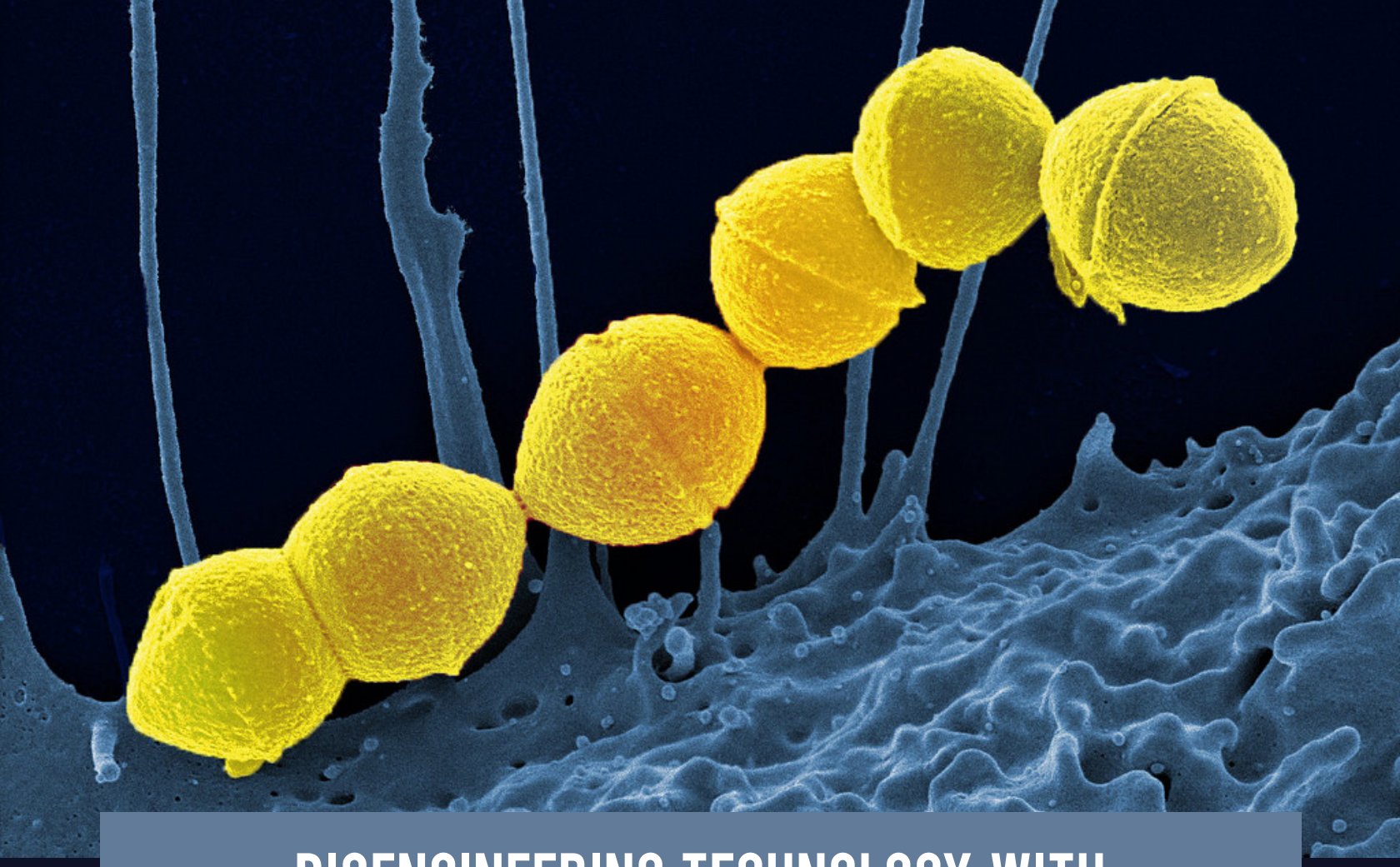

# BIOENGINEERING TECHNOLOGY WITH A SOCIAL RESPONSIBILITY

## *Interview with Professor Luke P. Lee*

BY MATTHEW COLBERT, CASSIDY HARDIN, MICHELLE LEE, ROSA LEE, MELANIE RUSSO, AND NIKHIL CHARI

***Dr. Luke P. Lee*** *is a Professor of Bioengineering, Electrical Engineering and Computer Science at UC Berkeley. He is a pioneer of technologies such as rapid microfluidic PCR and optofluidic spectroscopy. Dr. Lee is also a strong believer in tackling problems with broad-scale social implications. In 2016, he founded the Biomedical Institute for Global Health Research and Technology (BIGHEART) at the National University of Singapore. We spoke with Dr. Lee about his mission at BIGHEART, his work in establishing microfluidic platforms for rapid polymerase chain reaction (PCR) and waterborne pathogen detection, and the importance of bringing socially responsible technologies to market.*

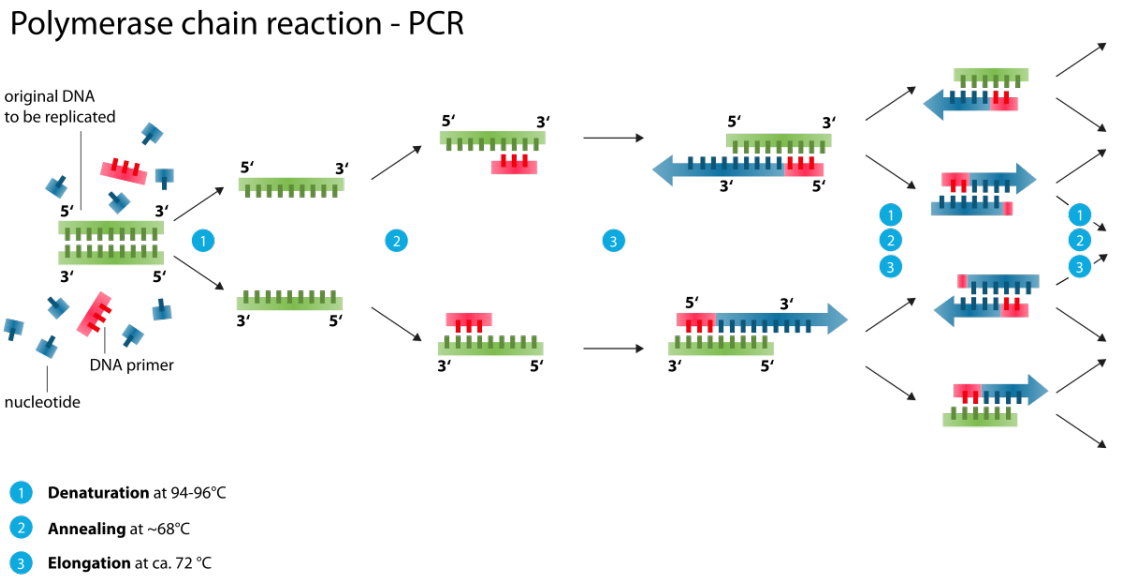


***Figure 1: Steps of PCR cycle.***[5] *Denaturation of double stranded DNA at high temperature, followed by annealing of polymerase at lower temperature, and elongation of new DNA strands.*

**BSJ: We'd like to start off by talking about BIGHEART. Can you briefly explain the purpose of BIGHEART and how BIGHEART uses multidisciplinary approaches to address modern humanitarian and medical challenges?**

LL: I originally started my career here in Berkeley, but back then, I didn't have much funding, physical space, or manpower to set up this institution. I wanted to create an environment where people from different disciplines could work together to solve one problem—global health. Of course, there are so many different issues within global health that need attention, but I wanted to focus on early infectious disease diagnostics and precision medicine using organoid chips—tissue cultures that can replicate the behavior and complexity of a real organ. Everyone's metabolic activity is different, but even if we assume there is a universal recipe for treating a patient for a certain disease, certain people may still not be able to handle that treatment. In the case of cancer in Third World countries, if the patients are malnourished, they might not be able to handle the same toxic compounds that are found in cancer medications. We were thinking about how to find the best personalized medicine for organoid-on-a-chip technologies. But molecular diagnostics was our first priority. That's why I spent a lot of time developing more efficient technologies to detect DNA and protein biomarkers. That's one of the reasons we're working on PCR-on-a-chip. If possible, we want to detect the pathogen directly without any labeling.

