RIMAPS analysis of waviness and roughness images of rice leaves

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This research work introduces the application of Rotated Image with Maximum Average Power Spectrum (RIMAPS) for describing the surface topography of rice (*Oryza sativa*) leaves, specifically the micromorphological pattern seen on the waviness and roughness images of the original scanning electron microscope (SEM) image. RIMAPS technique has been used to characterize the micro-relief of metallic and biological surfaces [1] [2]. This kind of analysis allows finding the preferential directions, determined by RIMAPS, of the leaf microstructure pattern according to different characteristic lengths (CL) of the surface topography. This technique consists basically in performing the following steps: A) rotation of the original image from 0º to 180º; B) calculation of the x-step of the two-dimensional Fourier transform for each y-line of the new image obtained after rotation; C) computation of the average power spectra for each angular position and D) plotting of the maximum values of the power spectra as a function of the angle of rotation [1].

A typical epidermis microstructure of grasses leaf panicoide type as the rice leaf (herbarium sample coated with gold) can be seen in figure 1. The micrograph was obtained by using a SEM (FEI Quanta 200). The micromorphology in longitudinal view of the adaxial side consists on two distinct areas: the costal zone (R) with rows of short silica and cork cells and may have trichomes (T) as micro hairs type; the intercostals zone (IR) constituted by rows of large cells covered by wax papillae (P) of different sizes that intermix with stomata and may have trichomes (T) as prickle hairs type. The biggest papillae form a hexagonal pattern, being the mean distance between two of them of 32 μm. The width of the costal zone is approximately 62 μm. There are other CLs, such as the width between costal zones (180 μm). The RIMAPS spectrum of figure 1 (figure 5, black line) shows an absolute maximum around 25°, in concordance with the preferential direction of the epidermis cells distribution. There is another maximum around 50°, determined by one of the hexagonal sides formed by the wax papillae. There are also secondary maxima indicating that other arrangements of papillae are present. The following step was to filter the original image with two of the CLs previously found, to study the arrangement of the pattern according to their dimensions and find which of the surface characteristics influence the RIMAPS spectrum of the original image. The respective waviness and roughness images (figures 2, 3 and 4) were obtained by using cut-off length of 62 μm and 32 μm and analyzed with RIMAPS. The waviness filter eliminates those features of the leaf microstructure smaller than the cut-off length. The roughness filter works in the inverse way. It is clearly seen that the RIMAPS spectrum of the roughness images with both cut-off lengths, is more similar to the RIMAPS spectrum of the original image, than the RIMAPS spectrum of the respective waviness images. The maxima found around 5°, 20°, 30°, 45°, 60°, 70° and 90° of the waviness image with a cut-off length of 62 μm are due to the main directions of the biggest characteristic of the topography and their arrangement (figure 5). Similar result is found for the waviness image with a cut-off length of 32 μm. The maxima in this case are 5°, 15°, 45°, 55° and 90° (figure 6). The RIMAPS spectra of the roughness images indicate that the main directions of the pattern coincide with those found in the original image. It can be concluded that the small features of the topographical pattern and their arrangement are responsible for the maxima found on the RIMAPS spectrum of a SEM image. However waviness and roughness analyses are both important for a complete characterization of the surface, which allows to distinguish the macro and micro features of the topography. This kind of studies can also be applied on non-biological surfaces (*e.g.* metallic surfaces), where the wavy component plays an important role.

References

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[2] E. A. Favret *et al*, *Micron*. **39** (2008) 985-991.
[3] The authors gratefully acknowledge Mr. Pablo Reynoso Peitsch (CNEA) for his work on the scanning electron microscope.

