

B3 - FLIM - Instrumentation & Algorithms

Time: Monday, 13.09.2010

Location: Humboldt-Building, Lecture Room 211

Chairman: D. Schweitzer (DE-Jena)

1:30 p.m.	Welcome Speech (Audimax) Jens Haueisen (DE-Ilmenau)
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1:35 p.m.	P. French (GB-London) Key Note Lecture: Multidimensional fluorescence imaging and metrology for high content analysis and label-free diagnosis This talk will review our development and application of multidimensional fluorescence imaging (MDFI) technology, with an emphasis on fluorescence lifetime imaging (FLIM) applied to microscopy, endoscopy and tomography. Applied to autofluorescence, MDFI can be used to provide label-free molecular contrast in biological tissue and is being investigated for ex vivo and in vivo imaging with a view to developing diagnostic tools. To this end we have developed a FLIM microconfocal endoscope and a multispectral multiphoton FLIM microscope based on the Dermalnspect platform. Applied to fluorescent labels, fluorescence lifetime imaging (FLIM) and Forster resonant energy transfer (FRET) can provide readouts of variations in the local molecular environment of labelled proteins (e.g. calcium transients) and of protein-protein interactions. To study cell signalling networks and mechanisms of disease, we have developed a range of microscopes ranging from stimulated emission depletion (STED) FLIM microscopy to study spatial phenomena with < 40 nm resolution through to multibeam multiphoton FLIM microscopy to map changes in metabolic pathways using cellular autofluorescence. For faster and more systematic investigations, we have developed an automated high-speed optically-sectioned FLIM multiwell plate reader to read out, e.g. membrane properties and protein interactions via FRET in fixed and live cells. We are also developing multiplexing strategies to simultaneously readout different protein-protein interactions. While this can increase the value of cell-based assays, for drug discovery, as well as for fundamental research, it is imperative to translate such assays to live disease models – both to further elucidate disease mechanisms and to enhance the identification of promising drug candidates while decreasing the time to failure of unsuccessful compounds. Noting that FLIM provides a robust readout, such as is required for translation to in vivo experiments, we are developing tomographic FLIM instruments based on optical projection tomography for transparent/optically cleared samples and diffuse fluorescence molecular tomography for mouse imaging.
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2:25 p.m.	W.Becker (DE-Berlin)
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Probing Molecular Interactions by Fluorescence Lifetime Imaging

Fluorescence imaging techniques have found broad application in live sciences because they are non-destructive, extremely sensitive, and deliver information about biochemical interactions on the molecular scale. Of all fluorescence parameters, it is the fluorescence decay function that yields the most direct insight into molecular processes within live cells and tissues. A fluorescence lifetime imaging (FLIM) technique for biological imaging has to combine high photon efficiency, high lifetime accuracy, resolution of multi-exponential decay profiles, simultaneous recording in several wavelength intervals and optical sectioning capability. We will show that the combination of multi-dimensional time-correlated single photon counting (TCSPC) with confocal or two-photon laser scanning meets these requirements almost ideally. Multi-dimensional TCSPC is based on the excitation of the sample by a high-repetition rate laser and the detection of single photons of the fluorescence signal. Each photon is characterised by its time in the laser period, its wavelength, and the coordinates in the scanning area. The recording process builds up a photon distribution over these parameters. We will demonstrate the application of the technique to ion concentration measurements, FRET experiments, and autofluorescence imaging.

2:50 – 3:10 p.m. Coffee break	
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3:10 p.m.	S. Orthaus (DE-Berlin)
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Recent Developments in Hardware and Data Analysis Schemes for FLIM

Time-resolved fluorescence microscopy has become very popular in the recent years and has enabled new measurement procedures such as Fluorescence Lifetime Imaging (FLIM). Today complete systems are directly available either as stand-alone units [1] or as upgrades to confocal laser scanning microscopes (CLSM) [2]. Such systems are typically based on Time-Correlated Single Photon Counting (TCSPC) electronics along with picosecond pulsed diode lasers as excitation sources. Typical FLIM applications nowadays include ophthalmology, environmental sensing or even single-molecule experiments. In this presentation we will summarize the recent developments in this field, which include e.g. a new truly parallel multi-channel TCSPC unit[3], that allows to perform FLIM measurements at a photon rate much higher than the classical pile-up limitations thereby minimizing data acquisition time [4]. We will also present a new solution in the field of compact picosecond pulsed diode lasers such as an excitation source in the green spectral range (532 nm) [5]. In addition to improvements in the area of hardware for FLIM, we will also summarize our latest results on data analysis concepts. While multi-exponential decay analysis has become a standard tool, we are working on new analysis schemes such as cumulant analysis and multidimensional visualisations to decrease the data analysis time and to identify and separate characteristic subpopulations. We will also present results that show how pulse pile-up distortions and dead time effects can be corrected in the data analysis using dedicated correction algorithms [6].

