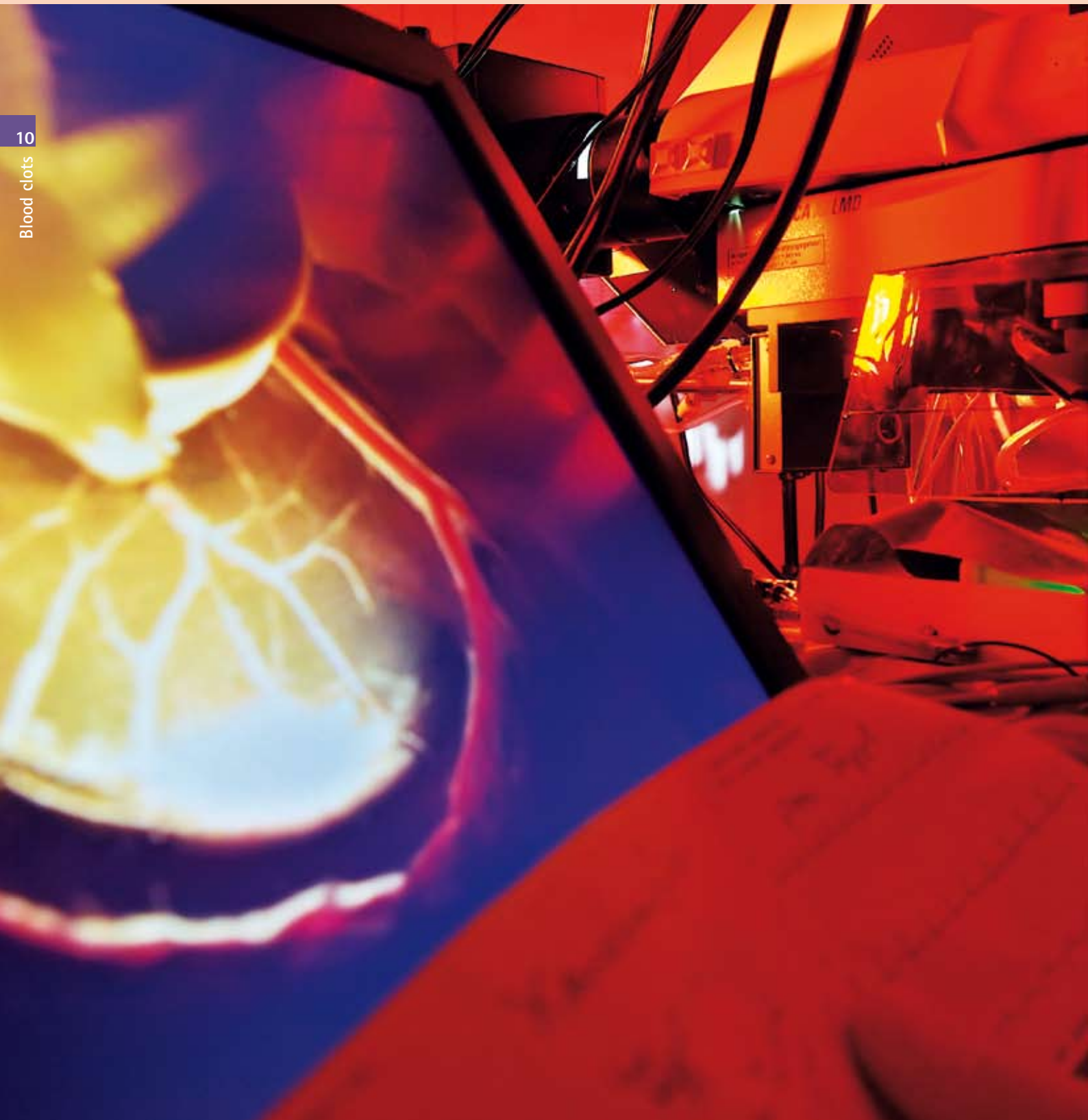
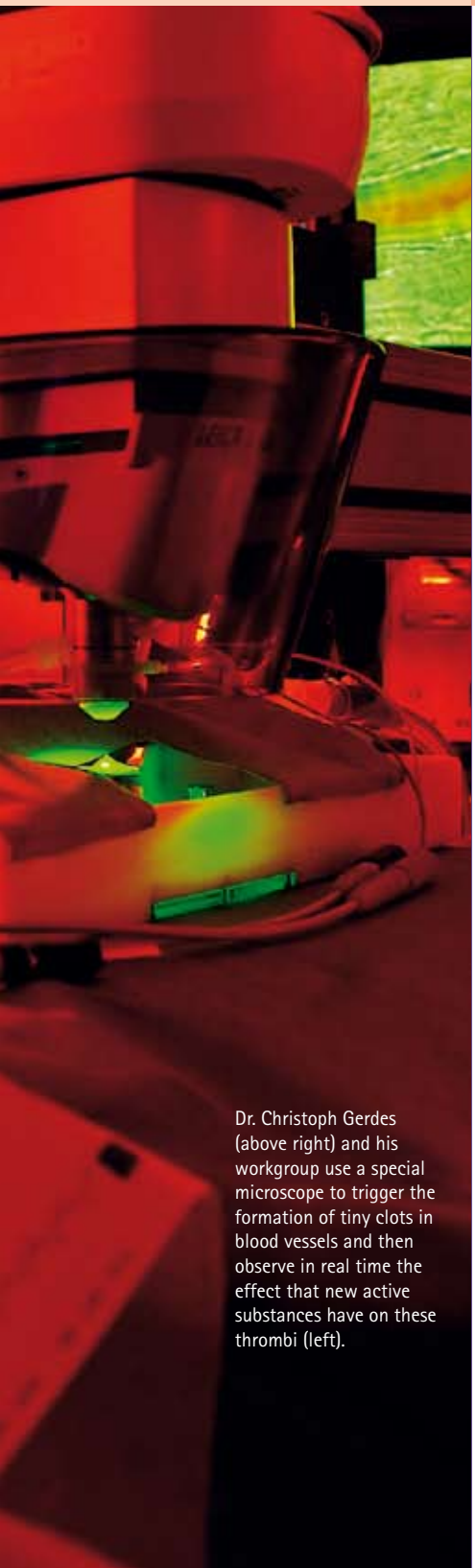
A new microscope lets scientists see the vascular system in fine detail

