46 - Fluorescence microscopy techniques to study actin dynamics and mechanical properties of actin cables in cells

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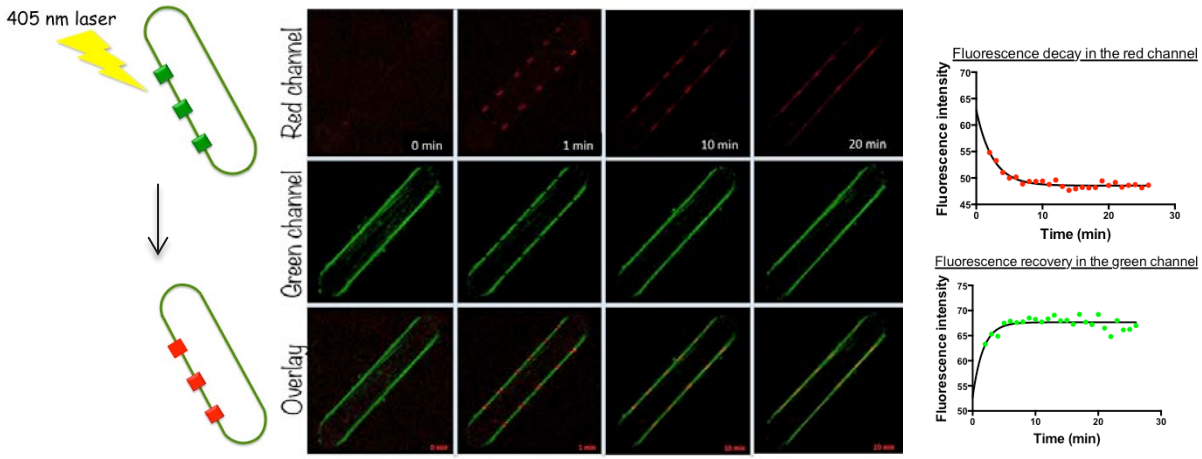
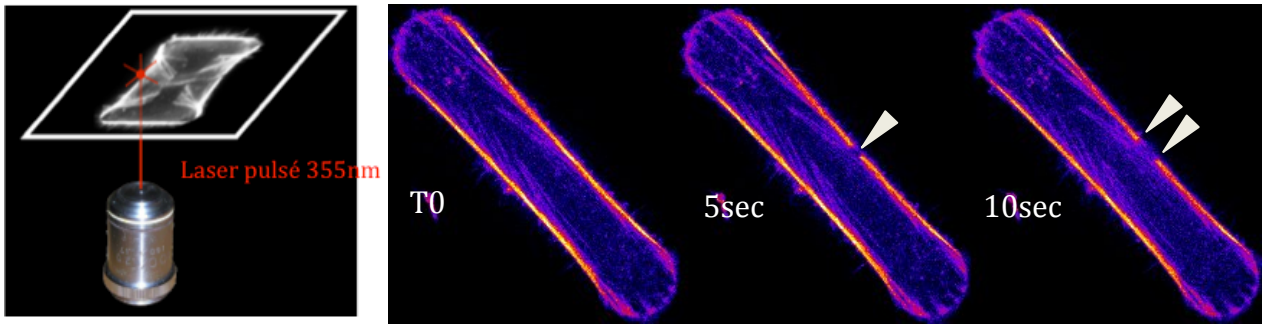
Cells have the remarkable ability to sense changes in their microenvironment and adapt their shape in response to these changes. In particular, they can sense mechanical cues from the substrate and remodel their microstructures accordingly. This process is mainly driven by the actin cytoskeleton that can both transmit and generate mechanical forces. In order to understand better the interplay between actin dynamics, cytoskeleton architecture and mechanical property of the cells, we are using different imaging-based techniques.

- In photoconversion experiments, photoconvertible proteins fused to actin can be switched between two fluorescent states, allowing to look at actin velocity and turnover in stress fibers.



- In laser nanosurgery experiments, a localized photodamage is induced on the contractile stress fibers leading to their severing. By coupling this technique to traction force microscopy, we can look at the fibers end retraction according to a viscoelastic model and measure the released mechanical energy.



We propose an introduction to these different techniques and their subsequent analyses during the practical work (2 days).

References:

Actin: Blanchoin L., Actin dynamics, architecture and mechanics in cell motility, Physiol Rev. 2014

Photoconversion : Dovas A., Visualization of actin polymerization in invasive structures of macrophages and carcinoma cells using photoconvertible β-actin-Dendra2 fusion proteins, PlosOne, 2011

Laser nanosurgery : Kumar S., Viscoelastic retraction of single living stress fibers and its impact on cell shape, cytoskeletal organization, and extracellular matrix mechanics, Biophys J., 2006

Traction force microscopy : Sabass B., High resolution traction force microscopy based on experimental and computational advances, Biophys J., 2008

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