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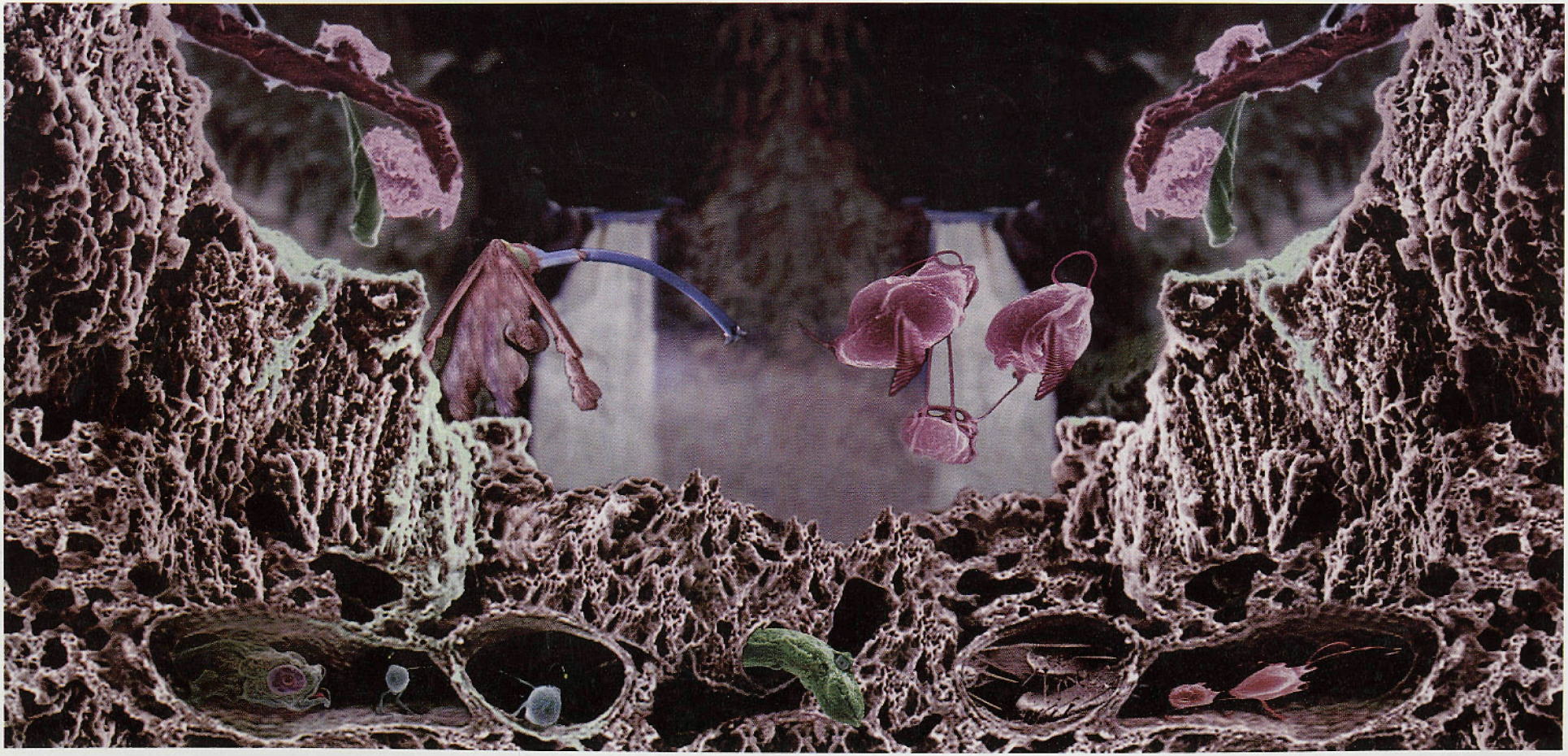

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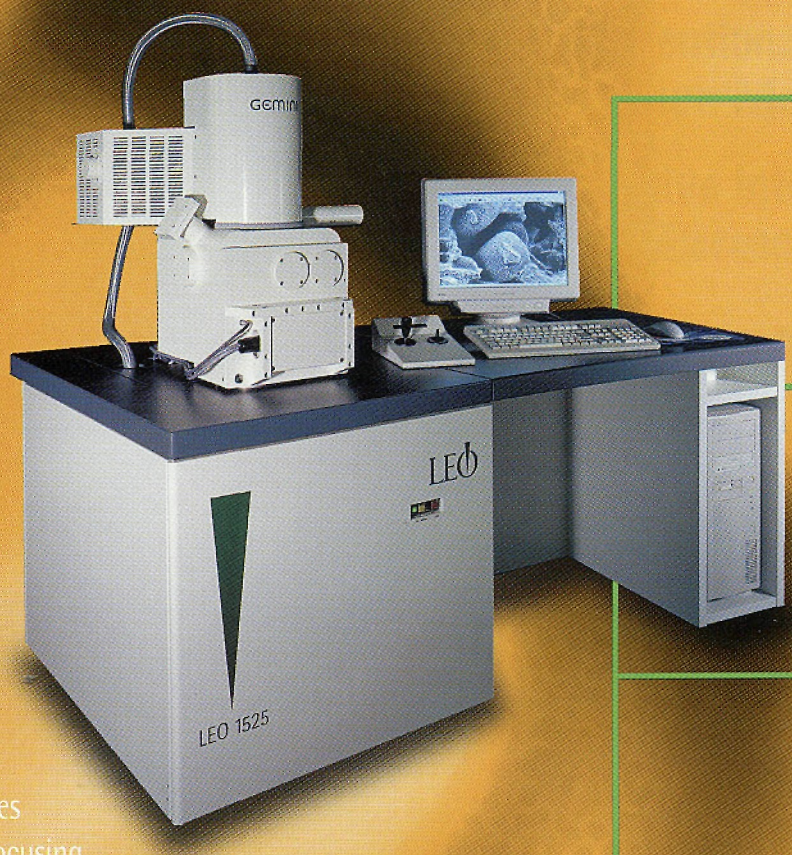