Thrombosis live



Arteriosclerosis is one of the most dangerous lifestyle diseases. In their quest for new medicines, scientists have to simulate the symptoms of the disease in animals. This is no easy task. Using a newly developed laser microscope, Bayer's experts can deliberately cause occlusions in blood vessels, monitor them live and test active substances.



Dr. Christoph Gerdes (above right) and his workgroup use a special microscope to trigger the formation of tiny clots in blood vessels and then observe in real time the effect that new active substances have on these thrombi (left).

Sometimes a successful invention combines two existing techniques: light bulbs, in which filaments in a vacuum tube glow when electricity passes through them, are a good example of this. Two scientists working for Bayer HealthCare have now exploited this principle: they managed to combine two types of microscopes into one, creating a brand new tool for research into vascular diseases: the intravital laser dissection microscope.

"The intravital microscope was a standard fixture of our laboratory. It is used to observe processes in blood vessels or other internal organs as they happen and is a key aid to research into new medicines," explains Dr. Christoph Gerdes, a veterinarian who plans and conducts disease simulation experiments on animals in Bayer HealthCare's cardiovascular research unit in Wuppertal, Germany.

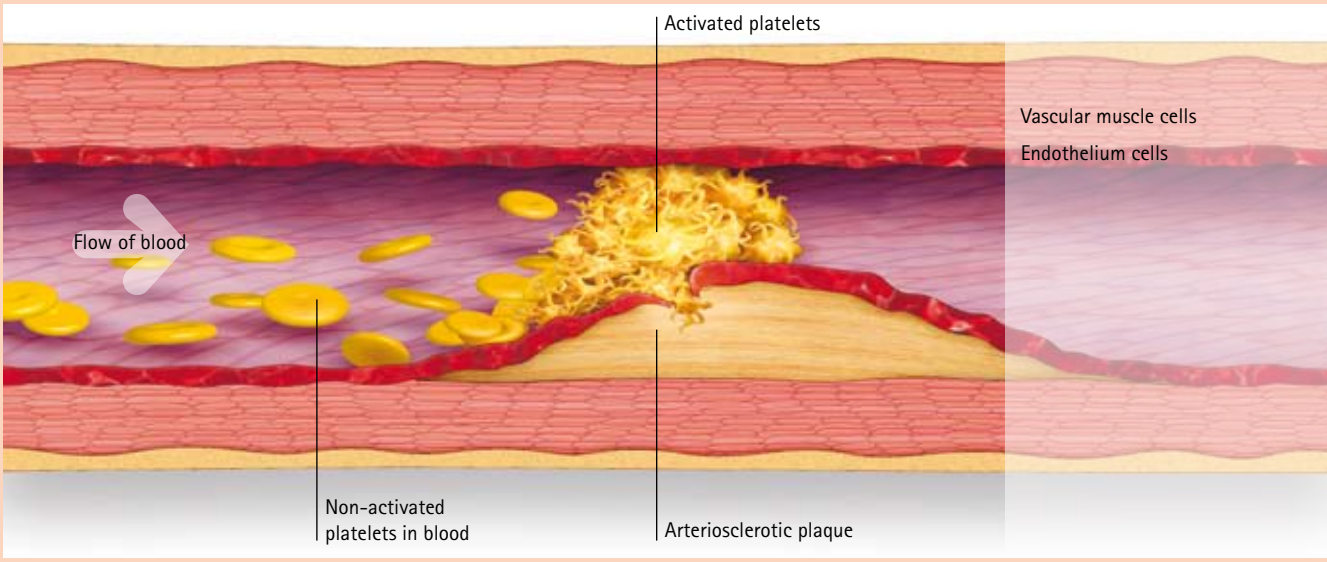
"Also of importance to our work are methods used to produce a blood clot (thrombus) in a blood vessel," continues the scientist. "This is the only way we have of testing the effect of our substances." Lasers, some more accurate than others, have also been used for this purpose for a few years. While seeking possibilities for optimizing existing methods, Dr. Gerdes came across the Leica laser microdissection microscope in spring 2003. However, this device was being used in a completely different field, for example in molecular pathology to isolate a small number of cells from an ultra-thin slice of tissue for closer inspection, as when tumor cells are taken from surrounding healthy tissue. These devices were not made for use on living tissue. The scientist was nevertheless inspired to combine the laser dissection microscope with the intravital microscope so that the accurate laser could be used for thrombus induction.

Two high-tech microscopes combined

