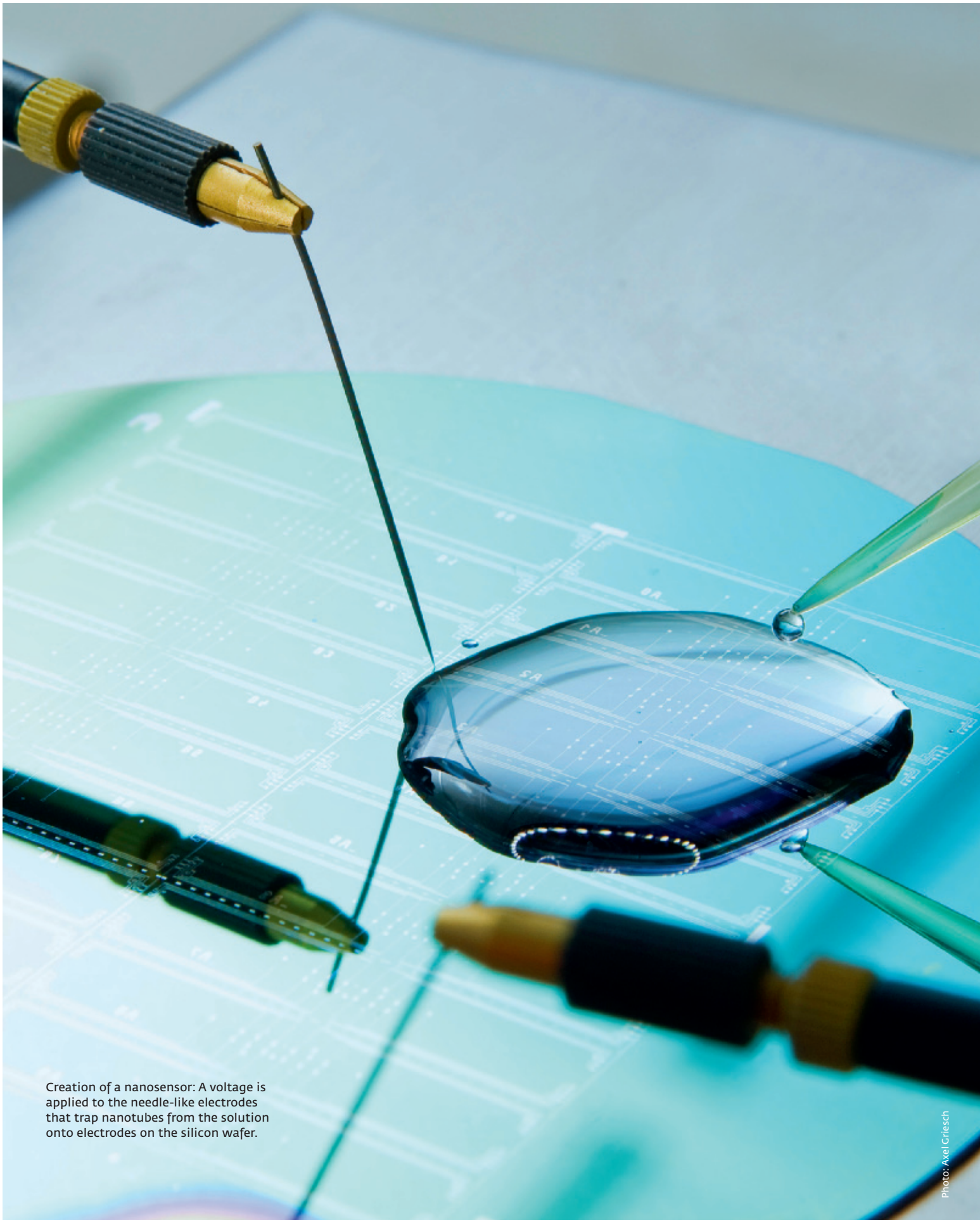


Creation of a nanosensor: A voltage is applied to the needle-like electrodes that trap nanotubes from the solution onto electrodes on the silicon wafer.

Photo: Axel Griesch

Blood Samples
Undergo Nanotesting

Handy, quick and very sensitive – that's how **Kannan Balasubramanian** imagines a device for carrying out blood analyses. At the **Max Planck Institute for Solid State Research** in Stuttgart, his team is developing nanosensors with which it would be possible to carry out a blood count or to detect pathogens.

