

FLUORESCENT SPECKLE MICROSCOPY – A TUTORIAL

P. Maddox¹, and G. Danuser²

¹*Department of Biology, U. North Carolina, Chapel Hill, NC,* ²*[Department of Mechanical and Process Engineering, ETH Zurich, Switzerland](#)*

INTRODUCTION: Fluorescent speckle microscopy (FSM) has been discovered in 1998 by the Salmon Lab at the University of North Carolina, Chapel Hill [1]. It allows the visualization of movement, assembly, and turnover of polymeric structures inside the cell. Initial applications studied by Salmon and co-workers have included measurements of microtubule movements in mitotic spindles and actin retrograde flow in migrating cells. Since then it has become one of the most powerful techniques to investigate different aspects of polymer dynamics *in vivo* and *in vitro* [3].

METHODS: The speckle pattern can be explained by random incorporation of labelled and unlabeled free monomers / dimers into a polymer. When only a small fraction of monomers / dimers have fluorescent label, the polymers tend to acquire a perceivable variation (contrast) in fluorescence emission along their lattice. Local intensity maxima are called speckles. At first approximation the speckle contrast increases with lowering the ratio between labelled and unlabeled monomers. Experimentally, it was determined that fractions of 0.1% to 0.5% give optimal contrast [2]. At such fractions speckles contain only 1 – 2 fluorophores per resolvable unit (Airy disc). Therefore, camera noise, background and out-focus fluorescence, limitations in fluorescence quantum efficiency, etc. are significant factors that have to be controlled. On the imaging side efforts have to be made in determining the best way of labelling monomers, in finding markers with high photostability, and in assembling stable, high-precision digital microscopes that can monitor the speckle dynamics.

Even under optimal imaging conditions the speckle signal is extremely difficult to analyse. Thousands of weak contrast spots move through the image. They can disappear any time, which indicates local polymer turnover while new spots may pop up indicating local polymer assembly. The density of image events is overwhelming for a human observer. Hand-quantitation of the positional and chemically kinetic information that is inherently contained in this type of image data is impossible. Thus, FSM can only be exploited in connection with specialized image analysis. Algorithms are needed for 1.) Measurement and representation of

directed speckle flow; 2.) Speckle lifetime analysis; 3.) Speckle modelling in order to connect speckle contrast to biophysical parameters, such as posterior estimates of the fraction of labelled and unlabeled monomers, polymerisation and depolymerisation rates, and ultimately the mechanics of the monitored polymer assembly.

RESULTS: Results will be shown from two applications. 1.) FSM is currently used to investigate the role of actin dynamics in regulating cell motility and shape formation. Software is developed to measure the position of the leading edge and actin translocation and polymerisation / depolymerisation rates. From this data we will build a mechanical model that couples spatio-temporal variations in f-actin flow with leading edge activity. In a next step we aim to apply the same techniques with a surface sensitive microscope in order to study the dynamic linkage between actin cytoskeleton and micro-patterned surfaces. 2.) The general power of the technique will be demonstrated with FSM studies on microtubule activity in mitosis. Microtubules attach via their plus ends to replicated chromosomes while the minus ends are forming the poles of a spindle-like structure. During anaphase, chromosomes are actively moved towards the spindle poles, resulting in equal division of the genome. We have used FSM and specific inhibitors to investigate the mechanism of this movement in the *Xenopus* egg extract system and found that chromosome movement is driven mainly by translocation of the microtubule lattice rather than polymer turnover at the plus end.

REFERENCES: ¹C.M. Waterman-Storer, et al. (1998). Fluorescence Speckle Microscopy: Visualizing the movement, assembly and turnover of macromolecular assemblies in living cells. *Current Biol.* 8:1227-1230. ²C.M. Waterman-Storer and E.D. Salmon (1999) Fluorescent speckle microscopy of microtubules: how low can you go? *FASEB J.* 13, S225 – S230. ³T.J. Keating, and G.G. Borisy (2000) Speckle microscopy: When less is more. *Current Biol.* 10 (1): R22-R24.

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