effect of crop production process as well as the feedback from the quality of environmental resources to crop productivity.

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SCIENTIFIC CORRESPONDENCE

‘Holes’ on erythrocyte membrane and its roughness contour imaged by atomic force microscopy and lateral force microscopy

Atomic Force Microscopy (AFM) is a powerful, recent technique which has been largely applied in the field of material sciences, but less so in the biological realm[1–5].

The first AFM image of erythrocytes was reported to reveal a large number of closely-packed nanometer size particles[6]. This has been further studied recently[7]. Another work[8] deals with the action of drugs on human erythrocytes, which opens up new horizons in the medical field.

We report here the presence of depressions or pits on the human erythrocyte membrane and the varying roughness, as revealed by the change of friction, of different areas of the erythrocyte with the help of Lateral Force Microscope (LFM) technique.

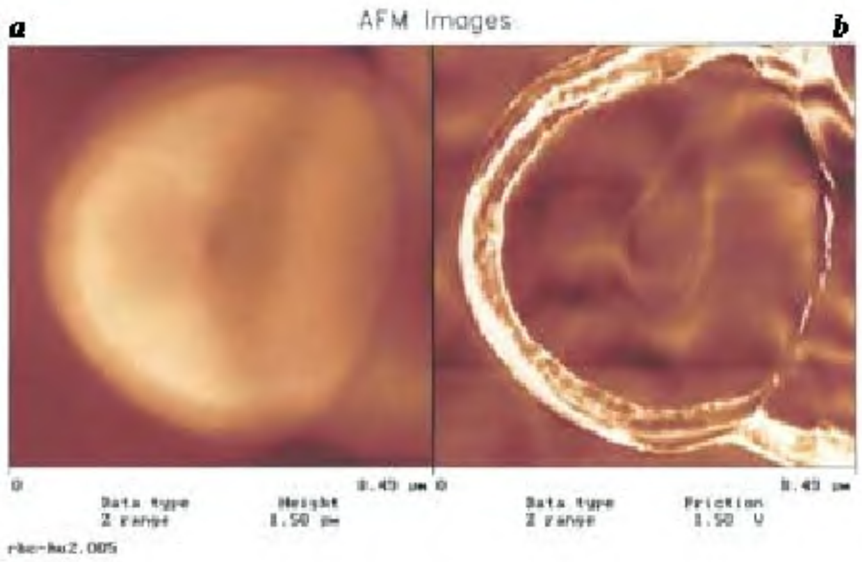

Figure 1 ***a***. AFM of erythrocyte image at lower magnification shows that normal shape has been retained after air-drying on a glass slide. White areas indicate highest regions and darker areas, lower regions; ***b***, LFM image of the same erythrocyte shows the roughness contour. White areas indicate the highest roughness, darker areas are smoother.

The AFM was a Digital Instruments Nanoscope, ESPM system. The cantilever was 100 µm, wide-legged tripod type. Imaging was effected in the contact mode in air after drawing blood (from a healthy subject) on a glass slide and air-drying.

Figure 1 *a* shows that this procedure maintains the normal shape of erythrocytes as evident in natural or near-natural conditions. Figure 1 *b* shows the ‘roughness contour’ of the erythrocyte shown in Figure 1 *a*. For this, LFM, i.e. using the horizontal component of the force has been utilized. This force reveals the variable friction (due to varying roughness) on the erythrocyte surface. Here the white portions show the roughest parts of the membrane and the darker (reddish) parts, the smoother region. This erythrocyte has of course been subjected to ‘unnatural’ stress/strain forces during air-drying, but interestingly, the whole circular rim is the roughest (with an inner, smoother band) and there is a sudden transition beyond this rim.

Figure 2, at higher magnification, reveals between the ‘closely packed particles of nanometer size’ at least nine

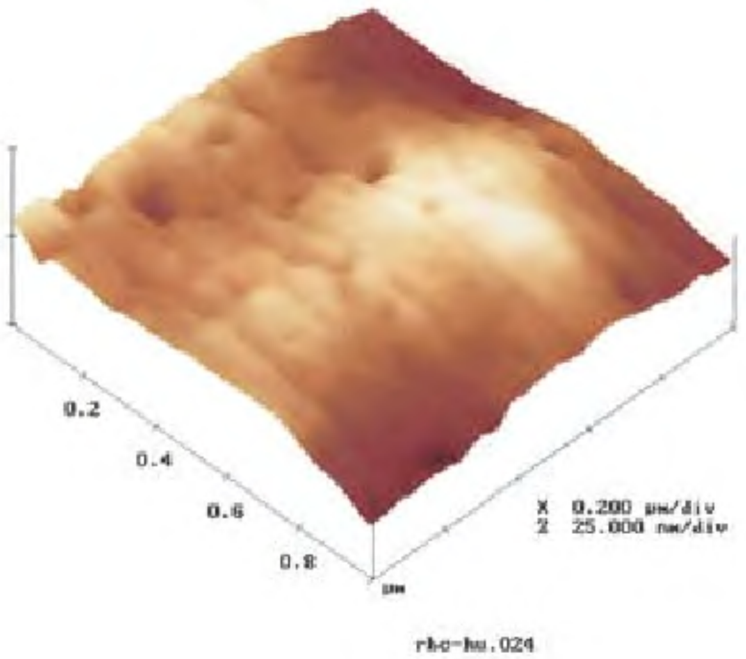

Figure 2. The pits or depressions on air-dried erythrocyte as revealed by AFM.

depressions or holes on the outer surface. Four of these are quite prominent. Thus in an area of 10 µm × 10 µm there are 4 to 9 depressions. The size of the particles is mostly in the 75–100 nm range. The pit-like depression near the upper edge of the white area is about 0.04 µm in diameter, as can be determined with the help of the scale furnished in the image. These pits are much smaller than the pores on the nuclear envelope[9]. These pits are not visible in SEM images up to about × 20000. (SEM images are not good at these or higher magnifications.)

An image[10] of the inner membrane obtained from a lysed erythrocyte at an effective scanning area comparable with ours, is of interest in this context. The authors presume that the holes are due to spectrin–actin network. This is complementary to our work and possibly the pits on the surface partially or largely overlap with the holes of the inner membrane. In our system the cells dried in air have been subjected to stress and shear, but such forces are not likely to cause rounded pits.

The significance of the pits in normal cells, and for invasion of malarial parasites may be worth exploring. Mean surface roughness of erythrocytes has been estimated[8] by measuring the standard deviation of membrane height and tabulating the results. A visually satisfying approach is the LFM reported here. This image reflects the varying roughness on the basis of variable friction offered by the different areas.

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