Dr. Gerdes discussed the idea with his colleague, the biology laboratory technician Manfred Schumacher. They decided to entrust the task to a specialist department of Leica Microsystems in Wetzlar, Germany. As Schumacher says, "We needed real specialists for this." They soon found their man: Wolfgang Pauly, a precision engineer who had been making special orders for the company for many years. The combination microscope was ready to use a few months later.

A few technical innovations were incorporated into the design, such as a motor to raise and lower the microscope workbench a few millimeters. "We just used a power windows motor, like you get in cars," says Pauly. The base on which the apparatus stands was also strengthened. This was done to prevent shocks, which would be very

Arterial thrombosis



Arteriosclerosis is a condition in which blood fats and white blood cells accumulate in inflamed vessel walls and cause them to thicken (plaque). The thin layer coating the vessel (endothelium) is torn at one point, allowing blood platelets (yellow) to come into direct contact with tissue factors. This immediately causes the platelets to activate, change their form and form a clot – a thrombus – which blocks the vessel.

New active substance Rivaroxaban

The active substance rivaroxaban is a novel, oral, direct Factor Xa inhibitor that reduces the risk of life-threatening thromboembolic events. It is intended for the treatment of deep vein thrombosis and for secondary prevention. Rivaroxaban inhibits a key enzyme in the clotting cascade, Factor Xa. This is a target enzyme that acts at the critical point in the clotting cascade, the process that leads to the formation of a clot. Results so far published show that rivaroxaban inhibits clotting reliably and predictably. The data also show that rivaroxaban does not interact with a number of drugs that are usually administered alongside an anticoagulant. In view of these good research results, a program of Phase III studies has been initiated.

Rivaroxaban is being developed jointly by Bayer HealthCare and Ortho-McNeil Pharmaceuticals Inc., a subsidiary of Johnson and Johnson.

damaging to the operation of the nitrogen laser. But precise preparation of the sample tissue was only possible thanks to the precision engineering work done by Hartmut Osterloh and Dirk Kohrsmeier in the Wuppertal pharmaceutical center's workshop.

