LIFETIME IMAGING AND FCS UPGRADE PACKAGE FOR CONFOCAL MICROSCOPES

U. Ortmann, F. Koberling, B. Kraemer, M. Wahl, M. Patting, P. Kapusta, A. Bülter, R. Erdmann
PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany,
www.picoquant.com, photonics@pq.fta-berlin.de

KEY WORDS: Confocal Laser Scanning Microscopes (LSMs), Fluorescence Correlation Spectroscopy (FCS), Fluorescence Lifetime Imaging (FLIM)

Confocal Laser Scanning Microscopes are widely used tools in biochemistry, cell biology and other related sciences today. However, the capabilities of these microscopes can be further enhanced by adding another dimension: time. The upgrade towards temporal resolution is easily done using picosecond diode lasers along with time-correlated single photon counting (TCSPC) for data acquisition. This upgrade allows additional measurement modes, like Fluorescence Correlation Spectroscopy or Fluorescence Lifetime Imaging.
The upgrade is based upon a special designed variant of TCSPC, which is the so-called Time-Tagged Time-Resolved (TTTR) mode, that is implemented in the TimeHarp 200 PC-board. Essentially, it is an elegant extension of the classical TCSPC preserving the full photon information content. There is no data reduction done by the electronics, nor limitation by on-board memory, because the records are continuously stored on the computer hard disk. The result is an information-rich TTTR datafile, where virtually all algorithms and methods for the analysis of photon dynamics or lifetime imaging can be applied.
In this presentation we show recent results of FLIM as well as FCS measurements, done on a FluoView 1000 from Olympus upgraded to lifetime capabilities. The FLIM examples are centered around measurements of hepatocytes (HepG2). It is shown that the determination of the relative hydrophobicity inside of hepatocytes (HepG2) is possible using the dye NBD (nitrobenzoxadiazol) as a marker, with the lifetime of NBD acting as an indicator for the hydrophobicity of the surrounding medium.
The FCS capabilities of the upgrade are demonstrated using freely-diffusing ATTO 655 molecules in solution. The software based method does not have the limitations and approximations inherent in classical hardware autocorrelators. On-line monitoring of the autocorrelation function is also possible for a fast control of the measurement parameters. However the ultimate strength of the TTTR based FCS is the combination of TCSPC timing and time-tags. For this method [1] we show separated FCS data of two non-interacting dyes in methanol (BODIPY / $DilC_{18}(5)$) mixtures utilizing a single detection channel setup.

[1] A. Benda, M. Hof, M. Wahl, M. Patting, P. Kapusta, R. Erdmann, "TCSPC Upgrade of a Confocal FCS Microscope", *Rev. Sci. Inst., accepted for publication (2005)*