OXFORD INSTRUMENTS

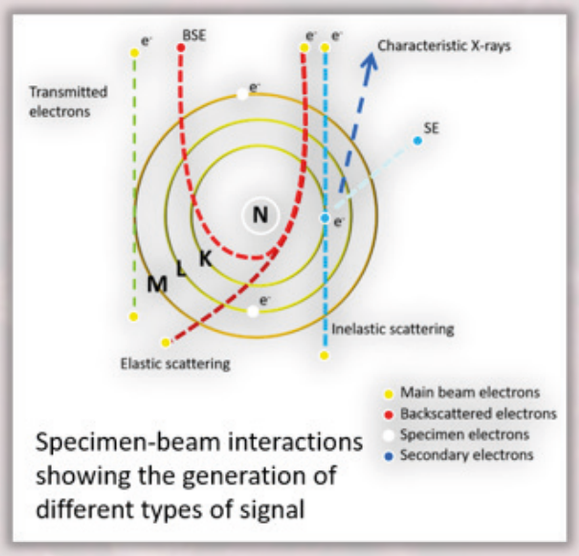
Energy Dispersive X-ray Spectrometry (EDS) for Biology

A brief introduction to Energy dispersive X-ray Spectrometry (EDS)

X-ray Signal Generation

EDS (also known as EDX) is the analytical technique used to detect x-rays that are generated when a charged particle beam (e.g. an electron beam) interacts with a specimen, as shown in the interaction diagram.

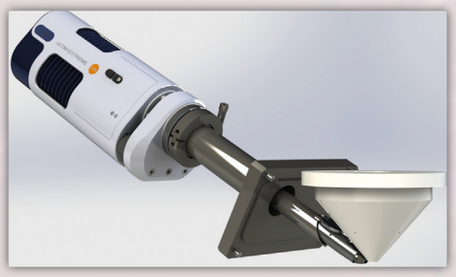
- Beam electrons transfer energy to a shell electron
- The shell electron is emitted from the atom as a secondary electron, leaving a "hole"
- An outer shell electron releases energy in the form of an x-ray (or other signals not discussed here) and moves into the hole
- The x-ray energy is the energy difference between shells, which is unique for each element.



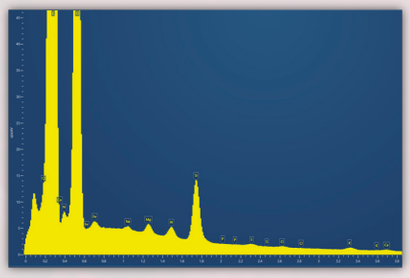
Specimen-beam interactions showing the generation of different types of signal

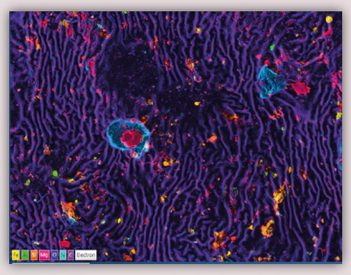
EDS Detectors and Signal Processing

EDS detectors today are silicon drift detectors (SDD), a significant improvement in sensitivity and stability compared to older generation detectors. X-ray energy is converted to a change in voltage, which is then measured and analysed in a pulse processor and sent to a software such as AZtec to present the signal in the form of a spectrum. A spectrum is collected in every position the beam interacts with the specimen, which could either be a single point or multiple positions to generate a map, similar to the way secondary electron and backscattered electron images are collected. Maps can be produced in a TEM using STEM modes.


