A Microscopic Movable Feat

Innovations in both microscope technology and cellular physiology are showing scientists what does and does not happen in the front-running part of a moving cell

By DIANE D. EDWARDS

For my part, I travel not to go anywhere, but to go. I travel for travel's sake. The great affair is to move.
— Robert Louis Stevenson

Unlike Stevenson and other inveterate travelers, cells normally don't "travel for travel's sake." They receive, instead, chemical signals as traveling orders, explicit itineraries to engulf an invading bacterium or help shape a developing embryo's heart. This directed motility is so basic to cellular function that scientists have for centuries peered through microscopes, looking at the movements of internal cellular parts and of cells themselves. What they see, in the words of Jeremy S. Hyams of London's University College, is "a bird's-eye view of Piccadilly Circus in rush hour" — a complex coming and going by structures inside each cell, some of which help the entire cell move. So, even for individual cells, moving becomes a great affair.

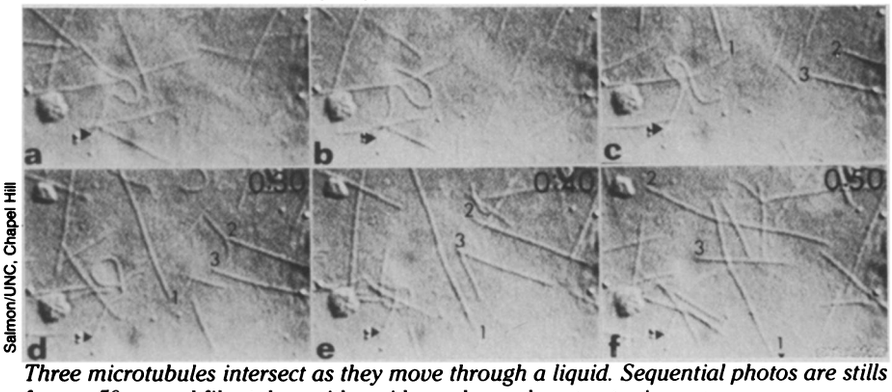
Some one-celled organisms are equipped with appendages like cilia and flagella that propel them through solutions. But others, like nearly all mammalian cells, must depend on a type of "self-propulsion" to creep along. What happens at the leading edge of these creeping cells is of particular interest to scientists looking for clues to the why and how of cell movement. Among them is D. Lansing Taylor of Carnegie Mellon University in Pittsburgh, site of the Center for Fluorescence Research in Biomedical Sciences. At a recent briefing organized by the Council for the Advancement of Science Writing, Taylor described his group's work using the latest in microscope technology.

"The head has to be different from the tail, or the cell wouldn't be moving," says Taylor. In his view, the edge is "the most exciting part of the cell, where all the action is."

To tell heads from tails, Taylor and others are using two recently developed techniques: video enhanced contrast microscopy and low light dose microscopy. Unlike the venerable electron microscope — which requires fixed material held in a vacuum chamber — these newer methods allow scientists to gaze upon living specimens.

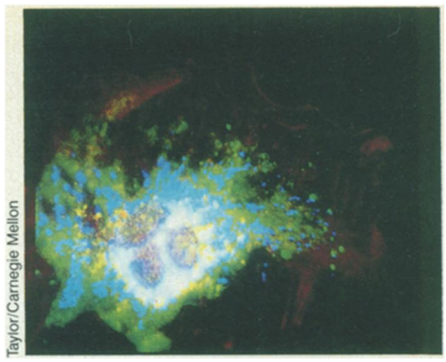
Combining computer capability and superior cameras, video enhanced contrast microscopy (VECM) shows smaller structures than previous light microscopes, because it improves the contrast between the black and the white images seen through the scope. The computer produces crisp images by combining multiple exposures captured on videotape, then subtracting "background" information that tends to make a picture less clear.

Unlike the more-illuminated VECM method, low light dose microscopy (LLLM) detects very weak fluorescent signals using sensitive light detectors and cameras. By using marker dyes that fluoresce under different wavelengths of light, scientists can design chemical probes that attach to specific components of a cell. Computers connected to the apparatus then enhance the fluorescent signals, allowing "biochemical dissection" of the machinery at work in living cells.



Salmon/UNC, Chapel Hill

Three microtubules intersect as they move through a liquid. Sequential photos are stills from a 50-second film taken with a video enhanced contrast microscope.



Taylor/Carnegie Mellon

A fibroblast cell, seen through low light level fluorescence microscopy, illustrates the "molecular sieving" that occurs during forward movement of a cell. With computer-enhanced imaging, actin fibers appear red, mitochondria blue, synthetic beads (engulfed by the cell) green and structures called endosomes yellow. While full of actin, the cell's leading edge (right) does not contain the larger beads or cellular structures.

With VECM to detect minute structures and LLLM to follow biochemical changes, the Carnegie Mellon scientists have focused on the cytoplasm at the edge of cells and found it to be a rather exclusive club. In a process called molecular sieving, the nearly solid cytoplasm of the moving area is too dense for larger objects to penetrate. But what *is* there, in great abundance, is the contractile protein actin.

