



Technology Core Facilities

Light Microscopy

www.london-research-institute.org.uk/lightmicroscopy

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This LRI facility provides services in laser scanning confocal microscopy, low light level wide field imaging, video microscopy and microinjection. We have also pursued research applications and development.

Available imaging technology

Confocal microscopes LSM 510 (Zeiss) and SP5 (Leica) including a multiphoton system with tunable Chameleon laser (Coherent), UltraVIEW confocal imaging systems (PerkinElmer), low light level imaging systems (Molecular Devices and Kinetic Imaging) including high content screening and Total Internal Reflection Fluorescence (TIRF) microscopy (Nikon), video microscopes (Olympus), and microinjection systems (Eppendorf) have been configured for contrast enhancement, high resolution 3D, spectral and dynamic imaging of cells in multiple fluorescence channels with optical sectioning using motorised focus at multiple fields. Image processing and statistical analysis can be employed for deconvolution, colocalisation, automatic or interactive segmentation of cells and intracellular structures, morphometry, and tracking using Huygens (SVI), Volocity (Improvision), Imaris (Bitplane), AQM (Kinetic Imaging), Metamorph (Universal Imaging), MATLAB (MathWorks) and Mathematica (Wolfram Research).

Research applications examples

We developed a novel direct viewing cancer cell invasion assay with shear flow *in vitro* using analysis of high-resolution images, illustrated in Figure 1. This assay involves a custom-made flow chamber, specially developed cell labelling, observation by inverted wide-field microscopy and image processing-based quantitation of cell invasion. We applied this assay to metastatic sarcoma cells, where the cells invaded monolayers of endothelial cells. The metastatic sarcoma cells were labelled with green Vybrant DiO (Invitrogen) and the endothelial cells were labelled with CellTracker Orange

CMTMR (Invitrogen) using a modified protocol (100 mM CMTMR in DMSO was incubated at -20°C for at least 2 weeks prior to use). Our findings showed that after adhesion, the cells initially invaded significantly faster under flow conditions compared to situations without shear stress. Later, however, the rate of invasion under flow decreased and the metastatic cells without shear stress achieved significantly higher levels of invasion. Our observations thus revealed the non-linear modulation of a tumour cell invasion process by

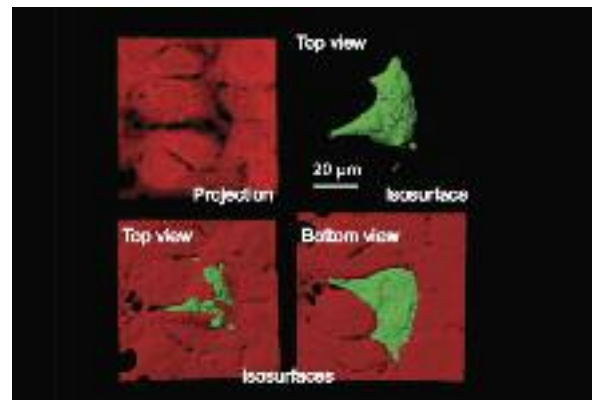


Figure 1. Images of a metastatic sarcoma cell labelled with Vybrant DiO (green colour) invading a monolayer of endothelial cells labelled with CellTracker Orange CMTMR (red colour) presented as Imaris (Bitplane) renderings of a z-stack of images acquired using Zeiss LSM 510 confocal microscope (Tamara Cavanna, Light Microscopy).

shear flow, demonstrating that tumour cells can respond to flow by enhancement of invasiveness similarly to white blood cells (Hagglund *et al.*, *Frontiers in Bioscience*; in press). We also collaborated in a project with the Leukocyte Adhesion laboratory (Nancy Hogg) where we analysed migration of T cells by Interference Reflection Microscopy and identified different patterns of cell adhesion determined by specific epitopes (Stanley *et al.*, *Embo J* 2008; 27: 62-75).

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