A Layer Based Approach for Multi-Exponential Fitting of Autofluorescence Data in the Human Eye

Purpose: To enhance multi-exponential fitting of fluorescence lifetime imaging (FLIM) measurements in ophthalmology by refined modeling of the measured data. Methods: To measure the fluorescence lifetime at the human fundus a modified Heidelberg Retina Angiograph was used. The endogenous fluorophores were excited by a diode laser with pico-second pulses at 446 nm and a repetition rate of 80 MHz. The auto-fluorescence was detected in two spectral channels: 490-560 nm and 560-700 nm using the time-correlated single photon counting method. A time resolution of approximately 12.2 ps was achieved by dividing the time between laser pulses (12.5 ns) into 1024 time channels. The acquired images cover 30° of the fundus with a lateral resolution of 40 x 40 μm². The same technical setup was used for cuvette measurements using a lens with 150 mm focal length. The here discussed method was first described by Schweitzer [Patent DE 10 2008 045 886]:

$$\frac{I(t)}{I_0} = \text{IRF} \cdot \sum_i a_i \cdot e^{-\frac{t-t_c}{\tau_i}} + b$$

(I - intensity, IRF - instrumental response function, € - amplitude, t - time, t_c - time-shift, € - lifetime, b - offset). It extends the classical multi-exponential model by a time-shift which allows each exponential to be moved on the time axis independent from the other exponentials. This allows for modeling of distances between fluorophores along the excitation laser beam which translate into a time lag between the fluorescence emissions of the fluorophores. Datasets from human subjects were approximated with the classic three-exponential model and with the layer based approach. Further on the algorithms were compared using simulated data which was generated by a Monte-Carlo-approach. Synthetic data was created based on given parameters ("parametric") as well as based measured data ("nonparametric"). Two regions have been analyzed: the fovea centralis and the optic disc which also affected the determination of the simulation parameters. Identical preprocessing (especially binning) is applied for both algorithms. The optimization was done with the help of a differential evolution followed by a simplex algorithm. Results: Measurements of fluorescent dyes in three cuvettes with a distance of 40 mm and 80 mm relative to the first cuvette show the principle of layered approach is valid as the calculated distances from the FLIM data are 43 mm and 80 mm. Lifetimes of the layered approach (167 ps, 1362 ps, 3347 ps) match lifetimes of single measurements (177 ps, 1272 ps, 3052 ps) while lifetimes of classic three-exponential model are significantly different (117 ps, 622 ps, 2052 ps). The "parametric" simulation with three exponentials (40 ps, 500 ps, 3500 ps) whereby the latter two are shifted by 100 ps and 200 ps respectively also show remarkable differences. The layered approach approximates 42 ps, 528 ps and 3582 ps whereas the common three-exponential model determines 39 ps, 536 ps and 4238 ps. Lifetime variances are smaller in all cases for the layered approach. Conclusions: The presented approximation algorithm is able to determine the lifetimes more accurately than the classic multi-exponential approach in layered structures such as the human eye.

4:00 p.m.	K. König (DE-Saarbrücken)
<p>Clinical fluorescence lifetime imaging in dermatology Multiphoton Tomography was performed on patients with dermatological disorders using femtosecond near infrared laser tomographs. The spatial resolution was less than one micrometer. Using time-correlated single photon counting, fluorescence lifetime imaging was performed. The temporal resolution was 270 ps. FLIM data from volunteers and patients will be presented.</p>	
<p>End of Lecture Session</p>	
4:30 p.m.	Guided tour through the Institute of Biomedical Engineering including Ophthalmology Laboratory
7:00 p.m.	Dinner in restaurant of hotel "Lindenhof" for all referents