BIGHEART's goal is to bring scientists together—whether they're engineers, biologists, physicists, even clinicians—to communicate and solve one project, such as malaria, as a group. But all those scientists are busy with their own work, so it's not always easy for them to work on a specific medical issue. We wanted to bring these people into one place and allow them to work together without any physical or mental barriers.

**BSJ: You spoke briefly about your work in PCR-on-a-chip technology. PCR is a technique that is commonly used in biology to amplify a certain section of DNA. What are the current applications of PCR?**

LL: There are many, many applications. One is rapid and accurate measurement of different diseases. In our microfluidic PCR device, each circle is a reaction chamber. You can do simultaneous tests of different biomarkers for different cancers or infectious diseases in each one. The PCR solution is automatically pumped into the chambers using a vacuum, and each chamber has an individual PCR reaction.

**BSJ: Could you briefly highlight the issues and potential inefficiencies with current PCR systems?**

LL: Current PCR takes hours. You have to use a heater to change the temperature of the reaction several times to denature the DNA and then allow the polymerase to bind (Fig. 1). Many companies build heater blocks, so you are heating up for denaturing and cooling down for annealing, and then you go back up a little bit for extension. You have to make a cycle, which takes a lot of power and time. We created a technique called ultra-fast photonic PCR using LEDs and so-called "plasmonic concept antenna" to heat up and cool down the reaction really fast. Instead of one hour for 30 cycles, you can do it in two to three minutes. The idea is to use a specific wavelength to resonate this plasmonic structure, like gold. The other issue with microfluidic PCR is when you heat up the PCR fluid, you generate bubbles. We are trying to remove these bubbles by pumping them out of the system.

This is not only important for infectious disease detection or cancer diagnostics. Everyone in the life sciences needs to use PCR to quantify gene information; it's the only way to amplify DNA. That is why PCR is so important and there are so many people competing with each other to claim that they made ultra-fast and accurate PCRs. Some labs might not mind waiting one hour, but if you want to screen a lot of samples or build a library and you have to wait one

hour for one experiment, it can take a lot of time. With microfluidic PCR, we can go from one experiment per hour to 100,000 experiments in five minutes, because we also have many wells or chambers in each PCR chip (Fig. 2). 15 years of work can be finished in 15 days. You can speed up an enormous amount of life science automations and capture more reliable biomarker discovery and detection.

A lot of people don't think we need fast PCR, but it's not so much about speed as it is about collecting massive amounts of data. If you really want to make a precision personalized medicine, you need to collect all the information about how cellular behaviors change with various types of cancers or diseases. We need to build reliable, high-density information about these biomarkers. Not only for disease, but also for our food and our environment. We can prevent disease by correlating it with the food we eat. For example, say I got cancer and the doctor hypothesized it was because I was eating a lot of junk food. If we are able to build these biomarker databases, we could trace my eating habits and my DNA information to the source and verify the doctor's hypothesis. In other places, water is very important. The DNA of the pathogens that introduce infectious diseases is hidden somewhere—in the water, the environment, the food—we need to find it.

**BSJ**: Going back to microfluidic PCR, we wanted to ask: how does the generation of bubbles hamper the efficacy of microfluidic PCR?[1]

**LL**: If there is air trapped in the reaction chamber, when you introduce the PCR solution into the chamber, bubbles will dominate the space and you will not have any reactions. This is because when you heat the chamber, the bubble will expand and push all of the DNA and PCR solution out of the chamber. You need to pump out the bubble before you begin. We built this ring (Fig. 3) to be intentionally gas-permeable so we can remove the bubble before the reaction begins, and we developed a degassing method that uses a vacuum battery to pump out all of the residual gas trapped inside the chamber.

**BSJ**: To aid in the prevention of bubbles forming in the PCR microfluidic machine, you used a layer of polyethylene (PE) and two layers of polydimethylsiloxane (PDMS) (Fig. 3). Can you explain how these two substances prevent the formation of bubbles?

**LL**: The gas permittivity of PE is much lower compared to PDMS. We made the top surface of the reaction chamber PE to ensure that all the gas would be pumped out laterally through the PDMS around the sides of the chamber.