TEXT **CHRISTIAN MEIER**

Often it's just a nuisance, but sometimes it's actually dangerous. Currently, if you have a blood test, the doctor's parting words will usually be: "You'll get the results in a couple of days." Kannan Balasubramanian wants to change all that. The physicist at the Max Planck Institute for Solid State Research in Stuttgart is pursuing the visionary objective of creating a diagnostics laboratory that can fit on a chip the size of a fingernail. Working with just a droplet of blood, the minuscule laboratory would be able, within minutes, to determine a patient's blood count, estimate risks of disease and even detect sinister virus infections.

Doctors could carry out the test in their practices, or patients could even do it in the comfort of their own living rooms simply by taking a drop of blood from their fingertip. A device the size of a mobile phone would quickly deliver all of the relevant measurements from this drop.

"In just five to ten years we would like to have developed mature chips with which such portable diagnostic laboratories can be made a reality," says Kannan Balasubramanian, who heads the "Nanoscale Diagnostics" Junior Research Group, which is funded by the German Federal Ministry of Education and Research. Over the past three years, the scientists have already cleared some of the major hurdles they were facing. Others still remain to be overcome.

VIRUS DETECTION STILL TAKES TOO LONG

Above all, it is the examination speed with a portable laboratory that would be a big step forward in medicine. Just one example: "In a hospital, it often takes days to detect a viral infection," explains the Max Planck researcher, who is originally from India. This is because the pathogen's genetic material, namely its DNA or RNA, is present in the patient's blood at only a very low concentration. And it cannot be detected using conventional analytical methods. As a result, any viral genetic material suspected of being present in a blood sample must be enriched to a level above the detection limit using

Coming to grips with the problem: Kannan Balasubramanian (center) and his colleagues (from left: Vivek Pachauri, Tetiana Kurkina, Nassim Rafiefard and Ashraf Ahmad) use a drawing and a model to discuss the measurement principle underlying their nanosensors.

polymerase chain reaction, or PCR for short. The entire test, from taking the sample to getting the result, thus takes several days.

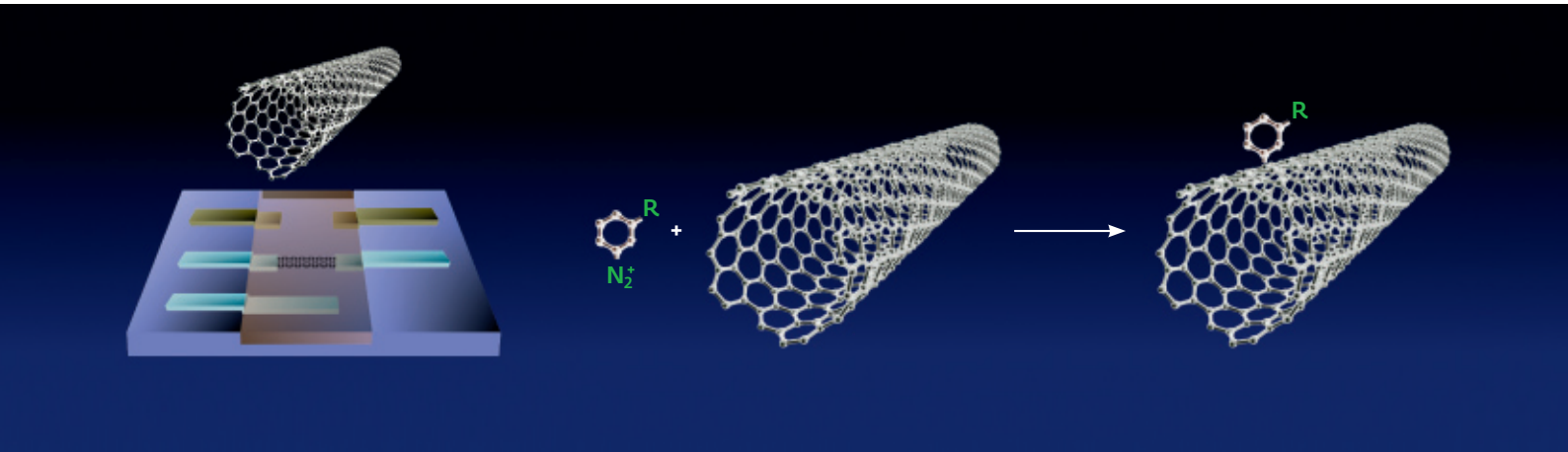
"Doctors want testing to become faster so that they can treat the infection as quickly as possible," Balasubramanian says. This is why his team is developing sensors that can forego PCR and are capable of detecting minuscule concentrations of viral RNA. Using a highly sensitive diagnostic chip, it would be possible to get closer to the end user, as analysis would be much simpler. "In an epidemic situation, rapid mass testing would be possible," says the 32-year-old researcher. Moreover, a patient would no longer have to have ten or more milliliters of blood taken for a blood analysis.