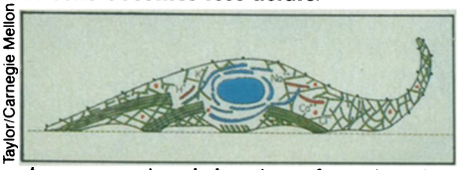
Actin's presence is no surprise. Scientists have known for decades that individual cells have structures akin to skeleton and muscle, with microtubules and microfilaments acting as "bones" and "muscles" — giving the cell support and motility. But the exact processes involved are still part of what Taylor calls "the black box" of cell motility and the subject of intensive study. In the Nov. 12 NATURE, a group at the Worcester Foundation for Experimental Biology in Shrewsbury, Mass., reported the discovery that a particular microtubule-associated protein can transport chemical-containing organelles from the edge of a cell toward the interior. As for the mysteries of microfilaments, Harriet Harris, of the AFRC Institute of Animal Physiology and Genetics Research in Cambridge, England, pointed out in the Nov. 26 NATURE that sorting out "what makes microfilament structure dynamic" will be a "considerable task" for biochemists.

As the main protein of microfilaments, actin helps the cell move by a dynamic process of continuous assembly and disassembly of actin filaments, which are much less than one-millionth of an inch in diameter. Found in places like the tip of

growing nerve fibers and in the tiny fingers of tissue that line the intestine, actin seems to be everywhere, flexing its "muscles" to get the job done. But without techniques like VECM and LLLM, it was impossible to study the contortions of individual microfilaments in living material. With these microscopes, says Taylor, researchers can repeatedly observe cells that are kept alive for days in special chambers, where temperature and other environmental factors can be manipulated.

During normal wound healing, various cells move in to seal the injured area. To study actin's role in this response, Taylor and his co-workers used a blunt instrument to "cut a swath of destruction," as he describes it, through a sheet of cells growing on a transparent surface. Seen through a VECM microscope, actin fibers begin appearing in the extended edge of cells, and as they lengthen and contract, the entire cell will follow. LLLM will also improve scientists' understanding of the chemistry of the leading edge, says Taylor. For example, he has found that, after wounding, the inside of cells becomes less acidic.

Taylor/Carnegie Mellon



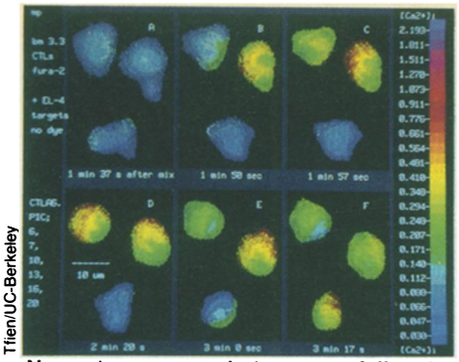
A cross-sectional drawing of a migrating mammalian cell shows networks of actin in green, with the undulating moving edge curled upward before attaching to the surface and contracting to pull the cell forward. Within one or two hours, cells can extend these edges 20 to 30 microns.

Other experiments show that the center of a migrating cell is relatively more acidic than the edges, where, at the leading edge in particular, the cytoplasm becomes more alkaline during movement. This and other biochemical characteristics make the migrating edge special, says Taylor. "Although there's no membrane delineation out there [to isolate the moving edge]," he says, "the cell has compartmentalized itself."

Taylor predicts that, given the sensitivity of the LLLM procedure, scientists will soon learn how different molecules interact at specific times in specific locations within a single cell. As few as 10 to 20 fluorescent signals in a cell can be detected with LLLM, he says, and the average cell has 1 billion copies of actin that might someday be individually labeled with fluorescent dyes. By labeling subunits of actin and other proteins and then injecting them into cells, scientists can track microfilament and microtubule formation with precision, says Taylor.

At present, it is possible to use five different fluorescent labels in the same cell or tissue, says Taylor. These measurements, he says, will not always be limited to two dimensions. Within a year, the scientists expect to produce three-dimensional images of cells dividing and migrating, with key biochemical changes represented by special markers. This use of cells as "living cuvettes" to study chemistry will take researchers far beyond the leading edges of cells, says Taylor. LLLM studies of immune-system cells being stimulated by foreign particles or other cells, for example, show rapid and drastic chemical changes inside the activated cells.

Tsien/UC-Berkeley



New microscopy techniques can follow internal responses to external stimuli in living cells. A series of images taken through a low light level fluorescence microscope shows that the concentration of calcium in "killer" T lymphocytes rose and fell within minutes after the T cells were mixed with target cells. The more red the color, the higher the calcium content. An important chemical signal in cells, calcium helps regulate the contraction of actin filaments.

Other studies under way are using the newer microscopy to look at cellular changes in very small embryos, the blood circulation in tumors and interactions among networks of nerves. Also being developed are genetically engineered fluorescent probes that will be native to cells, passed on from one generation to the next. And RNA and DNA tagged with fluorescent dyes will someday enable scientists to follow genes in living cells, says Taylor.

In addition to dissecting the normal machinery of a cell, the newer microscopes might help explain abnormal functions that lead to disease. "There's not a big gulf between wound healing . . . and following transforming cells [that are becoming cancerous]," notes Taylor. He says that monoclonal antibodies, used in combination with these and other new diagnostic techniques, will provide rapid cell identification. Although he estimates that the needed equipment will cost $220,000 when commercially available, Taylor says such microscopes will become at least as common as electron microscopes are today. "The future is very bright," he says. "[The technology is] literally changing on a monthly basis." One could say that the researchers are going from one leading edge to another. □