EDS Detector


Pulse Processor


X-ray Spectrum

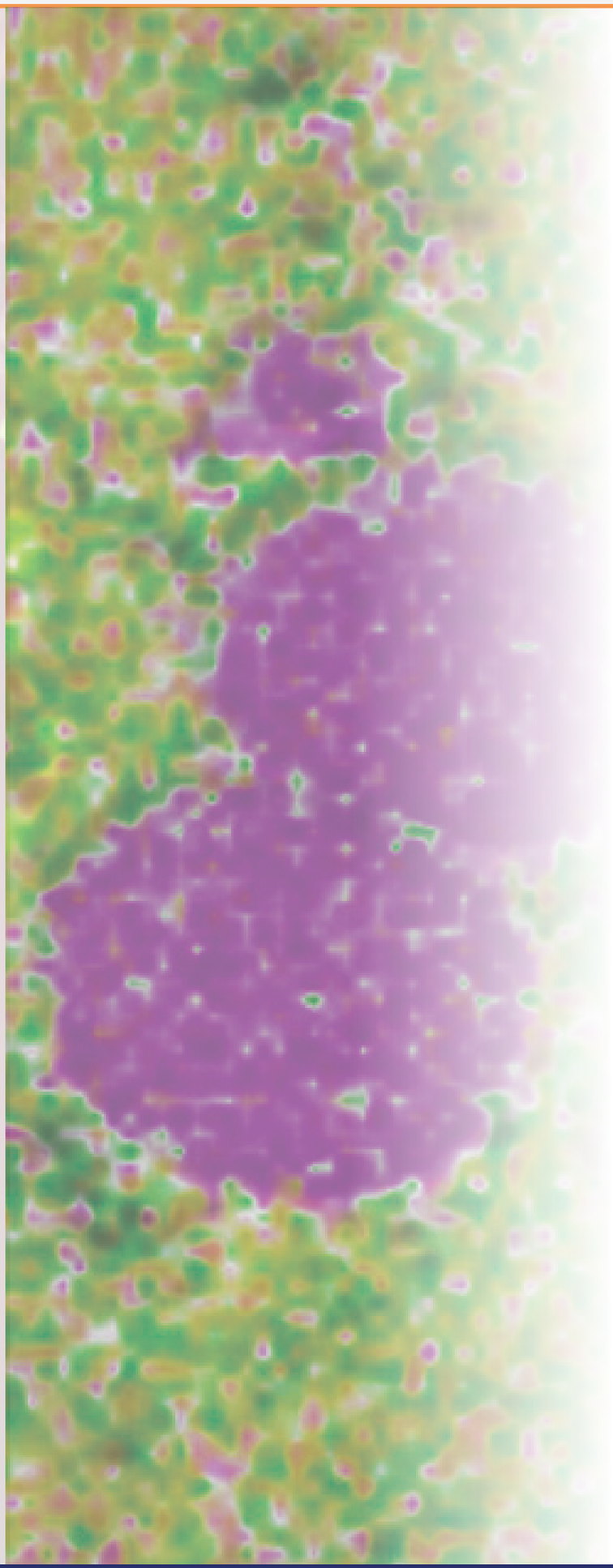

X-ray map & EM image

The spectrum is relatively easy to interpret, with x-ray energy along the x axis. The position of each peak in the spectrum indicates which element it originated from and AZtec can perform an auto identification of elements if desired. The height of each peak relates to the number of x-rays (counts) recorded for each element. If two x-rays hit the detector at the same time a higher energy would be recorded, creating a false peak. This is a common artefact in the data and AZtec has a number of algorithms to deconvolute spectra and maps, correcting for this and other known artefacts.

Maps are a visual representation of spectra across an area. A different colour is used for each element and the comparative intensity across maps reflects the relative abundance of elements. A layered map composed of several element maps and EM micrographs is used to create a colour EM image (see above right), providing ultrastructural information combined with composition.

Oxford Instruments produces a range of different detectors for TEM and SEM.

- Low kV, high resolution, cryo and low dose conditions and/or sensitivity to light elements may require windowless detectors such as the Ultim Extreme (SEM) and Ultim Max TLE (TEM), which are optimised for life science analysis.
- We also have a wide range of detectors and solutions for very fast data collection, heavier element analysis, operation of up to 30kV (SEM), high dose and high count rate conditions.



Differential staining in biological tissue

Energy dispersive spectrometry (EDS) can be used to analyse differential staining protocols, allowing access to new information about biological samples. Differential staining is an important step in generating contrast when preparing cells and tissues for electron microscopy. EDS produces qualitative data about the distribution of elements associated with the stain and comparative quantitative data about relative concentrations of elements.

The zinc-iodide-osmium (ZIO) staining protocol differentially stains the endomembrane system of plant cells in addition to other organelles. EDS shows that zinc and osmium have a differential distribution in Venus' fly trap gland cells, colocalising in some vesicles, and in others demonstrating different concentration ratios (Fig. 1). Osmium preferentially localises to fat droplets in liver tissue allowing rapid identification and differentiation from other vesicle-like structures such as lysosomes (Fig. 2).

