

Laser Scanning Multiphoton Microscopy

Focusing on Fluorescence Correlation Spectroscopy and
Fluorescence Lifetime Imaging for Biomedical
Applications

Laser scanning multiphoton microscopy (MPM) has great potential as a non-invasive optical tool for three-dimensional imaging of complex biological tissues. This thesis demonstrates how to adopt MPM in combination with fluorescence correlation spectroscopy (FCS) and fluorescence lifetime imaging microscopy (FLIM), focusing on both development and validation of methodology, as well as targeting biomedical applications. A practical guideline for MPM-FCS was developed. Proof of principle was demonstrated by measuring the diffusion of a fluorescent dye, i.e. Rhodamine B in different water glycerol mixtures having different viscosities. Biomedical applications were explored by performing MPM-FLIM investigations in sentinel lymph nodes *ex vivo*. The tissues were derived from melanoma patients. The study demonstrated the potential to differentiate atypical cells, healthy lymphocytes, blood vessels based on morphological features combined with fluorescence lifetime data. MPM-FLIM investigations of *in vitro* cell cultures of Keratinocytes revealed the complexity of intrinsic autofluorescence in data interpretation. Furthermore, the work highlights the finding that the contribution from other structural proteins, such as keratin, should not be neglected when performing MPM-FLIM. This thesis has made it one step closer to the utilization of the quantitative aspects of MPM. It is also emphasized that continued research for a fundamental understanding of underlying principles is required in order to translate the techniques clinically in the future.



Jeemol James received a master's degree in optoelectronics and laser technology from International School of Photonics, Cochin University of Science and Technology, India in 2017 and started the dissertation in Biomedical Photonics group, University of Gothenburg, Sweden. Her PhD focuses on adopting MPM together with fluorescence techniques like FCS and FLIM for different biomedical application

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