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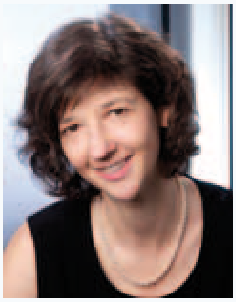
reSOLUTION

Metallography with Color and Contrast
The Possibilities of Microstructural Contrasting

Genuine or Fake?
Stereomicroscopy Exposes Counterfeiters

Vertical Resolution – Small Steps, Big Effect
3D Visualization of Surface Structures

EDITORIAL



Dear Readers,

The fact that we see different colors is purely because of physical laws concerning the absorption and reflection of different wavelengths at objects. Light itself has no color, as Isaac Newton discovered as long ago as 1730 ("The rays are not colored"). The sensation of "color" is triggered as light stimuli are processed by our eyes and brain. Why evolution taught us to see in color is still a point of contention among experts today. One of the key functions of color is that it organizes our perception and helps us to identify and differentiate objects. This effect plays a major role in light microscopy, too. The very first article in this issue vividly explains how color is used in metallography in the form of different contrasting techniques and color etching to give microscope images extra informative value.

Forensic scientists inspecting the authenticity of printing dyes in a document have quite a different view of color than metallographists. Restorers, on the other hand, are on the lookout for the tiniest traces of paint on antique sculptures to prove how magnificently colored the everyday life of the Greeks and Romans must have been.

Naturally, this issue of reSOLUTION also features new developments in microscopy technology, such as surface analysis with digital microscopes, high-definition imaging and microscopes for wafer inspection.

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Communications & Corporate Identity

Carola Troll
European Marketing Manager Industry

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The Possibilities of Microstructural Contrasting

Metallography with Color and Contrast

Ursula Christian and Professor Norbert Jost, University of Pforzheim

The examination of microstructure morphology plays a decisive role in materials science and failure analysis. There are many possibilities of visualizing the real structures of materials in the light microscope. The image samples shown in this article demonstrate the information potential of some of the techniques used.

A clean section

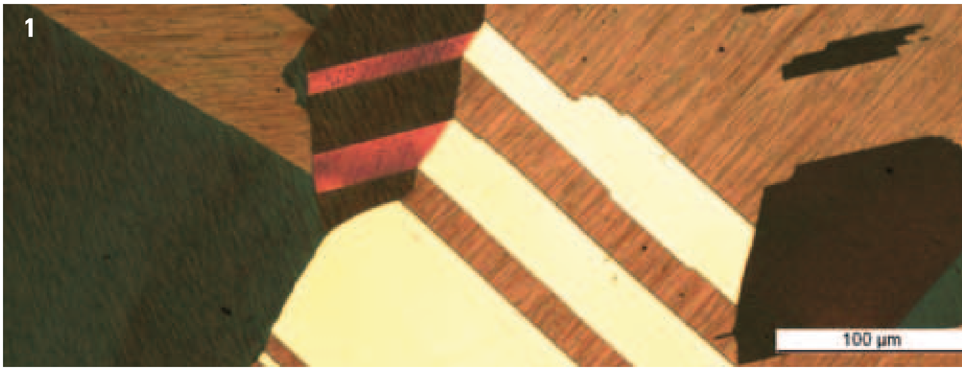
The first step is always to produce a polished metallographic section. However, the preparation of the real microstructure is only successful if the surface of the sample is completely clean and deformation-free. After the section has been produced it is normally immediately etched in acids, lyes or salt solutions to develop the microstructure. This attacks the grain boundaries or roughens certain grain and phase areas which then appear dark in brightfield.

