IBEC
Institute for Bioengineering of Catalonia

Core Facilities

Microscopy

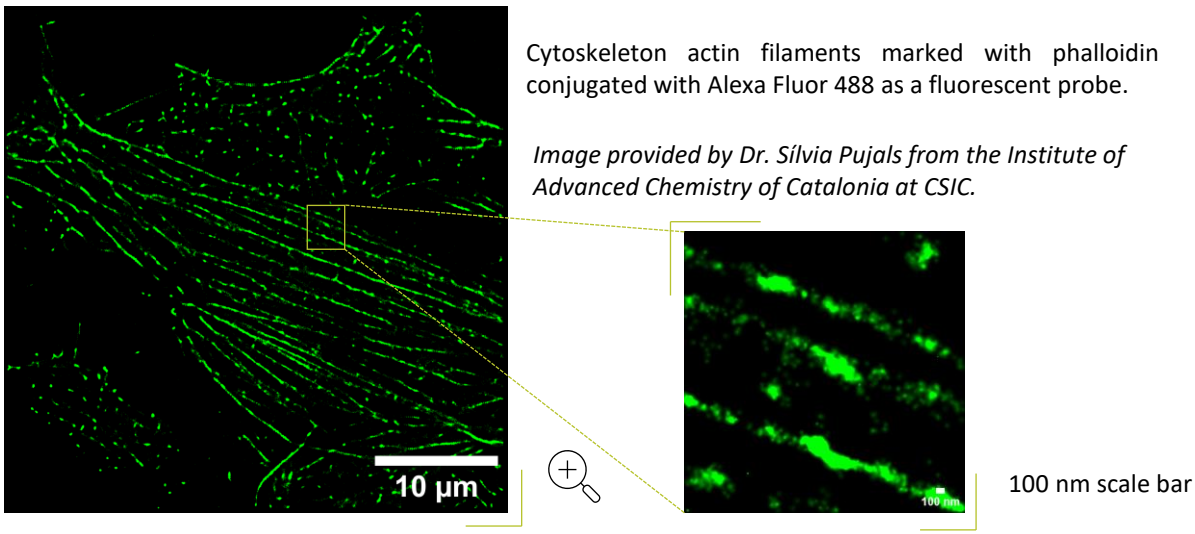
STORM Microscopy

The Microscopy Characterization Facility has a single molecule localization microscope. This is an advanced characterization tool in nanoscience, due to its unique attributes against other optical microscopy techniques, which allows achieving resolutions under Abbe's diffraction limit.

STORM Microscopy:

ST*ochastic* ***O****ptical* ***R****econstruction* ***M****icroscopy* is a single molecule localization technique based on the blinking properties of photoswitchable probes, when these are imaged under certain buffers and high-power laser conditions. The sample is covered in fluorescent probes by immunostaining procedures and a high-framerate camera is used to record the blinking of the probes on a large set of fast, consecutive frames. Since not all probes will blink at the same time in different frames, an algorithm is able to detect and assign a localization in the XY axis for every separate blinking with high precision. Afterwards, all these precise localizations are recombined to form a super resolution image of the sample.

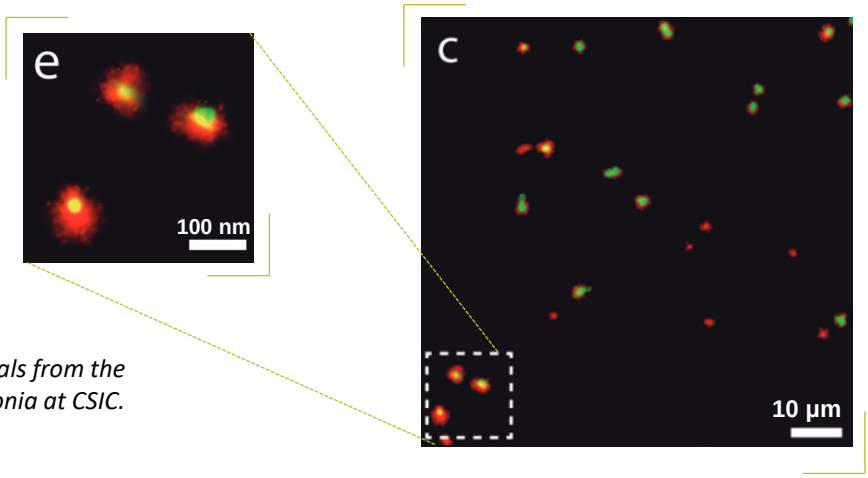
A stigmatic lens aberration piece can be placed in front of the camera to obtain 3D super resolution images.



