Abstract

This thesis guides through contemporary nonlinear optical microscopy. First, it introduces hurdles in microscopy and outlines the relevant works in the fields of super-resolution microscopy and imaging through scattering media. Then it presents the gold-standard technique of two-photon scanning microscopy and a homemade microscope based on Benjamin Judkewitz's design. The microscope can scan at 30 frames per second, and its flexible design supports additional modalities, such as Bessel-beam or two-photon ISM scanning. The microscope was used for end-user experiments for in vivo calcium imaging of dopamine-sensitive neurons.

Next, I describe another custom setup for 3D super-resolution optical fluctuation imaging with temporal focusing, introducing the key concepts of each method. The setup provides axial sectioning of ~2.4µm (temporal focusing excitation), improved to ~1.2µm in a temporal focusing line-scan variant. When combined with super-resolution optical fluctuation imaging, it delivers ~420nm of axial sectioning, enabling reliable volumetric reconstructions with reduced out-of-focus bleaching.

The central part of the thesis is a detailed description of Nonlinear Imaging with Speckle Excitation (NISE). The novel technique provides super-resolution through highly scattering media. The use of avalanching particles enables achieving a resolution of ~560nm, which is more than 2 times better than the 1.2µm theoretical limit in environments where no standard method can provide imaging. Such a result is possible due to uniting two fundamental properties of scattered light: speckle statistics and the angular memory effect, with a highly nonlinear optical response. This combination yields, rather surprisingly, super-resolution, low-background, non-invasive imaging of objects completely hidden behind a strongly scattering, opaque layer. Further developed NISE has a potential impact across fundamental and applied research: from bio-medical imaging and sensing (in vivo brain structure and activity studies) to optogenetics, smart drug delivery (targeted optical uncaging of drugs deep inside tissues), photodynamic therapy, optical computing, and material processing (where high nonlinearities occur naturally).

Throughout, the thesis emphasises reproducible, custom designs and provides practical build guides and instructions for using the specific experimental setups. Collectively, these results show that nonlinearity offers viable routes to deeper, higher-resolution imaging. More importantly, nonlinear microscopy is a perfect playground for interesting ideas, with significant implications when applied.

Keywords: Nonlinear microscopy, Two-photon microscopy (TPSM), Temporal focusing, SOFI (super-resolution optical fluctuation imaging), 3D super-resolution, Deep imaging, NISE (Nonlinear Imaging with Speckle Excitation)