NANOTUBES FOR SENSITIVE SENSORS

To achieve his goal, Balasubramanian needs sensors that respond to single or very small numbers of molecules of a specific substance – for instance, to one molecule of viral genetic material or to one protein indicative of a risk of heart attack. The researcher's five-member team focuses on a material that is currently used for reinforcing ultra-light tennis rackets and bicycle frames: carbon nanotubes (CNT).

These tubes have one fascinating property that makes them ideally suited for use as highly sensitive sensors: they are nothing but surface. Their walls could not be any thinner because they consist of a single layer of carbon atoms. The entire nanotube is thus ex-

Photo: Axel Griesch



posed to its environment; not a single one of its atoms is protected inside it. And if foreign molecules bind to the surface of a carbon nanotube, this influences its overall characteristics.

In particular, its electrical resistance changes sharply if molecules attach themselves to its surface. This is explained by the structure of a nanotube: its carbon atoms form a regular lattice reminiscent of a honeycomb. This regularity means that each carbon atom contributes an electron to an electron cloud that extends over the entire CNT and allows electrons to move through it unimpeded. The nanotube thus conducts electricity very well.

Should a foreign molecule bind to one of the nanotube's carbon atoms, it interrupts the honeycomb pattern at this spot. In this way, the attachment disrupts the free passage of electrons over the surface of the tiny carbon tube. Figuratively speaking, this puts a baffle in the track the electrons are racing around, and the tube's electrical resistance increases. The more foreign molecules stick to the nanotube, the more the resistance rises.

The change in resistance, and thus the concentration of foreign molecules, can be measured if the nanotube bridges the gap between two electrodes. This is the fundamental idea behind the carbon nanotube sensors that Balasubramanian's team is developing.

However, if a CNT sensor is to yield usable information, the nanotube has to be choosy – after all, it isn't intended to respond to just any old molecule in the blood, but only to specific molecules, such as viral RNA or the blood sugar molecule glucose. In specialist circles, this characteristic is known as selectivity. At first glance, it would seem impossible to give a carbon nanotube this property, since a CNT exposes its surface to all the molecules in a blood sample.

RECEPTORS TAILOR NANOTUBE PROPERTIES

However, nature gives the researchers a helping hand in making the nanotubes more selectively perceptive. Many biomolecules have a kind of companion, a second molecule into which they fit, like a key in a lock. DNA, for instance, the carrier of genetic information, consists of two single strands that together form a double helix. One of the single strands fits like a jigsaw puzzle piece with the opposite strand – and with no other. Another example: on the surface of foreign bodies in the bloodstream are proteins, known as antigens, that the body combats with the help of antibodies. An antibody is a protein that can be slipped over the antigen like a mold. Researchers generally call a molecule that selectively binds a sought molecule to itself a receptor.