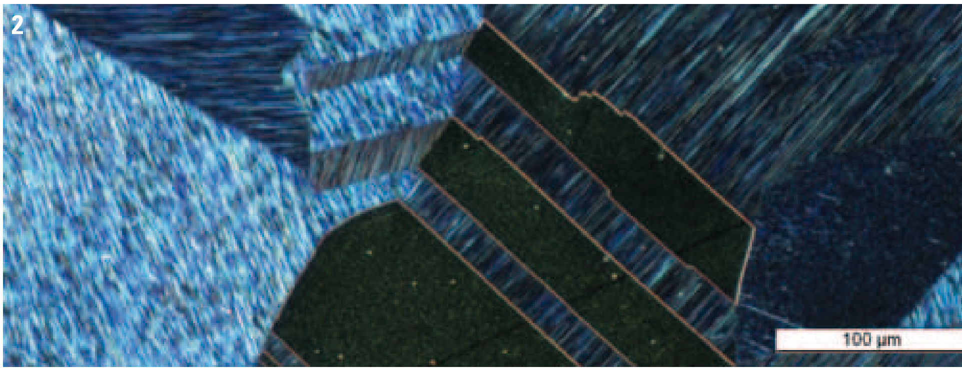
Combining the right methods

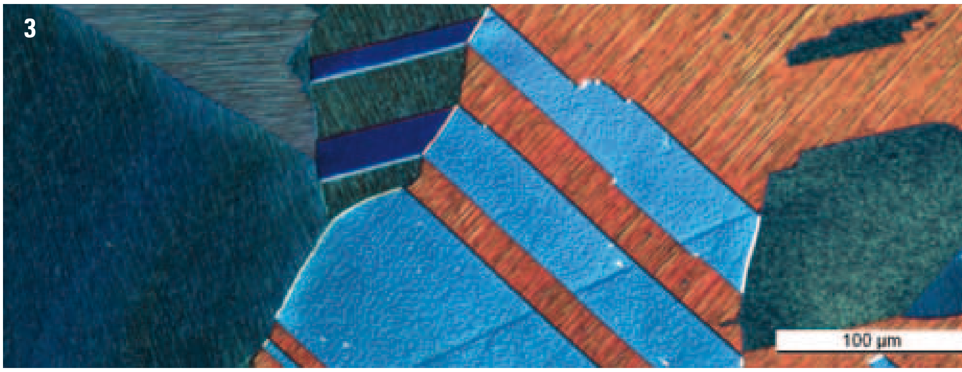
If these techniques are not sufficient to allow a full examination, if the etching result does not meet the specifications or if the material is etch-resistant, either color etching or other light microscopic techniques such as polarization, darkfield and interference contrast are used. Often, it takes a combination of color etching and optical contrasting to get the best results. The great variety of possible imaging techniques is shown by the photos of the same detail of a copper alloy sample (Figs. 1–6).

Figures 7 to 12 show different ways of contrasting microstructural constituents in different materials. The color etching technique applied here caused the formation of sulphate layers of different thicknesses on the grain or mixed crystal areas.

The sections were etched in Klemm (K) or Beraha (B) etchants, which are tint etches based on potassium sulphite. The composition is given in "Metallographisches, keramographisches, plastographisches







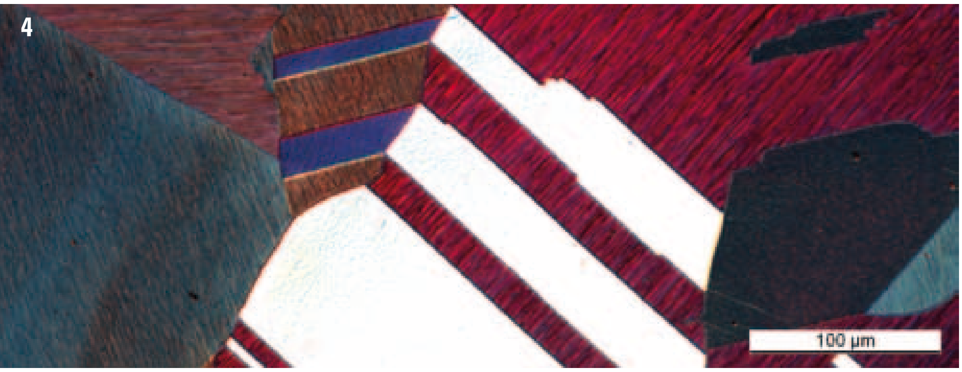
Figs. 1–3: Images of a face-centered cubic (fcc) lattice of a copper alloy using different contrasting techniques, 1: brightfield, 2: darkfield, 3: interference.

