Peer Review Notes May 2015

The Invention of Expansion Microscopy, a Diaper Compound, and the Power of Peer Review

Scientists can't break the laws of physics. This was a problem Dr. Edward Boyden faced at the Massachusetts Institute of Technology. The best light microscopes cannot bring key molecular structures into focus without losing track of the larger cellular context. But if scientists could see more deeply across a broader expanse of tissue—with nanoscale precision—they could potentially learn more about the abnormal biochemistry of disease, and come up with new targets for drug design.

With a positive assessment by CSR reviewers and a subsequent NIH Director's Pioneer Award for "high risk, high reward" research, a team led by Dr. Boyden turned this tough problem on its head, inventing expansion microscopy, a new approach to visualizing biological samples. The creativity and problem-solving potential of the strategy already has drawn the attention of scientists who investigate a variety of diseases, including neurodegenerative disorders and cancer.

"We found a way to magnify samples directly, rather than just trying to use a lens to magnify light from a sample," said Dr. Boyden, Associate Professor of Biological Engineering and Brain and Cognitive Sciences, at the MIT Media Lab and the MIT McGovern Institute. "It is turning out to really be useful."

To expand tissue samples and cells without altering their shapes, Dr. Boyden and graduate students Fei Chen and Paul Tillberg use a chemical polymer known for decades and popular as an absorbent in diapers.

To enlarge a sample the scientists first tag the proteins within with fluorescent antibodies, and then embed the sample in an expandable polymer gel, made of polyacrylate. The polyacrylate binds to the fluorescent tags, and remains bound even after the protein is dissolved away. Adding water causes the polyacrylate to swell, as it does in a wet diaper, enlarging the entire fluorescently labeled sample without distortion.

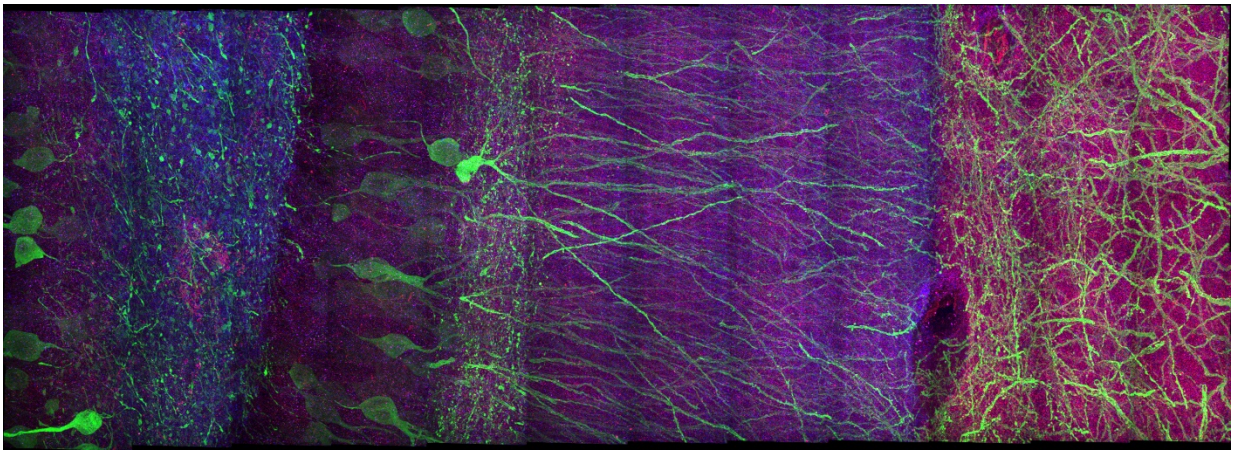
Dr. Boyden's goal is to probe what's going on at a molecular level within signaling pathways inside neurons, without losing sight of the bigger picture

that reveals what's happening within a larger network of nerve circuitry. So far, his research team has investigated expansion microscopy in lab-cultured brain cells and in slices of nerve tissue from mice.

"We have had a fantastic response from the neuroscience community, but the response from the cancer community also has been significant," said Dr. Boyden. "We now are engaged in collaborations to do cancer research, and the work also has generated interest in exploring how immune cells are arranged within organs in cancer and in autoimmune disease. That was a direct output from this high-risk, high-reward funding."

Dr. Boyden has a track record generating big ideas leading to major research success. A decade ago, he wanted to control and explore nerve signaling in living cells under the microscope on millisecond time scales. To accomplish this, as a Stanford University graduate student working on a side collaboration with Dr. Karl Deisseroth, now the D.H. Chen Professor of Bioengineering and of Psychiatry and Behavioral Sciences, he co-invented optogenetics, a technique in which researchers use light-sensitive, ion-channel proteins to convert light into electricity.

Dr. Boyden's continued success as a scientist who is skilled in developing new ways to record electrical activity from neurons and in devising other molecular techniques for learning more about what goes on in cells helped sway the scientists who reviewed his Pioneer Award proposal, noted Dr. Kip Ludwig, Program Director for Neural Engineering at the National Institute for Neurological Diseases and Stroke, which funds Dr. Boyden's Pioneer Award.



Expansion Microscopy of mouse brain tissue: Volume rendering of a portion of hippocampus showing neurons (expressing YFP, shown in green) and synapses [marked with anti-Bassoon (blue) and antibody to Homer1 (red)]

"The idea of the Pioneer Award and certain other NIH high-risk, high-reward

grant mechanisms is to support big and bold ideas from researchers with a history of turning big and bold ideas into success stories," said Dr. Ludwig. "There are disadvantages to requiring all the smallest details at the application stage. With big ideas applied to big problems, science does not always go the way you expect it to, and the focus needs to be on the impact of the idea and the proven ability of the investigator to solve problems as they arise."

The results to date are exciting, but not exactly what Dr. Boyden initially proposed. "I wrote my Pioneer Award proposal partly on neural recording in three dimensions, but I also mentioned that I would love to do super-resolution imaging of brain circuits," he said.

Peer review of Pioneer Award proposals includes an interview and presentation before an audience of scientists. By the time of his presentation, Dr. Boyden's lab team had acquired early data on expansion microscopy. "I showed them an image of one of the very first cells we had expanded, and we proceeded to talk about this new technology that was not part of the original written proposal," said Dr. Boyden. To his relief, the reviewers endorsed moving the groundbreaking research forward.

While he continues to work on other big ideas, he expects his lab's success in expansion microscopy to become, dare we say — more expansive. "We are looking for polymers that expand bigger, that preserve structures even better, and that are even more compatible with biomolecules," he said.

"Our role is to build technology, and technology development doesn't always fit within hypothesis-driven or disease-focused paradigms. We often simply set out to build tools that are going to help people solve a whole bunch of problems."

— *Jeffrey Norris*