**BSJ**: Could you briefly describe the concept of degas-driven flow?[1]

**LL**: The PDMS is very flexible and contains lots of nanopores. After we fabricate a PCR chip, we pump out all of the gas inside by putting it in a desiccator. You can remove even the small amounts of gas trapped in the nanopores. You cannot see them with the naked eye because they are so small—less than a nanometer. If

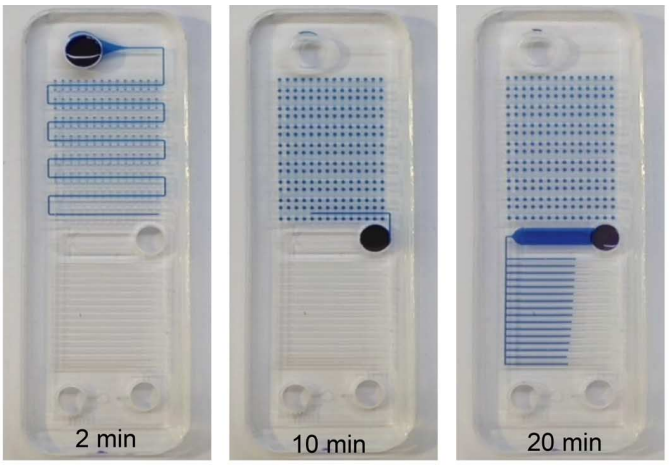


***Figure 2: PCR reaction chambers in one of Dr. Lee's microfluidic PCR chips.***[3] *The chambers are filling up over time.*

you pump out all of the gas from the PDMS, you can build a negative pressure environment, lower than the atmosphere. Then you package it. When you are ready to use it, you can open the package just like a potato chip bag—of course, you cannot store in in this negative pressure for a long time. Just like potato chips—if you eat the potato chips after a certain amount of time they're not crispy anymore, right?

Anyway, you pump it down, and then you package it. When you are ready, you drop in the PCR solution, and the negative pressure draws the solution into the chip. Building that negative pressure in the first place is what we call degassing. But our design also adds a vacuum battery. If we rely only on the properties of the material to degas, the operator of the chip only has about two minutes to drop in the PCR solution before the pressure returns to atmospheric. But with the vacuum battery, we increased the volume of space you can pump air out of, so it's much more than just the small nanopores in the PDMS. Now, we can wait for 15 minutes instead of two. This accounts for less efficient human operation of the chip, and the operator can take more time to drop in the solution.

**BSJ**: Could you describe what the function of the circumferential degas pump surrounding each chamber is?[1]

**LL**: The circumferential degas pump helps to pump out any gas within this chamber. As I mentioned, PDMS can pump out gas because there are small, invisible pores which you have already vacuumed. It works, but it takes time. Here, we intentionally made a space, the circumferential degas pump, where you can have more vacuum volume (Fig. 3). This makes it faster to pump out residual gas. Also, it's easier to pump laterally. If you have this kind of design,

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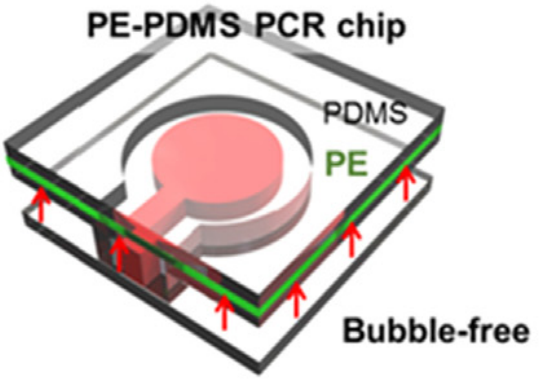


***Figure 3: The design of the microfluidic PCR chip features a thin layer of PE sandwiched between two thicker layers of PDMS.***[1] *The PE layer prevents PCR fluid (red arrows) from flowing out of the cell vertically, whereas gas flows out laterally through PDMS.*

all the gas bubbles are pumped to the outside.

**BSJ**: Earlier, you talked briefly about methods of detection for waterborne pathogens. Could you elaborate on what some of the limitations of the current methods of waterborne pathogen detection are?