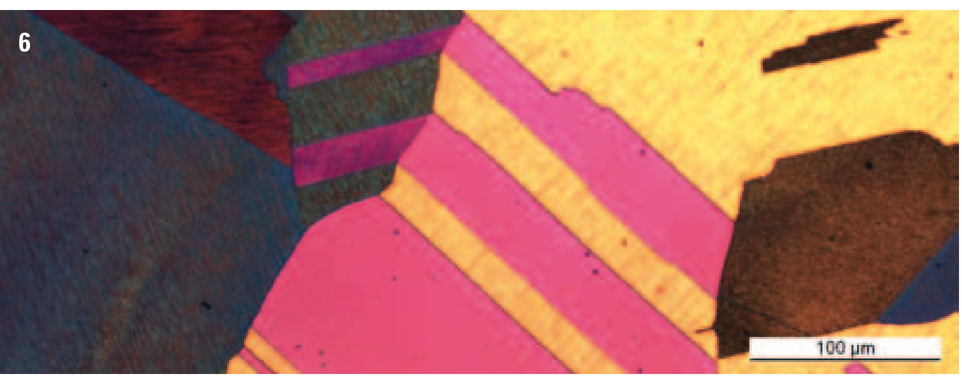
Ätzen” by Günter Petzow and Veronika Carle, published by Borntraeger, 2006. In Figs. 7 and 8, the ferrite in the steel is colored, while the iron carbide is kept white to achieve a clear contrast of the carbide precipitations. Weld layers of austenitic steel are shown in Figs. 9 and 10. The images highlight not only the cast structure, but also the segregation and heat-affected zones. Fig. 11 also shows a segregation in a tin bronze sample due to incipient melting. Fig. 12 is a good example of how such an etching can even be used to visualize subgrain formation.

Polarization with and without color etching

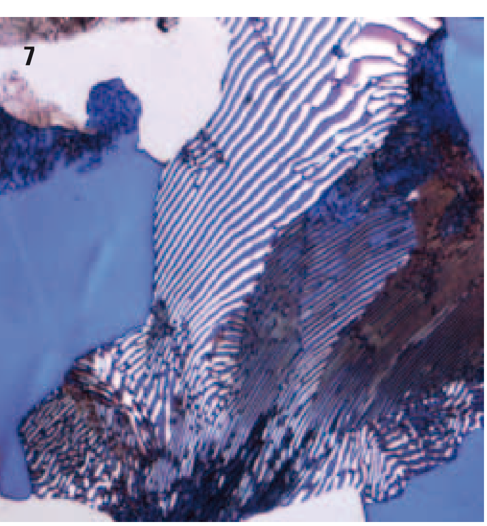
Color contrast and specific microstructure formations can frequently be enhanced by optical polarization of the etched samples under the microscope. In Figs. 13–18, this method is used to highlight different deformation mechanisms (mainly induced in the manufacture of semi-finished products or components) and ensuing specific deformation structures in the material microstructure.

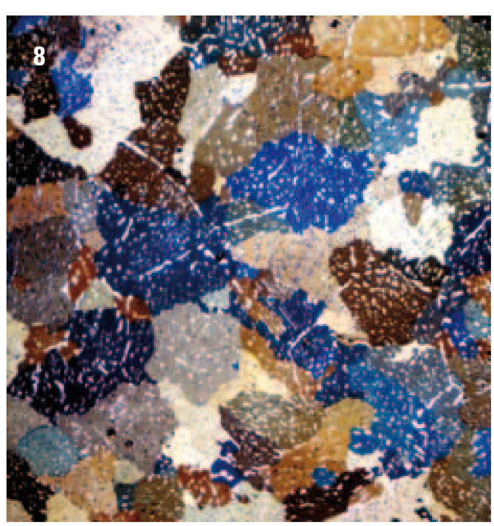
The examination of the sample in polarized light is also often helpful in cases where color etching fails to provide the desired contrast of individual microstructure components, or if only one phase is attacked in composite materials. Examples are shown in Figs. 19 to 21. Fig. 19 shows a much better image of the grain and twin structure in a 10 cent coin made of Nordic gold, while in Fig. 20, individual crystals and their needle structure can be seen in tungsten carbide. Fig. 21 shows the amount, size and shape of graphite fibers in black, carbon fiber reinforced plastic. If documentation is required of the different components of a composite material, additional optical contrasting is usually essential. Fig. 22 documents the excellent result that can be achieved by optical imaging of

