A THREE-CAMERA IMAGING SETUP AND NOVEL CELL-PREMEABLE DYSES FOR MULTIPLEXED SINGLE-MOLECULE LIVE CELL EXPERIMENTS

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Our aim is to develop quantitative single cell and single molecule assays to study when and where molecules are interacting inside living cells and where enzymes are active. To this end we present a simultaneous three-camera imaging setup for fast tracking of multiple interacting molecules simultaneously. We further present novel bright and cell-permeable dyes that have enabled live-cell multi-color tracking experiments [1]. This new color palette of bright and photostable fluorophores is a key component for multi-color experiments within the nucleus of a living cell. We also present algorithms that accurately track and co-localize interacting biomolecules. The new setup combined with our co-movement algorithms have made it possible to simultaneously image and track both nascent mRNAs and RNA polymerases, to image where and when mRNA may be translated by ribosomes, and how the chromosome environment affects diffusion kinetics (Figure 1). The novel dyes have facilitated multiplexed single-molecule measurements at a high spatiotemporal resolution inside living cells. Such experiments will constitute a major tool in testing models relating molecular architecture and biological dynamics.