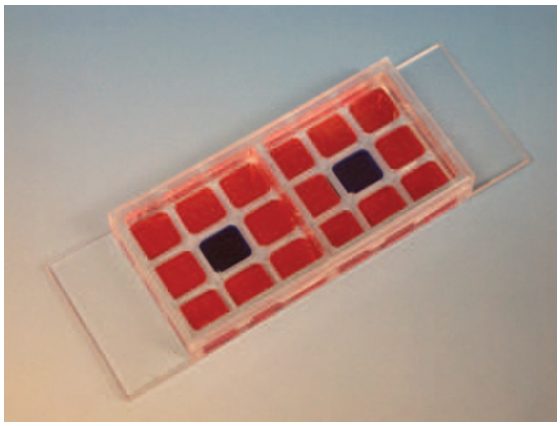
Cell migration: our protruding knowledge

Nathan Blow looks at some of the technologies used by cell biologists to unlock the mysteries of cell migration.

As people move from place to place to carry out daily activities, within their bodies cells also move to perform many critical biological functions. Development, immune response and wound healing all rely on the directed migration of cells. When cell migration goes awry, the effects can be very bad, indeed, ranging from tumor metastasis to vascular diseases.

Cell migration involves complex protein signaling cascades and changes in the structure of the cell's actinomyosin cytoskeleton. Thus biologists studying cell migration wear many hats in the laboratory, and embrace new methodologies, technologies and even dimensions in the quest to discover how and why cells move.



Ibidi is developing slides for long-term studies of cell migration. (Courtesy of Ibidi GmbH.)

New twists on one of the classics

Although some cell movements are random, others are directed toward a signal. One such process, chemotaxis—which occurs in response to a chemical gradient—has been extensively studied. A classical way to study chemotaxis is to use a two-chamber system: cells are placed in one chamber and the chemical signal, or chemoattractant, in the other.

Several variations of two chamber systems have been used including a top-and-bottom configuration known as the Boyden chamber, a side-by-side chamber configuration known as a Zigmond chamber and the Dunn chamber, which consists of concentric rings. More recently, Ibidi introduced the μ-Slide chemotaxis assay, which allows real-time imaging of chemotaxis on a microscope slide.

The Boyden chamber was the first two-chamber system to be described[1]. It is still used today to study many types of cell migration, including chemotaxis, although the original design has been modified and improved upon since the system was first described in 1962. Separating the two chambers is a porous membrane through which cells can actively migrate. The size of the membrane pores is critical when using a Boyden chamber, and companies sell membranes with a variety of pore sizes. A typical eukaryotic cell is 30–50 μM in size and can fit through pores in the 3–13-μM size range, but various companies have optimized pore size for specific cell types. For example, Millipore supplies the QCM Quantitative Cell Migration assay that uses an 8-μM pore size membrane, optimal for epithelial and fibroblast cell migration assays.

The company Neuro Probe, founded in 1970, has based a large part of its business on improving the Boyden chamber. "We simplified the design of the Boyden chamber for use and production, and further refined the design—creating the Blind Well chamber," says Mark Phillips, president of Neuro Probe, adding that its main advantage is the smaller required volume of cells and chemoattractants. Today, Neuroprobe is taking the Blind Well chamber into the high-throughput world with 96- and 384-well disposable ChemoTx systems and a 1,536-well variant in development. The use of a single membrane covering all the wells eliminates the need to handle many small, fragile membranes during the assay.

Scratching the surface

Another classic, in the low-tech category, is the 'wound healing' assay, which is used to study migration of cells in the absence of a chemoattractant. A confluent plate of cells is 'wounded' by scraping a specific area of the plate. Migration of cells can then be monitored over time by imaging the cells as they move from the untouched areas into the wounded area. Although it is simple, this assay is also cumbersome because imaging cells over long periods can be difficult, and at first glance the system does not appear to be amenable to automation.

Enter Applied BioPhysics, which has developed a wound healing assay based on the concept of electric cell-substrate impedance sensing (ECIS). One small electrode is placed on the bottom of a tissue culture well in the center, and a second electrode at the edge of the well completes the circuit. Cells that attach to the electrode in the culture well alter the current, and this effect can be measured directly. "Cells are basically insulating, so when you put the cells on the electrode, the current has more difficulty flowing, so the resistance and the impedance increase," says Ivar Giaever, president of Applied BioPhysics. By growing the cells to confluence and then applying an electric field for 10–15 seconds, one kills the

cells on the electrode. As the surrounding cells migrate into the wounded area, the associated change in current can be measured to calculate the cell migration rate.

