Imaging dynamic biological processes with ESEM – A proof of principle study on plant tissue

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Keywords: ESEM, dynamic processes, stomatal pores, beam damage, leaf tissue

The Environmental Scanning Electron Microscope (ESEM) differs from a conventional SEM in that a differential pumping system maintains a pressure of gas (typically H_2O) in the specimen chamber whilst the gun remains at high vacuum [1]. Ionizing collisions between electrons and these gas molecules create positive ions which drift down onto the sample providing a mechanism for neutralising specimen charge. It is therefore possible to image insulating samples without the need for a metallic coating. The presence of water vapour in the chamber also means that a high relative humidity can be maintained and therefore samples can be imaged in a hydrated state without the need for dehydration and fixation.

These features suggest that ESEM could be well suited to imaging dynamic processes occurring in living biological samples. Our current work in this area focuses on optimising the microscope to view stomatal movements in Tradescantia andersonia (spiderwort) leaf tissue and hence provide a proof of principle study on the feasibility of ESEM methods for imaging biological changes occurring in real time in the microscope chamber.

Stomatal pores in the lower epidermal tissue of plant leaves allow the intake of carbon dioxide necessary for photosynthesis. Evaporative water loss from these pores usefully cools the tissue and drives the transpiration stream; however, if the water loss is excessive, the plant may become water stressed. It is therefore vital that the plant can strike the correct balance between assimilation of carbon dioxide and water loss. Consequently mechanisms have evolved to control the size of the pore aperture. Each pore is surrounded by two guard cells (Fig. 2) which can swell and change shape, opening or closing the pore depending on their turgor pressure. Guard cell turgor is governed by a range of intrinsic and extrinsic cues which serve to ensure that the pores are open if conditions are favourable for photosynthesis but closed if water is limiting.

We present a protocol for imaging the closure process of these pores in response to a decrease in relative humidity. The secondary electron signal is collected using a needle detector [2] and we follow a custom pumpdown procedure outlined by Cameron and Donald [3]. Figure 2 shows a typical closure sequence. Challenges include reconciling the need for an adequate physiological temperature and a low gas pressure favourable for imaging, with the thermodynamic constraints on achieving a high relative humidity. The constraints on imaging imposed by beam damage considerations are discussed and possible damage mechanisms are proposed.

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4. The authors would like to thank T. Fairhead for his assistance with the XL30 ESEM, P. Franks for his practical advice and A. Webb, H. Griffiths and D. Waller for helpful discussions. We acknowledge support from the EPSRC.

