QUANTIFICATION OF KERATIN NETWORK DYNAMICS FROM CONFOCAL MICROSCOPY IMAGES

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Keratin intermediate filaments are major and highly dynamic components of the epithelial cytoskeleton, which protects epithelial cells against different forms of stress and regulates various cell functions [1]. We have observed a cycle of keratin filament assembly and disassembly in cultured cells: new keratin filaments assemble in the cell periphery where they integrate into the keratin network that is moving towards the nucleus. A part of the network forms a very stable cage around the nucleus, the other part disassembles into soluble subunits that can be reused for another cycle of assembly and inward transport [2]. A major challenge is measuring keratin cycling in quantitative terms, especially in response to growth factors and drugs.

We therefore developed image analysis tools to deduce keratin transport rates and polymerization/depolymerization from confocal time-lapse fluorescence images. Images were first denoised by thresholding the curvelet coefficients. If needed, global cell movement was compensated for by rigid registration. To make data sets from different cells more comparable, to compensate for cell shape changes and to allow topological mapping, image data were optionally converted into idealized round cells. Local keratin filament motion was detected by a maximum-a-posteriori motion estimation assuming stable brightness and using the sum of squared differences as data term and an elasticity model as regularization term. By coupling the information contained in the motion vector field with fluorescence intensity the keratin flux was determined. The keratin transport rates (bulk flow) then served to identify sources and sinks of keratin filaments corresponding to regions of keratin filament polymerization and depolymerisation, respectively. These data are used to develop models of keratin filament turnover and architecture.

Using the newly established work flow, we found that increasing time after seeding of cells correlates with down-regulation of inward-directed keratin filament movement and a reduction in keratin turnover. Conversely, treating cells with epidermal growth factor reverses these processes within a few minutes. These examples highlight the usefulness of the new tools to characterize keratin network dynamics and to study the influence of different cellular pathways on keratin network architecture.