Type of presentation: Oral

L-O-2400 The methodology of correlative microscopy between holographic incoherent quantitative phase imaging (Q-Phase) and laser scanning confocal microscopy (NIKON)

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The development of the novel correlative methodology of microscopy between Holographic Incoherent-light-source Quantitative Phase Imaging (hiQPI) and Laser Scanning Confocal Microscopy (LSCM) is carried out in the field of lung cancer. This methodology for correlative microscopy combining hiQPI and LSCM is suitable for pre-testing drugs and revealing information about mechanisms of action.
The established procedure of correlation microscopy between hiQPI and LSCM will be invaluable for other projects with a common goal in identifying the correlation between accurate quantification of cell behaviour and expression/localization of specific molecular species in individual cells in tissue culture. Lung cancer cell lines A549 and H1299 are treated with test substances (putative migrastatics) and their growth and motile responses will be measured by hiQPI. Cells from hiQPI analysis will be immunolabeled for putative molecular biomarkers (Focal Adhesion Kinase - FAK and protein 4.1B) and their expression will be correlated with dynamic measurements from hiQPI. Using this procedure, a methodology is established not only for preliminary testing of drugs but also for revealing the mechanisms of their action.
Cells were fixed and immunocytochemically labelled with FAK and 4.1B antibodies immediatelly after 20 hours of hiQPI time-lapse, and analyzed with LSCM. At the same time, we obtained images of FAK and 4.1B localisation at the level of the substrate and Interference Reflection Microscopy (IRM) images, revealing all focal adhesions. Arrows indicate examples of focal adhesions with increased FAK and 4.1B concentrations.
An in-house developed stage insert is transferred from hiQPI onto the stage of the LSCM, positions of the multi-field time-lapse are exported from the hiQPI software, transformed by a specially developed procedure for this project, imported into the LSCM software and thus cells automatically identified.

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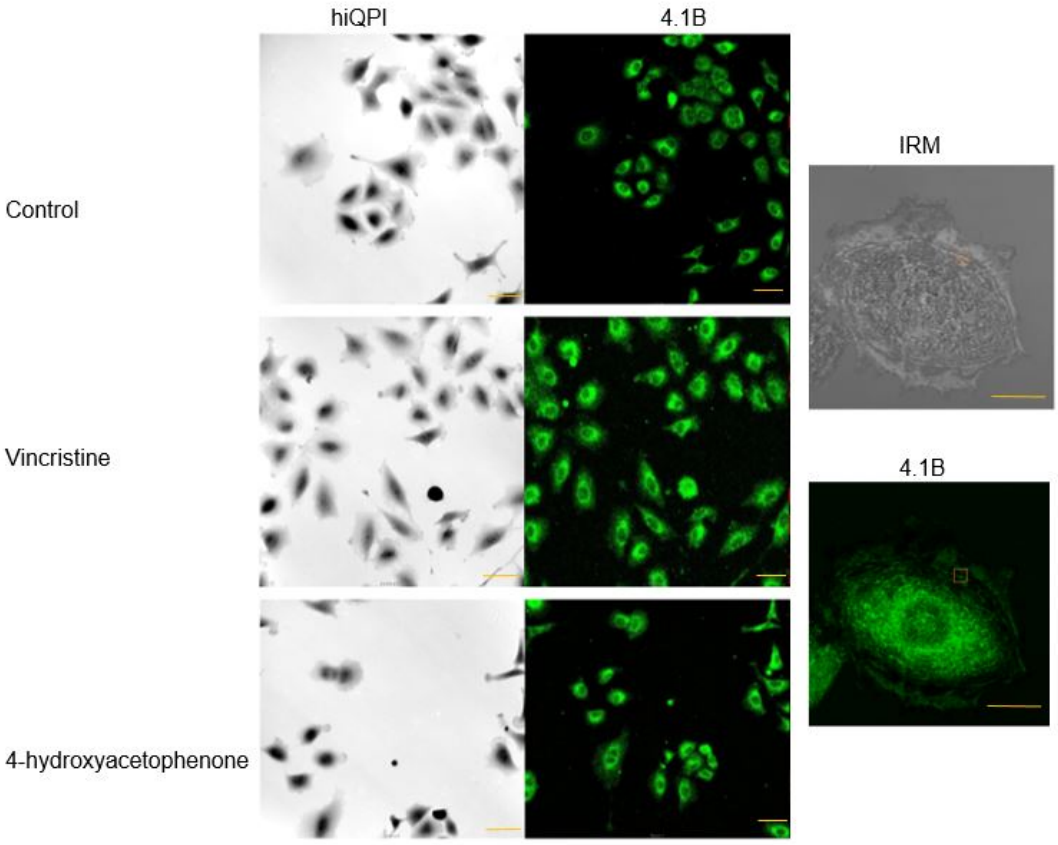

Fig. 1: Images of A549 cells visualised by hiQPI and subsequent immunocytochemistry of 4.1B and IRM. The same hiQPI position is found on the LSCM. Control and 2 treatments are presented. Arrows indicate examples of focal adhesions with increased 4.1B concentrations. Scale bar 50 µm. Objective lens 10x NA 0.3 and 20x NA 0.8.