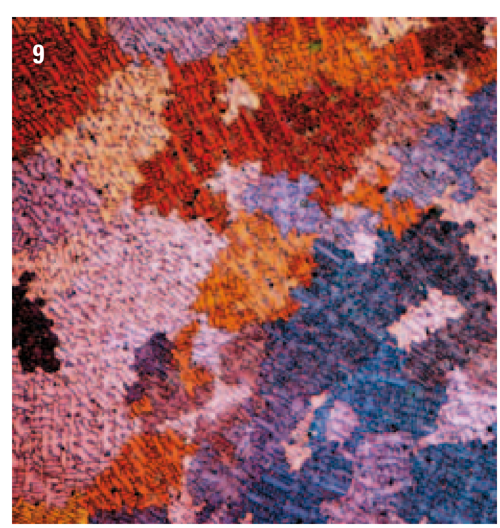




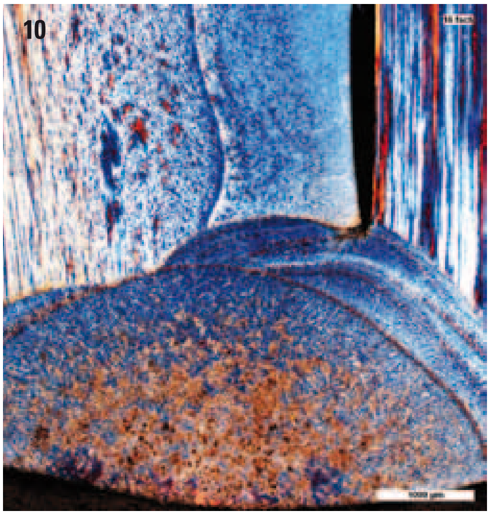
Figs. 4–6: Images of an fcc lattice of a copper alloy in polarized light and from different angles.

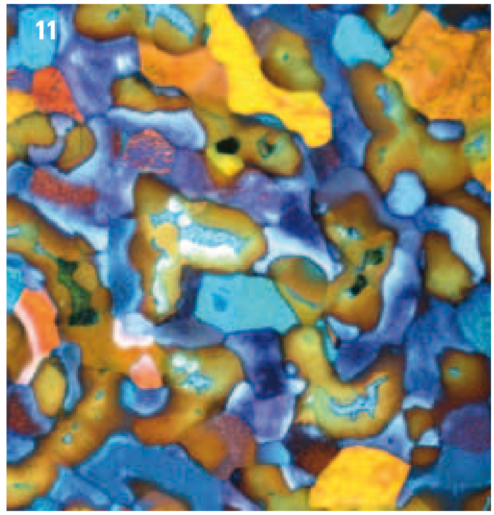


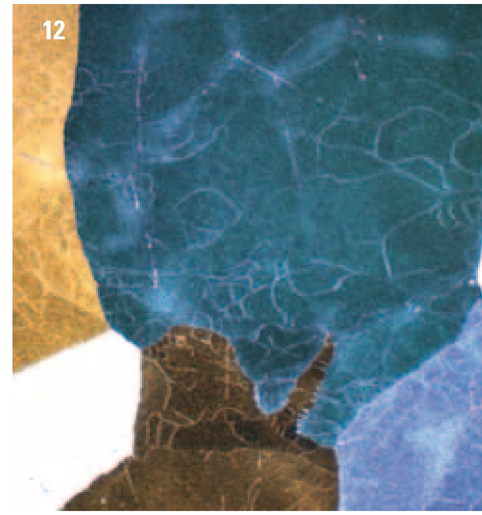




Figs. 7–9: Color etching of various grain or mixed-crystal areas and sulphate layers of different thicknesses. 7: Ferrite-perlite microstructure, the ferrite is tinted while Fe_3C is kept white, Klemm (K) etch, 8: This contrasting visualizes the quality of a soft annealing (K), 9: Microstructure of an austenitic cast produced by laser treatment, Beraha (B) etch.







