

## Assessment of actin fibers in the cell using fluorescent polarization microscopy

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### [Background]

Actin fibers, one of the cytoskeletons, maintain cell morphology and control the movement of the cells. In the research field of mechanobiology, proper assessment of the cytoskeletal response against the external force is important. Subcellular distribution of actin can be observed by normal fluorescent microscopy. However, it is still difficult to observe the dynamic changes of actin fibers due to mechano-stress with an ordinary optical microscope. Polarization microscopy is generally used in physical property analysis. It is also possible to analyze stress applied to a substance by polarization analysis. We propose a new method for measuring the mechano-stress on the cytoskeleton by polarization microscopy. To achieve this issue, we first confirmed if it is possible to observe the fluorescence-labeled actin fiber by self-customized polarization microscopy.

### [Methods & Results]

A custom-made polarization microscope was established to observe fluorescence polarization of actin fibers. A polarizing beam splitter was placed immediately after the DPSS laser with 532-nm wavelength, and an analyzer was placed immediately before the detector. HeLa cells were fixed with 4% paraformaldehyde, and then actin was immunostained with rhodamine. The fluorescence intensity of rhodamine-labeled actin fiber was periodically changed, while the polarizer was rotated. The time phase of the detection light change was shifted between the vertical fiber and the horizontal fiber with respect to the microscopic field, which suggested that this was not an artifact, but a change in the detected light accompanying the polarizer rotation. The polarization extinction ratio due to polarizer rotation was only 3.5, it was thought that improvements of the polarizer and optical system were still necessary.

### [Conclusion]

Fluorescent polarization microscope was established, and polarization dependency of fluorescent labeled actin fiber was confirmed. Fluorescent polarization microscopy could be a useful to analyze the actin cytoskeleton.

**Keywords:** polarization microscopy, actin