May mechanobiology work forcefully for you

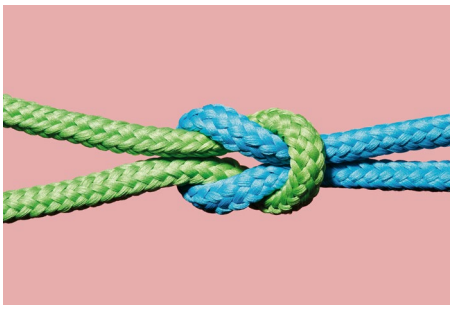
Mechanical measurements would be easy if cells were homogeneous objects — they're not.

Vivien Marx

Cells are shape-shifters; they stretch, compress and contort to fulfil functions in health and disease. During cytokinesis, an indent becomes a constricted furrow and eventually two daughter cells arise from one parent cell. In metastasis, tumor cells set out and squeeze through tight spaces in the extracellular matrix. As muscles move, nerves stretch and compress. Mechanobiology offers insight about such mechanics through numerous non-destructive techniques[1–3]. Yet existing methods are hard for non-experts to use well. Decades of research efforts have not led to a single routine application of cell mechanics in biological research or clinical labs, says Jochen Guck from the Max Planck Institute for the Science of Light[4]. It's still slow-going to democratize mechanobiology, says Emory University researcher Khalid Salaita. Guck and Salaita represent two sides of the mechanobiological coin. Some, like Guck, prod cells to infer their mechanical properties, whereas labs like Salaita's develop tools to measure the forces the cell exerts. "Those two things go hand in hand," says Salaita. Like genomic and proteomic changes, mechanical changes are information that cells harness and process. But mechano-information faces some prejudice.

Might biology have been "swept away" by the successes of molecular biology and lost interest in "seemingly trivial physical properties of cells?" asks Guck. He sees many biologists are keen to 'think physics', but what gets in the way are issues such as a lack of standards when using even established methods, including micropipette aspiration (MPA), nano-indentation with atomic force microscopy (AFM), and approaches with tracer particles and the application of magnetic or optical forces. Emerging approaches include the use of thermally excited sound waves in Brillouin microscopy.

"Essentially all methods for measuring cellular mechanical properties involve poking or squeezing the cell in some way," says MIT cancer researcher Scott Manalis. "I don't have a general favorite," he says. "The most attractive approach will very much depend on the question being asked."



Mechanobiological measurements are non-destructive ways to test cellular muscle. The methods are yet to be in routine use. Credit: R. Drury/Digital Vision/Getty

When applying one or several approaches in mechanobiology, says Douglas Robinson, a Johns Hopkins University cancer researcher who develops and uses mechanobiological methods, labs commonly stick to a single method as the one they are used to and, often, it's one they believe to be superior to others. He wishes scientists would kick both habits. "The methods all have niches to fill, and it does not really help the field to think a particular method is superior to others," he says.

Choices, choices

Complex, dynamic and heterogeneous all describe the way cells change their behaviors and properties when perturbed or stimulated, says Ning Wang of the University of Illinois at Urbana-Champaign. When making mechanobiological measurements, labs have much to heed: a probe's force, the amplitude and directionality of deformation, whether it's a shearing or compressive force, and the measurement duration. The measured mechanical properties of cells depend on the length and time-scale of the poking or squeezing, and the area over which the cell is deformed, which differ with each method, says Manalis. A mechanobiological property might be measured with an AFM tip or with tiny plates that apply shearing forces. When a lab is not well-versed in this area, he says, awareness of three parameters — length, time and area — can help reduce measurement variability.

Manalis and his team explore biophysical properties of cancer cells circulating in the bloodstream to find what could uniquely distinguish them from healthy blood cells. Those traits might be an improvement over cell-sorting using surface markers, he says. The team has developed microfluidic tools to assess the mechanobiology of cancer cells. Microfluidics has higher throughput than other methods; throughput is another issue Guck says is holding back mechanobiology.

Mechanobiology lets researchers track the pathways connecting force to function, says Robinson. The cortex, which is the 'skin' covering cells, is replete with mechanosensors. That's true for the plasma membrane, its sugary coating (the glycocalyx) and the cytoskeletal network. Each layer's mechanosensors recognize different magnitudes of force and each one bears on the readings of others.

His lab uses MPA and laser-based particle tracking microrheology (LTM), and they have compared their results to AFM data. Lately, the team has been using microfluidics approaches such as real-time-deformability cytometry. "Each approach has its strengths and weaknesses, but we have found that major observations are reproducible across methods," says Robinson. The team has measured a cell's elastic modulus using MPA and the values agree closely with values measured by other labs using AFM on the same cell types.

