measure molecular interactions in cellular systems using the principle of Förster resonance energy transfer (FRET). Broadly speaking, two approaches are currently in use – time-domain FLIM, and frequency-domain FLIM. The former approach requires the use of expensive pulsed laser systems, and is essentially a point-scanning method that is inherently time-consuming. The frequency-domain method, in contrast, can be exploited in wide-field mode, is significantly more photon-efficient, and uses commonly available continuous wave laser systems or, as in this experiment, light-emitting diodes (LEDs).

## 2. FREQUENCY DOMAIN FLIM ON A MULTI-BEAM CONFOCAL MICROSCOPE

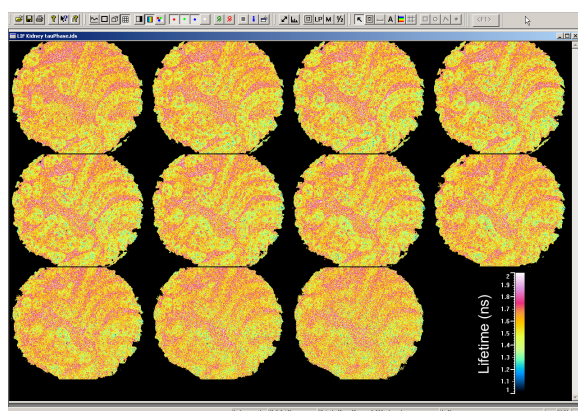
Experiments were done on an Olympus IX71 Inverted Fluorescence Microscope with Olympus Disc Scan Unit (DSU) and with the Lambert Instruments LIFA system for Fluorescence Lifetime Imaging Microscopy (FLIM) attached. The Olympus lamphouse was used with a mounted 470nm 3W LED as the modulated lightsource of the LIFA system.

The aim of the experiments was to:

1. Show the feasibility of generating a cross-sectional 2D lifetime image from confocal fluorescence intensity images recorded at any Z-defined cross-section in the sample.
2. Acquire 3D confocal lifetime image stacks by applying Z-control of the microscope.

## 3. RESULTS

We used a fluorescently stained mouse kidney cell slide (Molecular Probes) as a test sample. The sample thickness was approximately 20 microns. Eleven sections were recorded at 2



micron intervals. For each of the 11 z-positions 12 phase images were recorded, from which 11 lifetime images were computed. Averaging the 12 phase images in each z-position yielded an intensity image stack. Both stacks (ics/ids format), intensity (not shown) and lifetime (fig.1), can be viewed by using a suitable viewer such as the EZ FLIM-viewer. Comparison with images taken in the non-confocal mode shows a clear improvement in contrast.

Figure 1 : Lifetime image stack, 11 z-positions, interval = 2 $\mu$ m