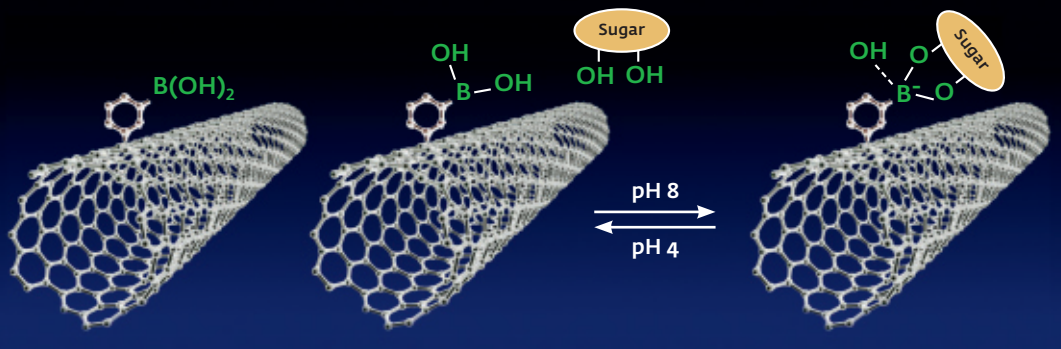
"We make the CNTs selective by attaching a receptor to them, for instance a receptor for blood sugar," explains Balasubramanian. The presence of the receptors themselves increases the nanotube's resistance. The researchers define this increased resistance as the new baseline. If the substance sought – called an analyte – then binds to the receptor, the distribution of electrons on the carbon nanotube changes more strongly than due to the receptor alone, and conductivity falls further.

In this way, the researchers can make sensors for specific substances. Balasubramanian, however, would like to build a chip that simultaneously tests for several analytes in one drop of blood, for example to carry out a complete blood picture in a single test. To achieve this, the chip needs several pairs of electrodes connected by carbon nanotubes, the current flow between which can be separately measured. The nanotubes between the electrodes would in each case have to carry different receptors.

But how can the nanotubes be provided with different attachments in such a way that the ultimate location of each kind of receptor is still known? Immersing the chip in a solution containing all the receptors at once would be pointless, as the receptors would react at random with the carbon nanotubes and no one would know where each one is located. As a result, it would be impossible to tell which analyte is bringing about a change in resistance. Balasubramanian and his team, however, found a surprisingly easy way to arrange the receptors at a specific location.

The researchers immerse a chip comprising numerous pairs of electrodes that they have already bridged with nanotubes into a solution of one receptor. They then touch one of the electrodes with the tip of an electrical-

Graphic: designergold, based on original material from the MPI for Solid State Research



Step by step to a nanosensor: The nanotubes are first placed between pairs of electrodes and then provided with a receptor that, thanks to the diazonium group (N_2^+), bonds strongly with the carbon nanotube. The receptor may, for instance, be sensitive to sugar. If sugar comes into contact with the sensor, the sensor's electrical conductivity changes.

ly charged metal needle so that electrons flow from the needle into the nanotube and onward into the receptor. The electronic stimulus makes the receptor particularly reactive, and it bonds very easily with the nanotube that provided the charge boost. The researchers then change receptor solution and tailor the next sensor using the same electrochemical method.

MEASURING DEVICES FOR PH AND BLOOD SUGAR LEVEL

However, the Stuttgart-based physicists haven't yet solved all the problems, as the nature of carbon nanotubes puts another hurdle in their way: not all nanotubes are the same! Depending on the angle of the carbon lattice cells relative to the tube axis, the nanotubes act as a metallic conductor or as a semiconductor. Production usually results in a mixture of metallic and semiconducting nanotubes.

While both kinds may, in principle, be used to build sensors, metallic nanotubes require special treatment, as they respond to an analyte only if they enter into strong chemical bonds, or covalent bonds, with their receptors. Balasubramanian's researchers have thus been looking for a method to achieve this.

Electrochemistry again provided the solution, as it makes it possible to control whether electrons are supplied or removed during the reaction between CNT and receptor – that is, whether it is a reduction or an oxidation reaction. "If we provide the receptor with a diazonium salt and have the reaction proceed as a reduction, a covalent bond is formed between the receptor and the metallic CNT," explains Balasubramanian.

Using electrochemical methods, the scientists have already built a nanosensor that measures the pH value of a solution and another that measures blood sugar concentration. The latter serves primarily as a demonstration because easy-to-use test strips for measuring blood sugar levels are already commercially available.

