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Imaging Uncoated Plant Seeds with In-lens and Secondary Electron Detectors in a High Vacuum Field Emission SEM

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Scanning electron microscopy (SEM) is routinely used for surface characterization of many nonconductive biological specimen. The imaging is primarily performed using a secondary electron (SE) detector. Non-conductive specimen can be made conductive by coating them with a thin layer of metal (mainly gold or gold-palladium), called sputter coating[1]. This prevents "charging", i.e. the build-up of electrons on the surface of the specimen by conducting the charge to the ground[1]. However, with the advancement of technology and significant improvement in modern day SEMs, dry non-conductive biological specimen can be viewed under a high vacuum SEM without any sort of sputter coating and minimal sample preparation. The University of South Carolina Electron Microscopy Center (EMC) acquired a Zeiss Ultra plus field emission SEM (FESEM) in January 2010. This equipment is outfitted with an Everhart-Thornley type secondary electron detector (SE2) and an annular in-lens detector. The in-lens detector is located directly in the beam path above the objective lens and the detector efficiency of this detector results from its geometric position in the beam path and from the combination with the electrostatic/electromagnetic lens[2]. The SE2 is an Everhart-Thornley type detector, not located in the beam path, and detects SEs as well as BSEs[2]. Electrons moving to the detector are attracted by the collector and directed to a scintillator.

Angiosperms, also known as the flowering plants, are the more advanced type of seed plant in that their seed coats are more resistant to environmental threats. Seeds usually have an external seed coat that is resistant to damage, which protects the embryo within the seed. Seeds with hard and dry textured surfaces provide a detailed SEM image. Three types of seeds (tobacco, *Arabidopsis* and poppy) representing three different plant families were selected for imaging using the in-lens and SE2 detectors of the Zeiss Ultra Plus FESEM. The surface structures were analyzed for best image quality and overall structural details. The two detectors were compared on the basis of reduced or zero charging and maximum overall surface details. All imaging of uncoated dry seeds was done in high vacuum, using accelerating voltage of 3-5 KV with a working distance of approximately 4-9mm. Images were collected in relatively low magnification to show larger areas of the seed coat. The in-lens detector indicated more charging effect than the SE2 detector, however, the surface details were same for the two detectors (fig 1-6). We also used the built-in charge compensator to reduce charging in uncoated seeds. However, the SE2 without the charge compensator, at relatively low kV, produced superior images with minimal charging effect.

References:
1. Bozzola J.J. and Russell L.D. 1999 Electron Microscopy; Principles and Techniques for Biologists; Jones and Burtlett Publishers, Boston, MA
2. Zeiss Ultra plus FESEM Instruction Manual 2010; Carl Zeiss NTS GmbH, Germany

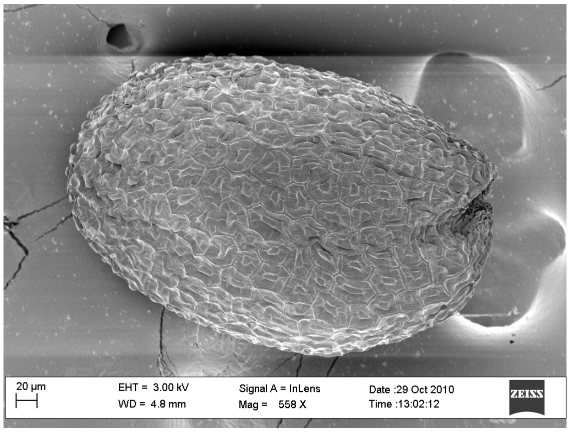

Fig 1. In-lens image of *Arabidopsis* seed showing charging effect. See bright areas and horizontal lines across the image.

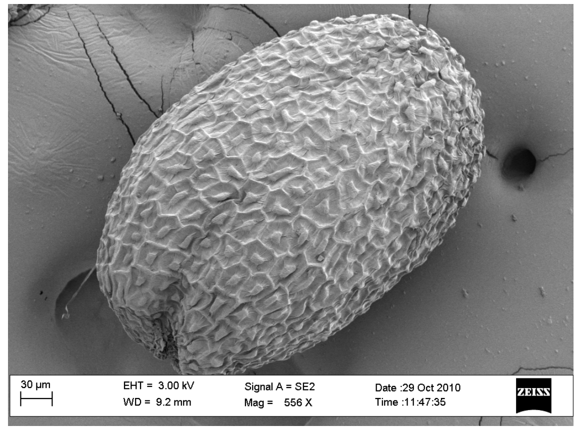

Fig 2. SE2 image of *Arabidopsis* seed showing no charging effect.

