We present methods and areas worth watching in the coming years.

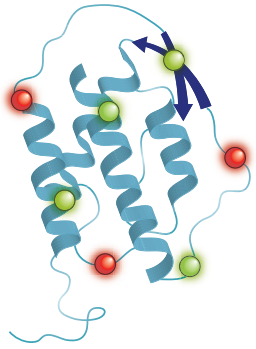
Structure via super-resolution

Fluorescence nanoscopy is extending its reach into structural biology.

It has been some years since super-resolution imaging first contributed to understanding macromolecular complex organization, a subject classically within structural biology. Borrowing particle-averaging methods from cryo-electron microscopy, researchers used single-molecule localization microscopy (SMLM) to map the locations of fluorophore-labeled proteins to the structure of the nuclear pore complex (NPC) (*Science* **341**, 655–658, 2013).

This work made use of the known symmetry of the pore. Although it was not strictly needed in that case, there remain few if any fluorescence-based structures that do not rely on prior knowledge. Several developments now raise the intriguing possibility that fluorescence may be used increasingly for structural studies.

First, super-resolution approaches are improving in resolution, down to the molecular scale. In the MinFlux method from the group of Stefan Hell, an intensity minimum is used for fluorophore localization, achieving resolution on the low-nanometer scale (*Science* **355**, 606–612, 2017).The group of Vahid Sandogdhar reported Angstrom resolution with SMLM on proteins at cryogenic temperatures (*Nat. Methods* **14**, 141–144, 2017).



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Fluorescence can illuminate protein structure.

Second, CRISPR-based gene editing is making it more possible to fluorescently label all endogenous copies of a protein. Together with brighter probes and smaller affinity reagents, this will help achieve the more complete labeling needed for fluorescence-based structural mapping.

Improved analytical methods to reconstruct structure from fluorescence data will probably also be needed. Methods that do not require structural templates and that are explicitly designed for fluorescence data may prove beneficial.

We predict that super-resolution fluorescence microscopy will continue to brighten and elucidate biological structure, even down to the molecular scale.

Natalie de Souza

Spatial transcriptomics

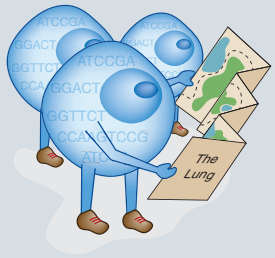
It will soon be commonplace to localize gene expression in tissues.

Space has been a formidable, if not final, frontier in gene expression. But that frontier is eroding as methods developers put transcripts onto various tissue maps. The variety and creativity of these approaches makes this a fascinating area to watch.

Spatial gene expression is critical for understanding cell identity and function in the tissue context. The popularity of model organism expression atlases and the Allen Institute for Brain Science's mouse and human brain atlases attest to the power of spatial gene expression. However, existing atlases were largely created using reporter genes or *in situ* hybridization—low throughput methods that make it painstaking to construct references and that limit the ability to assess multiple samples.

A bevy of recent tools offer greater flexibility and scale; highly multiplexed fluorescence *in situ* hybridization, *in situ* sequencing of imaged sections or three-dimensional tissues, and algorithmic methods that project gene expression onto limited existing spatial information, among others, offer very different solutions.

There is good reason to expect the innovation to continue. Many methods are currently being optimized, and each has unique strengths and limitations with respect to ease, speed, spatial resolution, quantitative accuracy, and the number of genes than can be profiled. Recent initiatives, including the Human BioMolecular Atlas Program of the US National Institutes of Health and the Human Cell Atlas, a major international undertaking, have strong technological components and define spatial mapping as an explicit goal.



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There are many ways to map cellular transcriptomes.

How to integrate gene expression data into a spatial coordinate system, and how to visualize and compare these kinds of data sets are difficult open questions for the computational community, which has had little data to work with so far. We anticipate that improvements in data generation and analysis will bring spatial transcriptomics into wider practice and will be transformative for biology.

Tal Nawy