Figs. 10–12: Color etching of various grain or mixed-crystal areas and sulphate layers of different thicknesses. 10: Laser welding connection of various austenitic steel wires (B), 11: Concentration differences in a bronze wire (K), 12: Grain area etching and subgrain formation in a tin rod (K).

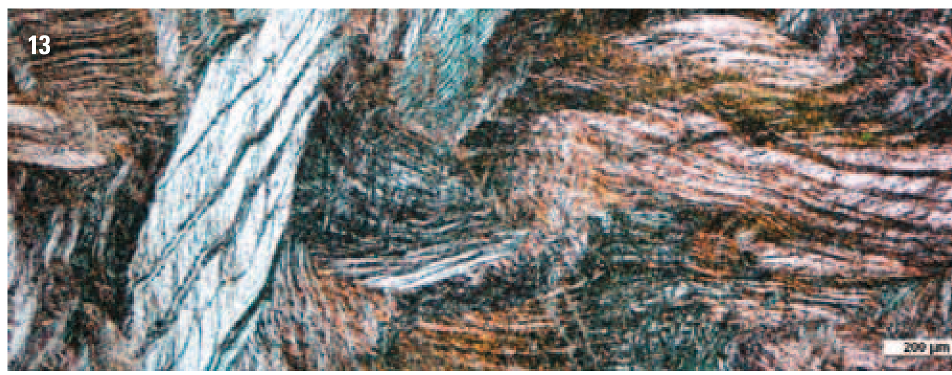
the microstructure of special brass and, at the same time, the glass fiber braid coating. In the photo of a severed capacitor, the glass fiber core can be seen in its thin copper sleeve welded onto a conductor track of tin bronze (Fig. 23). The last photo of this series shows an anti-wear sintered layer of tin bronze with graphite components and ceramic particles (Fig. 24).

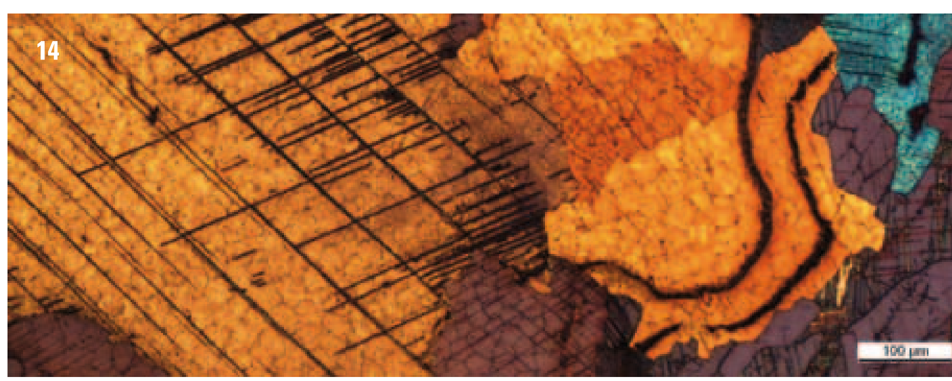
These examples clearly demonstrate that the distribution and formation of different phases are of great, if not overriding, significance for the properties of the material. This is why clear differentiation with the method presented here is particularly important.

Contrasting with interference

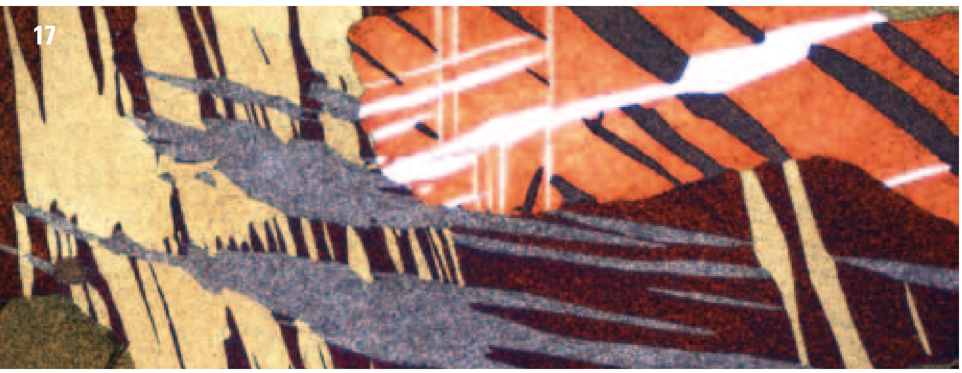
Figs. 25 to 28 show that a microstructure that has already developed due to etching reveals an additional dimension when imaged in interference contrast. This is particularly noticeable in the cast brass wire shown below (Fig. 27), where the crystal structure and also the dendritic solidification typical of a cast can be seen in far greater detail.

