SIMPLE AND COMPACT MICROSCOPE FOR TIME-LAPSE PHASE AND FLUORESCENCE IMAGING BASED ON CHROMATIC ABERRATION

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We propose a surprisingly simple microscope combining phase imaging with multi-colour fluorescence. This compact setup can be easily inserted in a standard biological incubator and allows observation of cellular cultures over several days. The phase image is reconstructed from a single, ~50 µm defocused image taken under semi-coherent illumination [1, 2]. Fluorescence is recorded in-focus in epi-fluorescence geometry. The phase and fluorescence images are taken sequentially using a single CMOS camera. No mechanical movement of neither sample nor objective or any other part of the system is required to change between the imaging modality. The only change is the wavelength of the illumination and excitation light for phase and fluorescence imaging, respectively. The defocus needed for phase imaging is achieved due to specifically introduced chromatic aberration in the imaging system.
We present time-lapse movies of cellular cultures observed over several days in physiological conditions inside an incubator. A field-of-view of 3 mm^2 allows observation up to several thousands of cells with micro-meter spatial resolution in quasi-simultaneous phase and fluorescence mode. We believe that the simplicity, small dimensions, ease-of-use and low cost of the system make it a useful tool for biological research.

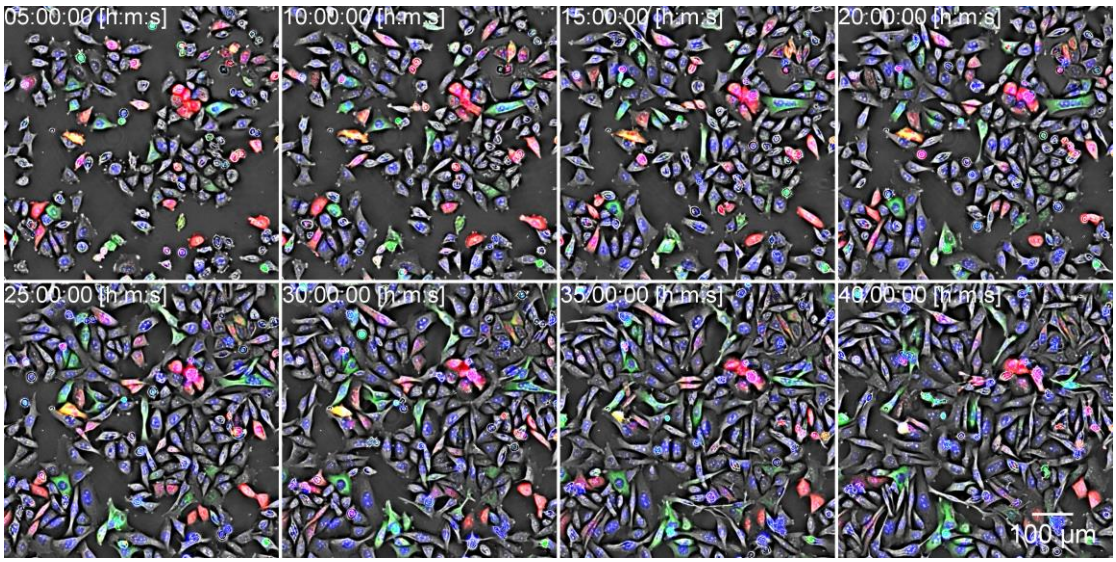

Figure 1: HeLa cells triple stained with Hoechst (blue), GFP tubulin (green) and mCherry (red) merged with reconstructed phase (grey). The cells on a petri dish were observed for 40 hours with recording every 10 mins directly in a standard biological incubator. ROI of 0.3 mm^2 from a whole region of 3 mm^2 containing ~10^3 cells.

[1] Allier, C. et al., "Imaging of dense cell cultures by multiwavelength lens-free video microscopy," *Cytometry Part A* 91(5), 433 (2017).
[2] L. Herve et al., "Multispectral total-variation reconstruction applied to lens-free microscopy," *Biomed. Opt. Express* 9, (2018).