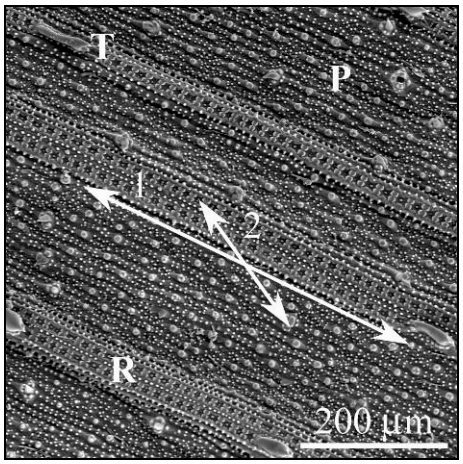

Figure 1. *SEM image of rice leaf. Arrows 1 and 2 indicate main directions of the topographical pattern T: trichomes; P: papillae; R: costal zone*

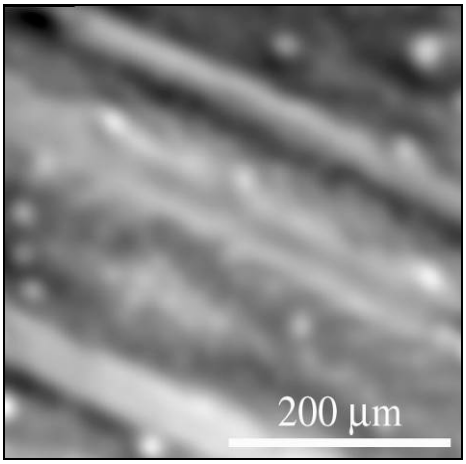

Figure 2. *Waviness image of figure 1. Cut-off length: 62 µm*

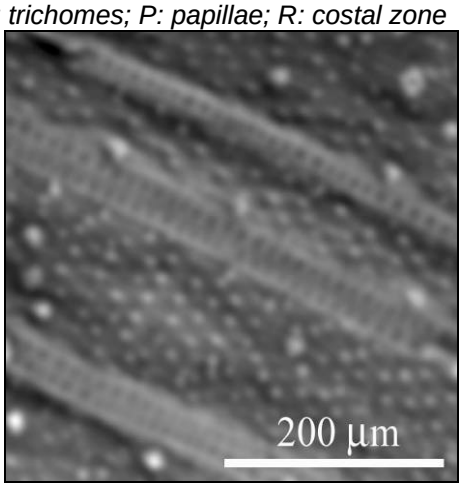

Figure 3. *Waviness image of figure 1. Cut-off length: 32 µm*

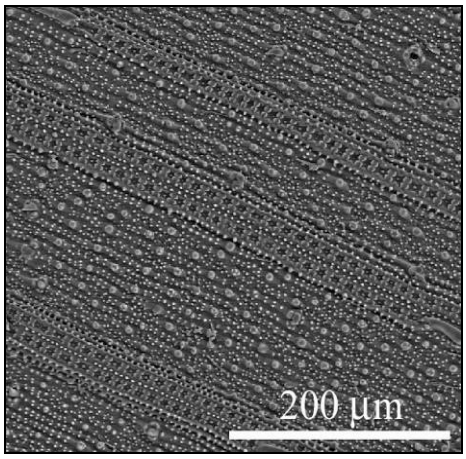

Figure 4. *Roughness image of figure 1. Cut-off length: 32 µm*

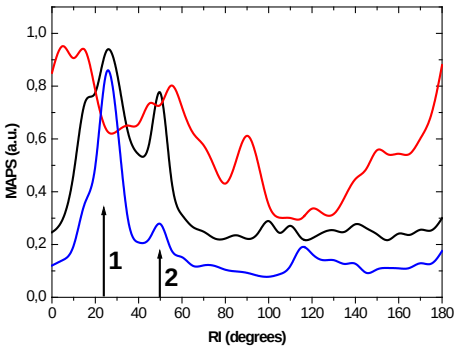

Figure 5. *RIMAPS spectra of the original image (black line), the waviness image (red line) and roughness image (blue line) for a cut-off length of 62 µm. 1 and 2: main directions*

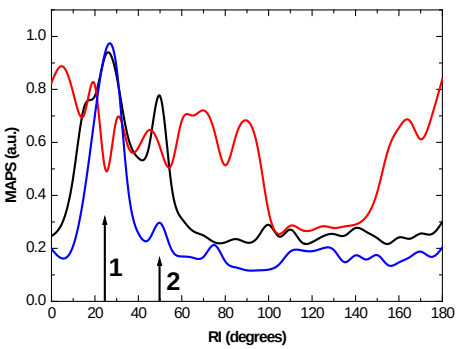

Figure 6. *RIMAPS spectra of the original image (black line), the waviness image (red line) and roughness image (blue line) for a cut-off length of 32 µm. 1 and 2: main directions*