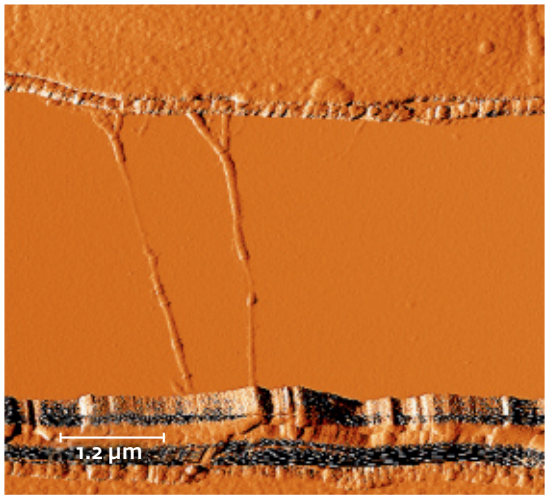
"However, the search is still on for sensors that can monitor blood sugar levels over an extended period," says Balasubramanian. This could then be built into devices that automatically inject insulin as required. "So far, only test systems that work for less than a week are available," explains Balasubramanian. This is because the sensors are based on enzymes, which do not have long-term stability. Nanosensors made from CNTs, on the other hand, last significantly longer. They may thus possibly be suitable for continuous insulin monitoring.

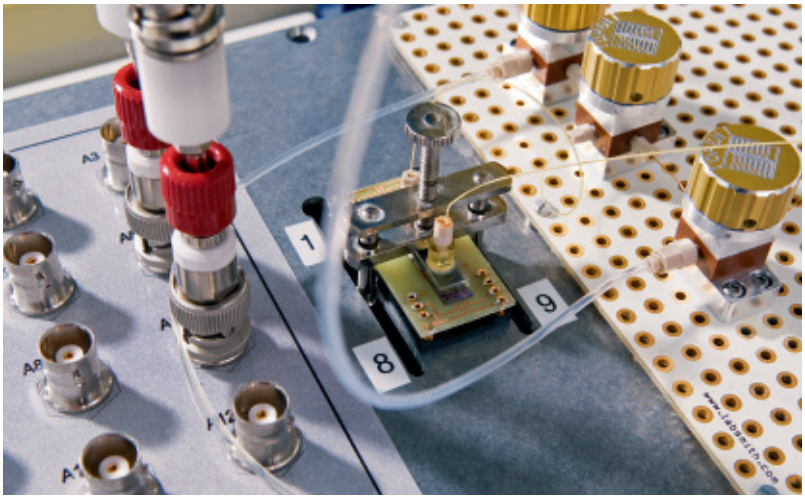
Mass-producing blood testing devices, however, means more than just being able to build laboratory demonstration models. "We wanted to develop our process further so that it is suitable for industrial nanosensor production," says Kannan Balasubramanian. The first step is no problem in this respect. Platinum electrodes can be placed on a silicon wafer using standard chip production methods.

After that, however, the process enters new technical territory: at least one CNT must be located between each pair of electrodes so that there are no dead sensors. Industry expects a process that has the lowest possible reject rate. According to Balasubramanian, "100 percent throughput is absolutely essential for industry."