Reproducibility issues persist in the literature, such that labs face a flurry of sometimes contradictory cell-mechanics-oriented measurements, says Tufts University researcher Igor Sokolov. It led him, Wang, and colleagues at 11 institutions in the United States, France and Germany,

Many biologists are keen to 'think physics', says Jochen Guck. But cell mechanical measurement should be higher throughput. Credit: MPI for the Science of Light

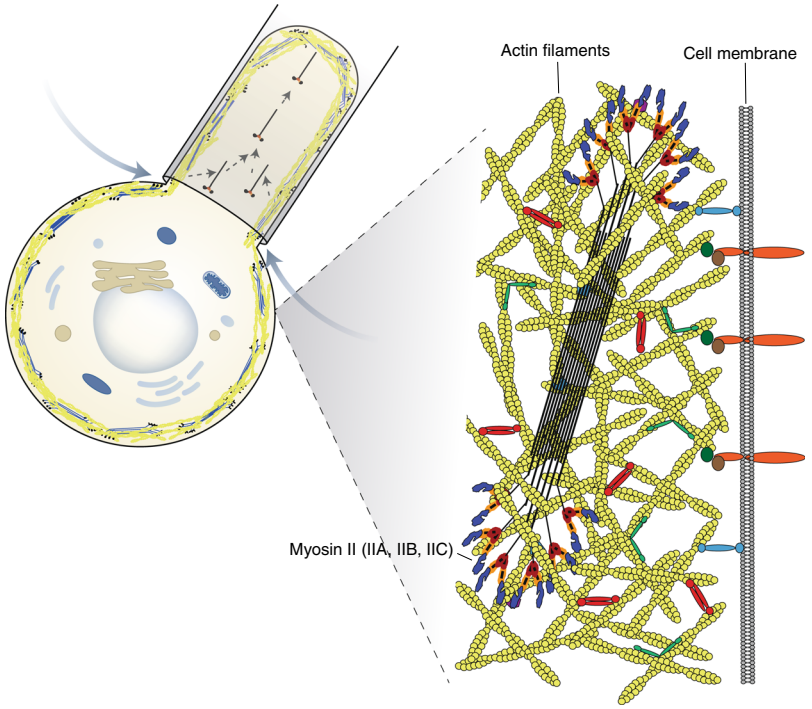
to compare widely used methods, including AFM, magnetic twisting cytometry, parallel plate rheology and optical stretching, to measure the mechanobiology of MCF-7 human breast cancer cells all prepared the same way[5].

Among their results, they found that elasticity measurements of these cells can vary up to 1,000-fold and viscosity values can vary 100-fold. Measurement confounders include the probe's geometry and the probed cell location. MCF-7 cell elasticity measured by optical stretching (OS) was more than two orders of magnitude below the elasticity measured by AFM or magnetic twisting cytometry. One factor is that OS is generally applied to freely flowing cells such as circulating tumor cells, which are perhaps more biomechanically 'relaxed' than when measured with the techniques that involve cells adhered to a substrate.

Differing viscoelasticity measurements can arise when different parts of the cell are measured and because cells are inherently heterogeneous, says Sokolov. Another, more hidden, issue: "Virtually all techniques are based on the approximation of the cell as a homogeneous and isotropic object," he says. But cells are far from homogeneous. One of the many issues the team point out in their comparison is that many biological materials are quite poroelastic: under compression or stretching, fluid leaves cells or tissues and the stresses relax. This dynamic tends to not affect quick rheological measurements but can affect the consistency of indenting techniques.

When using large AFM probes, says Sokolov, labs can achieve results comparable to whole-cell measurements with parallel-plate rheometry, which applies shearing forces to cells. But to see this measurement-level agreement between AFM and rheometry, labs need to use a model that takes the cell's pericellular layer into account. It's softer than the cell body, thus the AFM viscoelasticity measurements will be smaller than the rheometry ones. Measurements made assuming isotropy are not useless, they capture relative change of mechanical properties. But, in Sokolov's view, labs are advised to examine their assumptions.

Sokolov was glad that the group compared multiple methods to study cell mechanics. Given the large number of techniques and scientific questions, "everyone may have different take-home conclusions, specific to each technique." It reminded him of some caveats to AFM, his favorite mechanobiological method. For example, using sharp AFM probes leads to an overstretched cell response. It's a measurement with high spatial resolution. "However, the entire result will be an artifact of overstretching," he says.



