Real-Time Fluorescence Lifetime-Resolved Imaging

Peter C. Schneider, Oliver Holub & Robert M. Clegg, Department of Molecular Biology
Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany

Abstract:
We present a new instrument based on a SUN UltraSparc Computer with Creator 3D graphics processor that is capable of acquiring, processing and displaying fluorescence lifetime-resolved images at a very high speed. The frame refresh rate is dependent upon the available amount of light, the data acquisition mode, the algorithms used for processing data and the sophistication of the display. Refresh rates of up to 15 Hz have been achieved, although refresh rates of 1-7 Hz are typical.

The system uses the phase and modulation technique in homodyne mode. According to the operator’s requirements several methods of data processing can be applied to yield different information. Using an additional photomultiplier non-imaging lifetime measurements in a microscope are also possible.

The programs, including hardware control, have been written using AVS/express with newly developed C modules. We show examples from three major fields of applications: real-time FLIM, rapid fluorescence lifetime micro assays and medical endoscopy.

Instrumentation:
The light of an Ar+-Laser is modulated by means of standing wave acousto-optical modulators and coupled into single-mode optical fibers. Thus, the excitation light can be guided by appropriate optics to several measurements:
— a fluorescence microscope
— a medical endoscope
— a long working distance low magnification optics in combination with an computer-controlled XY scanning stage.
The 2 dimensional fluorescence emission is imaged onto the cathode of a modulated image intensifier (directly or via an imaging fiber bundle) or focused onto the cathode of an modulated photomultiplier.
The phase sensitive homodyne output of the intensifier is captured by a fast, chilled 10 bit CCD camera synchronized to the computer-controlled phase switching of the control voltage driving the intensifier.
The camera output is digitized and read out to the computer at a rate of 10 MHz using a DMA digital frame grabber.

Medical Endoscopy:
The modulated fluorescence image can also be transmitted via an imaging fiber bundle.

Data Analysis:
1) Digital Fourier analysis
$$K^{-1}\sum_{k=0}^{K-1} \sin \left(\frac{2\pi k}{K} \right) S_i (\psi_k, \Delta \phi^{c, G} + k \frac{2\pi}{K} )$$
$$K^{-1}\sum_{k=0}^{K-1} \cos \left(\frac{2\pi k}{K} \right) S_i (\psi_k, \Delta \phi^{c, G} + k \frac{2\pi}{K} )$$
3-8 images needed

typ. frame rate: 1-3 fps
3 output images
$$F_{ij} = \frac{1}{K} \sum_{i=0}^{K-1} S_i (\psi_i, \Delta \phi_j^{c, G} + k \frac{2\pi}{K} )$$

2) Normalized Difference
$$P_N \left( S_i (\psi_k, \Delta \phi^{c, G} + k \frac{2\pi}{K} ) - S_i (\psi_k, \Delta \phi^{c, G} + k \frac{2\pi}{K} ) \right)$$
2 images necessary

Rapid Micro Assays:
Fluorescence lifetime-sensitive micro assays can be analyzed rapidly and with a minimum amount of sample solution.
Examples shown:
1) 2 micro cuvettes (~125 nl ea.) with Rhodamin 101 and TMRh
2) 3 micro cuvettes (~125 nl ea.) with solutions of TMRh and Fluorescin labelled oligo nucleotides showing different amounts of FRET (= Förster Resonance Energy Transfer) (FRET: 0%, 30%, 55%)

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