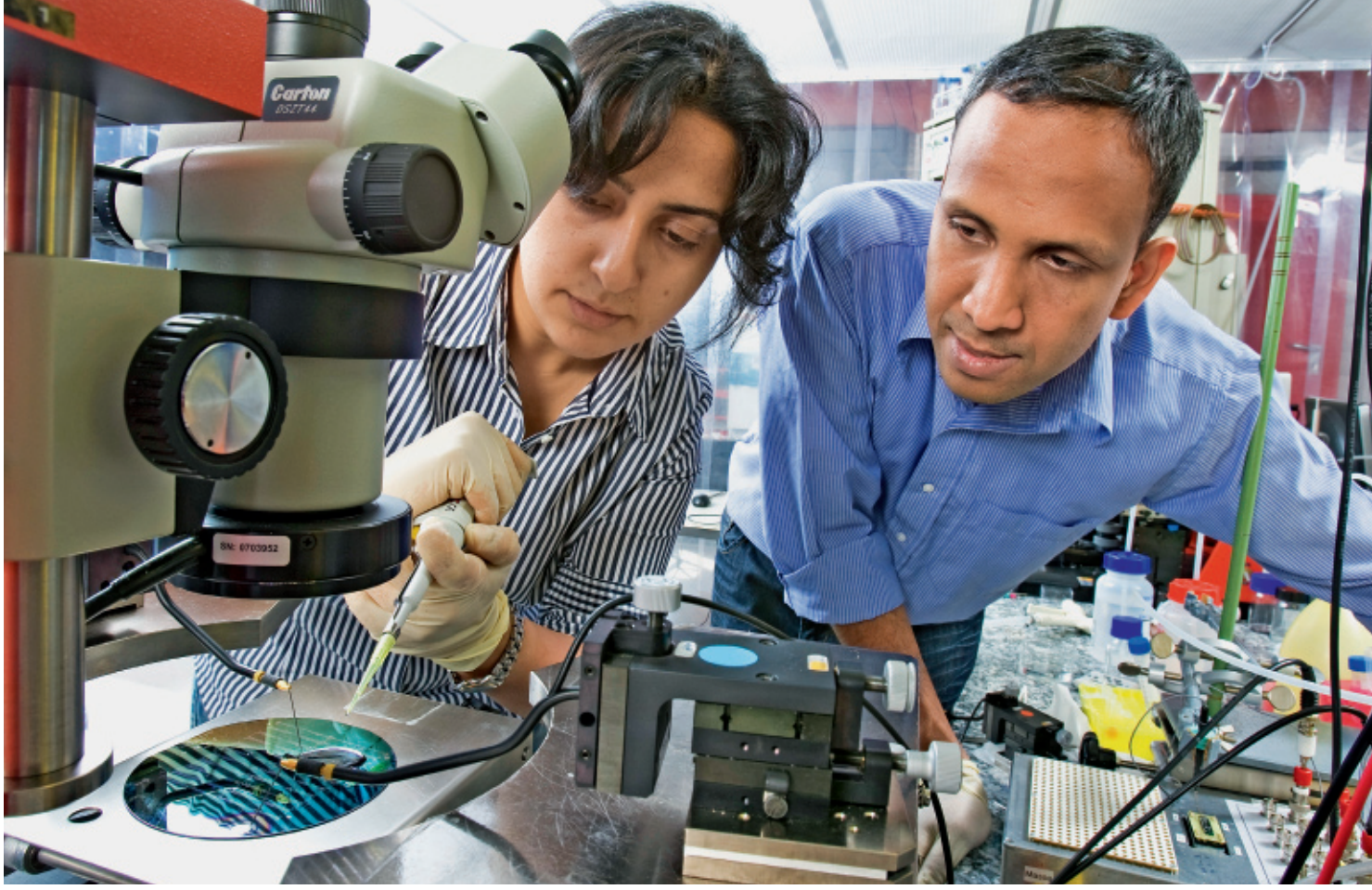
Placing the CNTs is similar to hunting with a shotgun: like a hunter shooting a spray of pellets at the prey and hoping that one will hit its target, the

left | Under the atomic force microscope, the nanotubes (vertical lines) can be seen between the electrodes.
right | Device under test: Sensors are fitted into this apparatus and investigated.





Photos: MPI for Solid State Research (left), Axel Griesch (right)



Nassim Rafiefard and Kannan Balasubramanian trickle a nanotube dispersion onto a wafer so that the nanotubes can then be directed to pairs of electrodes via dielectrophoresis.

researchers try to place nanotubes between the electrodes. The "shot" corresponds to the mixture – a dispersion – of carbon nanotubes and water, while the "gun" the researchers use is a method known as dielectrophoresis. A non-uniform electric field generated in the solution drives the nanotubes toward the electrodes. By chance, some of them are caught between the electrodes in such a way that they exactly fit into the gap.

Since the nanotubes are not uniformly distributed in the dispersion, but instead have a tendency to clump together, not every pair of electrodes receives a nanotube. "Until a short time ago, a throughput of only around 30 to 60 percent could be achieved with dielectrophoresis," says Balasubramanian. In other words, at least one third of the sensors were dead. "But then we found a way of producing a dispersion without nanotube agglomerates," explains the researcher. This allows the nanotubes to be distributed so uniformly that at least one is guaranteed to land between each pair of electrodes.

In this way, 100 percent throughput can routinely be achieved. At present, up to 40 sensors can reliably be produced on a 10 centimeter diameter wafer without any rejects. This means that the nanosensors have made an important step toward mass production.

