Multi-angle projection method allows rapid volumetric visualization and reconstruction.

Reto Fiolka[1,2], Bo-Jui Chang[1], Kevin M. Dean[1,2], Etai Sapoznik[1], Philippe Roudot[3], James Manton[4], Kayley Hake[5], Lachlan Whitehead[6], Andrew York[5]

1) Department of Cell Biology
2) Lyda Hill Department of Bioinformatics
UT Southwestern Medical Center, Dallas, TX, USA.
3) Institut de Mathématique de Marseille, Marseille, France
4) Laboratory of Molecular Biology, Cambridge, CB2 0QH, UK
5) Calico Life Sciences LLC, South San Francisco, CA, USA
6) Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia
Reto.Fiolka@UTsouthwestern.edu

Key words: Projection imaging, calcium dynamics, light-sheet microscopy, confocal microscopy.

Traditional 3D image formation in optical microscopy involves the serial acquisition of 10's to 100's of 2D image frames. This procedure is time consuming and limits one's ability to observe rapid 3D dynamics. Various methods have been devised to extend the depth of focus of an imaging system, such that a projection of a 3D object can be formed on a 2D image frame, which can be orders of magnitude faster than traditional 3D image acquisition approaches. As a drawback, a projection obviously loses information along the third dimension and typically, methods that extend the depth of focus also deteriorate lateral resolution and only provide a single viewing direction.

Here we introduce a novel multi-angle projection method based on optical shearing (Figure 1). The method allows any camera-based microscope to form projections under varying viewing angles from a three-dimensional sample while maintaining the spatial resolution of the parent imaging technology. As each projection is integrated during a single camera exposure, our method speeds up 3D imaging by ~hundred-fold, allowing the observation of dynamics that would otherwise be too quick for conventional 3D stack acquisitions. Further, the ability to produce projections under different viewing angles opens the possibility for 3D reconstructions from a few rapidly acquired projections.

We evaluate our method with careful comparisons between ground truth z-stacks acquired traditionally, and our projection method, using both light-sheet and spinning disk microscopy. We demonstrate its application to rapid projection imaging of biological dynamics at frame rates of up to 250Hz. This includes imaging of cellular morphodynamics, detection of calcium potentials in neurons, and the beating heart in a zebrafish embryo viewed simultaneously from two orthogonal directions. We furthermore highlight the potential for 3D reconstructions and visualization, as we localize nanoparticles in a cancer cell and demonstrate virtual reality viewing of cellular protrusion dynamics. Given its simple nature and broad utility, we anticipate that this new method for generating projections from multiple viewing angles at camera limited rates will dramatically accelerate volumetric imaging, both in microscopic as well as macroscopic optical systems.

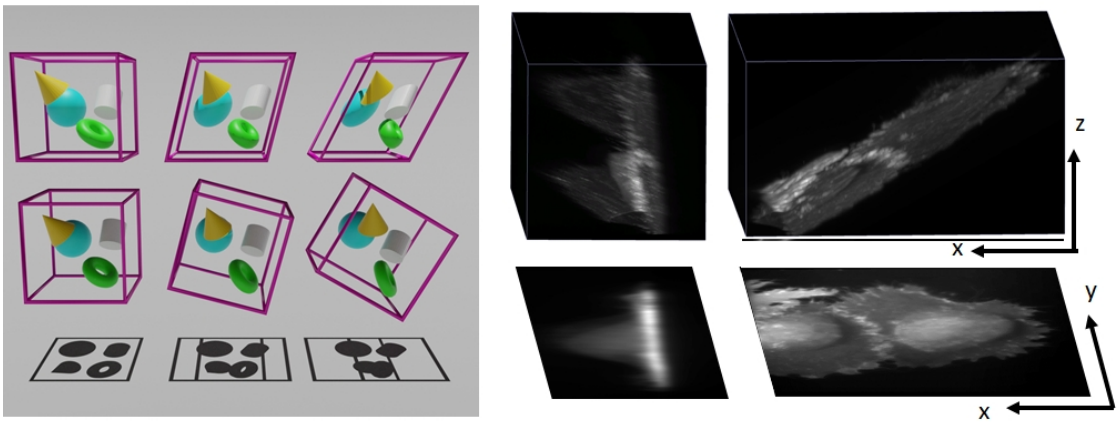

Figure 1 Left: simulation of rotated (top row) and sheared volumes (middle row) and their projections (gray, bottom row). Right: XY projections (bottom row) obtained from a normal and a sheared volume (top row), resulting in different views of the sample.