Podcast interview: Vadim Backman

PNAS: I’m Brian Doctrow, and welcome again to Science Sessions.

Imagine being able to look inside a living cell, see every molecule in that cell and watch the processes carried out by all of these molecules in real time. Although well beyond our most advanced microscope technology, such an ability would greatly expand our understanding of how cells work. The advent of superresolution microscopy, which was awarded the 2014 Nobel Prize in Chemistry, was a significant advance in biological imaging. Superresolution microscopy circumvents the limits on the resolution of conventional optical microscopy imposed by the laws of physics, allowing objects on the scale of nanometers to be visualized. However, the technique has limitations of its own.

In 2016, Vadim Backman, a professor of Biomedical Engineering at Northwestern University, along with his colleagues, published in PNAS a method for avoiding some of these limitations, which earned Backman and his co-authors the 2016 Cozzarelli Prize for excellence and originality in Engineering and Applied Sciences. I spoke with Backman at the 2017 NAS Annual Meeting. He explained the limitations of conventional microscopy, and how superresolution microscopy overcomes those limitations.

Backman: If you have a fluorescent molecule, you can always pinpoint the location of the molecule very, very precisely. The problem becomes when you have two molecules close together. Each of them gives rise to what is called a diffraction-limited spot, which is a few hundred nanometers across. When you have two molecules or more close together they essentially all merge into one big amorphous blob, their emissions. So the trick here was that if you excite one molecule at a time, and then you collect multiple frames, you localize each molecule with 20 nm precision. So eventually, you can build up the whole image. Just like, imagine you have a Christmas tree, Christmas lights, and one light bulb lights up at any given point in time. You take a picture of that, and eventually you can reconstruct the whole Christmas tree.

PNAS: What limits the application of superresolution microscopy is the need to attach fluorescent labels to the molecules of interest in order to make them visible.

Backman: It’s hard to label each and every molecule in the cell and still keep the cell functioning in a normal way, not to mention that it probably won’t be alive for that long. So using label-free approaches would allow for the first time to see things as they really are—the “ground truth,” so to speak. The problem is—what everybody used to know before the publication of our first paper in PNAS—was that endogenous macromolecules that build up the cell, such as DNA, proteins, lipids, etc., they don’t fluoresce in the visible range.
**PNAS:** Backman, in collaboration with Hao Zhang, another biomedical engineer at Northwestern, discovered a previously undocumented fluorescent process in biological macromolecules, which allowed them to image these molecules at high resolution without the use of labels.

**Backman:** Let me bring up the running analogy here—the DNA, proteins, and other macromolecules in the cell, they’re sprinters. They shine very brightly for a short period of time, and then they go to the dark state, so they don’t fluoresce for a while. So if you image them for long enough, it looks like the signal is extremely weak. But if you look at the bursts of emission, they are actually as bright as some of the best exogenous fluorophores. We were able to captures these bursts of emissions, and pinpoint the locations of these emissions with sub-20 nm resolution. The paper which was published in PNAS talks about 20 nm resolution, we are down to six now, without the harm of labeling the cell, and without the need to label each and every molecule.

**PNAS:** In the PNAS paper, Backman applied this technique to examine how DNA is folded up and packed inside the nucleus of the cell, which could in turn have implications for how cells regulate gene expression, and possibly for the development of cancer.

**Backman:** What we have been learning is that the change in the structure of the genome—three-dimensional structure of the genome—allows cells to change, explore their genomic landscape, do things transcriptionally that normal cells would never be able to do. And that’s what in a sense cancer is all about. We don’t have answers to all the questions yet, but I think the important thing I would like to emphasize is for the first time, we actually have a tool, which can lead to these answers. It can also allow us for the first time to ask questions, which is as important as answering questions, because for the first time we can see the whole structure of chromatin. And who knows what we’re going to find?

**PNAS:** Backman envisions that the technique could have wide-ranging applications, beyond observing the structure of the genome.

**Backman:** Almost every application that you can think of, which is amenable to more conventional label-based microscopy, should be potentially addressable using this technology as well. If you look beyond the cell nucleus, we have lots of other interesting things: we have mitochondria, which are of course the lungs of the cell; cytoskeleton, which keeps the cell together. We can potentially look at diffusion and transport of molecules. That’s another place where label-free ground truth can be quite significant. If you want to study dynamics of a runner, going back to the running analogy, and you put on a label, which is the size of a truck, you’re probably going to get a little different understanding of what the dynamics really is.
**PNAS:** Backman emphasizes that the technique is still in its infancy, and its full capabilities have yet to be explored, but that hasn’t stopped him from imagining what the technique might lead to in the future.

**Backman:** Can we image the whole cell and with nanometer precision identify the location of each and every molecule, which exists in that cell, each and every gene, each and every protein? That would be the kind of “science fiction” vision where this technology may eventually go.

**PNAS:** You can find more Science Sessions podcasts at [www.pnas.org](http://www.pnas.org). Thanks for listening.