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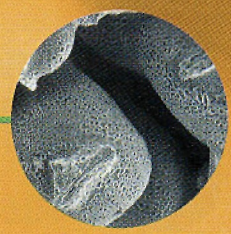
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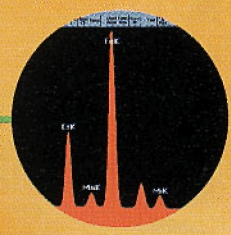
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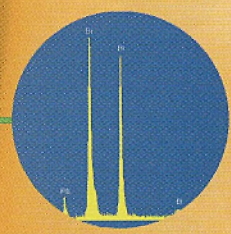
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TYPING BLOOD CELLS, ONE AT A TIME

Stephen W. Carmichael[1] Mayo Clinic

We are all familiar with the 4 groups of human blood types; O, A, B, and AB. Each group is characterized by specific molecules on the surface of the red blood cells (RBCs). Recently, Michel Grandbois, Wolfgang Dettmann, Martin Benoit, and Hermann Gaub have modified the atomic force microscope (AFM) to use the different affinities of these molecules to produce an image.[2]

A key step was to specifically functionalize the scanning probe tip of the AFM. What this means is to covalently attach an active molecule to the tip. The active molecule they used was a lectin derived from the snail Helix pomatia (this is the same snail valued by the French as escargot). This lectin binds specifically to molecules found on the surface of RBCs in blood group A (N-acetylgalactosamine-terminated glycolipids), but these molecules are not found in blood group O. But if the lectin was bound directly to the AFM tip, nonspecific adsorption to the specimens would occur. Grandbois *et al.* avoided this by tethering the lectin to the AFM tip with an amylose polymer that allowed the discrimination of specific unbinding events.

The specimen was a monolayer of RBCs from the A and O blood group. The concept was to sample the specimen with the functionalized tip so that a specific binding/unbinding event produced a bright pixel, and the lack of an event produced a dark pixel. The binding/unbinding event produced a force curve that yielded additional information. The first part of the curve correspond to the contact of the tip with the cell surface. At that point specific recognition between the tip and the cell surface occur but no adhesion signal is recorded. As the tip was subsequently withdrawn, the cantilever that the tip was mounted on deflected, producing the adhesion signal observed in the force curve. After it was withdrawn a certain distance, the bond between the lectin and the molecule would rupture, and the cantilever would spring back to its equilibrium position.

However, Grandbois *et al.* were concerned that the unbinding event was actually what they were observing. The problem was the molecule on the A cells was a sugar-coated lipid, that was not firmly anchored in the surface membrane. Imagine that your hand was coated (functionalized) with a substance that stuck only to carrot tops; not turnips or beets, or anything else. As you tapped your hand over the ground in your garden, you could feel that sometimes it would stick to a carrot top. As you raised your hand, you could feel a force that would cease by the time your hand was a foot or so off the ground. Did the coating on your hand release the carrot top, or did you pull the carrot out of the ground? Likewise, the lipid molecule could have been pulled from the surface relatively easily. Grandbois *et al.* concluded that if this problem occurs in the experiment, it may well be avoided by the high off-rate of the lectin-sugar pair during the acquisition time of one picture, meaning that the dynamics of the experiment indicate this is a non-destructive detection of the specific molecule on A cells.

Of course this demonstration of affinity imaging with the AFM was not meant to type RBCs. In fact, Grandbois et al. used conventional fluorescence microscopy to show a labeled lectin binding to A cells, and not O cells, with much better resolution than by affinity imaging. However, they also pointed out that the binding of the labeled lectin to the A cells was a destructive process since the lectin remained on the RBCs, altering the surface. In contrast, the functionalized AFM tip moved on, leaving the surface in its original condition.

Imaging with the AFM based on affinity, as demonstrated by Grandbois et al., has the potential to become a valuable tool. Specific surface molecules may be imaged as they are expressed on cells under different conditions and at different stages of development.

1 The author gratefully acknowledges Dr. Michel Grandbois for reviewing this article.

2 Grandbois, M., W. Dettman, M. Benoit, and H.E. Gaub, Affinity imaging of red blood cells using an atomic force microscope, J. Histochem. Cytochem. 48:719-724, 2000.

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