The advantage of a system like this is clear: it can be automated. "You can grow cells in tissue culture and then say, 'I want to wound them at midnight'. The system will do that, and you get all the data when you get back in the morning," says Giaever. Applied BioPhysics also offers this assay in a 96-well culture dish format, providing a high-throughput solution.

Moving in a 3D world

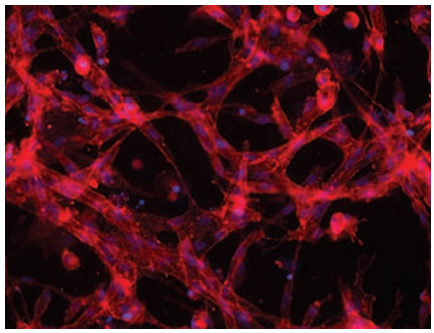
The classical tissue culture dish is a two-dimensional (2D) environment in which cells lack the components and structure of the three-dimensional (3D) extracellular matrix they would see *in vivo*, composed of collagens, proteins and proteoglycans.

Migration of some cell types differs dramatically between 2D and 3D worlds (see also **Box 1**). Kenneth Yamada at the US National Institutes of Health realized this quickly after analyzing his group's early results studying migration of human fibroblasts in three dimensions. "Rates of migration are moderately higher," he says of the 3D culture experiments, "but what was striking was the cells were migrating more directionally, and that was not related to an intrinsic pattern in the matrix".

Yamada's group and others have since shown that it the matrix structure itself is likely responsible of the effect[2]. "It is something about the three-dimensionality, not a particular component," says Yamada. He points to work by Edna Cukierman in his lab and the work of other groups suggesting that the stiffness of the extracellular matrix might be important in directed migration, and that making a matrix stiffer makes cells behave more like in two dimensions.

Yamada thinks that the matrix composition and compliance are important in the ability of cells to migrate and therefore needs to be considered when designing experiments. "In fact, it is pretty clear that 3D matrices are different in different tissues and that you will need to match, in terms of stiffness and composition, your particular tissue."

To help understand cells in 3D environments, researchers have made up a variety of home-brewed matrices, and commercial companies now also supply matrices for cell culture studies. These matrices can be either synthetic or derived from animal products, allowing researchers to tailor an extracellular matrix to their needs.



Human umbilical vein endothelial cells (HUVEC) cultured in the 3D PuraMatrix Synthetic Nanofiber Scaffolds and stained with rhodamine-phalloidin (red) and DAPI (blue). (Courtesy of Alisha Sieminski, Olin College.)

PuraMatrix Synthetic Nanofiber Scaffolds from 3DM Inc. are amino acid–based hydrogel scaffolds suitable for 3D cell culture. Developed by Shuguang Zhang from the Massachusetts Institute of Technology, PuraMatrix is based on 16-mer oligopeptides that self-assemble into nanofibers that are 10–50 nm in diameter, to form a synthetic extracellular matrix at physiological salt concentrations. PuraMatrix can mimic many aspects of the 3D cellular environment, without the need for animal materials. "PuraMatrix is customizable. You can change the biophysical or biochemical characteristics of the material by either altering the concentration of the matrix, which alters stiffness and porosity, or by adding in components such as extracellular matrix proteins or growth factors," says Lisa Spirio, director of research and development at 3DM. Additionally, this matrix is transparent, allowing visualization of cell movement. "Some of the harder scaffolds or membrane filters are opaque, which is a challenge for microscopy and analysis," says Zen Chu, president of 3DM.

Another matrix that has found wide use in the scientific community is Matrigel from BD Biosciences. Matrigel is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells. At room temperature, Matrigel polymerizes into a three dimensional structure that is useful for both cell culture and studying

BOX 1 A DEADLY DIFFERENCE

"Migration and invasion are related, but they are not the same," says Mina Bissell from Lawrence Berkley National Laboratory. "You cannot invade without migrating, but you could migrate without invasion." The two processes also can have dramatically different outcomes.

Bissell's recent work focuses on characterizing the invasion properties of malignant cells in three dimensions, properties that allow these cells to break through the basement membrane and enter the bloodstream. This process relies on proteins such as matrix metalloproteases to 'chew' through the membrane. "We needed a new assay for invasion, that includes migration, to explain how normal cells invade but respect tissue boundaries, whereas tumor cells invade and destroy tissue structure," says Bissell.