**LL**: All pathogen detection nowadays is culture-based. It takes time. For example, let's say your kid has an infection. You don't know whether it's from the water or if it's an airborne pathogen. It happened to my daughter. She had an infection, but we didn't know what happened, so we just drove down to the best children's hospital—at the time, I thought that Stanford's Children's Hospital was better. Doctors just kept injecting her with different antibiotics without knowing what the real problem was. It was really disastrous; she was hospitalized for many months. Thankfully, she survived, but some people can die. If you just keep on adding antibiotics without knowing what the infection is, it's actually damaging the patient. It's better to treat it with the proper drug that will kill that particular pathogen. I had food poisoning the last four days, I couldn't even eat. I don't know what caused it, but it would've been nice. It is critical for certain diseases to detect pathogens precisely.

How can we identify pathogens right now? Either ELISA, which is protein-based detection, or PCR, which is DNA-based. We are trying to quickly detect the specific fingerprint of the pathogen from its outer surface. It's challenging, but we're trying to do our best.

**BSJ**: Could you briefly describe what plasmonic bacteria are? How did you modify water-borne pathogens to create plasmonic bacteria?[2]

**LL**: This kind of solution we use is nothing but gold nano-plasmonic particles. Actually, it's not very expensive; it's a salt solution—it costs less than a few cents! We wanted to be able to detect pathogens without lysing them, which you have to do to detect the DNA inside. Our idea was to detect a chemical signature on the surface of the pathogen. Just like how your faces all exhibit different phenotypes, the surfaces of pathogens exhibit unique chemical structures. Unfortunately, the surfaces of all pathogens look quite similar. There are a few vibrational spectra that are different, but they're difficult to differentiate because the signal is very weak. Wrapping the bacteria in gold nanoparticles helps to amplify the chemical signal (Fig. 5).

All chemicals have a vibrational spectrum, and we're using vibrational spectroscopy called surface-enhanced Raman scattering (SERS) to detect these different spectra. Since these vibrations are normally so weak, we have to use the plasmonic bacteria to amplify the vibrational scattering signal. We also measure the signal from the

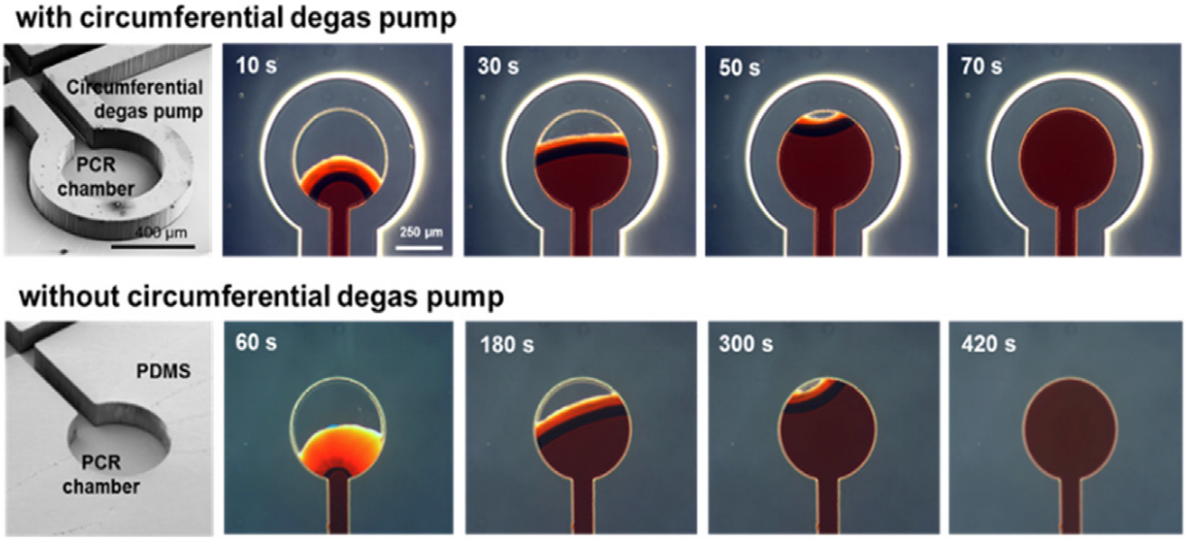


***Figure 4: The role of the circumferential degas pump.***[1] *It ensures uniform flow of the PCR fluid into the reaction chamber.*

