

## LIGHT SHEET BASED MICROSCOPY AND A REFINED APPROACH TO MODERN BIOLOGY

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Modern bio-photonics provides a wealth of technologies that operate in a *Nanodomain*. The resolution of optical microscopes is in the range of several 100 nm, the precision of optical tweezers can be as low as a single nm [1], laser cutters such as the laser nanoscalpel generate incisions a couple of 100 nm wide and in three dimensions cause severing that is barely 700 nm deep [2,3]. More recently, extremely efficient light microscopes require only nanoWatts of power per volume element to induce a reasonably detectable fluorescence emission [4]. However, most optical technologies are mainly applied to 2D cellular systems, i.e. in a cellular context that is defined by a hard and flat glass or plastic surfaces. However, obtaining meaningful information requires the morphology, the mechanical properties, the media flux and the biochemistry of the cell's context found in live tissues [5]. Such a physiological context does not consist of single cells cultivated on cover slips. It is found in more complex three-dimensional constructs of cells that are grown on patterned surfaces, cultivated in an ECM-based gel and in embryos of flies and fish or directly in live tissue. The observation as well as the optical manipulation of thick and optically dense biological specimens suffers from at least two very severe problems. 1) They tend to scatter and absorb light. The delivery of the probing light as well as the extraction of the signal light tends to become inefficient. 2) Many biochemical compounds apart from the fluorophores also tend to absorb light and to suffer degradation of some sort (photo toxicity), which induces malfunction or death of a specimen. The Light Microscopy Group at EMBL continues to develop technologies for the observation and manipulation of large and complex three-dimensional biological specimens. The basic technology of choice seems to be the use of light sheets, which are fed into the specimen from the side and observed at an angle of 90° to the illumination optical axis. The focal volumes of the detection lens and the volume of the light sheet overlap. Thus, optical sectioning and no photo damage outside the common focal plane become intrinsic properties. EMBL's single plane illumination microscope (SPIM) takes advantage of modern camera technologies and can be combined with essentially every contrast and specimen manipulation tool. However, it operates in a truly three-dimensional fashion. The straightforward optical path in SPIM is designed to allow high flexibility and modularity. We have already successfully integrated a UV-laser nanoscalpel into one of our SPIMs and intend to make SPIM a complete toolbox of photonic nanotools. It will allow us to investigate the influence of localized mechanical forces on cell function, and to induce targeted perturbations in cellular systems. Possible starting points for relaxation type of experiments are cutting actin fibres and microtubules, optical ablation of cell-cell contacts, manipulation of sub-micrometer particles within cells, stimulation of selected cell compartments with optically trapped probes. In addition FRAP can be used to monitor the system response with improved specificity.

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