Multiview Imaging in Two-Photon Microscopy

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Multiview imaging for confocal microscopy was proposed almost 30 years ago [1]. However due to demanding computational needs it did not proliferate in the biomedical community. Later it was reintroduced for Light Sheet Microscopy where it was shown to offer improved resolution and contrast compared to single view [2]. Fundamentally, there are no restrictions in applying Multiview in conventional microscopies such as two-photon or confocal. Multiview imaging consists of imaging the sample from different views and afterwards registering all views in one 3D image.

We applied Multiview imaging in two-photon microscopy fluorescence and Second harmonic Generation (SHG) from collagen and muscle tissue. Multiview deconvolution was further applied to increase contrast and resolution. Effective isotropic resolution was achieved in 3D images. We showed that there was a 3-fold improvement in axial resolution compared to conventional techniques. This methodology retains the advantages of classical methods such as high resolution, and easy sample mounting, while it provides 3-5 fold improvement (depending on the objective) in axial resolution. There are however restrictions regarding the sample thickness as it should not exceed the objective's working distance. Multiview imaging and deconvolution was applied in zebrafish embryo heart based on its SHG signal, in order to visualize that morphology cardiac tissue in 3D (Figure 1). This method can be successfully applied to thick samples for improvement of axial resolution.

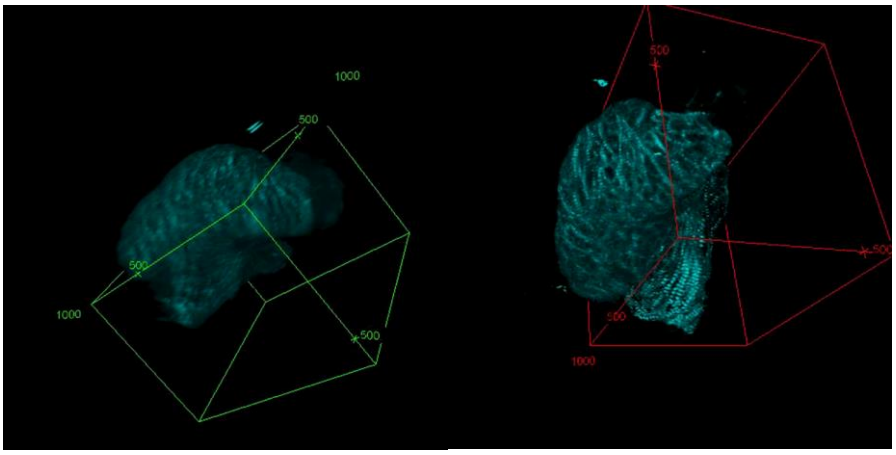

Figure 1: SHG from zebrafish embryo heart. Left: single view, Right: Multiview deconvolution.

References

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