Phenospot (Photonic hyperspectral no-label spotter); label-free, real-time in-situ monitoring of live-cell protein specific secretion.

A.G. Kay[1], I. Barth[2], A.H. Cull[1], L.M. Miller[2], C. Reardon[2], S. Johnson[2], P.J. O'Toole[1], T.F. Krauss[2]

[1] Department of Biology, University of York, York, UK; [2] School of Physics, Engineering and Technology, University of York, York, UK.

Abstract

Cells coordinate behaviour and function through secretion and response to signalling molecules, such as cytokines and chemokines. To gain a deeper understanding of complex signalling events, tools can be applied that quantitatively assess signalling related to known cellular responses. Current tools, like ELISA, multiplexed bead assays, or mass spectrometry, only measure signalling from many cells at a single time point.

A new imaging technique called "Phenospot" is being developed at the University of York in a collaboration between physicists, chemists and biologists. The Phenospot uses a highly sensitive resonance sensor chip integrated into a standard microscope with a wavelength-tuneable light source. Surface-immobilized antibodies bind secreted proteins from individual cells, resulting in a localised change in refractive index and shift in resonant wavelength. By monitoring the shift in sensor resonance wavelength over time, it is possible to create maps of the spatial distribution, onset, and kinetics of single-cell protein secretion, capturing the full heterogeneity of a cell population.

We used Tet2 knockout mice as a model species for blood disorders such as Myeloproliferative Neoplasms (MPN) where C-X-C motif chemokine ligand 10 (CXCL10) is thought to be an indicator of disease severity. Phenospot has been successfully applied to the detection of CXCL10 in wild type control versus Tet2 knockout mice. Heterogeneous cell populations were isolated using peritoneal lavage and adhered to poly-l-lysine coated Phenospot sensors. Cells were activated with an interferon-gamma/lipopolysaccharide activation cocktail and individual cell CXCL10 secretion monitored over a 16 hour period to determine heterogeneity in cell populations. Activated macrophages isolated from Tet2 knockout mice demonstrated increased CXCL10 expression in comparison to macrophages from wild type C57Bl/6J or non-activated controls. This result will inform biomarker studies for blood disorders such as MPN and highlights the versatility of Phenospot for monitoring cell secretomics.

The novel Phenospot technology demonstrates significant potential to address a variety of health-related questions in immunity, cancer progression, cell signalling and wound healing.