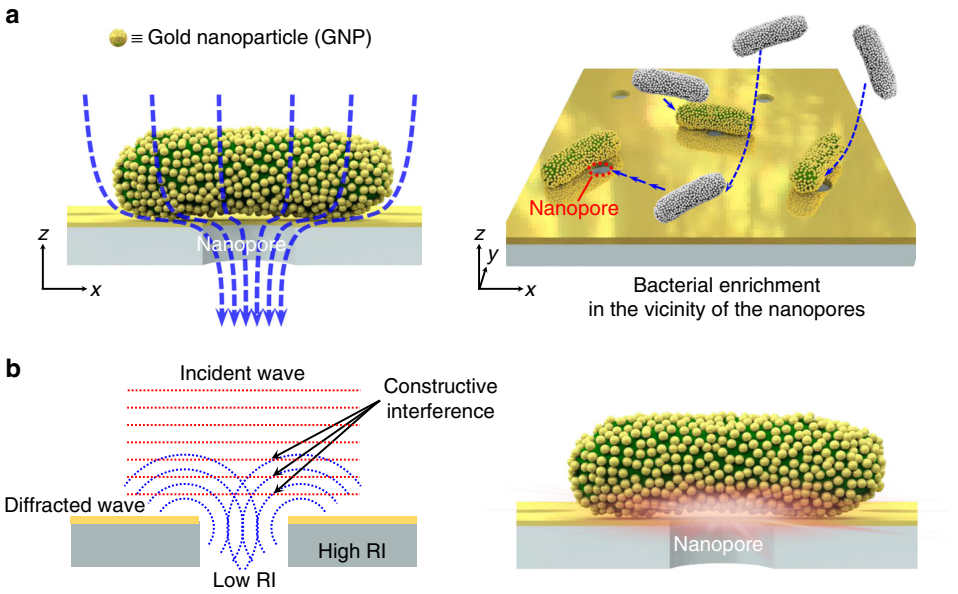


***Figure 5: Lee's optofluidic platform.***[2] *It aims to enhance surface signals from pathogenic bacteria using GNP coatings and constructive interference with nanopores.*

plasmonic bacteria on the surface of a gold mirror, because we're trying to amplify as much as possible. We're doing all kinds of different things to increase the vibrational peak of surface proteins on these pathogens. It's not an easy job, but we want to figure out whether we can accumulate enough data to distinguish from pathogen to pathogen. But without the gold we cannot get any signal.

**BSJ**: You explained that you measure the plasmonic bacteria signal on a thin gold mirror dotted with nanopores. How do these nanopores contribute to trapping plasmonic bacteria?[2]

**LL**: In our system, polycarbonate plastic is cased with a thin gold layer, which is dotted with nanopores. Around the edges of the nanopores, we see what are called "hot spots," which are generated by more electron oscillation (Fig. 4). More electron oscillation means more radiation. It's like an antenna—which is nothing more than a simple rod structure with oscillating electrons. What happens if you oscillate electrons in the surrounding area? An electromagnetic field is generated, which we can detect. But we want to get as much information as we can out of that field. Many people have tried to make nanopillars or nanostructures to enhance this signal - something with a sharp tip. But here, instead of using a sharp tip, we use nanopores because we want to trap more of the pathogen. If we weren't trying to filter out bacteria, we wouldn't use nanopores —we would use a sharp tip structure.

**BSJ**: How may rapid detection of waterborne pathogens enabled by your optofluidic mechanism contribute to our understanding of the pathogenic genome?

**LL**: We need to mass-produce these technologies to disseminate to the Third World. That was my dream. It's a "chicken and egg" problem because many venture capitalists want to make money right away, and this kind of thing doesn't make money right away. They value a winner's market. Waterborne pathogen detection is not really a big market. That means we have to invest in technologies with a social responsibility. That's why I started BIGHEART. After all, we are in Berkeley and we shouldn't give up. Social responsibility is important, because these pathogens are connected with real health problems. You may think that the health problems in the Third World will not affect us, but actually, these pathogens circulate through the air, or traffic, or even the food that we import. We have to think about how to use our resources wisely to prevent and mitigate these global health problems.

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