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Review of the dissertation entitled "Microscopy behind Turbid Media" submitted by Archana Malavalli

Dear dean of studies, dear Thierry

In her thesis, Archana Malavalli describes different approaches to achieve high resolution microscopy behind turbid media. On the one hand, this comprises a method to increase the field of view of a method developed in my group, which allows for diffraction limited imaging behind completely turbid media. On the other hand, Archana shows a method to apply structured illumination microscopy behind turbid media, which allows a surpassing of the diffraction limit by a factor of almost two.

The thesis starts with an introduction of multiple scattering of light and how this determines the properties of imaging systems that are supposed to work behind or within such turbid media. This introduction is succinct and to the point, while still showing that she has grasped the subject in several key aspects that are important for her project. This includes a description of the distribution of speckle intensities in the presence of a random medium and how a random walk of photons can be used to model highly scattering media, where the optical properties are simply described by the scattering cross-section and the absorption coefficient. Similarly, the origin of the diffraction limit due to the wave nature of light is explained in detail, which turns out to be useful in the later chapter concerning super-resolution microscopy.

After this introductory chapter, Archana describes the theory and experiments behind the possibility of "un-doing" multiple scattering using spatial modulation of the incoming wave-front to counteract the phase-shifts incurred during the transport through the turbid medium. Using this method of wave-front shaping, it is possible to obtain a diffraction limited-focus behind a turbid layer by iterating on a fluorescent signal. Around this point one can then obtain an image by scanning the focus using the optical memory effect. The angular range of scanning is however limited by the thickness of the turbid layer by the memory effects, such that typically only small fields of view can be imaged effectively behind such a turbid layer. Together with a Master student, Archana has now created a method, described in chapter 2, that can increase this field of view by iteratively refocussing and re-scanning the area of interest. With this it was possible for them to at least triple the field of view achieved by this method, such that a wide field imaging behind completely turbid materials becomes a viable possibility. A manuscript about this project is in preparation.

Archana's main project is however described in chapters 3 and 4, where structured illumination microscopy behind turbid media is discussed. In chapter 3, the ground work is laid ensuring the structured illumination is possible by creating suitable phase mask and adding them to the spatial light modulation used to create a focus behind turbid media. For this she describes the theory as well as several experimental methods of verifying this. This work has already been published and shows that all kinds of periodic patterns can be created at will behind turbid media. Such periodic patterns are the necessary ingredient for the creation of structured illumination microscopy, where the periodicity of the illumination is used as a way to obtain sub-wavelength information via imaging.

This is what is described in chapter 4, where Archana presents not only the theory behind structured illumination and how a resolution breaking the diffraction limit is at all possible, but also presents an experimental realisation of this behind turbid media. The main part of the work described in this chapter concerns the restoration of the phases of the different illumination patterns, which are needed to establish a complete coverage of the optical transfer function in extended Fourier-space. Only in this case is it possible to obtain a better spatial resolution due to the increased coverage in k-space. The thus developed algorithms are then applied on experimental realisations of fluorescent beads, where the imaging takes place behind turbid layers, which in combination with the light modulation act as a "scattering lens" that can produce the required illumination patterns. Taking fluorescent images with a combination of such illumination patterns Archana is then able to estimate the phase shifts and hence reconstruct the images with an increased resolution. The obtained increase compares well with that predicted by theory and is almost a factor of two relative to the diffraction limit. Thus Archana has shown that high resolution imaging is possible using turbid media and light modulation, something which may be of interest for relatively low cost super-resolution microscopy. A manuscript describing these results is currently in preparation.

In conclusion, Archana Malavalli presents a thesis describing clear advances in several aspects of imaging behind turbid media. The theoretical background is always well described and the theory corroborated by experimental results, which advance the field of microscopy to the realm of turbid media, such as biological tissues. While some work still needs to be done for this to be applicable in biological tissues to the long time scale of the experiments, the work clearly points the way that such imaging is possible. Based on these considerations, I can strongly recommend acceptance of this thesis to the faculty of sciences without any reservations.

Sincerely

Christof Aegerter