In vivo imaging and quantification of the continuous keratin filament network turnover

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Keratin polypeptides are major components of the epithelial cytoskeleton forming a filamentous 3D-network. Like intermediate filament polypeptides of other cell types, keratins make up a stable, but elastic network that is responsible for mechanical stress resilience. At the same time the keratin network is able to change its shape during development, cell division, metastasis and cell migration.

Our long term goal is to elucidate and characterize mechanisms that control the formation, shaping and remodelling of the keratin network to establish a general model of dynamic intermediate filament network organisation. Therefore we established a workflow to monitor fluorescently labelled keratins in living cultured cells under standard and experimental conditions. We isolated several epithelial and non-epithelial cell lines stably expressing fluorescent protein-tagged keratins alone or in combination with other fluorescently-tagged proteins for simultaneous monitoring. Time lapse series of fluorescence microscopical images were generated with the help of widefield or confocal fluorescence microscopy.

Unexpectedly and in contrast to the existing text book model, a topologically-defined constant turnover of the keratin cytoskeleton was observed [1]. Detailed high resolution analyses of z-stacks in combination with fluorescence recovery after photobleaching (FRAP) experiments and excitation of photoactivable keratin-GFP fusions led us to propose a dynamic model of the keratin network turnover cycle that is independent of protein biosynthesis. Central to this model is the continuous and regulatable polymerization of keratin filaments which is primarily restricted to the cell periphery. After nucleation filament network precursors further elongate and move from the cell cortex to the cell interior by continuous, actin-dependent transport thereby reaching the peripheral pre-existing network into which the precursors are integrated. Filaments continue to migrate toward the perinuclear region where they dissolve giving rise to soluble subunits that can be re-utilized in the cell periphery for another round of the keratin turnover cycle.

To substantiate the model and to work out aspects of individual steps of the proposed keratin network treadmill, we developed and applied image processing tools.

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In the first set of analyses, the conspicuous network flow was examined. To this end, quantification was performed on a time-lapse series of 69 z-stacks (22 focal planes at 1024x256 pixels) recorded at 60 s intervals. Global cellular movement was compensated for by an image intensity-based method [2] using the normalised sum of squared intensity differences (SSD). Robustness was increased by embedding the registration in a multiscale Gaussian pyramid framework. Visual assessment of the results revealed no misalignments. Based on the aligned image series, analyses could be restricted to intracellular keratin motion. A z-axis projection of each volume was superimposed by a grid of regions of interest (ROIs) at a 20 pixel spacing. The overlapping ROIs were of 40 pixel width. Each ROI was rigidly registered to the previous frame by the SSD criterion using exhaustive search for translations of ±20 pixels. SSD was modified by a weighted window function on the ROIs which dropped linearly from the centre to zero. Thereby the non-rigid character of the data was accounted for while enough structural information was captured by the ROIs, similarly to [3]. Assuming long-term local stability of the keratin flow, the medians of the translation vectors were computed for each grid point over time revealing coordinated centripetal keratin flow all the way to the nucleus (Fig. 1). Different zones characterized by different transport rates could be defined with incremental decrease from the periphery to the cell centre. Taken together, these data strongly support our proposed centripetal keratin treadmill model.

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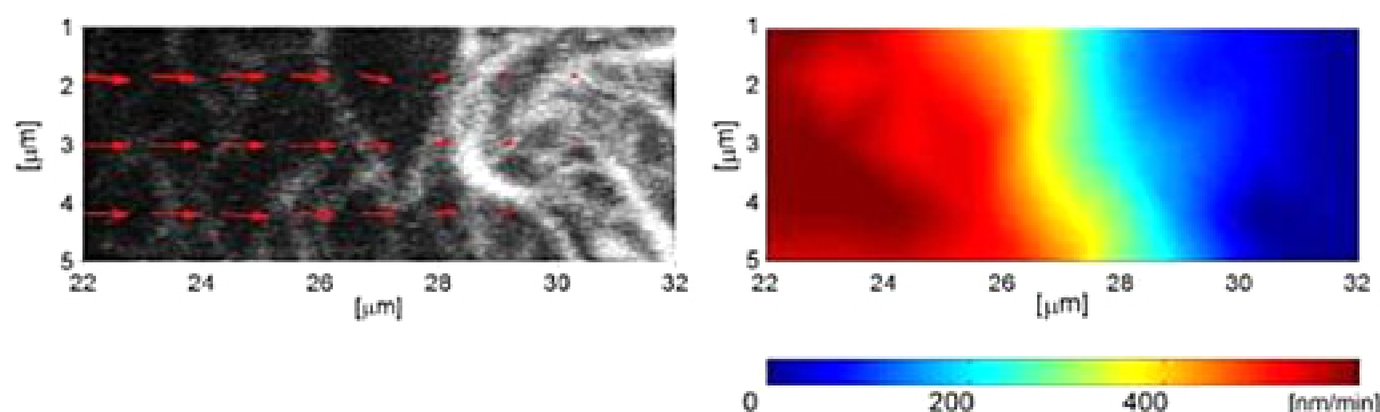

Figure 1. Determination of direction and speed of keratin flow from a time-lapse fluorescence image series. (Left) Vector visualization superimposed on projection of initial fluorescence images at time point 0. (Right) False colour representation of velocities determined in different regions (cell periphery at left, perinuclear region at right).