Differential localisation of lead and osmium in maize root cells demonstrates the different affinity stains have for structures, even when there are similar levels of contrast in the backscattered electron data (Fig. 3). This highlights the importance of using post-section staining in order to visualise as much ultrastructure as possible. Information on stain distribution can be utilised when examining new structures or developing new staining regimes. It is also possible to combine several stains that provide similar levels of electron contrast and use EDS to differentiate ultrastructural information.

These techniques provide further information about staining profiles which may occur in biological samples, facilitating our understanding of specimen preparation procedures and the distribution of the structures stains are binding to.

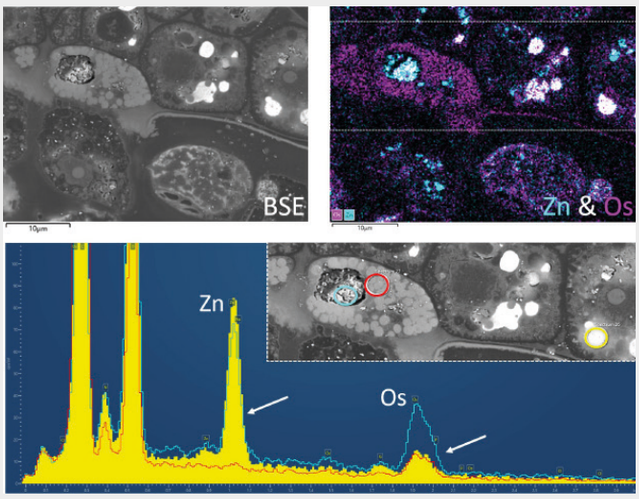


Fig. 1 - Venus' fly trap gland stained with zinc-iodide-osmium. The sample is embedded in resin and coated with carbon. Images were collected of the sample block face using scanning electron microscope with Ultim® Extreme EDS detector and AZtec® software.

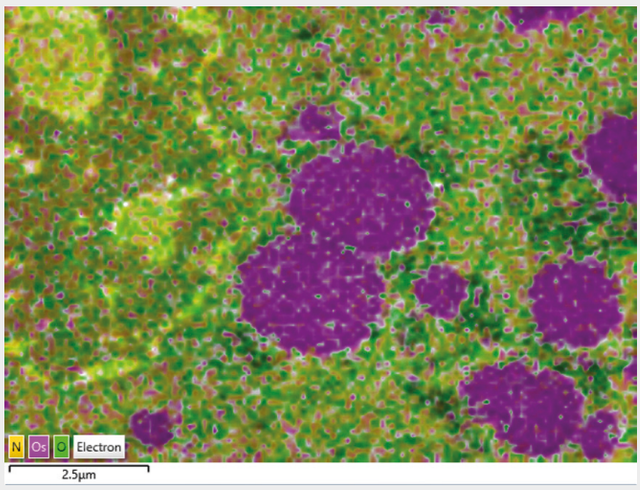


Fig. 2 - EDS map showing fat droplets in a liver cell stained with osmium tetroxide (pink). The sample was sectioned at 100nm thickness and placed onto a copper grid. Images were collected on a a scanning electron microscope with an Ultim Extreme EDS detector and AZtec software.

