ENHANCED ALGORITHMIC APPROACH IMPROVES THE SPATIAL RESOLUTION OF STRIPED-ILLUMINATION MICROSCOPY IN INTRAVITAL SUPER-RESOLUTION DEEP-TISSUE IMAGING OF ADULT MICE

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Super-resolution and nanoscopy technologies have become key methods for biosciences and biomedicine. They augment our microscopic vision all the way to the single molecule level, both in fixed and live samples. Adaptive optics and light-sound-interaction-based technologies have similar achievements by extending the range of observations deep into the tissue. Intravital microscopy applications in whole animal models are also becoming increasingly important in our quest of understanding pathogenesis, investigating drug effects and finding cures for human ailments. With fluorescence intravital microscopy, we always have to compromise between spatial and temporal resolution. Thus, further enhancements of fluorescence microscopy techniques in supporting the needs of medicine to resolve the details of life-supporting mechanisms in adult mammals are essential. We present here a newly developed customized evaluation algorithm for multi-beam striped-illumination multi-photon microscopy, previously developed by our group [1,2], which takes into account high-frequency tissue scattering and wave-front distortions. By combining this new algorithm with the use of longer excitation wavelengths in multi-beam striped-illumination multi-photon microscopy, we are able to expand the tissue depths that are accessible for dynamic super-resolution intravital imaging in adult animals. In whole spleen, 300 µm imaging depth was achieved. The increase of lateral resolution in this tissue was 1.7 fold when comparing the new algorithm (Figure, right column) to the raw CCD data (Figure, left column), whilst providing better XY resolution than the previously applied MinMax algorithm (Figure, middle column). The widening of the Fourier spectra (Figure, middle row) and the sharpening of the line profiles (Figure, bottom row) show the enhanced lateral resolution by the new algorithm, even compared to the MinMax method. The 3-fold improvement of axial resolution was maintained. We will further demonstrate the power of our approach in various tissue types.