Micropipette aspiration is a way to probe how cells react to mechanical forces and to study cytoskeletal proteins. Studying non-muscle myosin II might reveal a way to thwart metastasis. Credit: D. Robinson, Johns Hopkins University, E. Dewalt/Springer Nature

He and his team study the physics of cancer and aging, and the methods comparison has led him to want to explore how to capture more cancer cell features. He sees support for his "brush model," which includes measuring mechanical properties of the cell body and its pericellular coat. Among his ongoing projects is to apply this approach to aging cells. The comparative study is by no means "the end of the story." Among the new methods to watch is Brillouin microscopy, in which light in the gigahertz range is used to probe a material's viscoelasticity.

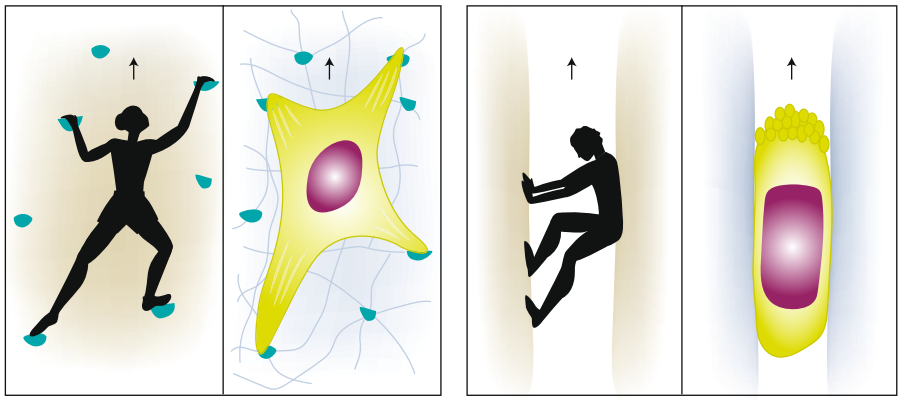
As the authors discussing this technique note[6], to use Brillouin microscopy for precise measurements, labs need familiarity with the material they are measuring, and should know its refractive index and density. It's an "ongoing quest" to combine this technique with other approaches such as tomographic phase microscopy, which measures refractive index and density in situ. The team believes Brillouin microscopy offers "unique abilities" to measure spatial and temporal modulation of mechanical properties within intact cells and tissues.

Cancer mechanics

Tissue-level mechanics play roles in pathology, be it hardening of the arteries in arteriosclerosis, or the weakening of blood vessels that lead to an aneurysm, says Wang. And yet, he says "there is a huge gap between the tissue-level and cell-level understanding of how these occur and what really is the underlying mechanism." There are also plenty of unknowns about the forces that shape nuclear events. Using 3D magnetic twisting cytometry, Wang and his team applied shearing forces to Chinese hamster ovary cells that carried a green fluorescent protein (GFP)-labeled housekeeping gene. They found that the stretching induced gene expression. Perhaps stretching by around 20 to 100 nanometers decondenses chromatin, giving access to transcriptional machinery.

By studying how mechanics drives biological changes, labs can get many layers of cell biological understanding, he says. With cancer cells, "mechanics plays a huge role in metastasis and malignancy." Many tumors lay down extra-cellular matrix (ECM) that is more internally cross-linked than is typical in tissue, and cancer cells are adept at moving through it, says Andrew Holle, a postdoctoral fellow in the lab of Joachim Spatz at the Max Planck Institute for Medical Research.

As the cells burrow through the ECM, they rearrange their cytoskeleton. A cancer cell, typically around 15–20 micrometers (μm) in diameter, squishes itself to a



Cancer cells move with attachment and traction force (left). In a tight space, they switch to moving like amoebas. That's akin to rock-climbers who switch from hand-holds to 'chimneying' (right). Credit: A. Holle, MPI for Medical Research, E. Dewalt/Springer Nature

width of three µm. To study cancer cell invasion, labs use the Boyden chamber assay, in which cells follow chemoattractants through a barrier with different-sized pores. But it's hard to use with single cells, says Holle. He and colleagues built a narrow microfluidic channel that mimics the crawl space cancer cells use[7]. As the cells travel through, he captured cytoskeletal changes with laser scanning confocal microscopy. It's a view unlike studying cancer cells in a 96-well plate, says Holle. "Confinement mechanobiology" captures the forces that shape cancer cell behavior — its "mechanoscape."

