Towards high accuracy µCT-LM-EM correlative workflow with fluorescent µCT contrast

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Abstract

Visualization and precise quantification of the vasculature on micro- and macro-scale samples are essential for many anatomical and physiological applications. Recent research shows that µAngiofil, a fluorescence polymer-based contrast agent for µCT vascular imaging [1], is highly suitable for multi-scale correlative imaging combining µCT with wide field transmission and fluorescence light microscopy of thin histological sections. The existing correlation accuracy of several microns can be further increased in lateral and axial directions by employing confocal microscopy. Furthermore, application of advanced microscopy modalities, such as fluorescence lifetime imaging (FLIM) and potentially super-resolution modality can provide further insights into tissue structure and its composition. Here we report on the investigation of spectral and lifetime fluorescence properties of µAngiofil contrast for the purpose of further improvement of accuracy in existing µCT-LM correlative workflow.

We used 5 µm thick sections of murine kidney perfused with µAngiofil and stained with H&E for routine histological observation. The imaging was done using a Leica STELLARIS 8 FALCON confocal microscope with a HC PL APO 20x/0.75 IMM CORR CS2 objective. We used the Lambda-lambda scan mode in the range of 460-795 nm for the evaluation of excitation and emission spectra of µAngiofil. For FLIM-based sample characterisation we used the FALCON module equipped with a pulsed white light laser (WLL). We worked with excitation at 488 nm and the detection in the range of 520-590 nm, the accuracy of lifetime measurement is 0.1 ns. The phasor diagram analysis was used to distinguish and separate different lifetime populations.

We found that µAngiofil contrast has a strong autofluorescence within a broad spectral range between 450 and 650 nm with an emission maximum at 570 nm. The excitation maximum for measured wavelength range is at 525 nm. The FLIM analysis shows a significant difference in lifetime between µAngiofil (2.7 ns) and H&E-stained tissue (0.2 ns). Confocal microscopy with better resolution and significant improvement in discrimination of out-of-focus signal, allows for improved accuracy of correlation for the sample features. Although for the sample of murine kidney, FLIM information does not give an additional advantage for distinction between µCT contrast and the tissue sample compared with normal fluorescence, it has been shown [2] that FLIM can be beneficial for discrimination of different tissue types, e.g. healthy and diseased. Furthermore, as the µAngiofil has no significant autofluorescence in the far-red spectral range, we expect that usage of super resolution methods, for example STED with 775 nm depletion or SMLM with far-red fluorophores, can further increase the accuracy of the spatial characterisation of the sample. Introduction of additional fluorescence markers in the sample, not only increases the accuracy of correlation, but also allows for an extension of existing µCT-LM correlative workflow with different EM modalities, e.g., with SBF-SEM or even with cryo-EM.

References

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