Celeste Nelson, a bioengineering fellow in Bissell's laboratory, developed a quantitative assay using micropatterns of cells sandwiched between two layers of collagen. The model, recently described in Science[4], is based on the process involved when the mammary gland invades the fat pad during puberty, a normally very well controlled process. Once cells become malignant, in contrast, this tight spatial control is lost, and the cells invade beyond the fat pad. This gave Bissell an idea for a way to study invasion and tumor-cell metastasis. "If we understood the invasion and control of the cells into the fat pad, then we could find out how it is that tumor cells hijack this pathway."

Using their model, Nelson, Bissell and her group showed that the tissue architecture surrounding the cell has critical effects on mammary cell branching into the mammary gland structure, and how these cells migrate and invade collagen.

Bissell insists that the difference between invasion and migration needs to be understood and made clear. As Bissell points out, "if you have a wound in your skin, the fibroblasts migrate but they don't invade, because if they are able to invade, sooner or later, you will end up with a tumor!"

cellular processes in three dimensions, including cell migration. The rigidity of the matrix formed by Matrigel can be varied, allowing user-defined stiffness to more closely resemble natural microenvironments.

Trevigen supplies its Cultrex products, three matrix formulations that vary according to the concentration of basement membrane. Derived from EHS mouse sarcoma cells, like Matrigel, the Cultrex matrices can be used for tumorgenicity, migration, growth, differentiation and neurite-outgrowth assays. The ECMatrix from Millipore, which is used in the company's cell migration kits, is also a reconstituted basement membrane matrix of proteins derived from EHS mouse tumor cells.

An image worth a thousand words

Another important challenge in studying cell migration is to understand what happens in a cell when it moves. Imaging the cytoskeleton proteins, for example, during cell migration on 2D surfaces requires some form of labeling in addition to specialized microscopy such as confocal and total internal reflection fluorescence (TIRF) systems.

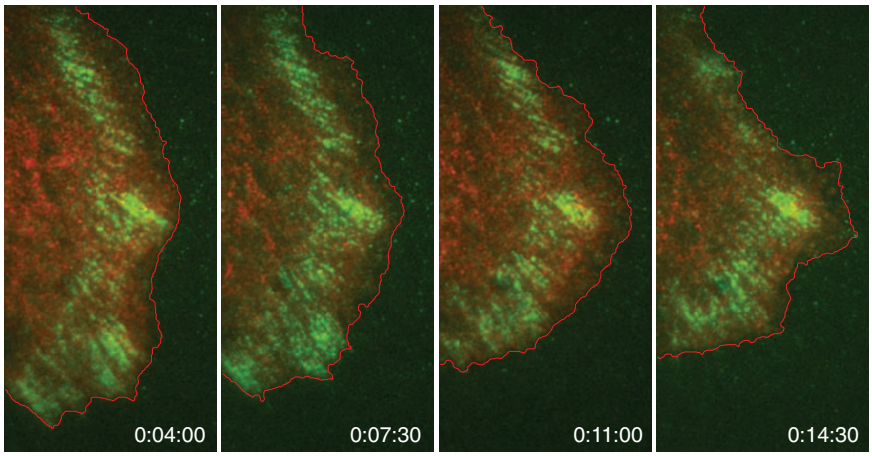
TIRF seems to be the microscopy method of choice for studying interactions and structures at the edges of cells. The great advantage of TIRF for this purpose is that the illumination does not penetrate very deep into the cell. Therefore fluorophores inside the cell do not contribute to the signal, and the background tends to be very low. Overall, using TIRF "you get good discrimination of things that are at the membrane surfaces," says Jennifer Peters from Nikon Instruments, which offers the TIRF-2 illuminator system.

Among the most popular combination of labeling method and microscopy system is speckle microscopy. This visualization method was established by Clare Waterman-Storer of the Scripps Research Institute, with the help of a small dose of serendipity, as her Scripps colleague Gaudenz Danuser recalls. "Clare wanted to label actin in a continuous mode, and she injected a pool of labeled monomers, which was really, really poorly labeled, so only a few of these monomers had a label at all," says Danuser. When Waterman-Storer cranked up the camera amplification, a few labeled molecules could be seen. But interestingly, these labeled molecules showed movement and could be tracked. This gave Danuser and Waterman-Storer the idea to use speckle microscopy to examine molecule movement in cells.