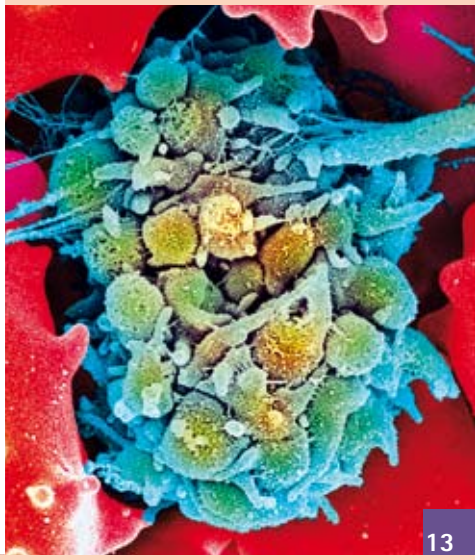
Tiny arterial lesions simulate vascular disease

This is because the lesions which the scientists wanted to create in the blood vessels of the animal models in order to produce blood clots are very fine. "We can home in on individual cells in the endothelium, the innermost layer of the blood vessel," explains Dr. Gerdes. "This shows us very much what the conditions are like in a patient's artery when an arteriosclerotic plaque breaks off and a blood clot forms at the site." Such plaques form in human vessels as a result of abnormal deposits of blood fats and white blood cells in the vessel wall.

And the blood vessels that Dr. Gerdes' team is studying are not exactly thick. They are fine arterioles from the transparent abdominal fur of a rodent. These vessels are 20 to 50 micrometers in diameter: as Schumacher says, "about a third as thick as a human hair."

Using the intravital microscope, scientists are able to see inside the blood vessel and can watch as a clot develops and then generally breaks down again before their eyes. They can also test conventional drugs as a positive control to see how they affect this process. "We can even use one blood vessel both for the test and as a control," says Dr. Gerdes. "First we do the test without the trial substance; that's the control. Then a few micrometers further on we cause a new injury and this time we use the substance we are investigating to affect clot formation." This kind of approach helps to reduce use of lab animals.

"We basically try to use animal experiments as little as possible for the purposes of our research," explains Dr. Gerdes. However, animal testing remains essential to prove that new substances are safe and effective. This



Thrombosis on film: Manfred Schumacher (left) and Dr. Christoph Gerdes discuss a video recording of the development of a thrombosis they triggered with the new Bayer microscope. At the moment, the growing thrombus (grainy bulge) has not yet occluded the blood vessel, so that the red blood cells transported by the flow of blood appear as reddish streaks. The photo on the right shows a thrombus is it appears when viewed with an electron microscope.

is because the complex interactions between organ systems in the body and the physiological and pathological effects of drugs can only be studied in a living organism. "That is why animal testing is not only sensible and necessary from a scientific point of view, but also required by law," continues Dr. Gerdes. "Potential ingredients must be shown to be safe and effective in animals before testing them on humans is permitted and morally justifiable."

The scientists can not only watch the artificially induced blood clots growing and shrinking in minutes before their eyes, they can film the whole process as well. A DVD is used to record events. "Specially designed software can be used to assess various quantitative parameters, like the thickness of the clot, the rate at which blood flows, the passage of time and so on."

New microscope proves itself in active substance research

The new microscope, in use at Bayer HealthCare in Wuppertal since early 2004, has already proven to be a valuable addition to the investigators' toolkit. The very promising new substance rivaroxaban, which is thought to protect against venous clots, was examined under the intravital laser dissection microscope to validate the experimental system (see text box).

The experts working for the firms involved have received recognition for their efforts. Dr. Christoph Gerdes and Manfred Schumacher from Bayer HealthCare, along with their colleague Ralf Vetter, won Bayer's internal pharmaceutical research prize. And Wolfgang Pauly of Leica Microsystems has now made two more intravital laser dissection microscopes, as cardiovascular scientists in Würzburg and Basel also wanted one. Only one problem still has to be resolved: thinking up a catchier name for this handy device.

www.animalstudies.bayer.com
This website provides comprehensive information on the use of animal studies at Bayer.