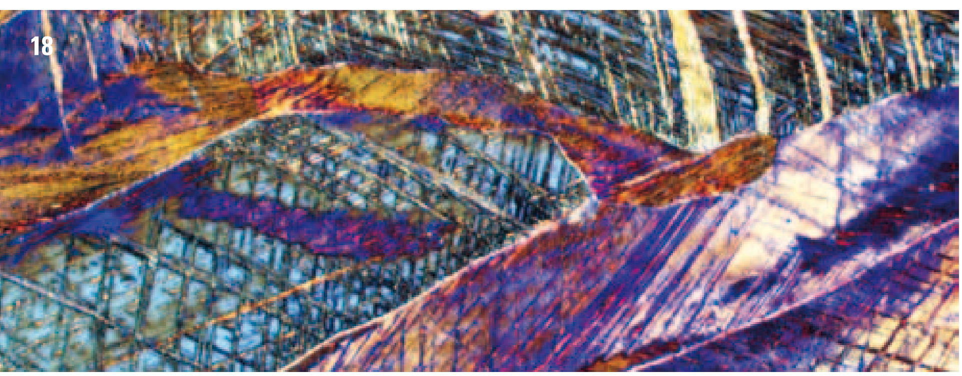
Figs. 29 to 31 are further impressive examples of the potential of interference contrast imaging. Fig. 29 shows the material behavior of tin, in which sudden stress leads to new grain formation and crystalline Umklapp processes due to twinning. Fig. 30 clearly shows the orientation of slip bands in the grain microstructure in accordance with the grain orientation. This technique can be used for most etch-resistant hard metals to obtain a much better image of spherolithic carbides with their secondary adhesions – here embedded in a two-phase nickel-based alloy (Fig. 31).





Figs. 13–15: Polarization with and without color etching. 13: Niobium, cold-formed (B), 14: Cobalt, cold-rolled (B), 15: Zinc with twinning due to dynamic deformation (K).





Figs. 16–18: Polarization with and without color etching. 16: SnPb tin solder, twinning shows deformation at the solder point (K), 17: Sn dynamically deformed, the pronounced formation of deformation twinning is a sign of a dynamic load (K), 18: Slip bands due to pronounced deformation on a CuZn wire with fcc lattice (K).

Combinations of extremely different substances in one material are illustrated in Figs. 32–34. Fig. 32 shows a silver solder ceramic/copper connection. Fig. 33 depicts a composite of glass-plastic layer and glass fiber braid coating stuck to a ceramic substrate. A cross-section of an electronic component can be seen in Fig. 34, with the glass fiber-reinforced plastic on one side of the copper conductor and the ceramic structure on the other.