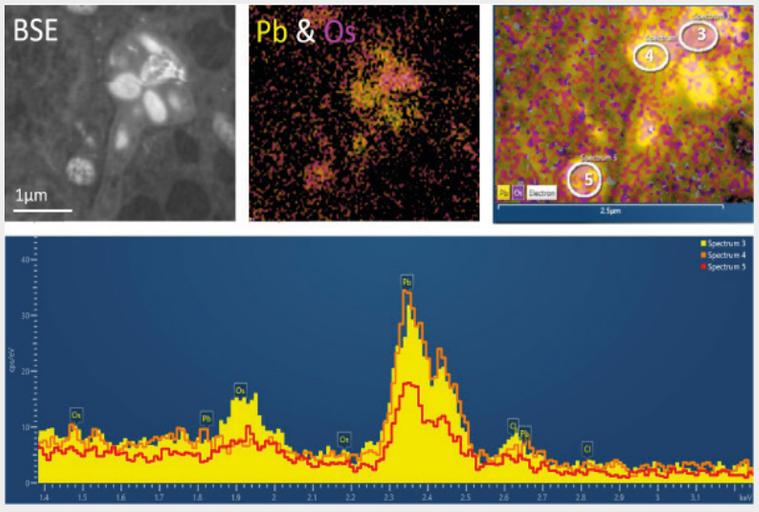


Fig. 3 - EDS and electron images showing differential lead and osmium staining within organelles inside a maize root cell. Images were collected of sectioned material mounted onto a copper grid using scanning electron microscope with an Ultim Extreme EDS detector and AZtec software.

Mapping essential elements in biological samples

The distribution of native elements in biological samples and their association with ultrastructure is a growing area of interest. Specimen preparation is crucial as every procedure the sample encounters has the potential to change distribution.

EDS can detect essential elements in stained samples. EDS can be used to reveal or identify structure using elemental maps even if there is no stain present (see example of thylakoids in a chloroplast in Fig. 4 and nucleus in Fig. 5). EDS can also be used to detect and map essential elements in stained samples (Fig. 6).

Stained samples were fixed using a standard Karnovsky's fixative protocol under a mild vacuum and stained using the ZIO technique prior to dehydration and embedding. Unstained leaf samples were fixed using a standard Karnovsky's fixative protocol under a mild vacuum, dehydrated in ethanol and embedded in LR white.

Both samples were sectioned at 100nm thickness. No post-section staining was used. Samples were on a copper grid and data was collected in a TEM using an Ultim Max TLE EDS detector and AZtecTEM software.

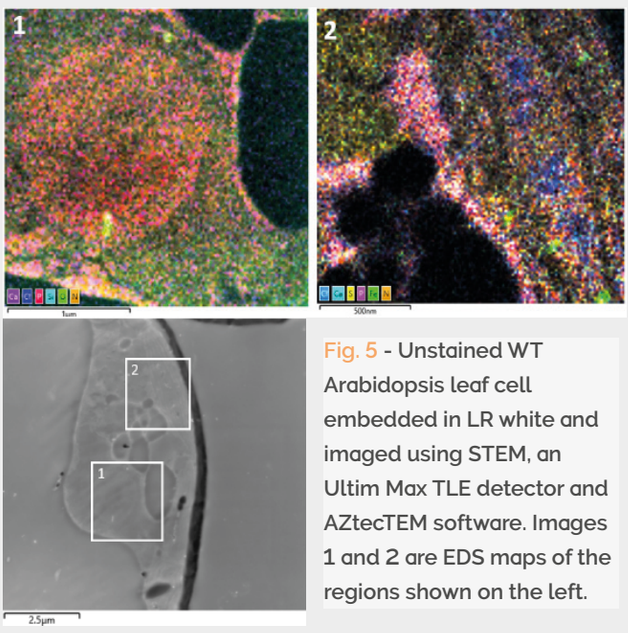


Fig. 5 - Unstained WT Arabidopsis leaf cell embedded in LR white and imaged using STEM, an Ultim Max TLE detector and AZtecTEM software. Images 1 and 2 are EDS maps of the regions shown on the left.

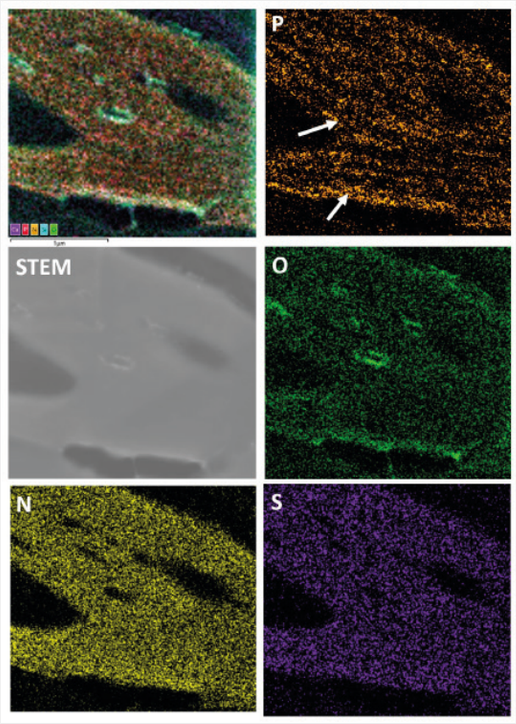


Fig. 4 - Unstained WT Arabidopsis leaf chloroplast, embedded in LR white and imaged using STEM, an Ultim Max TLE detector and AZtecTEM software. Arrows indicate the thylakoids in the phosphorous EDS map.

