Nanoparticles give a more reliable diagnosis

A show of lights in the interests of medicine

Dr. Werner Hoheisel plans to use fluorescent powders composed of nanophosphors (inset) to make medical diagnosis more reliable.

Fluorescent dyes frequently serve as an indispensable tool for detecting disease pathogens, diagnosing cancer or carrying out genetic tests. These dyes, which are usually organic substances, do not always provide a diagnosis that is both reliable and quick. This is why researchers have been trying for many years to come up with alternatives. Bayer scientists have now found what they were looking for in the nanocosmos: nanophosphors are in many ways far superior to the dyes used up to now.

Subjecting the nanophosphors (shown here is a dispersion) to special UV light causes them to fluoresce in different colors.

Before viruses and bacteria can be effectively controlled in the human body, they have to be made visible. Unfortunately, it is not possible to simply daub them with paint. Instead, special diagnostic tests are carried out in the laboratory using fluorescent dyes. When these substances are stimulated by light of a particular wavelength, they reemit the absorbed energy in the form of light of a different wavelength. For example, with the help of fluorescent dyes, experts can spot antibodies to certain disease-causing agents in a patient's blood. This process involves adding special test antibodies to a blood sample. If the blood really does contain the suspected pathogens, the test antibodies latch onto these proteins. And since the test antibodies have been marked with special fluorescent dyes, doctors can measure the fluorescent light to establish the quantity of the specific proteins in the patient's blood sample. Fluorescent dyes are also being increasingly used in a DNA analysis test called polymerase chain reaction (PCR) to allow quicker and more reliable diagnosis of a range of conditions, from viral infectious diseases and cancer to genetic defects. Last but not least, many future biochips will also be based on fluorescent dyes, allowing them to identify and quantify antibodies, hormones, genetic material and the like.

"These fluorescent tubes contain the material we are using for diagnostics research," says Dr. Werner Hoheisel, Project Manager in the Biophysics Competence Center at Bayer Technology Services in Leverkusen, pointing at the ceiling. Phosphors, which light up offices across the globe, may soon be taking up residence in medical diagnostics laboratories. The Diagnostics Division at Bayer HealthCare aims to tap into their fluorescent properties for use in tests, for example to detect cancer at the earliest possible stage.

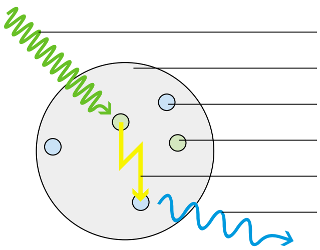
Nanophosphors as mini-lights for blood samples

Experts discovered the new method by exploring the nanocosmos. They came across what are known as nanophosphors, a miniature form of the inorganic fluorescent phosphors used in lighting. The new "mini-lights", measuring no more than a few nanometers, may one day allow samples of blood, saliva, urine and hair to be analyzed more quickly and more reliably in the laboratory. Although doctors are already using fluorescent dyes for laboratory tests, these molecules are organic substances and come with a number of drawbacks. One problem is that they soon fade, which makes it difficult to archive tissue samples, for example. They also show no clear difference between the stimulating light and the emitted light, which leads to deceptive overlaps when several dyes are used at the same time. This is because the emission light of one dye has the same wavelength as the stimulating light of another dye. An evaluation is often impossible under these

Fluorescence of organic dyes

Organic fluorescent dyes, such as fluorescein or rhodamine, are composed of several aromatic rings. These molecular structures are stimulated by light of a certain wavelength: what happens is that electrons are elevated from their normal state to an unstable level of energy – comparable with water being pumped from a lower reservoir into a higher one. If the electrons fall back down, the previously "stored" energy is released in the form of light – in the same way that a cascade of water can drive a turbine and generate electricity. The number of times these cycles of stimulation and emission can be repeated depends on the dye in question. If the molecule is destroyed or the electron is captured in a stable intermediate state, the cycle may be broken, in which case even the best organic dyes will fade after a relatively short period of time.

Fluorescence of nanophosphors
The molecular lattice of phosphors contains individual embedded lanthanide ions, like europium or terbium. The crystal lattice – or sometimes "activator ions" such as cerium ions used especially for this purpose – absorbs the stimulating light and transfers the energy to the lanthanide ions, which are the true source of fluorescence. The color emitted depends mainly on the lanthanide ions used. Terbium, for example, gives off a yellowish green color, while europium produces a red fluorescence. As shown by the "microparticles" in fluorescent lights, the cycle of stimulation and emission can be endlessly repeated, which means that the dye never fades.



Nanoparticles < Viruses

Dr. Karlheinz Hildenbrand (left) and Helmut Kruells show the phosphors they successfully encapsulated by means of gel electrophoresis.

circumstances since it is very difficult to distinguish between the two types of light.
This is why, for the last ten years or so, scientists around the world have been investigating inorganic fluorescent nanoparticles as an alternative. Initially they focused on semiconductor materials measuring no more than ten nanometers. This set the ball rolling in a quest that could ultimately revolutionize detection methods in medical diagnostics.
"One characteristic of such tiny semiconductor nanoparticles is that the wavelength of the fluorescent light they emit depends not only on the material but also on the size of the particle," explains Werner Hoheisel. Since they can all be stimulated at one and the same wavelength in the UV range, various antibodies or genetic material can be detected in a sample at the same time without any problems of overlaps between the stimulating and emission lights. "This multiplex ability would make diagnostic tests quicker and cheaper and would also require less sampling material," explains Werner Hoheisel.

