Light sheet microscopy technologies for quantitative 3D imaging with high spatiotemporal resolution

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It has become increasingly clear that the three-dimensional microenvironment critically influences pathways that govern cellular functions. Therefore, it is important to study cell biology under physiologically relevant conditions, such as xenograft models or artificial microenvironments that closely mimic conditions encountered *in vivo*. In contrast, our most detailed knowledge of subcellular architecture and dynamics stems from high resolution microscopy of cells plated on glass coverslips. To dispense with such artificial 2D culturing conditions requires significant progress in spatiotemporal resolution, optical penetration depth and volumetric coverage of 3D microscopy techniques.

Here, we present emerging light sheet microscopy techniques that enable rapid subcellular imaging over large volumes [1, 2]. We have used these microscopes to study focal adhesions, signaling, and cytoskeletal organization in a variety of cancer cells embedded in reconstituted collagen matrices (see also Figure 1). Using advanced computer vision, we quantify cell morphodynamics and the arrangement of the extracellular matrix surrounding the cell. A new approach to parallelize 3D image acquisition is presented which boosts the volumetric acqusition rate without compromising sensitivity and viability. This enables 3D particle tracking of rapid vesicular dynamics (>10 microns/s) and calcium waves in cultured neurons at volumetric image acquisition rates of 7 and 14 Hz, respectively.

Finally, the use of adaptive optics combined with two-photon based excitation improves the optical penetration depth of light sheet microscopy. These advances are necessary for *in vivo* imaging in xenograft models with subcellular resolution.

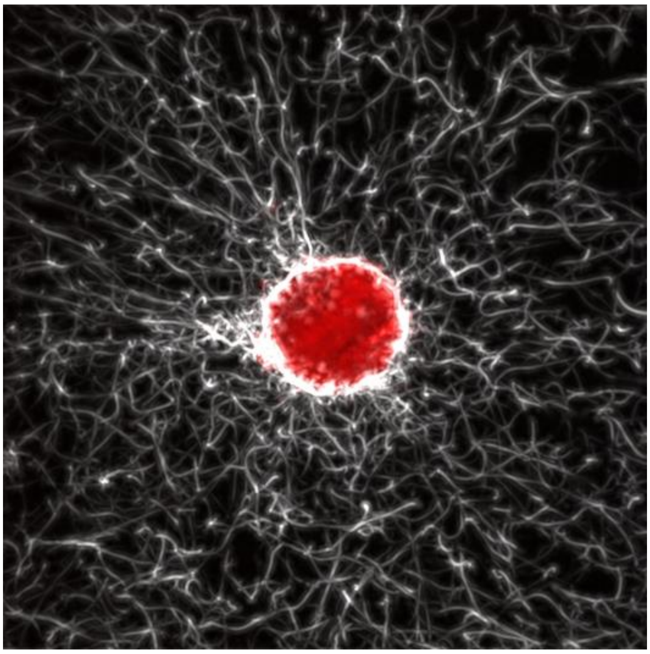

Figure 1. MV3 cancer cell embedded in a collagen matrix. Image size: 84x84 microns.

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