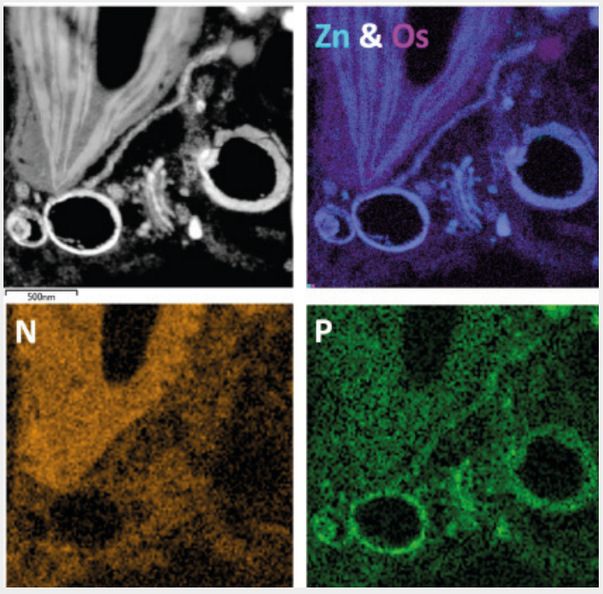


Fig. 6 - Stained WT Arabidopsis leaf cell with chloroplast and Golgi body imaged using STEM, an Ultim Max TLE detector and AZtecTEM software.

EDS & Biological Sample Preparation Checklist

Flat or very limited surface topography

- Resin/cryo block sectioned or trimmed using a diamond knife
- FIBSEM used to create a flat surface
- Very small or naturally flat sample (e.g. surface or a leaf)

Conductive sample

- Mounted onto conductive substrate (i.e. copper grid/ carbon tab)
- Coated with conductive coating (e.g. carbon)
- Contains conductive materials (e.g. osmium tetroxide stain)

Stability under electron beam

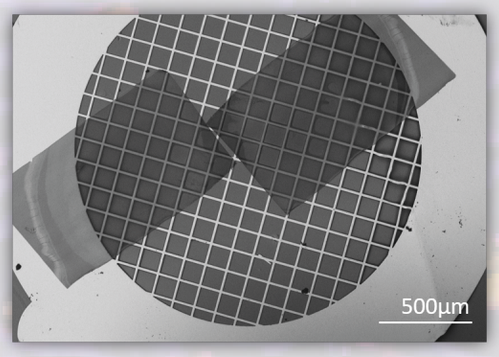
- Dehydrated and dried/embedded in resin for room temperature EM
- Vitrified (frozen) and imaged under cryo-conditions
- Coated when required

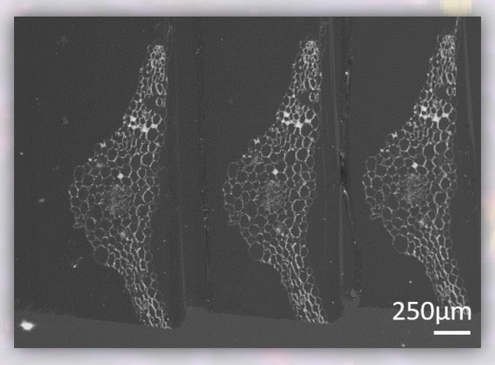
Contains elements of interest

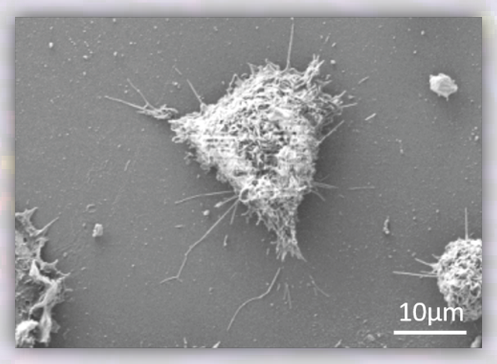
- Cryo-fixation for fastest specimen preparation
- Rapid fixation/embedding/freeze-substitution techniques to minimise extraction
- Non-biological materials at adequate concentration (e.g. pollutants and nanoparticles) (>0.1%)

