

Point Spread Function Measured in Human Skin Using Two-Photon Fluorescence Microscopy

Stina Guldbrand¹, Carl Simonsson², Maria Smedh¹, Marica B. Ericson¹

¹Department of Physics, University of Gothenburg

²Department of Chemistry, University of Gothenburg

contact: stina.guldbrand@physics.gu.se

Scanning fluorescence microscopy using two-photon excitation [1] has become a powerful tool for performing high resolution imaging of optically dense material such as the human skin. For example, the technique can be used for skin cancer diagnostics [2] and to study transdermal drug delivery [3]. Two-photon fluorescence microscopy can ideally be used for qualitative as well as quantitative analysis of the skin; however, the measurements are strongly limited by the variable optical properties when performing imaging at different tissue depths. The resolution is therefore not only determined by the excitation wavelength and the numerical aperture of the objective, but also of the optical properties of the skin and errors of the experimental set up such as aberration and misalignments. To be able to correct for these variable optical properties and misalignments, the point spread function (PSF) needs to be determined. The PSF is generally measured by using subresolution fluorescent beads giving an estimate of the lateral and axial resolution. However, the effective barrier properties of the skin make it difficult to introduce the beads into the tissue. PSF measurements on beads placed on top of skin samples of different thicknesses, have been published [4], but these data do not yield a true PSF in epidermis and dermis. Therefore, in this study fluorescent beads (diameter $0.175 \pm 0.005 \mu\text{m}$, ex/em: 505 nm/515 nm) were injected in human skin biopsies with a syringe.

The two-photon microscope used for imaging is a Bio-Rad Radiance 2100MP Rainbow system built on an inverted Olympus IX71 microscope. The excitation light is provided by a pulsed fs Ti:sapphire Tsunami laser, tunable in the range 700 – 1000 nm. A 40x/0.8 water-immersion objective and an excitation wavelength of 780 nm was used. The PSF, measured at varying depth in the skin specimen, is presented and its full width at half maximum (FWHM) is compared to the calculated width [5]. E.g. a PSF with a size and shape close to the calculated PSF was found close to the skin surface. At larger depths, on the other hand, refractive index mismatch and aberrations alter the size and shape of the PSF. Therefore, it is important to consider the PSF in order to visualise and quantify fluorophores in skin samples correctly.

Ref:

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