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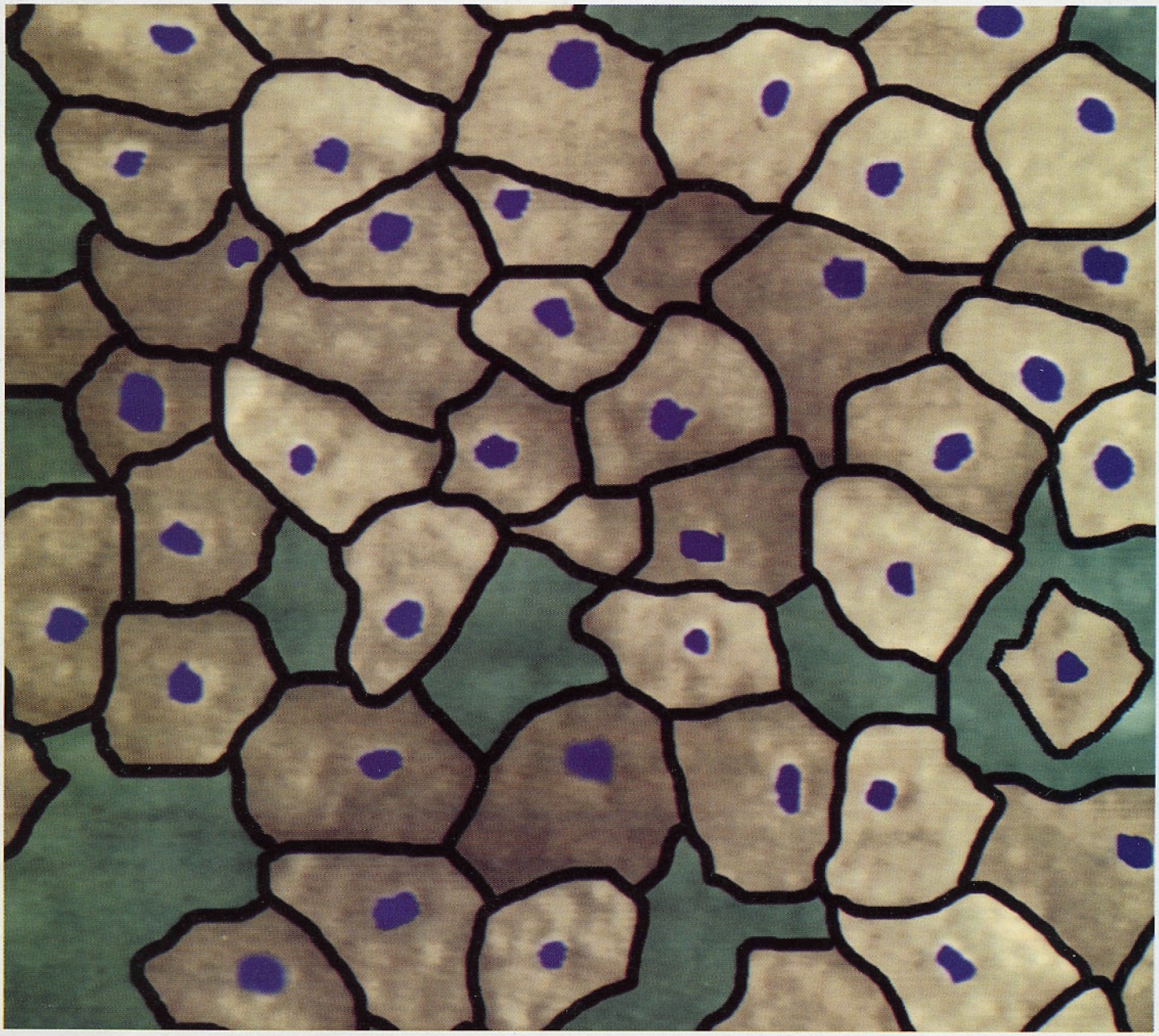

