# SNOM fluorescence techniques applied to cytoskeleton study in cell cultures

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**Introduction** Scanning near field optical microscope (SNOM) is a tool which overcomes the diffraction limit of conventional optical microscopy and allows optical imaging with a resolution of less than 100 nm without having to go through any special sample preparation. Moreover one of the best advantages of SNOM is to produce topographic, transmission and reflection images simultaneously [1]. Several studies have been carried out by SNOM in the last years on biological specimens, but the fluorescence SNOM technique has been rarely applied on cell culture investigations. Here we set up a TriA-SNOM with a specific fluorescence equipment to develop the performance of this probe microscope and identify cytoskeleton elements in cell cultures, mainly primary rat podocytes cultures.

**Materials and methods** The microscope used were a TriA-SNOM (APE Research, Trieste, Italy) equipped with a specific fluorescence set-up that allowed to obtain different SNOM images of the samples simultaneously: topography, SNOM fluorescence in reflection and SNOM-fluorescence in transmission. Cell cultures: Primary culture of rat podocytes were treated by immunofluorescence techniques with TRITC-phalloidin to visualize cytoskeletal elements such as actin filaments. Actin filaments were analyzed by SNOM-fluorescence and compared with conventional fluorescence.

**Results** SNOM- fluorescence, unlike all other techniques, may reconstruct a fluorescence mapping of the sample, and obtain a direct measure in height of the fluorescence structures. The major technical problem in this kind of microscopy is to reduce of the optical background noise. We actually obtained a SNOM-fluorescence set-up to adequately visualize the specimens, producing images with low background noise and high resolution. SNOM moreover allowed to discriminate the superficial actin filaments from the deep filaments by reflection system.

**Conclusions** SNOM with fluorescence technique may be suitable method for detection of specific molecules in cell cultures, providing an overlapping of the fluorescence image on the 3D mapping of the sample. These studies open further possibilities for application of SNOM to biological materials, especially in cells observed in liquid environment.

## Reference

[1] Neuroimage 49 (2010) 517-524.

Key words
SNOM fluorescence, podocytes, actin filaments