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EDS Mapping of Sub-Surface Plant Materials Using Non-Traditional Operating Conditions

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Microanalysis at non-traditional sample specific SEM operating conditions is useful when studying characteristics of the organism that are not readily apparent using traditional methods. The operating conditions are typically tailored to the tolerances of the sample being investigated, and in the case of biological plant specimens, a low kV SEM beam is routinely employed for sample surface imaging and to reduce beam damage to the tissue. This work explores SEM EDS of biological plant materials using moderate SEM beam conditions to characterize sub-surface inorganic materials within the plant tissue.

Traditional biological analysis emphasizes two main aspects of the electron beam, namely the kV and the beam current. A low kV beam, below 5 kV, and sometimes as low as 1 kV or lower, is used to limit the electron probe depth within the sample to the uppermost surface, revealing surface detail and structure. Also, a low beam current is used to reduce sample beam damage and charge retention and discharge within the non-conductive tissue. These conditions allow detailed image collection, however, they pose a disadvantage to x-ray microanalysis since there is low ionization potential and therefore a weak x-ray signal. The analytical depth is also limited to the material's surface, not gaining the benefit of the deeper escape depth advantage of x-ray analysis.

A benefit of x-ray analysis is a deeper x-ray escape depth, due to higher energy x-rays and non-charged particle escape compared to electrons. Therefore, x-ray analysis can be used to study sub surface features within a material. A higher kV electron beam is required to penetrate to these depths, so sample preparation or handling is necessary to counteract the beam damage and charging potential to the material. One method is the traditional sample coating with conductive materials to carry away the beam charge to ground and a second is introducing a gas into the SEM chamber which is used to conduct away the electron charge from the material.

In this investigation, the initial SEM EDS analysis of *Cladium masriscus* ,a saw-tooth sedge grass with a rough serrated edge, at 5 kV in high vac mode, revealed little information about the suspected presence of Silicon [1]. The results at these conditions failed to highlight the presence of silicon with both spectrum and map analyses along the flat surface of the grass blade. Further, at this operating condition, the sample showed excessive charge, preventing quality imaging and analysis. However, a follow up study at 10 kV and 40 Pa gas pressure in a Hitachi S3400N Variable Pressure SEM with an EDAX Octane Pro (10mm^2) Silicon Drift Detector highlighted the presence of silicon while preventing sample charging and damage. The presence and location of silicon was further confirmed with x-ray maps of various elements, including the organic materials of carbon and oxygen, and the inorganic silicon. The maps shown in Fig 1 confidently identify the expected dumbbell shaped silicon bodies [2] in red, and oxygen in blue. Due to the appearance of these bodies only at higher kV, this confirms that their location is further within the plant material and located sub dermally.

Of further interest to this study, the serrated edge of the grass was mapped to visualize the distribution of the silicon and oxygen as transported from the storage bodies to the edges.

Moderate beam energy is necessary when the materials of interest are located below the surface of the materials being investigated. X-ray analysis has a unique benefit in that the signal is of sufficient energy to escape from these depths, and therefore spectra showing sub dermal elements, and maps showing those elemental distributions are possible. Analysts working at moderate kV gain this benefit of microanalysis at non-traditional SEM conditions.

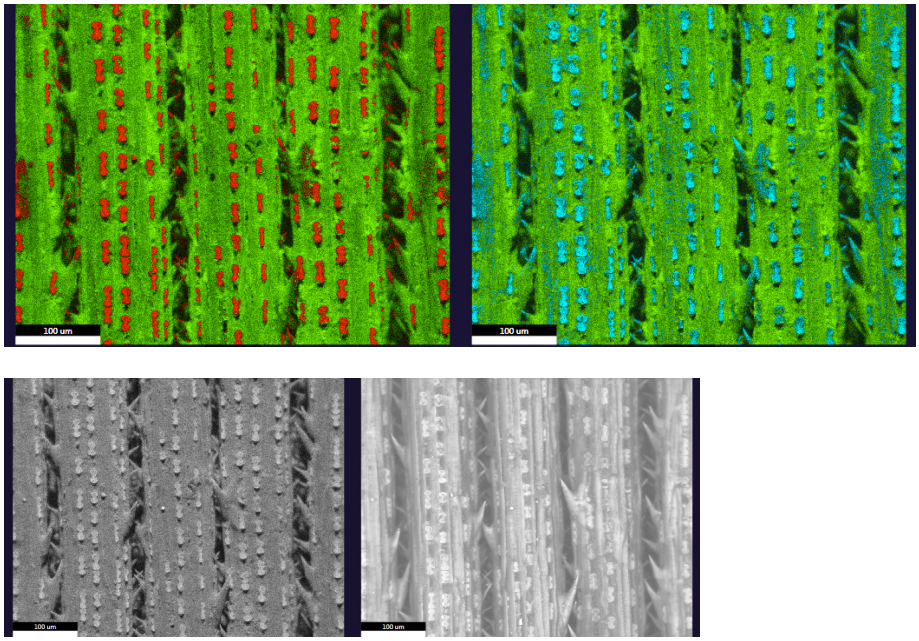

Figure 1 shows the elemental maps with organic carbon (green) and silicon (red) in the top left, carbon (green) and oxygen (cyan) top right. The X-ray Counts Per Second (CPS) map in the bottom left and the SE electron image in lower right.

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