On a microscope cover slip, cells advance with attachment and traction force. In a tight space, they switch to amoeboid progression for which they do not attach to a substrate. They generate internal pressure gradients: a squeeze in the back leads to forward flow. This is like rock-climbers who switch from hand-holds to stemming themselves against walls. "In the rock-climbing world, it's called chimneying," he says. When cancer cells sense tightness around them "they stop climbing and they start chimneying."

Mechanobiological findings could help labs find ways to hinder this behavioral transition, says Holle, perhaps by targeting the distinct mechanosensitive signaling pathways. Among his next projects, he plans to build microfluidics channels in softer materials and he is working on a traction force microscopy (TFM) system with beads embedded into the channel wall. One could then measure the walls deforming as the cells exert pressure, he says, and map "stiffness-sensitive cell behavior."

In TFM, cells are seeded on an extracellular matrix decorated with fluorescent beads. As cells move, they can nudge the beads. With imaging, changes in bead distribution are used to calculate exerted forces. In such set-ups, says Holle, one could closely watch which manipulation stops cells from exerting the hydrostatic pressure gradient they need to keep moving. Lessons learned from cancer cell invasion, says Holle, can be applied to studying stem cells in different mechanoscapes.

Biologists decipher the relationships between the genome and the proteome, the behavior of cells and tissues, and up the biological levels of hierarchy, says Robinson, but all of these events occur in the context of a physical environment. "In fact, one could say that the first cells had to cope with changing nutrient supplies, temperature, tonicity, pH and mechanical inputs before they had to deal with genetically encoded signaling factors," he says. "Perhaps then, it is quite natural for mechanical inputs to be integrated with these other pathways that we traditionally think of as controlling growth, migration and cellular decision making. "

Salaita says that relative to the genome and proteome, mechanics can seem "a black box." But mechanical changes can be just as profound as, say, enzyme activity. It's another form of information that cells have evolved to harness.

Both in cancer and in development, cells frequently change their "mechanical state," says Robinson. Tumor cells can be more or less deformable. Labs find that with shifting mechanical states comes a shifting ability, compared to healthy cells, to sense mechanical and force inputs. Perhaps this altered mechanical sensitivity drives cancer cells to be more adaptive to a changing physical landscape. This shift might contribute to their changed behavior as they disseminate and metastasize to a new site in the body.

Overcoming prejudice

Non-muscle myosin II is a protein that binds actin, is embedded in the cell's cytoskeleton, is found throughout the body and is well-known for the contractile forces it generates in cytokinesis. Beyond contractility, says Robinson, myosin II contributes to at least seven other features, for example the cell's viscoelasticity, cortical tension and mechano-responsiveness, and it plays a role in growth and signaling pathways. Oversimplifying myosin's role as "contractility-only, one can easily miss how the system is actually behaving and responding," he says.

Cellular shape-shifting behavior is the result of internal and external influences, such as compression, shear or tension. The behavior involves the 'mechanobiome', a set of macromolecules, such as cytoskeletal proteins, that sense and accumulate in response to stress and that lend cells the ability to react to the stimuli. When cancer cells move, they can look a little like amoebas as they protrude and contract. In amoebas, mammalian cancer cells and dividing eukaryotic cells, this movement or protrusion and contraction is partly enabled by motor proteins, mainly non-muscle myosin II.

Perhaps the cytoskeleton can be a target for anti-cancer strategies[8]; but there are mechanobiological prejudices. Many ideas about how the cytoskeleton and myosin II work originated from insight about muscle and its contractile system, says Robinson. Muscle is plentiful in the body, highly organized and it lends itself to study. That initial research led many labs to believe that targeting this protein in cancer would affect muscle, such as body muscle or cardiac muscles. But non-muscle myosin is not identical to those proteins, he says.

Some cancer researchers are concerned, given that myosin II is the essential force producer in cytokinesis, says Robinson, that inhibiting myosin II might induce cytokinetic failure and lead to major side effects, such as anemia or additional cancers. But, he says, people have three types of non-muscle myosin II — myosin IIA, IIB and IIC — that his lab and others are characterizing. In cancer research and other fields, labs are also identifying distinct roles for each paralog of non-muscle myosin, which set them apart from the usual activity of this protein class in cytokinesis and cell motility. He and others have found that hyper-activating one protein, myosin IIC, may have anti-cancer activity without inducing cell division failure. "Not every strategy has to be focused on inhibition of a target enzyme — sometimes activation may be the way to go," he says.

