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Metabolic imaging at the single-cell scale – recent advances and future challenges in mass spectrometry imaging

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Super-resolution optical microscopy using fluorescent labels has been transformational in allowing the machinery of life, e.g. proteins, to be seen at the nanoscale. There is a great desire in the life-sciences to achieve this level of insight for metabolites. This will allow unprecedented ability to understand rewiring of metabolic networks involved in disease, understanding of the uptake of drugs in cells and construct mechanistic understanding in fundamental biology. However, this is a monumental challenge since fluorescent labelling strategies cannot be used because of the dynamic processes in the creation of metabolites and because the fluorescent labels themselves radically alter the chemistry of the metabolite.

A strategy for metabolic imaging at the single-cell scale

Mass spectrometry allows label-free (or with stable isotope labelling) identification of endogenous and exogenous (e.g drugs) metabolites and when combined with high-resolution ion beams in secondary ion mass spectrometry (SIMS) allows sub-cellular resolution imaging [1]. Substantial barriers need to be overcome to achieve a super-resolution goal (< 250 nm) including increasing sensitivity, increasing specificity (accurate identification of molecules), sample preparation methodologies (e.g. cryo-SIMS) and improvements in ion beam resolution. A road map to achieve metabolic imaging at the single-cell scale is shown in Figure 1.

The roadmap shows the importance of correlative analysis with low invasive techniques having poorer spatial resolutions (e.g. MRI and DESI) and techniques requiring complex sample preparation and labelling stragies (e.g. NanoSIMS) have high spatial resolution. This provides a gap in capability for label-free metabolic imaging between approximately 5 micrometres and 100 nm. We have led the development of a powerful new hybrid instrument, the 3D OrbiSIMS[2], combining an Orbitrap™-based Thermo Scientific™ Q Exactive™ HF instrument and a dedicated ToF-SIMS 5 instrument. The instrument is equipped with high-resolution ion beams including a new micron resolution argon cluster ion beam for biomolecular imaging and 3D analysis of organics and an ultra-high resolution Bi cluster focussed ion beam with < 100 nm resolution. We demonstrate the unparalleled ability for 2D and 3D metabolite imaging with sub-cellular resolution. Recent developments have expanded the ability to cryo-OrbiSIMS analysis for 3D imaging of biological materials in their native state.

For imaging at the organelle scale, e.g. mitochondria and lysosomes, then a resolution of 50 nm or better is needed. This is achieved with the CAMECA NanoSIMS 50L [3]. Normally, the solvent based sample preparation procedures used wash out drug molecules. We have developed a method that traps molecules within the organelles and use this to demonstrate the first direct evidence of drug induced phospholipidosis caused by amiodarone uptake in rat alveolar macrophages [4].

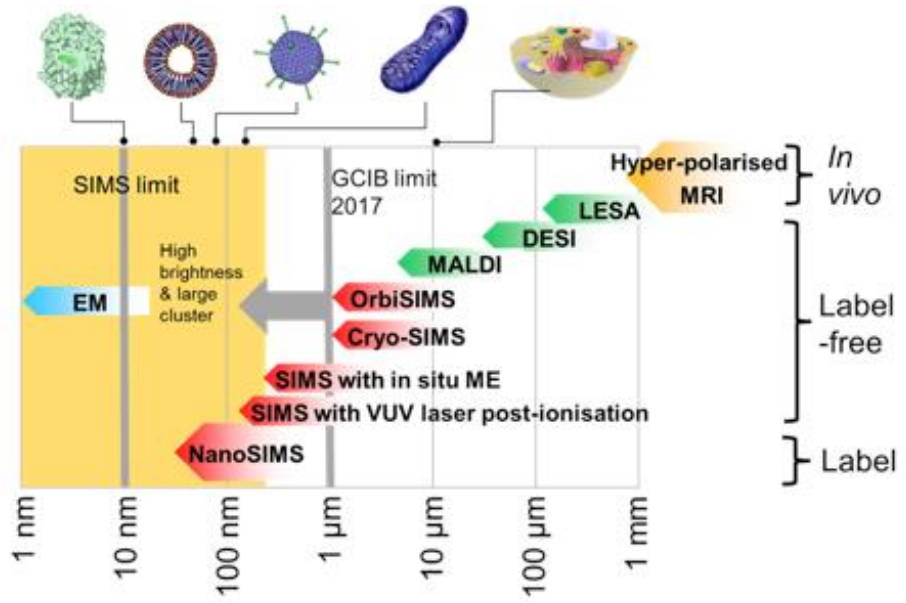

Fig. 1 A roadmap for achieving super-resolution label free metabolic imaging of cells. The map shows techniques categorised by their progressive invasiveness (*in vivo*, label-free and labelled) and the spatial resolutions that can be achieved. At present label-free mass spectrometry imaging is limited to 1 micrometre (OrbiSIMS[2]) and new technological advances to increase sensitivity such as matrix enhancement (ME) and laser-post ionisation are needed to achieve the super-resolution limit, shaded in gold. Biological structures are displayed above at positions corresponding to their dimensions (lower scale). Magnetic resonance imaging (MRI), Liquid extraction surface analysis (LESA), Desorption electrospray ionisation (DESI), Matrix assisted laser desorption ionisation (MALDI), Electron microscopy (EM).

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