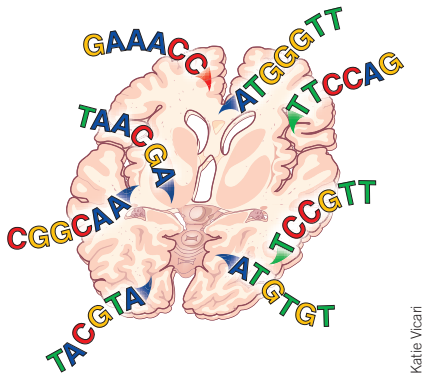
In situ sequencing

Biologists need methods for sequencing genetic material directly from intact tissues.

Technology has expanded the scope of sequencing to encompass entire genomes or transcriptomes, and pushed sequencing resolution down to the level of single cells. What has been missing is spatial context—the ability to 'read' sequence directly from intact tissue. *In situ* sequencing could provide a deeper understanding of the relationship between a cell's genotype or gene expression program and its morphology and local environment. Although it is too early to predict the ultimate form and potential of such a technology, some initial steps towards *in situ* sequencing have already been taken.

Methods such as *in situ* hybridization have long been used to pinpoint sequences in intact cells and tissues, but they are limited by the need to know the target sequence. On the sequencing side, many technologies use a light-based readout, suggesting that they could be compatible with imaging in intact tissues. But amplification and sequencing reactions require a special substrate or need to be separated from each other in an emulsion.



In situ sequencing maps sequence data directly onto morphology.

An alternative strategy is rolling circle amplification, which has been used to boost signal for *in situ* genotyping in cells. The method relies on a 'padlock' probe that hybridizes on either side of a target sequence to form a circular template that can be copied repeatedly as a long string. Because the product is tethered to the template, it provides reliable localization and is amenable to *in situ* sequencing by successive rounds of ligation-based oligonucleotide probe incorporation (*Nat. Methods* **10**, 857–860, 2013).

This form of *in situ* sequencing was used to interrogate four-base sequence variation in transcripts directly in tissue sections; however, it is a targeted approach that still requires knowledge of flanking sequence. It will be important to increase the number of transcripts and length of sequence that can be interrogated in a single cell, as well as balance image resolution with the ability to image tissue-wide. Image registration methods are a key component of this scaling up, and tools to quantify associations with morphology will be needed.

The ultimate goal is to sequence many loci—and even a significant fraction of the transcriptome or genome—but this will require a breakthrough in solving the signal-density problem: cells contain too much information to be imaged in such a small space. We look forward to further steps that join sequencing with biological context.

Tal Nawy

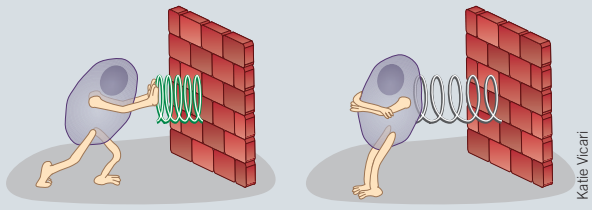
Tiny tools to measure force

Imaging-based sensors are used to map mechanical forces exerted by cells.

Cells both sense and exert mechanical force. *In vivo*, force is likely to be important, in many and varied ways, in the development and maintenance of tissue function. Toward understanding this as-yet relatively unexplored biology, methods to map cellular forces are of great interest.

Adhesion molecules are major players in the ways cells push and pull on each other and on the extracellular matrix. Imaging-based methods with designed sensors are now being used to measure force exerted by these molecules.

Earlier versions of such tools were variations on the theme of force-sensitive fluorescence resonance energy transfer (FRET) sensors. For instance, researchers placed a sequence encoding a force-sensitive domain from a spider silk protein between genes encoding two fluorescent proteins that undergo FRET and then expressed the entire cassette spliced into the sequence encoding vinculin (*Nature* **466**, 263–266, 2010). Upon calibration, the FRET signal provided a measure of the forces across vinculin at the cell surface. Another group constructed a slightly different tool: a ligand of interest was linked via a tension-sensitive polymer to the extracellular substrate; tension on the cognate cell-surface receptor could then be measured by monitoring the extent of quenching of a conjugated fluorophore by quencher at the substrate (*Nat. Methods* **9**, 64–67, 2011). Force sensors based on imaging can be combined with other high-resolution imaging to map forces to cellular structure.



Tools to measure biological forces.

In a recent twist, researchers dispensed with the fluorescence readout altogether. They constructed cell tethers by conjugating a ligand of interest to DNA, exploiting its well-studied mechanical properties to tune the tether strength (i.e., the force at which it ruptures). By simply monitoring whether a cell can adhere to a surface presenting the ligand with a tether of a given strength, the researchers measured the force exerted at a single receptor-ligand pair (*Science* **340**, 991–994, 2013).

Also very recently, cellular force measurements have moved *in vivo*. Using tiny ligand-conjugated oil droplets with known mechanical properties, researchers measured, via imaging, the forces exerted by cells in living tissues (*Nat. Methods* doi:10.1038/nmeth.2761).

Microscale and nanoscale imaging tools to monitor biological forces will undoubtedly continue to develop. Unanticipated biological vistas are likely to open up as a result.

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