IMAGING

Seeing DNA

DNA and chromatin structures can be visualized *in situ* with electron tomography.

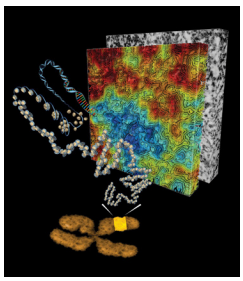
Electron microscopy (EM) is a powerful tool for high-resolution imaging. However, contrast in an electron micrograph relies on the presence of elements with high atomic numbers, making it difficult to see structures within biological tissue without heavy metal staining. While specific labeling for fluorescence microscopy is well established, similar methods for EM are still in their infancy.

The textbook picture of chromatin as 30-nm fibers that fold into 120-nm fibers was based on studies of purified DNA *in vitro* and lysed cells. However, "these structures are based largely on *in vitro* data," recalled Clodagh O'Shea from the Salk Institute in San Diego. A team by O'Shea and Mark Ellisman at the University of California in San Diego has developed a protocol to label and image DNA in cells. Their method, ChromEMT, first selectively stains DNA and then visualizes the DNA with electron tilt tomography. ChromEMT has now revealed chromatin structures *in situ* in human interphase nuclei and mitotic chromosomes.

Building on previous work, the team searched for a DNA-binding fluorescent dye that, when optically excited, would catalyze the polymerization of diaminobenzidine (DAB). DAB can then be stained with OsO_4 and visualized with high contrast in the EM. A challenge with DAB is that it autopolymerizes when excited with ultraviolet light. Thus, it is preferable to initiate the polymerization reaction with a fluorescent molecule excited in the red spectrum.The team found that DRAQ-5 fit the necessary criteria.

With the DNA labeled, "the next trick was really to reconstruct the chromatin," says O'Shea. Even with 70-nm sections, the two-dimensional projections measured with standard EM could not resolve chromatin. The two meters of DNA in each human cell nucleus are assembled by histones and proteins into chromatin structures which appear as a disordered and interwoven mesh. Given the small diameters of structural elements such as naked DNA or chromatin fibers with an average diameter of 13 to 14 nm, each section required an eight-tilt tomographic reconstruction using 800 images.

Once the tomographic reconstructions were possible, the challenge of analyzing the chromatin in each cell remained. "We suddenly realized we couldn't see any of these 30-nm fibers, which was a bit terrifying," O'Shea remembered, referring to previous chromatin studies. The team developed methods to first bin the raw high-resolution data and then segment and trace disordered chromatin fibers of constantly changing diameters weaving throughout up to ten sections. The team found that chromatin fibers fluctuate between 5 and 24 nm in diameter.



A map of the *in situ* chromatin ultrastructure in human mitotic chromosomes (colors represent volume density, increasing from blue to red). From Ou, H.D. *et al*. ChromEMT: visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science* **357**, eaag0025 (2017).

The lack of 30-nm chromatin fibers deviated from the textbook picture, but the team thoroughly validated their findings. First, they took advantage of artifactual microtubule staining, which provided an internal size reference to ensure that there is no sample shrinkage or systematic error in the analysis algorithms. Second, they repeated previous chromatin measurements on hypotonically lysed chicken erythrocytes, which revealed the expected 30-nm chromatin fibers.

With ChromEMT, O'Shea says "the main challenge is going to be how to analyze these kinds of data sets." They are large and complex and, unlike in fluorescence microscopy, much of the cell is partially labeled, giving a holistic view of the cell and "revealing both knowns and unknowns." In the future, O'Shea sees multicolor EM as a path to simultaneously visualize proteins and DNA. Using a fluorescent molecule such as miniSOG (excited at 488 nm) to label the protein, and DRAQ-5 to label the DNA, it will be possible to polymerize different compounds and use different metals for contrast.

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RESEARCH PAPERS

Ou, H.D. *et al*. ChromEMT: visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science* **357**, eaag0025 (2017).