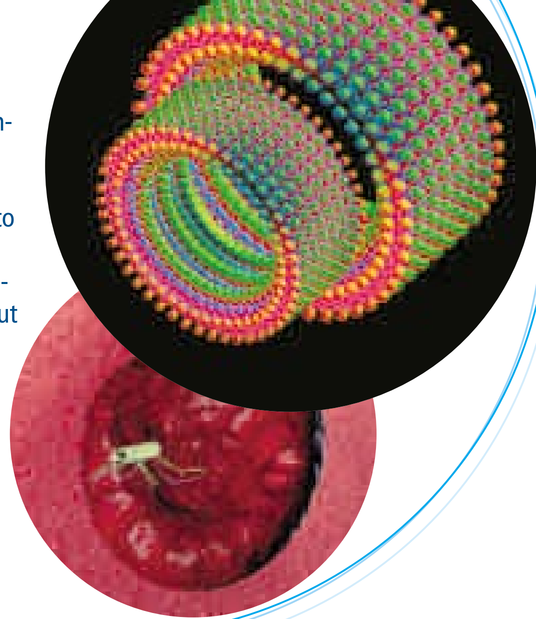
Lanthanide ions determine the wavelenth of the fluorescence

The synthesis of semiconductor nanoparticles, also known as quantum dots, is quite a costly and complex process, and the heavy metals they often contain are not very environmentally friendly. This is why Bayer researchers are pinning their hopes on a new class of materials: nanophosphors. Phosphors are chemical compounds which are made up of substances like silicates, oxides, sulfates or phosphates and whose molecular lattice contains embedded lanthanide ions. "Nanophosphors have a lot of potential: they have many of the advantages of quantum dots and fewer disadvantages," says Hoheisel. And he should know: before he joined Bayer, he worked as a postdoctoral researcher at the University of California in Berkeley where he helped to develop quantum dots. Nanophosphors hardly fade and can also be used for multiplex or multipurpose testing. And the major advantage they have over quantum dots is that the wavelength of their emitted light does not depend on particle size but on the type of lanthanide ions used. For this reason, their particle size, which is also no more than ten

Tiny detectives

The diameter of nanophosphors is one hundred times smaller than that of their "cousins" in fluorescent lighting. This is why they are much more suitable for detecting biological structures.

	Dimensions (diameter) in nanometers (nm)
DNA	2.5
Protein	5
Antibody	10
Phosphor in fluorescent lighting	1,000
Nanophosphor	10



Nanogears
In the future, nanomachinery with gears (above) made out of single molecules could help doctors to treat patients. They could even be used to expel diseased blood corpuscles out of the blood stream (below).

nanometers, does not need to be monitored so precisely. As a result, the manufacturing process is simpler and less expensive. Moreover, most ions of lanthanides, also called rare earths, are considered less harmful to the environment, and this facilitates their manufacture and disposal.
"In addition, the new dye boasts another advantage which ultimately improves detection," says Hoheisel. A lot of the biological components of cells are themselves slightly fluorescent, in other words they give off light without prior marking. This effect, also known as background fluorescence, makes it difficult to interpret the signal, for example the positive result of a diagnostic test for cancer. Nanophosphors are able to get round this problem because for many types of nanophosphor, the life span of the fluorescence – in other words the time between stimulation and emission – extends to several milliseconds. Accordingly, when the nanophosphor is exposed to a brief impulse of light, the background fluorescence disappears before the test result is displayed. This considerably enhances the sensitivity of the fluorescent marker in its various applications. Another important advantage of the nanophosphor system, particularly where medical diagnostics are concerned, is its ability to transfer fluorescent energy to a closely related dye. This allows biochemical reactions, like the coupling between antibodies, to be detected without the need for any additional procedures. So the relevant antibodies in the patient's sample can be detected immediately after the dye has been added to the test solution.
The interdisciplinary research team of physicists, chemists and biologists has already initiated the next step in the development process: coupling with biomolecules. Before the nanophosphors can be used to track down certain segments of DNA, for example in future cancer tests, they themselves need to be attached to suitable DNA segments. "This is one of the most difficult tasks in the project. It is always a major challenge to achieve stable coupling of small organic molecules or larger biomolecules with unique, inorganic nanoparticles. After all, the particles have to be painstakingly adapted to the properties of the organic molecules and prevented from lumping together themselves in the process. If we succeed," declares Werner Hoheisel, "then nanophosphors will give us a very sound technological basis for meeting the demanding challenges of medical diagnostics in the future."

www

www.accessexcellence.org/AB/IE/PCR_Xeroxing_DNA.html
This website contains further information on the topic of polymerase chain reactions.

Smooth operators

Nanoparticles also provide indispensable assistance when it comes to manufacturing storage chips. In the production of these highly sensitive components, a smooth surface is absolutely vital. Modern storage chips consist of up to 20 layers, whereby conductive tracks and insulating layers alternate with each other. Every one of the tracks is applied and processed individually. Any unevenness quickly adds up and can cause short-circuiting, so each layer must be polished absolutely smooth before the next one is applied. And for Dr. Arno Nennemann from Bayer Technology Services in Leverkusen, smooth means "eroding unevenness of less than one millionth of a millimeter." He and his team got together with members of the research department at Bayer subsidiary H.C. Starck to develop fine polishing pastes containing nanoparticles of silicon dioxide measuring between 5 and 50 nanometers. Their hardness can be varied through the addition of individual molecules such as aluminum oxide or by coating the particle surface with organic compounds. Both the size and the hardness of the particles govern the rate of abrasion and the roughness – or smoothness – of the polished layer.

Finely polished finish: Miniaturized polishing cell for smoothing the surface of the wafers.