In his lab, the team uses MPA to apply physiological levels of mechanical stress across a few tens of μm² of cell surface area, such as a cleavage furrow in a dividing cell. They look at how proteins redistribute in response to the stress and create a cell-scale equivalent of an optical trap to monitor how a single molecular motor, like myosin, generates force as well as how it responds to it. With a collaborator, they studied myoblast fusion using AFM and "again achieved similar results and identical interpretations between the methods," he says. Labs should choose a mechanobiological method based on the question they are asking, says Robinson. They can include, for example, the desired measurement time-scale, the amount of cell surface area under study and which physical parameters they seek to measure. To study a mechanical process unfolding over seconds to minutes, an approach that makes mechanical measurements on a millisecond-scale may not be needed. "The faster time-scale measurement might be nice for understanding the system, but it might not give you the necessary information for answering the question you are asking."

Tug and bend

Cells use mechanics to organize structure and architecture but also to increase the fidelity of information transfer, says Salaita. With a 12–19 piconewton tug, the T-cell receptor 'samples' peptides of the class I major histocompatibility complex displayed on the surface of other cells. This specificity filtering, he says, assures that a T-cell receptor can "distinguish friend from foe" and determine that what is being bound is "the real deal signal."

Thermodynamics and kinetics govern these interactions, such as how tight a binding interaction is. The tug between a T-cell receptor and a ligand happens in "a blink of an eye," a few milliseconds, says Salaita. To better map these forces, his lab built DNA-based tension probes[9]: stem-loop DNA hairpins that unfold when a certain force acts on them. Their unraveling triggers a fluorescent signal. With these probes, the team can track the history of many individual T-cell receptor-interactions. They are also looking at affixing the sensors on soft substrates to better mimic the biological context.

With every heartbeat, the nerves that innervate the aortic arch stretch and compress to regulate heart muscle contraction. Miriam Godman, from

"I'd love to be able to see that," says Miriam Goodman about a sensor that might help reveal how neurons stretch and compress over and over.

Credit: Stanford Medicine

Stanford University School of Medicine, seeks to understand how neurons can undergo these repeated cycles of stretching and compression without being destroyed, as part of her studies on mechanical neuroprotection and touch sensation. The team uses *Caenorhabditis elegans* to explore this resilience to persistent mechanical stress. They label neurons with GFP, and observe changes under the microscope as the worm moves.

The lab looks at axons in *C. elegans*, which as in humans, contain proteins such as spectrin and tau as well as microtubules. Instead of shrinking smoothly under compression, the axon can buckle like an accordion. Add another defect to the tau protein and the axon coils like a phone cord. These observations feed into the lab's models that capture the neuron's mechanical abilities to compress well without breaking or fracturing.

An intact spectrin network appears to confer protection from mechanical stresses, she says. "Whether it's elastic or viscoelastic, this helps us to develop mechanical models," she says, which may be molecular-level models involving cytoskeletal proteins, or force-oriented models about materials' properties.

To make in vivo measurements of the effects of mechanical stress, her team integrates a genetically encoded optical sensor into spectrin. She wishes she had a sensor with a wider dynamic range and greater sensitivity. She would like to understand how neurons sense mechanical changes. "We believe that involves a change in the local tension in the neuron," she says. "I'd love to be able to see that."

For Goodman's needs, Salaita says he doesn't have a sensor ready to go but he might be able to help if the cell surface has a receptor such that his probes could measure force transmitted through a ligand-receptor interaction. Speaking more generally, Salaita says genetically encoded tension sensors have issues, such as "miserable signal," which risk misinterpretation. The sensors are also large, which might not agree with a cell.

In collaboration with a materials scientist, Goodman's lab is developing a sensor that includes rare-earth nanoparticles, which emit light that changes color based on pressure. The particles absorb infrared light and emit visible red and green light. The red/green ratio changes when the particles are under pressure. Worms eat these particles and "are no worse for the wear," she says.

What's next

"I really think that the field has a lot of potential and requires some consolidation and push to deliver on promises that have been casually made in the past," says Guck. Robinson wishes he could do a high-throughput MPA-style experiment, which would capture several mechanical parameters not easily extracted with most other methods. He has high hopes for emerging microfluidics approaches and looks forward to linking mechanical manipulation to other readouts, such as single-cell multi-omics assays. After all, labs increasingly find that proteins and subcellular systems not thought to play mechanical roles actually do so.

Robinson hopes that researchers in the mechanobiology field can start recognizing that the cell is "an intact, well-integrated machine", and more than a system of linear pathways with little cross-talk. The concept of 'mechanobiome' starts capturing this deeper view of the cell as an integrated machine, he says. "We like to think that the cell is more of a gizmo where the parts click together, creating the functional system." ❐

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