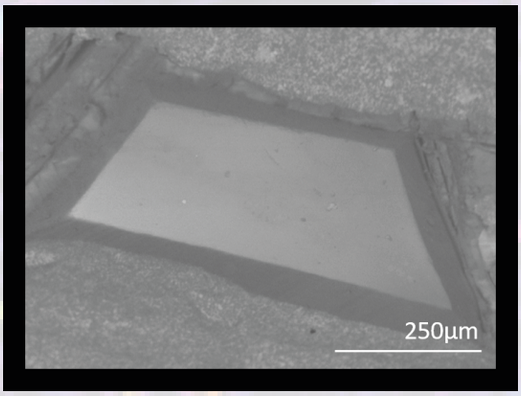
Optimise microscope operating conditions

- Tilt the sample towards the detector in TEM
- Low kV for unstable samples or high-resolution analysis of light elements
- Enough kV to activate x-rays from elements of interest (usually 2.6x for optimal x-ray production)
- Adjust apertures/mode/probe current/dwell time/ resolution to improve data generation without destabilising the sample
- Use a suitable EDS detector (e.g. Ultim Extreme for low kV applications, Ultim 170 for >10kV or Ultim Extreme combined with immersion lens for up to 30kV)
- Use the recommended working distance (this has been optimised by our engineers and is listed in AZtec)
- Increase number of frames and/or dwell time to improve signal for EDS maps (provided sample is stable enough). Reduce resolution or bin EDS maps to improve contrast during data collection.









From top to bottom: sections on a grid, serial sections for array tomography, cells cultured on plastic coverslips, trimmed block for serial block-face scanning electron microscopy.

A spatial resolution and sensitivity breakthrough for EDS in the FEG SEM

Extreme light element sensitivity

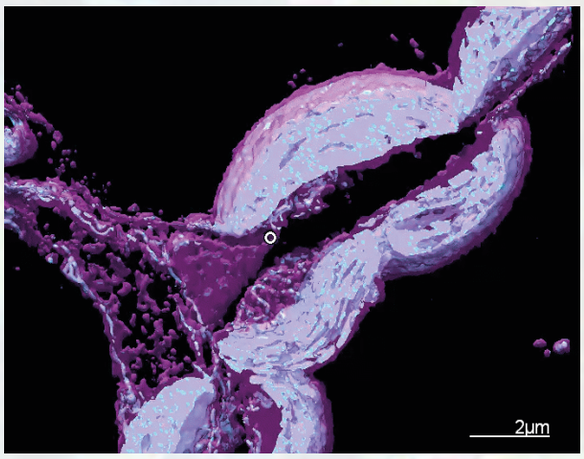
The windowless configuration and ultra high sensitivity of Ultim Extreme offers the most sensitive light element detection.

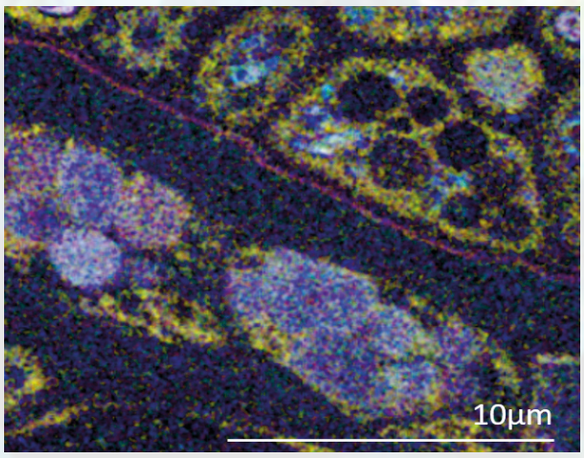
- Up to 15x increase in signal over conventional detectors
- Work at lower kV to minimise sample damage and charging
- New potential for the detection and characterisation of difficult elements, such as nitrogen
- New ability to analyse polymers and soft biological materials