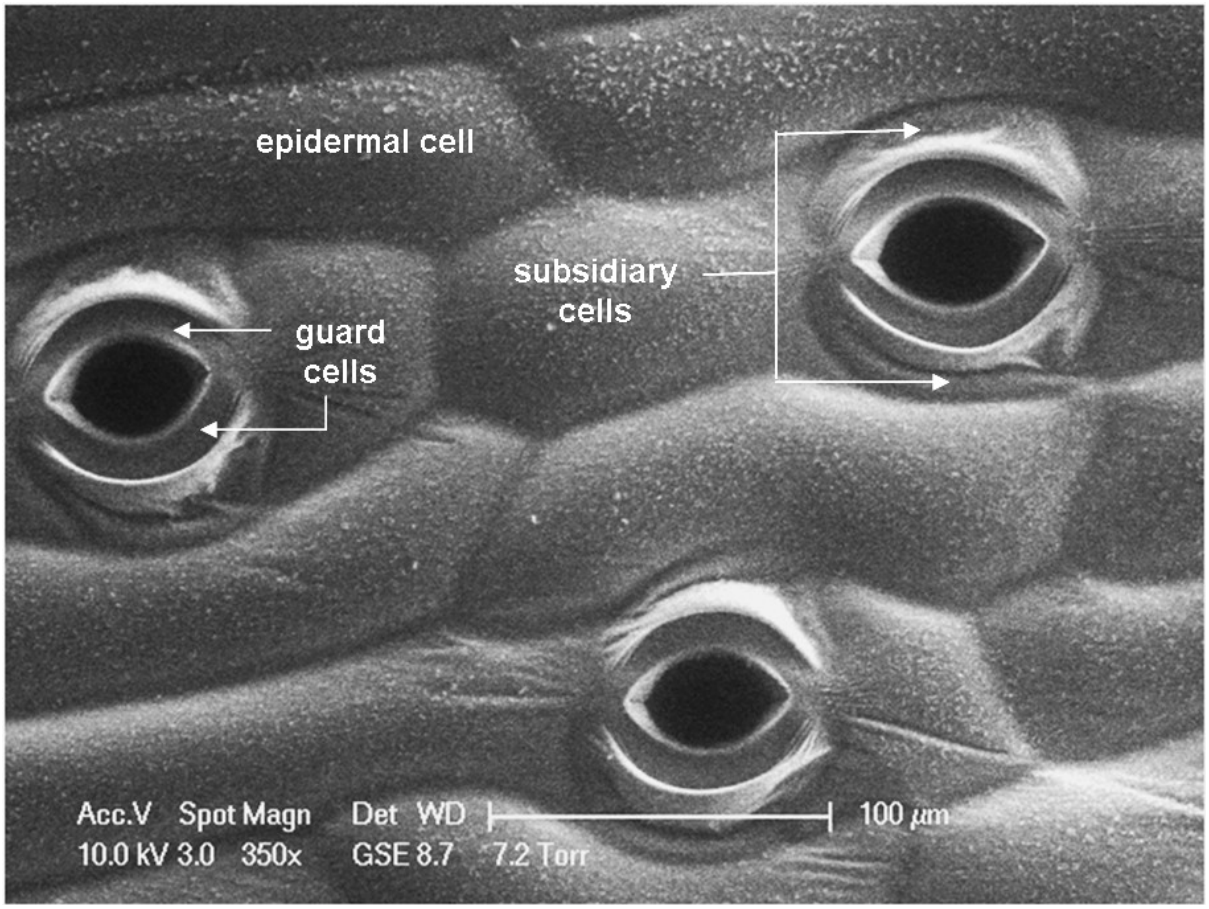

Figure 1. An ESEM image showing the character of the lower epidermis of *Tradescantia andersonia*. The three stomatal pores are fully open. Note the pair of guard cells forming the pore are flanked by supporting subsidiary cells. Accelerating voltage 10 kV, beam current 0.09nA, water vapour pressure 7.2 Torr, 7ₒC.

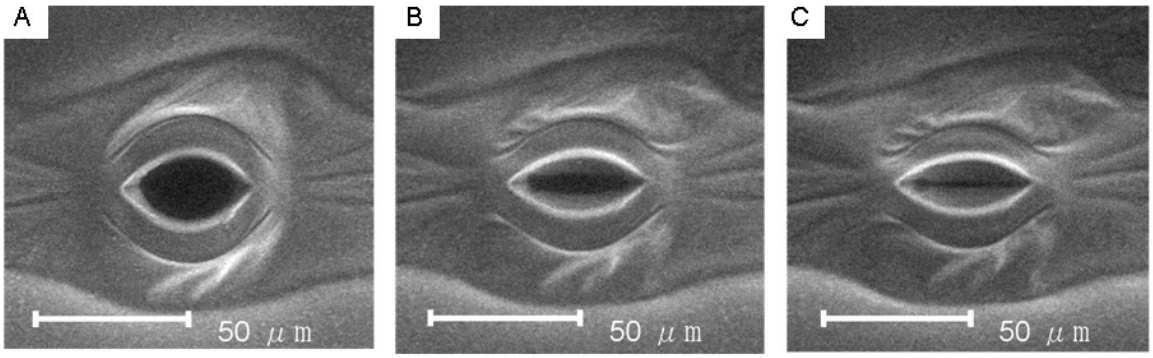

Figure 2. A sequence of three ESEM images following the closure of a single stomatal pore in response to the reduction in chamber relative humidity from 97% to 91%. **(A)** 12 minutes, **(B)** 25 and **(C)** 32 minutes after cutting. Accelerating voltage 10kV, beam current 0.09 nA. Water vapour pressure 7.3 Torr, temperature 7ₒC m in image (A), subsequent pressure 7.2 Torr, temperature 8 ₒC , in (B) and (C).

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-212, © Verlag der TU Graz 2009