Ultrastructural & Analytical Methods in Life Sciences

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The use of a color palette for TEM images to recognize cuticular channels in the epidermis of *Litchi chinensis* (Sapindaceae)

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The human eye can see more colors than grey tones due to the features of the photoreceptors (cones and rodes) of the retina [1] and should allow to improve the observation of fine details of the image. The Transmission Electron Microscope (TEM) images are formed as grey scale in relation to the different electron density of the material encontered by the electrons beam. For this reason we implemented a method to transform TEM images in false colors images. The software TEMPALETTE was written in Python 2.7.3 and is available under GPL licence at www.unifi.it/caryologia/PapiniPrograms.html.

The images were equalized with GIMP software (www.gimp.org) and then the grey tones were transformed to RGB colors with the software TEMPALETTE. This program works by maximizing the distance between 256 RGB colors (each corresponding to a grey scale tone) in the RGB cube, formed by the three axes R, G, B. *Litchi chinensis* is a plant belonging to family Sapindaceae of high interest for its commercial value. One of the main problems related to the commercialization of *Litchi* fruit is the relatively easy deteriorability of its epidermis. For this reason we began an in-depth study of the epidermis of the *Litchi* fruit, with particular reference to the structure of its cuticle.

One hypothesis about the water permeability of plant cuticle (mostly composed of hydrophobic substances, such as cutin and waxes), was that that the diffusion of water occurs along polysaccharide strands across the cuticle [2]. We investigate the ultrastructure of the cuticle of *Litchi chinensis* fruit, a cuticle of relatively notable thickness and whose resistence to water loss/entrance, is related to the fruit deteriorability. *Litchi chinensis* fruit were fixed overnight in 1.25 % glutaraldehyde at 4° C in 0.1 M phosphate buffer at pH 6.8. The samples were then post-fixed in 1% OsO_4 in the same buffer for 1 hr. After dehydration in an ethanol series and a last step in propylene oxide, the samples were embedded in Spurr's epoxy resin. Transverse sections approximately 80 nm thick were cut with a diamond knife and a Reichert-Jung ULTRACUT E ultramicrotome, stained with uranyl acetate and lead citrate. The images were recorded with a PHILIPS EM 300 Transmission Electron Microscope (TEM) at 80Kv. The main observed result is the observation of what appear to be channels crossing the cuticle of the external epidermis of *Litchi chinensis* (Fig. 3, arrow). The application of a Laplace contouring filter [2] apparently provided complimentary results (Fig. 4, arrow). One hypothesis is that such channels observed in the cuticle of *Litchi chinensis* fruit pericarp epidermis may be related to the polysaccharide strands across the cuticle observed by Riederer [3]. Reticulated structures in the thin cuticle of *Halodule* was also observed by Papini et al. [4]. Conclusion: the application of a color TEM palette appear to be of interest to improve the observation of fine structures in TEM images. Further investigation will be needed to understand the correspondence of the observed ultrastructure with the proposed function.

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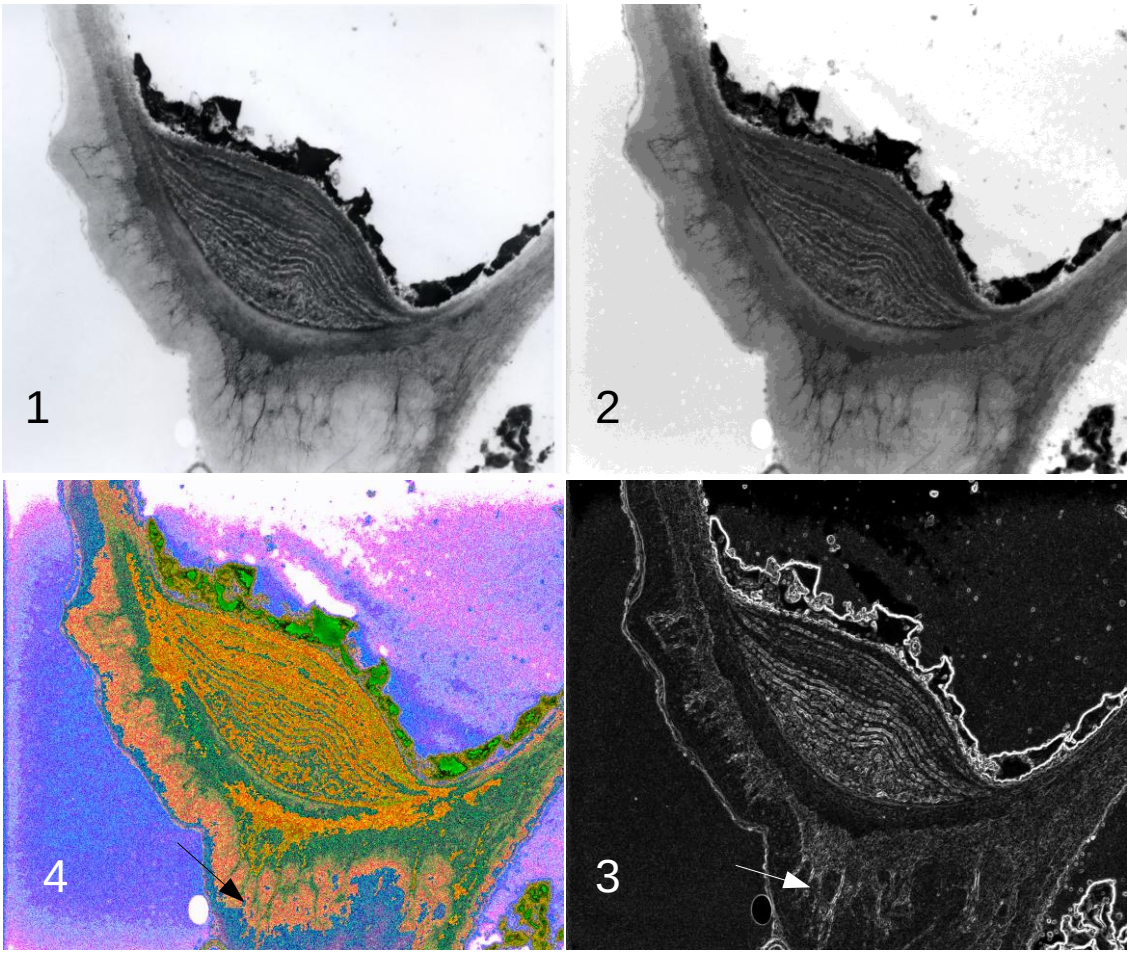

Figure 1. TEM image (magnification x14850) as it is scanned from a negative film.
Figure 2. same image as Fig. 1 but equalized with GIMP.
Figure 3. shows the result of the application of TEMPALETTE on Fig. 2.
Figure 4. shows the effect of the application of a Laplace contouring filter implemented in the GIMP package.