

## Subcellular Mapping of Trace Elements in Plants Using High Resolution Secondary Ion Mass Spectrometry (NanoSIMS)

Katie Moore<sup>1\*</sup>, Sadia Sheraz<sup>1</sup>, James Dinsley<sup>2</sup>, Mary Burkitt-Gray<sup>3</sup>, Peter Shewry<sup>4</sup>, Janneke Balk<sup>5</sup>

1. Department of Materials, University of Manchester, Manchester, UK.
  2. Department of Earth and Environmental Sciences, University of Manchester, Manchester, UK.
  3. Department of Physics, Kings College London, London, UK
  4. Department of Plant Sciences, Rothamsted Research, Harpenden, UK
  5. Department of Biochemistry and Metabolism, John Innes Centre, Norwich, UK
- \* Corresponding author: katie.moore@manchester.ac.uk

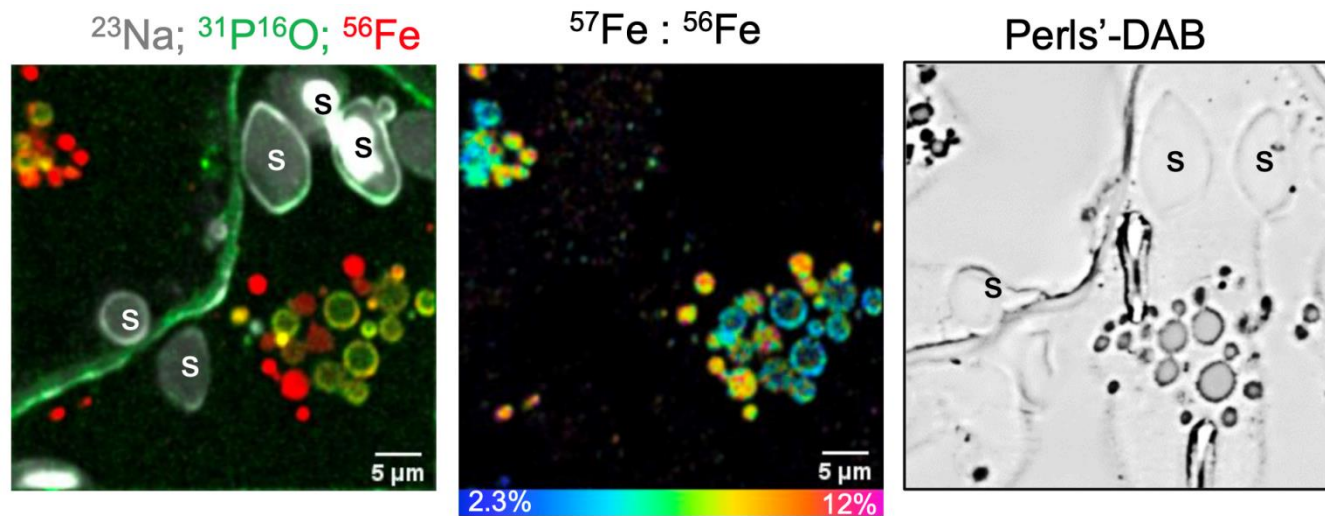
Mapping the distribution of trace elements at the subcellular scale is analytically challenging but necessary to understand the mechanisms of uptake of toxic and beneficial elements into plants and crops which can affect the human diet [1]. With subcellular localisation of the trace elements, it is possible to infer the pathways by which these important elements are taken up by the plant and how they are stored and accumulated in edible tissues.

The NanoSIMS is capable of high spatial resolution chemical imaging (down to 50 nm) and detecting very low elemental concentrations, making it ideally suited for subcellular trace element localisation in biological materials. It uses either an oxygen or caesium ion beam to generate secondary ions from the sample surface which are collected and analysed in a magnetic mass spectrometer. A wide range of elements in the periodic table can be detected, from hydrogen to uranium, allowing correlation of the trace elements of interest with other elements which can indicate storage and uptake mechanisms. Furthermore, as this is a mass spectrometry technique, it is possible to detect and image stable isotopes allowing for the possibility of pulse-chase experiments which can provide temporal information about uptake and mobilisation [1].

The analysis of biological samples with the NanoSIMS, which operates under ultra-high vacuum, is complicated by the need to preserve not only the *in vivo* structure of the cellular components but also the elemental distribution. Plant samples therefore require specialist preparation including high-pressure freezing, resin embedding and microtomy to create a sample that is flat and high-vacuum compatible, while preserving the water-based cell contents. Sample preparation routes will be discussed.

This presentation will show how the NanoSIMS has been used to localize a range of important trace elements in many different plant tissues including uptake of trace levels of iron into wheat grain using <sup>57</sup>Fe stable isotope labelling (Figure 1) [2] and the localisation of uranium in plant roots colonised with arbuscular mycorrhizal fungi. This presentation will also show how the NanoSIMS has been used to map nanoparticle distributions in plant roots.

Throughout this presentation, complementary and correlative imaging will be emphasised to show how it has been used to gain a deeper understanding of the samples than could be obtained from one technique alone [3].



**Figure 1.** NanoSIMS images from a wheat grain showing iron-rich vesicles in the endosperm (white flour) region of the grain labelled with  $^{57}\text{Fe}$  after a 72-hour feeding period. Correlative Perls'-diamine benzidine (DAB) is shown on the right indicating iron rich regions (dark) [2].

#### References:

- [1] PM Kopittke, et al., *Plant Physiology*, **82** (2020) p.1869 doi:10.1104/pp.19.01306
- [2] S Sheraz et al., *New Phytologist* **231** (2021), p. 1644 doi:10.1111/nph.17440
- [3] The authors acknowledge funding from the Biotechnology and Biological Sciences Research Council, grant award BB/P019072/1