Although they could tell the speckles moved, Danuser says it took over six years to develop the necessary computational algorithms to quantitatively track their movements. "We have developed over the past six years a comprehensive mathematical model that allows you to take the stochastic fluctuations of the speckles, track their motion, interpret the motion, enter mechanical events in the cell and also measure the rates of assembly and disassembly of your structure, *in situ*."

Recently, Danuser and Waterman-Storer applied speckle microscopy to better

Nikon provides TIRF-based imaging systems. (Courtesy of Nikon Instruments.)



Time lapse speckle microscopy images of actin (red) and vinculin (green). (Courtesy of the Waterman-Storer lab.)

understand the proteins that interact with actin during cell movement[3]. By labeling proteins with GFP, labeling actin monomer with a different dye and using TIRF microscopy and software they had developed, they could study interactions and movements of these molecules during cell migration.

So it turns out that speckle microscopy is an excellent tool for studying the molecules involved in cell migration, but the technique does have drawbacks. "The limitation of speckle microscopy is that we are limited to processes that happen at a time scale of half a second or slower, while methods like fluorescent correlation spectrometry can detect binding of molecules at a millisecond time scale," says Danuser. The cause of this is mainly technical as Danuser explains. "Signals are extremely low and the [charge-coupled device (CCD)] camera needs a certain amount of time to integrate enough photons."

Cell biologists have come long way in the study of cell migration. With advances being made on many fronts, it is only a matter of time until cell migration really comes into focus.

1. Boyden, S. *J. Exp. Med.* **115**, 453–466 (1962).
2. Pankov, R. *et al. J. Cell. Biol.* **170**, 739–802 (2005).
3. Hu, K. *et al. Science* **315**, 111–115 (2007).
4. Nelson, C.M. *Science* **314**, 298–300 (2006).

Nathan Blow is technology editor for *Nature* and *Nature Methods* (n.blow@boston.nature.com).

SUPPLIERS GUIDE: COMPANIES OFFERING SYSTEMS AND REAGENTS TO STUDY CELL MIGRATION

Company	Web address
3DM	http://www.puramatrix.com
Accuri Cytometers	http://www.accuricytometers.com
Agilent	http://www.agilent.com
Andor Technology	http://www.andor-tech.com
Apogee Instruments	http://www.ccd.com
Applied BioPhysics	http://www.biophysics.com
Applied Cytometry	http://www.appliedcytometry.com
Applied Scientific	http://www.asiimaging.com
BectonDickinson	http://www.bd.com
Beckman Coulter	http://www.beckmancoulter.com
BioCat GmbH	http://www.biocat.de
Biomedical Photometrics	http://www.genefocus.com
Bio-Rad	http://www.bio-rad.com
Cambio	http://www.cambio.co.uk
Cell Biolabs Inc.	http://www.cellbiolabs.com
Chroma Technology	http://www.chroma.com
Clontech	http://www.clontech.com
EMD Biosciences	http://www.emdbiosciences.com
Eppendorf	http://www.eppendorf.com
Fujifilm	http://www.fujimed.com
Guava Technologies	http://www.guavatechnologies.com
Harvard Apparatus	http://www.harvardapparatus.com
Inbiolabs	http://www.inbiolabs.com
Innovative Cell Technologies	http://www.innovativecelltech.com
Invitrogen	http://www.invitrogen.com
Ibidi	http://www.ibidi.com
Kodak	http://www.kodak.com
La Vision Biotec	http://www.lavisionbiotec.com
Leica Microsystems	http://www.leicamicrosystems.com
Lightools	http://www.lightools.com
Micro Video Instruments	http://www.mvi-inc.com
Millipore	http://www.millipore.com
Molecular Devices	http://www.moleculardevices.com
MP Biomedicals	http://www.mpbio.com
Nanoptek	http://www.nanoptek.com
Neuroprobe	http://www.neuroprobe.com
Nikon Instruments	http://www.nikon-instruments.jp/eng/
Olympus	http://www.olympus.de/microscopy
Omega Optical	http://www.omegafilters.com
Perkin Elmer Life Sciences	http://las.perkinelmer.com
Pierce Biotechnology	http://www.piercenet.com
Princeton Instruments	http://www.princetoninstruments.com
Qimaging	http://www.qimaging.com
Trevigen	http://www.trevigen.com
Sigma Aldrich	http://sigmaaldrich.com
Synthecon	http://www.sythecon.com
Zeiss	http://www.zeiss.com