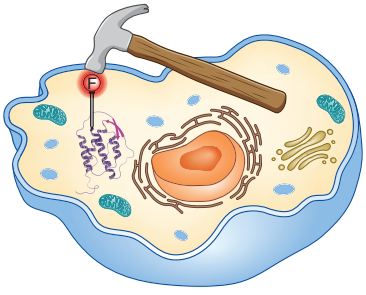
We present a selection of methods and areas of methodological development worth watching in the coming years.

Protein labeling in cells

Better protein-labeling strategies will improve imaging.

Fluorescent chemical dyes are relatively small, can have good photophysical properties and span the color spectrum, making them attractive alternatives to fluorescent proteins for labeling proteins. Researchers are actively developing tools for labeling proteins with dyes in living cells.

To be useful for most applications, methods for incorporating dyes into proteins must allow specific labeling of proteins. On this front, several tools already exist, such as SNAP and Halo tags, FlAsH and ReAsH, and hexahistidine tags. These tools involve genetic tagging of target proteins with a small protein or peptide that can specifically bind appropriate dyes. An alternative method involves the incorporation of non-natural amino acids into proteins during translation; here the non-natural amino acids either are fluorescent or can be made fluorescent via click chemistry.



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Improving tools for protein labeling.

Although these methods are gaining popularity, they suffer from issues such as limited utility in multiplexed imaging, low labeling efficiency and the number and quality of dyes that can cross the membrane of a living cell. Such dyes are an active area of research. Future work will undoubtedly improve labeling efficiency, which will enable enhanced quantitative imaging; improve available dyes, which could increase multiplexing and decrease the light dosages necessary for imaging; and reveal entirely new methods for protein tagging.

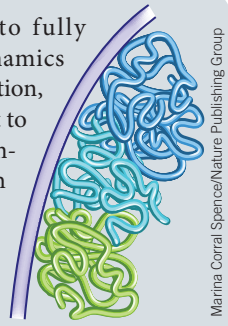
Specific protein labeling will also enhance super-resolution imaging in fixed and living cells. As resolving power approaches tens of nanometers, labeling issues come to the fore. For example, labeling with an antibody adds ~10 nm to the structure of interest, and using a secondary antibody doubles this added size. As an alternative, researchers are developing nanobodies, which are smaller, single-chain antibodies for use in labeling, as well as using the alternative strategies described above.

Rita Strack

Unraveling nuclear architecture

New approaches are needed to see the dynamics of 3D chromatin structure at high resolution and throughput.

The real estate mantra that emphasizes location over all else is also applicable to mammalian genomes. Gene regulation depends on the organization of chromatin and its regulatory elements in three dimensions within the nucleus. Classical methods that capture chromatin conformation (3C) either at select loci or genome-wide have provided a glimpse into the intricate architecture of the genome. But to really understand this 3D organization, how it evolves over time and how it contributes to disease, researchers need new methods. 3C-derived techniques depend on cross-linking of interacting chromatin loci and on the ligation of these loci prior to amplification and sequencing. These steps limit the throughput of the method and favor interactions in *cis* rather than in *trans*. Recent improvements in multiplexing *cis* interactions that involve two sequential capture steps have increased throughput and resolution and allow the relative quantitation of weak and strong interactions, as well as the elucidation of their respective biological roles (*Nat. Methods* **13**, 74–80, 2016).



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Chromosomes interact in *cis* and *trans*.

For scientists to fully appreciate the dynamics of nuclear organization, it will be important to combine population-based models with data on individual cells at high resolution. This will require a multidisciplinary approach bringing together genomics, biophysics and imaging. The 4D Nucleome Program, recently funded by the US National Institutes of Health, is one initiative that seeks to combine such expertise and includes consortia to map genomic structure, apply high-resolution imaging and study the properties of subnuclear compartments. The goals for tool improvement include improved experimental and computational methods for single-cell nucleome resolution and for the visualization of genome folding.

Once the mapping of nuclear architecture becomes a more routine, quantitative procedure, we will be better able to determine and predict the effects of mutations—whether linked to disease or introduced during genome editing—on gene regulation and the mechanisms by which they act. We may even be able to specifically alter genome architecture to bring about desired changes in a cell.

Nicole Rusk