

QUANTITATIVE FLUORESCENT SPECKLE MICROSCOPY OF LAMELLIPODIAL ACTIN MESHWORK ACTIVITY

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INTRODUCTION: Cell morphological activity is dictated by a constant, dynamic rearrangement of the actin cytoskeleton. Recently, we have discovered a direct interdependence between the geometries of lamellar actin retrograde flow and leading edge [1]. This finding led to the hypothesis that the actin meshwork acts as a mechanical transducer, translating spatial flow variations promptly into morphogenetic responses. One of the key factors controlling the flow geometry are cytosolic and extra-cellular cross-links of transmembrane proteins (integrins) which establish a molecular coupling between the cytoskeleton and adhesive surface proteins. Thus, we may speculate that the distribution of surface proteins has an immediate impact on the actin dynamics, which, in turn, affects cell shape formation and motility. To test this line of arguments we have started to implement techniques that allow us to quantify actin dynamics as a function of adhesive and non-adhesive patterns. Here, we report first results in applying fluorescent speckle microscopy (FSM) as a means to measure actin assembly, translocation, and turnover in living cells.

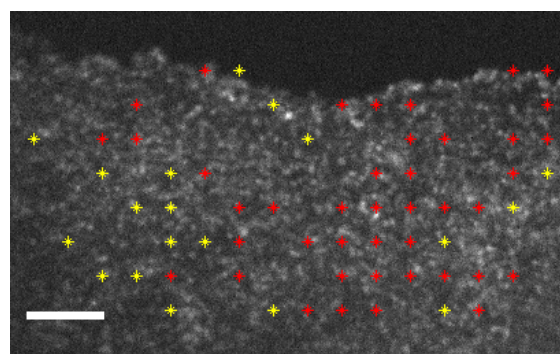
METHODS: FSM is a new technique for visualizing cytoskeleton activity [2]. In time-lapse FSM, movement of the speckles represents polymer translocation, while changes in speckle intensity and number density represent polymer assembly and turnover. However, the speckle signal is extremely complex and the density of image events is overwhelming for a human observer. The full power of FSM can only be exploited with specialized image analysis. Thus, our first effort is to implement software that enables us to extract the sought mechanical and chemical characteristics of f-actin from speckle image sequences.

RESULTS: To develop this program we have imaged actin speckles in two classes of living newt lung epithelial cells:

- 1.) Contact-inhibited cells within epithelial sheets do not exhibit obvious actin translocation. Thus, they are ideally suited for life-time analysis of spatially stationary actin speckles.
- 2.) In contrast, migrating cells at the periphery of the sheets exhibit actin flow from the leading edge towards the cell body. This provides us with a

system for developing tools that measure speckle field translocation.

Time lapse sequences were acquired for up to 3 hours at a frame-rate of 2 – 30 s with a cooled CCD camera (ORCA II) on a Nikon inverted microscope with 100X / 1.4NA optics. First results indicate that our FSM implementation indeed is capable of providing quantitative spatial information about cytoskeleton assembly, dissociation (Fig. 1) and translocation from inside



living cells (Fig. 2).

Fig. 1: Map indicating sites of polymerization (red) and depolymerization (yellow) in the lamellum of a contact-inhibited epithelial cell. Scale bar: 2.5 μ m.

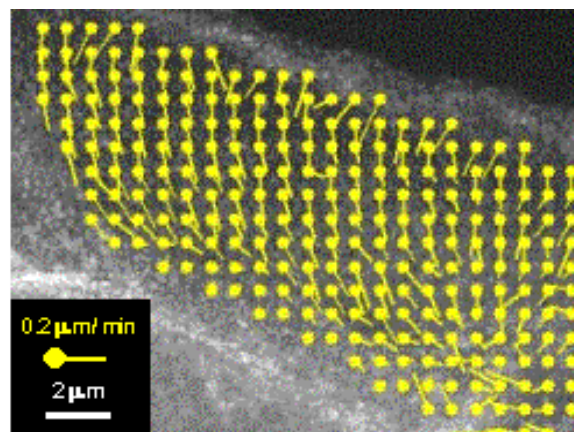


Fig. 2: Map of f-actin retrograde flow in a cell at the border of an epithelial sheet.

REFERENCES: ¹G. Danuser, and R. Oldenbourg (2000) *Biophys. J.* **79**:191-201. ²C.M. Waterman-Storer, et al. (1998). *Current Biol.* **8**:1227-1230.