2.5D microscopy: Fast, high-throughput imaging via volumetric projection for quantitative subcellular analysis

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Imaging-based single-cell analysis is essential to study the expression level and functions of biomolecules at subcellular resolution. However, its low throughput has prevented the measurement of numerous cellular features from multiples cells in a rapid and efficient manner. Here we will introduce 2.5D microscopy (2.5DM) that significantly improves the throughput of fluorescence imaging systems while maintaining high-resolution and single-molecule sensitivity. Instead of sequential z-scanning, volumetric information is projected onto a 2D image plane in a single shot by engineering the emitted fluorescence light. Our approach provides an improved imaging speed and uniform focal response within a specific imaging depth, which enabled us to perform quantitative single-molecule RNA measurements over a 2×2 mm^2 region within an imaging depth of ~5 μm for mammalian cells in <10 min and immunofluorescence imaging at a >30 Hz volumetric frame rate with reduced photobleaching. Our microscope also offers the ability of multi-color imaging, depth control and super-resolution imaging.

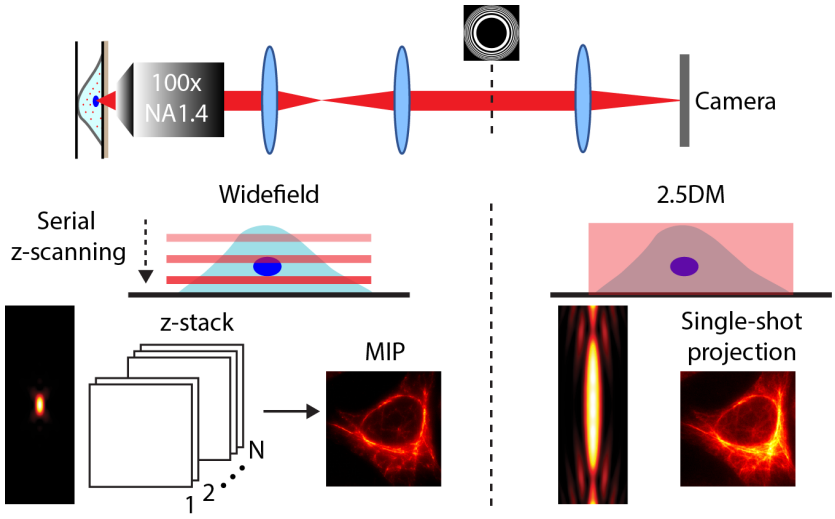

Figure 1: Fast high-throughput and high-content 2.5D microscopy for sub-cellular analysis.