HOW TO SLAM A CELL

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Many important discoveries in cell biology have been made by introducing specific compounds into a certain cell and observing the effect. Several methods have been used for the introduction of the compounds. For example, permeabilizing the cell by electroporation (an electric shock that has been demonstrated to poke holes in the plasma membrane), the use of biologic molecules (such as streptolysin O) to create channels in the plasma membrane, or detergents to dissolve holes in the plasma membrane. Several laboratories have shown these methods to be effective, but one questions the physiologic state of a holey cell. Liposomes or similar vehicles can be packed with compounds and fused with the cells of interest, but this method of introduction has drawbacks as well. Probably the most widely used method is impaling a stationary cell with the tip of a micropipette and injecting the compounds into the victim. As has been demonstrated throughout the world, this is an effective method, but from the cell's point of view it certainly is violent. More often than not, the cell of interest dies in the process. In this technique, appropriately nicknamed the "stab" technique, the micropipette is advanced at a rapid rate (around 700 µm/s) and its compounds quickly injected at high pressure (100 mbar or more). Let's scale a 10 µm cell up to the size of a basketball (about 30 cm). The "stab" comes into the ball at about 40 mph. The fastest a human being (I'm thinking of a top major league baseball pitcher) can advance an object is on the order of 90 mph. For you and me, we're rarin' back and stabbing a basketball just about as rapidly as we possibly can. How careful can you be not to hit something the size of a softball (representing the nucleus) inside? Then quickly inject a liquid under high pressure. Scaling up 100 mbar gives quite an explosion!) and what happens? No wonder so many "stabbed" cells don't survive the experience. There must be a kinder, gentler method.

Iraj Laffafian and Maurice Hallett from the University of Wales College of Medicine have developed such a method[2]. Although the acronym SLAM (for Simple Lipid-Assisted Microinjection) doesn't sound so gentle, it is. Their method is a hybrid of the liposome fusion techniques and the "stab" technique. First the micropipette was loaded with an aqueous solution that included Lucifer yellow as a marker. Then the tip of the micropipette was coated with a lipid, phosphatidylcholine-oleyl-palmitoyl (POPC), and dried. The lipid was "tagged" with 1,1'-dioctadecyl-3,3,3',3'-tetra-methylindocarbo-cyanine perchlorate (DiC_{18}). This "tag" has the property of fluorescing strongly when in lipid bilayers, but not when in aqueous solution. The micropipette was slowly advanced toward a cell (a neutrophil in the experiments described). While in the aqueous solution bathing the cell, the POPC swelled, closing off the tip. This was shown by applying a small pressure (about 10 mbar) and noting that the lucifer yellow did not leak out. After the lipid-coated tip touched the cell, but not before, some of the DiC_{18} could be seen by fluorescence microscopy to transfer to the plasma membrane of the neutrophil, and later distributed around the cell surface. About one second after contact, with the pressure maintained at about 10 mbar, Lucifer yellow was seen to enter the cell. The amount of liquid transferred into the neutrophil was equivalent to about 1% of the cell volume. In a control experiment, Lucifer yellow was not transferred to a neutrophil when an uncoated tip was used. Using the cell survival assay of trypan blue exclusion, all of the neutrophils treated with the SLAM technique survived. Typically, less than 5% of "stabbed" neutrophils survive.

This gentle SLAM technique has two important possibilities for future studies. The introduction of Lucifer yellow into the cytoplasm demonstrated that compounds dissolved in an aqueous medium can be reliably introduced into a small, mobile cell that is loosely adherent. As if this was not enough reason to get excited, the DiC_{18} experiments showed that molecules in the lipid phase can be transferred to the plasma membrane. The SLAM technique can be used to manipulate the cell surface, as well as the cell interior. This will be a useful technique!

1 The author gratefully acknowledges Dr. Maurice Hallett for reviewing this article.

2. Laffafian, I, and M.B. Hallett, Lipid-assisted microinjection: Introducing material into the cytosol and membranes of small cells, *Biophys. J.* 75:2558-2563, 1998. Also see the "Techsighting" by R. Peters and R. Sikorski in *Science* 282:2213-2214, 1998.

Front Cover Image
Cornea Epithelium In The Living Eye

The image is that of the cornea epithelium in the living eye. The foreground cells are gray and the background cells (one cell layer down) are green. The cell borders are in black and the nuclei of the foreground cells are blue. The original image was printed on a Kodak DS 8650, this was then scanned at 600 dpi (optical) with a Umax 2400 and moved into Adobe PS 5.0. Four layers were created: 1) nuclei, 2) foreground cells, 3) background cells, and 4) cell borders. Nuclei were revealed by thresholding.

The image was supplied by Roger Beuerman, Juan Reynaud and Stephen Kaufman of the LSU Eye Center.