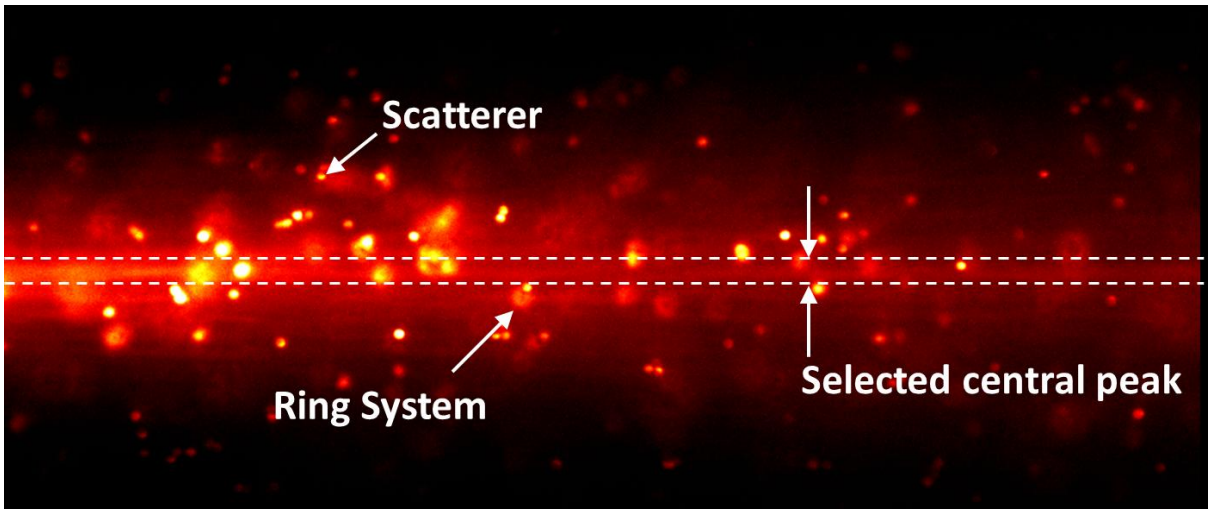
LIGHT SHEET MICROSCOPY OF SCATTERING MEDIA USING SELF-RECONSTRUCTING BESSEL BEAMS

Florian O. Fahrbach, Alexander Rohrbach
Laboratory for Bio- and Nano-Photonics, University of Freiburg



Light sheet based microscopy (LSBM) provides wide-field optical sectioning in large samples by exciting fluorescence only in a thin volume around the focal plane of the detection lens. The sample is therefore illuminated by separate optics oriented orthogonally to the detection optical axis. The image quality is best for very thin light-sheets that homogeneously illuminate the imaged plane. However, these parameters are strongly degraded due to diffraction and scattering by the sample.

Illumination by scanned diffraction-free Bessel beams strongly reduces scattering artefacts offers increased penetration depth thereby enabling the examination of details deeper within large, scattering samples [2,3]. However, the thin central lobe of a Bessel beam is surrounded by an extended ring system that carries a significant amount of energy. When the beam is scanned across the field of view, the rings smear out thereby effectively creating a light-sheet much thicker than the central lobe, resulting in images with reduced contrast. As the energy in the rings is crucial to the beam's self-reconstruction ability it cannot be reduced. However it is possible to minimize the influence on the image. In this talk, I will discuss contrast enhancement techniques regarding important parameters like resolution, photo-toxicity and speed with special emphasis on the suitability to imaging thick scattering samples.

For example, one alternative method consists in using a line-confocal detection scheme, where the image is recorded line-wise only along a thin line at the beam's central peak [4] (see Figure). By comparing illumination with Gaussian and Bessel beams for both standard and line-confocal detection it was found that our method leads to increased contrast for both beams, but is also able to provide increased axial resolution only for Bessel beam illumination. We compared line-confocal detection to structured illumination and found that it is more robust to strong scattering.

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[2] F.O. Fahrbach, P. Simon, A. Rohrbach, „Microscopy with self-reconstructing beams“, *Nat. Photonics* **4**, 780-785 (2010)
[3] F. O. Fahrbach, A. Rohrbach, “A line scanned light-sheet microscope with phase-shaped self-reconstructing beams”, *Opt. Express* **18**, 24229-24244 (2010)
[4] F.O. Fahrbach, A. Rohrbach, “Propagation stability of self-reconstructing Bessel beams enables contrast-enhanced imaging in thick media”, *Nat. Commun.* **3**, 632 (2012)