EFFECT OF DISPERSION COMPENSATION ON EXPERIMENTAL MPM SET UP FOR BIOMEDICAL APPLICATIONS

Jeemol James[1], Jonas Enger[2] and Marica B. Ericson[1]

[1] Biomedical photonics group, Department of Chemistry and Molecular Biology, University of Gothenburg, Sweden

[2] Department of Physics, University of Gothenburg, Sweden

Kemivägen 4,
Inst. for Chemistry and Molecular Biology
412 96 Gothenburg, Sweden

Email: jeemol.james@chem.gu.se

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ABSTRACT

The invention of multiphoton microscopy (MPM) by Denk et al in 1990 was a breakthrough in the field of biomedical imaging [1]. One of MPM's strengths lies in imaging as well as studying molecular interactions. The objective of this work is to show how an experimental MPM set-up can be optimized by quantifying the dispersion of laser pulses. The optimized MPM setup is used for studying the effect of pulse width compensation on quantitative techniques such as fluorescence correlation spectroscopy (FCS) and fluorescence life time imaging (FLIM).

MPM experimental set up contains a fs-pulsed (~80 fs) commercial Ti:Sa laser (Tsunami, Spectra physics). Pulse width measurement and dispersion compensation is done by using Carpe autocorrelator and a femtocontrol unit (APE, Berlin, Germany). The carpe autocorrelator is used to measure pulse width at laser output as well as at the sample stage. The femtocontrol unit containing a prism pair is used to compensate the pulse dispersion in the optical path between laser and the microscope part. This set up is suited for imaging purpose as well as quantitative measurements as it is equipped with a time correlated single photon counting module (TCSPC, Becker&Hickl, Berlin, Germany). Also, MPM is equipped with two GaAsP detectors (Hamamatsu) which are interfaced to TCSPC and makes it suitable for performing FLIM as well as FCS.

In this paper, the effect of pulse width compression on FLIM measurements will be presented comparing data for the relationship between photon intensity on life time images and pulse width. Diffusion time measurements using MPM-FCS at different pulse width will also be performed, together with a theoretical argumentation of the relation between pulse width dispersion and excitation volume. The first goal is to use the optimized experimental MPM system to investigate the diffusion of nanoparticles inside complex biological systems like biofilms, as well as implement FLIM in biological tissues and samples such as 3D skin models and lymph nodes.

1. Denk, W.; Strickler, J. H.; Webb, " Two-Photon Laser Scanning Fluorescence Microscopy", *W. W. Science (New York, N.Y.)* **1990,** 248, 73.