EACH SENSOR MUST BE INDIVIDUALLY CALIBRATED

However, there is another problem that nanotechnology must address in principle, and for which even the Stuttgart-based researchers have not yet found a perfect solution. Each sensor is unique: the nanotubes are not positioned in a fully controlled manner, as could perhaps be achieved with the arm of a nanorobot. Instead, how and in what numbers they stick between the electrodes is more or less left to chance. The result is an absolute jumble: sensors with different numbers of CNTs, some with a greater or lesser proportion of metallic CNTs and others with a tangle of overlapping or branching CNTs.

"As a result, each sensor has to be individually calibrated," says Balasubramanian. However, this may well be less troublesome than it sounds. "I estimate that it will be feasible to produce a diagnostic device using CNT sensors for around twice to three times the price of today's systems that operate with lasers," says the researcher. Such a device would, on the other hand, be small and highly sensitive, would operate quickly and work with only a little blood. Moreover, it would be easy to operate, as the analysis is based on a simple electrical measurement.

These advantages of devices with nanosensors could be of benefit not only in medical practice, but also in basic research, for instance for testing enzyme activity. This is because conventional methods investigate the effect of a very large number of enzymes in a solution. Using nanosensors, it is now possible to investigate the level of activity of individual enzymes. The conductivity of a carbon nanotube to which an enzyme is joined changes when a molecule attaches itself to the

Photo: Axel Griesch

Mixture with and without lumps: Ashraf Ahmad and his colleagues have found a way to make a fine dispersion of nanotubes (on the right), thus increasing the yield of functioning sensors.

enzyme. "It is thus possible to observe in real time when molecules bind to the enzyme," explains Balasubramanian, "thus making it possible to determine the activity of an individual enzyme."

JUST HOW IS THE SIGNAL PRODUCED?

The Stuttgart-based team nonetheless has some important tasks to tackle before nanosensors can be used in practice. While it has been found that nanosensors function and can be produced in processes under near-industrial conditions, the researchers have not yet gained a detailed understanding of the physical processes that take place during signal generation on the nanotubes. "We need a theoretical model to explain the dependency of the sensor signal on analyte concentration," says Balasubramanian. Only then will it be possible to prove that what is intended to be measured is actually being measured.

Not least, developing the diagnostic devices to market maturity means that the scientists must look beyond their own horizons. "It's not enough to build a nanosensor," says Balasubramanian. "We have to ask ourselves today what system our sensor will be working in tomorrow." And the technology must be flexible, and allow subsequent addition of interfaces.

Turning a vision into reality thus means tying up a lot of loose ends. As long ago as the 1990s, when Kannan Balasubramanian was studying computer science at the Birla Institute of Technology and Science in the northern Indian city of Pilani, he already had a dream: he wanted to work on building a nanocomputer. He was fascinated by the vision of a computer consisting of molecular-scale switches – a computer that would be visible only under a microscope, but that would still have the performance of a personal computer.

That's why he went to Germany and became a physicist at the Max Planck Institute for Solid State Research. Here, he has fine-tuned his original dream so that it can be turned into a reality within a decade. But who knows: perhaps there will be nanocomputers in a few decades. Perhaps they will contain nanosensors so that they can communicate with their environment. And perhaps some of these nanosensors will bear the signature of these Stuttgart-based Max Planck researchers. ◀

GLOSSARY

Superposition
Particles do not adopt a single state, but rather all possible states simultaneously, until measurement destroys the superposition.

Entanglement
Two or more particles form an overall system and measurements made on one particle have an instantaneous effect on the entangled partners, irrespective of how far apart the particles are.

Superconductivity
Below the "transition" temperature, which is usually below minus 260 degrees Celsius, many metals conduct electricity without resistance. Physicists have a very good understanding of this form of superconductivity – but not of the unconventional form of superconductivity. This occurs in, among other things, copper oxide ceramics, the record holder losing its electrical resistance at a temperature as high as minus 110 degrees Celsius.

Superfluidity
This phenomenon was first observed in two isotopes of helium. Quantum mechanical effects result in a liquid or gas flowing without friction.

Photo: Axel Griesch