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Live-cell imaging of invadopodia formation with simultaneous phase-shifted laser feedback interference microscopy and fluorescence microscopy

Background

Metastatic cancer cells form invadopodia, which are subcellular structures that degrade the extra-cellular matrix. Invadopodia invade their surrounding tissue, and hence their formation is closely linked to the metastatic nature of cancer cells. A mature invadopodia requires the controlled assembly of actin, intermediate filaments and microtubules [1]. Several key proteins such as cortactin, cofilin, and Arp2/3 regulate and shape the cytoskeleton during this process [2].

Methodology

We have combined phase-shifted laser feedback interferometry microscopy [3] with fluorescence microscopy to measure the formation of invadopodia on the ventral surface of metastatic rat mammary carcinoma (MTLn3) cells after epidermal growth factor stimulation. We plate MTLn3 cells either on fibronectin-coated surfaces or on gelatin matrices. From our label-free stage scanning laser-feedback interference microscopy we independently obtain the topography of the cell surface with nanometer precision (Fig. 1A) as well as a map of the reflectivity of newly forming invadopodia (Fig. 1B). Furthermore, the inherent *confocality* of our interference technique greatly extends the dynamic topographical range, and by optical sectioning we obtain detailed height maps of the invasive protrusions over a range of several hundred nanometers.

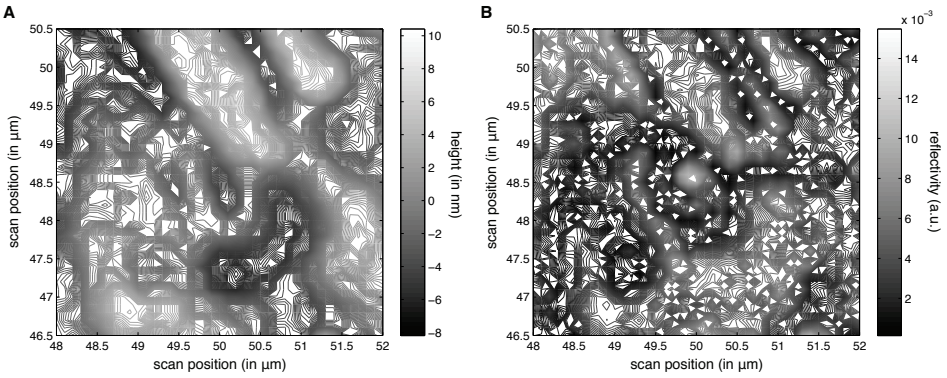

Figure 1. One frame acquired from a live-cell moving south-east using phase-shifted interferometry (A) Height image. (B) Reflectivity image.

We combine this label-free technique with simultaneous fluorescent high-speed time-lapse microscopy with stroboscopic laser excitation [4]. This allows us to track key proteins such as fluorescently labeled cortactin molecules in living MTLn3 cells. The specificity of the fluorescent imaging, combined with the topological maps obtained from our interferometric technique provides unique insight into the assembly process and earliest formation of invadopodia.

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[3] E. Atilgan and B. Ovryn, B, *submitted.*

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