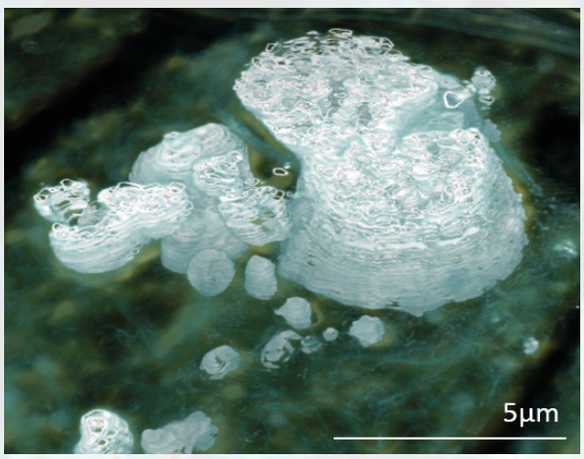
Sub 10nm element characterisation

Achieving practical EDS count rates and spectral quality at 2 kV or less, combined with short working distance for optimum beam size means unrivalled spatial resolution for element characterisation is now possible

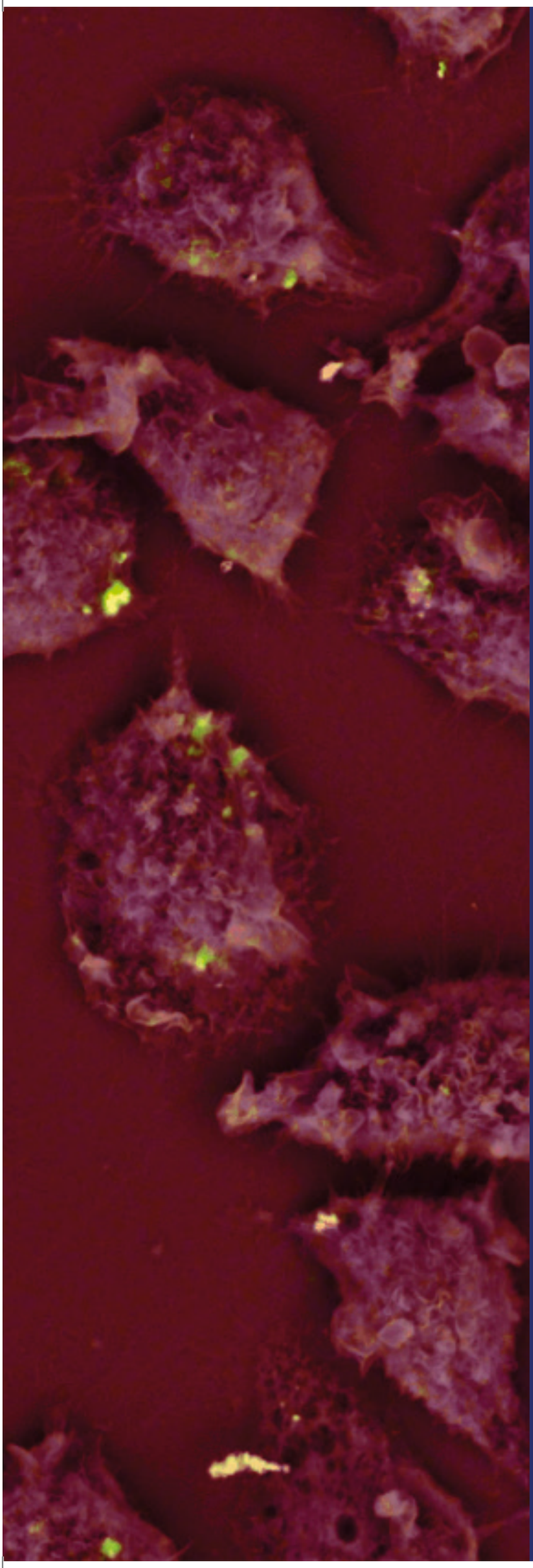
- Practical sub 10nm element characterisation on real materials in the FEG / FIB-SEM
- X-ray map resolution close to SEM image resolution
- Characterise smaller nano-structures, particles and materials
- Use bulk samples to reduce workload on TEM and sample preparation time







From top to bottom: 3D chloroplast data, Venus fly trap leaf glands, 3D EDS combined with BSE data of sample above.



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