

DISTINCT REGIONAL FOCAL ADHESION DYNAMICS EXPLORED BY AUTOMATED QUANTITATIVE ANALYSIS

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Background: Focal Adhesions (FAs) play a key role in cell-substrate adhesion and migration. Modern high-throughput live cell microscopy provides experimental data on FA's behavior with increasingly high temporal resolution for an ever-expanding variety of cell cultures and experimental conditions. For this kind of data, manual analysis is challenging in terms of reproducibility and sheer volume of it, so fully automated software-based FA tracking algorithms become crucially important[1].

Materials and methods: Cell cultures with fluorescently labeled FA proteins (vinculin-RFP and paxillin-EGFP) were obtained, live cells were imaged by spinning disk confocal microscopy, and FAs were quantitatively analyzed via custom MATLAB-based software.

Results: We optimized the algorithm for tracking FAs all the way to microscope resolution limit, accessing dynamics (growth, disassembly and lifespan) for events starting from half a minute in duration, while also tracking through FA splitting, merging, and translocation. After initial validation on a panel of 6 cell cultures, we used this algorithm to quantify differences in regional FA behavior in A549 cells. Newly-formed small FAs underwent frequent merging/fusing events, consistent with

liquid-liquid phase condensation. FA formation in the regions of rapid edge protrusion was upregulated by an order of magnitude in respect to other cell edges. On retracting edge long-range FA translocations were observed. By simultaneous tracking of vinculin and paxillin in double transfected cells differences in the dynamics of these proteins inside FAs were highlighted, including the phenomenon of FA layer separation.

Conclusion: Our automated algorithm proved to be a powerful tool for high-throughput analysis of FA behavior in cells and highlighted several previously poorly documented features of FA dynamics.

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Key words: cytoskeleton, focal contacts, cell migration, confocal microscopy, automated analysis.

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