INSTITUTIONEN FÖR FYSIK

Multiphoton microscopy enhanced:
Exploring Annular beams and Gold Nanoparticles for improved imaging

Johan Borglin
Institutionen för fysik
Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i fysik, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras fre den 27de maj 2016 kl. 10 i KE, institutionen för kemi och molekylärbiologi, Kemigården 10, Göteborg.

ISBN: 978-91-628-9787-1
ISBN: 978-91-628-9786-4
Tillgänglig via http://handle.net/2077/42385

Abstract

Laser scanning multiphoton microscopy (MPM) has emerged as a powerful tool for non-invasive three-dimensional imaging of biological tissue. The localized focal region enables confocality without the use of a physical pinhole while providing less photobleaching and photodamage compared to confocal laser scanning microscopy. As imaging depth increases, the capabilities of MPM becomes significantly limited by overwhelming background fluorescence and decreased contrast, particularly within highly light scattering tissue. This thesis presents two routes for improving the signal in MPM, involving beam shaping of the excitation laser, and using functionalized gold-nanoparticles (AuNPs) as contrast media. In addition, an experimental MPM system is presented. This system was set up to conduct proof-of-principle experiments.

Theoretical calculations, performed as part of the project, show that annular laser beams could reduce out-of-focus fluorescence when performing MPM, especially in optically dense media. This novel technique is evaluated both mathematically and experimentally. Computer simulations have been performed to predict the theoretical viability of the technique, and proof-of-principle experimentally performed in tissue phantoms and excised tissue samples have been conducted. Initial results demonstrate that the background signal can be reduced by the use of annular beams, which will lead to an increased imaging depth. Further refinements are required to gain full potential of the approach.

As an additional approach to improving MPM, spherical AuNPs were explored as contrast mediators, through the use of multiphoton induced luminescence (MIL). Investigations of AuNPs deposited on gradient substrates show that particle aggregation is required in order to give rise to a detectable signal in far-field MPM. This insight led to the application of a system of 20 nm AuNPs functionalized with synthetic peptides in solution. Upon addition of Zn^{2+}, the particles aggregate which enables the MIL process. Thus, this system is of interest for future development of a switchable contrast media, which will further enhance the capabilities of MPM.

An experimental MPM developed setup was designed and assembled to implement the annular beams. It will allow exploration of fluorescence life-time imaging and multiphoton induced photodynamic effects in a systematic manner. Initial data from the experimental platform are presented. This thesis comprehensively demonstrates the potential of improving and expanding the possibilities for MPM in biomedical research.

Keywords: Multiphoton microscopy, Annular beams, Gold nanoparticles, Multiphoton induced luminescence