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Light Sheet Fluorescence Microscopy: A Pathway into New Worlds

Jueves 10 de marzo 11:00 horas, Institut Pasteur de Montevideo

Light sheet fluorescence microscopy is a relatively young light microscopic technique, which features optical sectioning, low photo-toxicity and rapid image acquisition. The technique is based on illuminating the sample orthogonally to the detection pathway with a thin, focused sheet of light. The basic principle of the method will be introduced, and current lines of methodological developments in our lab will be outlined: confocal light sheet imaging, 2-photon excitation, large volume imaging and combination with expansion microscopy.

Real Time Observation of Single Molecule Pathways in Biological Systems

Viernes 11 de marzo 11:00 horas, Institut Pasteur de Montevideo

Observation and tracking of fluorescently labeled molecules and particles in living cells reveals detailed information about intracellular processes on the molecular level, e.g. the nuclear export of RNA particles. Whereas light microscopic particle observation is usually limited to two-dimensional projections of short trajectory segments, we report here image-based real-time three-dimensional single particle tracking in an active feedback loop with single molecule sensitivity. We tracked particles carrying only 1–3 fluorophores deep inside living tissue with high spatio-temporal resolution. Using this approach, we succeeded to acquire trajectories containing several hundred localizations. We present statistical methods to find significant deviations from random Brownian motion in such trajectories. The analysis allowed us to directly observe transitions in the mobility of ribosomal (r)RNA and Balbiani ring (BR) messenger (m)RNA particles in living *Chironomus tentans* salivary gland cell nuclei. We found that BR mRNA particles displayed phases of reduced mobility, while rRNA particles showed distinct binding events in and near nucleoli.

