IMAGE CORRECTION FOR INTRAVITAL MICRO-ENDOSCOPY OF FEMORAL BONE MARROW

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1. OBJECTIVE

With LIMB - our intravital two-photon fluorescence micro-endoscopy based imaging approach - we are able to investigate complex osteo-immunological cell processes inside the bone marrow over months.[1][2] The integrated Gradient Index lenses have intrinsic optical aberrations, which have to be corrected to allow quantitative analysis and reliable spatial information. Therefore optical specifications and aberrations of the lenses were quantified, and a post-processing algorithm was designed to correct for optical confounding factors.

2. IMAGE CORRECTION ALGORITHM

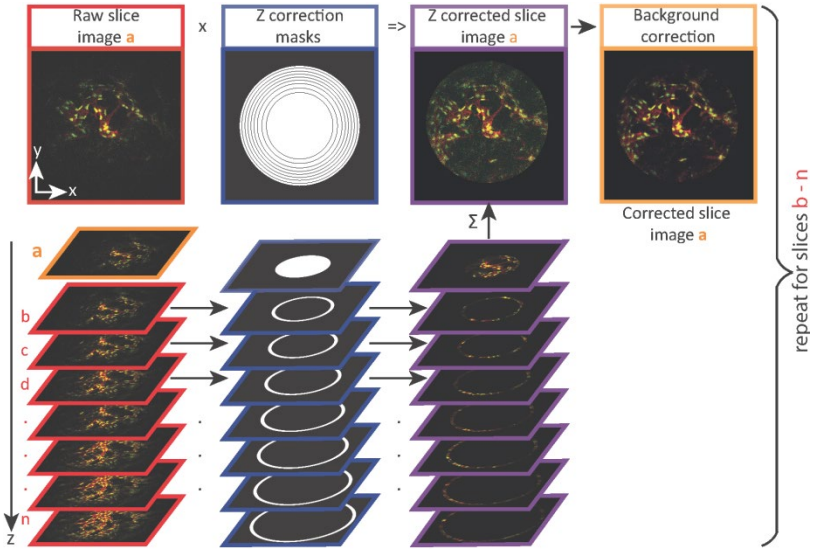

Figure 1: Image correction algorithm – flowchart

In order to correct for the chromatic and wave-front aberrations an image post-processing algorithm was designed consisting of two main steps. The first step sequentially corrects the slices of a raw stack (Fig.1, red frame) for image plane deformation introduced by the wave front aberrations in the axial dimension. This is done by cropping concentric rings of lower slices (b to n) with binary masks (Fig.1, blue frame) and summing them up (Fig.1, purple frame). The second step corrects for inhomogeneous illumination and background signal (Fig.1, yellow frame).

[1] D. Reismann, J. Stefanowski, "Longitudinal intravital imaging of the femoral bone marrow reveals plasticity within marrow vasculature." *Nature Communications* (2017).
[2] J. Stefanowski, A. Fiedler, "LIMBostomy: Longitudinal intravital microendoscopy in murine osteotomies" – *in revision*

3. SUMMARY

Experimental validation of image quality showed minimized image curvature after image correction. In addition, the extraction of cellular and nuclear structures of cells involved in the immune response to an injury (osteotomy) as well as newly forming vessel structures (neoangiogenesis) inside the femoral bone marrow yielded more reliable and accurate results in the corrected images[2]. Consequently, due to increased contrast as well as decreased noise and distortions, image quality was significantly increased.