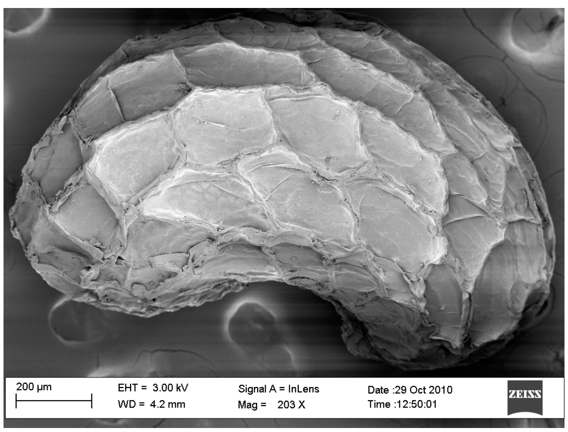

Fig 3. In-lens image of poppy seed showing charging effect. See bright areas and horizontal lines across the image.

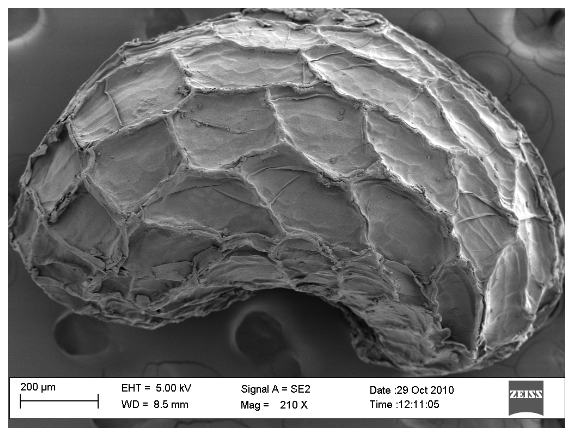

Fig 4. SE2 image of poppy seed showing no charging effect.

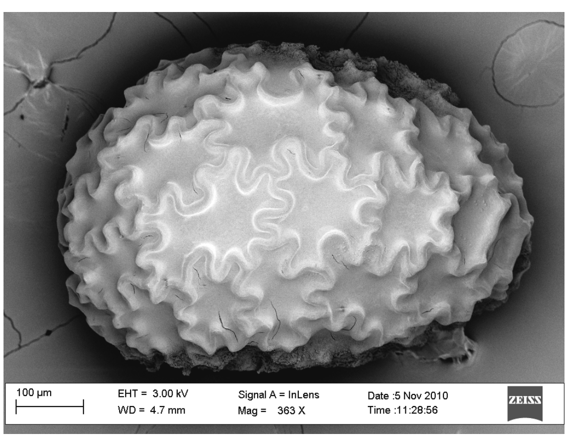

Fig 5. In-lens image of tobacco seed showing charging effect. See bright areas.

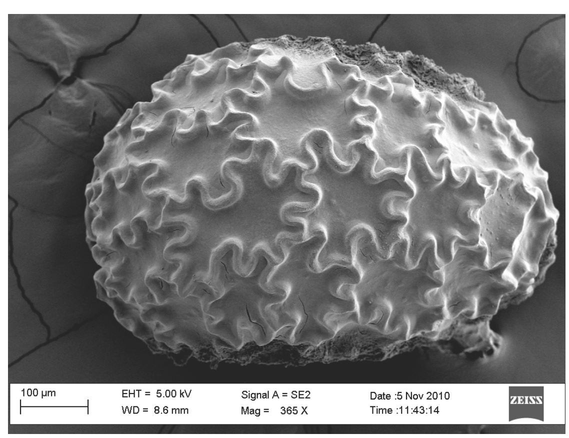

Fig 6. SE2 image of tobacco seed showing no charging effect.

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