Electron Optica Shows Low-Energy Electron Microscopy Can Distinguish Unlabeled Bases

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By Julia Karow

Startup Electron Optica and its collaborators have demonstrated that sequencing DNA by electron microscopy without labeling should be possible in principle because the four base types provide sufficient contrast and improvements in resolution seem feasible. The company is now looking for funding to build an instrument and to show that it can sequence DNA.

Two years ago, the Palo Alto-based company won a $500,000 grant under the National Human Genome Research Institute's Advanced Sequencing Technology program to explore the feasibility of using low-energy electron microscopy, or LEEM, for DNA sequencing (*IS 8/23/2011*). Since then, it has collaborated with groups at the Stanford Genome Technology Center and the National Center for Electron Microscopy at Lawrence Berkeley National Laboratory to show that this should be possible.

Electron Optica uses so-called monochromatic aberration-corrected dual-beam low energy electron microscopy, where two beams illuminate the sample with low-energy electrons, and the reflected electrons yield a magnified image.

While LEEM has been around for several decades, it is much less popular than transmission electron microscopy or scanning electron microscopy, according to Marian Mankos, founder and CEO of Electron Optica. "There are maybe 30 to 40 of these microscopes in the world, compared to maybe tens of thousands of SEMs or TEMs," he said, partly because of the lower resolution of LEEM.

Typically, LEEM is used to study the surface of materials, and it is not applied in the life sciences at all today, he said, mainly because most biosamples are insulators that charge up under the microscope, which affects the low-energy electrons. Previously, Mankos and his colleagues developed a charge-control technique to get around this, originally to inspect semiconductor chips with insulating material on them. "Now, I'm trying to see if we can expand the application of this microscope by using this charge-prevention technique in fields which have been a no-man's land, like biology," he said.

Two other companies, ZS Genetics and now-defunct Halcyon Molecular, have been working on reading the sequence of DNA directly off an electron microscopy image, with the prospect of obtaining very long reads (*IS 7/16/2013* and *IS 9/10/2013*). However, Electron Optica's approach will differ from theirs in several key aspects.

Electron Optica will use LEEM, where the electrons have energies up to several hundred electron volts, rather than transmission electron microscopy, where the electrons have about a thousand times more energy. Because of this, there will be no need to label the DNA, and damage to the molecule will be smaller, which could reduce sequencing errors.

Mankos explained that in TEM, the high-energy electrons are not scattered enough by the elements that make up DNA, so the molecule cannot be visualized directly. To "see" the DNA, scientists need to attach heavy atoms to the bases — usually as part of a larger organic complex — and from an image of these heavy atoms, they can deduce the DNA sequence.

That approach, he said, has several problems: one is that the labeling reaction might not go to completion, meaning some DNA bases will remain invisible. Also, scientists can only label one type of base at a time right now because the labels are difficult to distinguish and interfere with each other when they get too close, so the researchers need to reconstruct the sequence from four separate images. In addition, high-energy electrons often damage the DNA, causing the labels to move around, so their position is no longer aligned with the base they are attached to.

"By getting rid of the high energy, we basically get rid of the damage, so we don't blow the DNA away anymore," Mankos said. Also, because the low-energy electrons are slower, they get scattered by the elements of DNA – mostly carbon, oxygen, and nitrogen – so there is sufficient contrast to visualize DNA directly, which the researchers demonstrated in a publication in the *Journal of Vacuum Science and Technology B* last year.

However, to make LEEM suitable for DNA sequencing, two problems need to be solved: the resolution, which is lower than in TEM, needs to be improved, and scientists have to demonstrate that they can distinguish the four bases.

"The typical LEEM has on the order of a few nanometers resolution," Mankos said. "That's not enough to actually see the bases," which are less than a nanometer apart.

Others have already employed so-called aberration correctors to enhance the resolution of LEEM by a factor of two or three. "We tried to push it a little bit further," he said, designing a new aberration corrector that can bring the resolution to about half a nanometer. "We have very detailed simulations on the design, which, if built, should be able to deliver this resolution," he said.

His team has also collaborated with Ron Davies' group at the Stanford Genome Technology Center and Andreas Schmid's team at the National Center for Electron Microscopy to show that they can discern the four bases.

They designed small homopolymer oligonucleotides of all four bases, ranging in size from 5mers to 100mers, which they put on four different kinds of substrates. They found "several contrast mechanisms which we can clearly use to distinguish the individual bases," Mankos said. Specifically, they used "fairly well-known" spectroscopy techniques called X-ray photoelectron spectroscopy, XPS, and Auger electron spectroscopy that can be implemented in the LEEM imaging mode.

"We basically established that there is indeed contrast between the bases that can be used to sequence," he said. "When we put in a certain substrate with a given type of homopolymer, we know which one it is by just looking at the signature that comes out of the spectrometer." The main reason for the contrast is differences in the nitrogen content of the bases, he added. "Finding this contrast was really a big thing for us," he said, adding that the scientists recently submitted a paper on their findings to a journal. "It was a big unknown, and nobody knew whether this contrast exists."

Right now, Mankos and his colleagues are writing proposals to obtain additional funding that will allow them to build a microscope that contains the new aberration corrector, and to demonstrate that they can sequence DNA with it.

Building the microscope will be "a big task," he said. Another challenge will be to stretch out the DNA on the surface "in a very controlled manner," which already works "pretty well" for single-stranded but not yet for double-stranded DNA. "Those are the two big risks," he said.

If successful, Electron Optica plans to provide the microscope to early-access adopters, such as the Stanford Genome Technology Center, to compare it with existing sequencing technologies. After that, it wants to commercialize the instrument, possibly in partnership with an established electron microscope manufacturer and with a "full-scale sequencing company that will integrate the sample preparation, imaging, and data analysis," Mankos said.

Today, a LEEM costs on the order of $0.5 million to $1million, and the price of a sequencing microscope will be "at the high end of the typical sequencing instrument price range," he said.

The platform would compete with other technologies, such as nanopores, on read length – the aim is to obtain reads up to 50 megabases – as well as cost per base, accuracy, and throughput. Mankos said the goal is to have an error rate of 10^{-6} and a throughput of one genome per day. Besides DNA sequencing, it should also be useful for related applications, including RNA sequencing and methylation sequencing.

In the meantime, Electron Optica is working on other projects. Mankos founded the company, which focuses on building novel electron microscope instrumentation, in 2010 and has developed prototype instruments for several high-tech companies.

New funding for the DNA sequencing project would allow the firm to increase its staff of three to six or seven employees.

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