Cytoskeleton actin filaments marked with phalloidin conjugated with Alexa Fluor 488 as a fluorescent probe.

Image provided by Dr. Sílvia Pujals from the Institute of Advanced Chemistry of Catalonia at CSIC.

100 nm scale bar



Nanoparticles in green and protein corona formed on the surface in red.

Images provided by Dr. Sílvia Pujals from the Institute of Advanced Chemistry of Catalonia at CSIC.

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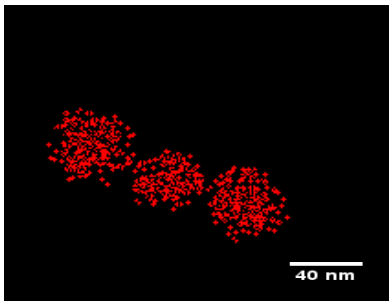
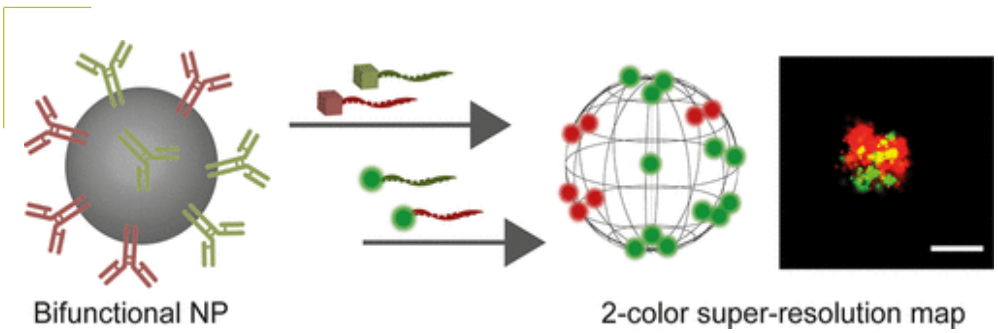
PAINT Microscopy:

***P**oints **A**ccumulation for **I**maging in **N**anoscale **T**opography* is another single molecule localization technique that can be performed with the STORM microscope. In PAINT, the sample is set on a medium filled with ligands that have affinity for the sample and transiently bind to it. These ligands are linked to a fluorescent probe that emits fluorescence when a transient bind happens. A high-framerate camera records these bindings on a large set of consecutive frames and an algorithm reconstructs the detected localizations into a super resolution (SR) image.



80-nm nanoruler beads imaged through DNA-paint
Image provided by Guillem Romero and Martí Milozzi from the Microscopy Characterization Facilities of IBEC.



A bifunctionalized nanoparticle
Image provided by Dr. Sílvia Pujals from the Institute of Advanced Chemistry of Catalonia at CSIC.

Particle tracking:

The high-framerate camera of the STORM microscope can record a set of thousands of consecutive frames or images with an exposure of, at minimum, 1 millisecond. As the system works with a *Perfect Focus System*, the optimal distance where it registers tracking is at 200 nm near the coverslip surface (it could also track particles in the 2-5 µm range, but with a huge increase in background noise). This allows for surface particle tracking experiments involving nanoparticles and other nanoscopic compounds. Thus, it is possible to calculate parameters such as velocity, type of diffusion and diffusion coefficient.

a) Set of trajectories obtained with the hexavalent monomannoside probe; c) Trajectory of two different hexavalent monomannoside probes by PAINT multicolor technique

Representations provided by Dr. Sílvia Pujals from the Institute of Advanced Chemistry of Catalonia at CSIC.