# TOWARDS SUPER-RESOLUTION METABOLIC IMAGING OF CELLS USING THE 3D ORBISIMS

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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, the molecular products and messengers involved in metabolism. 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.

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. 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. This lecture will review our research at NiCE-MSI using three SIMS instrument types; ToF-SIMS, 3D OrbiSIMS and a CAMECA NanoSIMS 50L.

A significant focus of our research is to image the uptake of drugs with sub-cellular resolution. We have discovered that in ToF-SIMS, the logarithm of sensitivity is directly proportional to the Log P value for many different drugs and that some drug molecules exhibit severe matrix effect resulting in no detection. Since the pharmaceutical industry is focused on making low Log P drugs ≤ 3 (Lipinski rule of five) this limits the current applicability. We have recently developed a new method for in situ matrix deposition that significantly increases sensitivity to help overcome this. Furthermore, initial experiments using laser post-ionisation show encouraging results to potentially circumvent some of these issues.

Many metabolites are of low mass (e.g. dopamine) and are in a highly congested region of the mass spectrum. Here, ToF-SIMS is inadequate to resolve the peaks especially at high-spatial resolution. We have led the development of a powerful new hybrid instrument, the 3D OrbiSIMS, combining an Orbitrap™-based Thermo Scientific™ Q Exactive™ HF instrument and a dedicated ToF-SIMS 5. 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.

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. 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.

Significant progress has been made towards the goal of super-resolution metabolic imaging but there are many challenges ahead. A technology roadmap will be presented.