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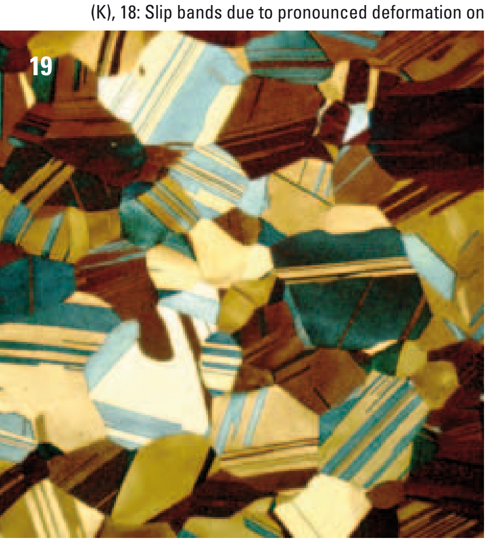
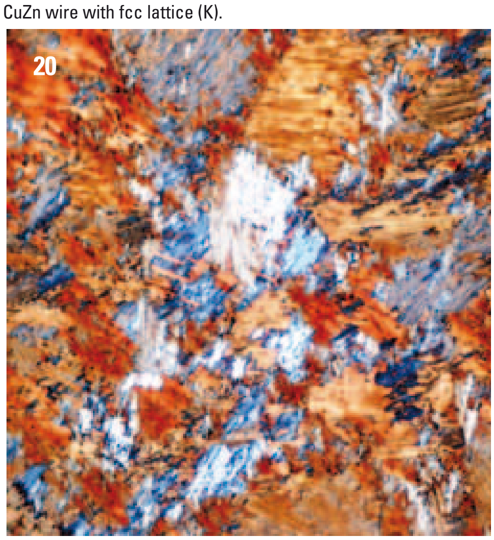
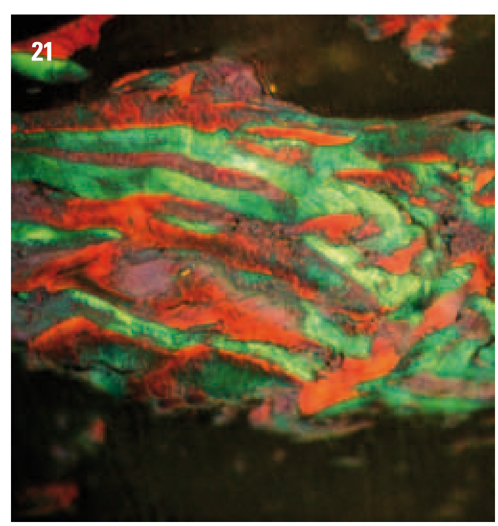
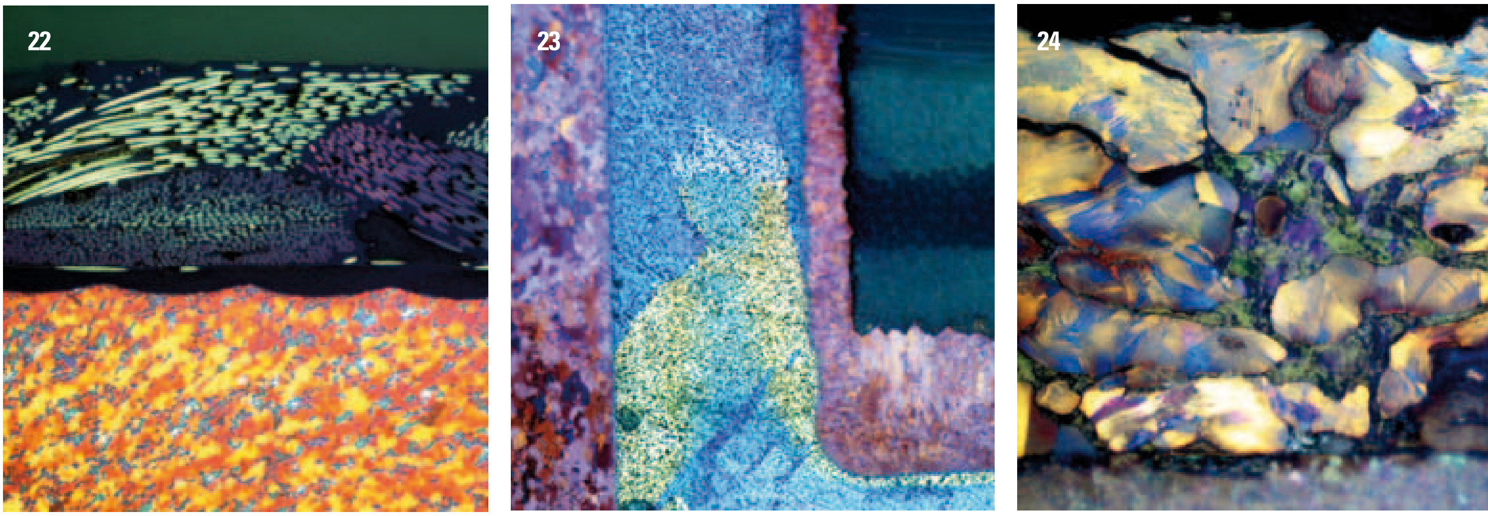


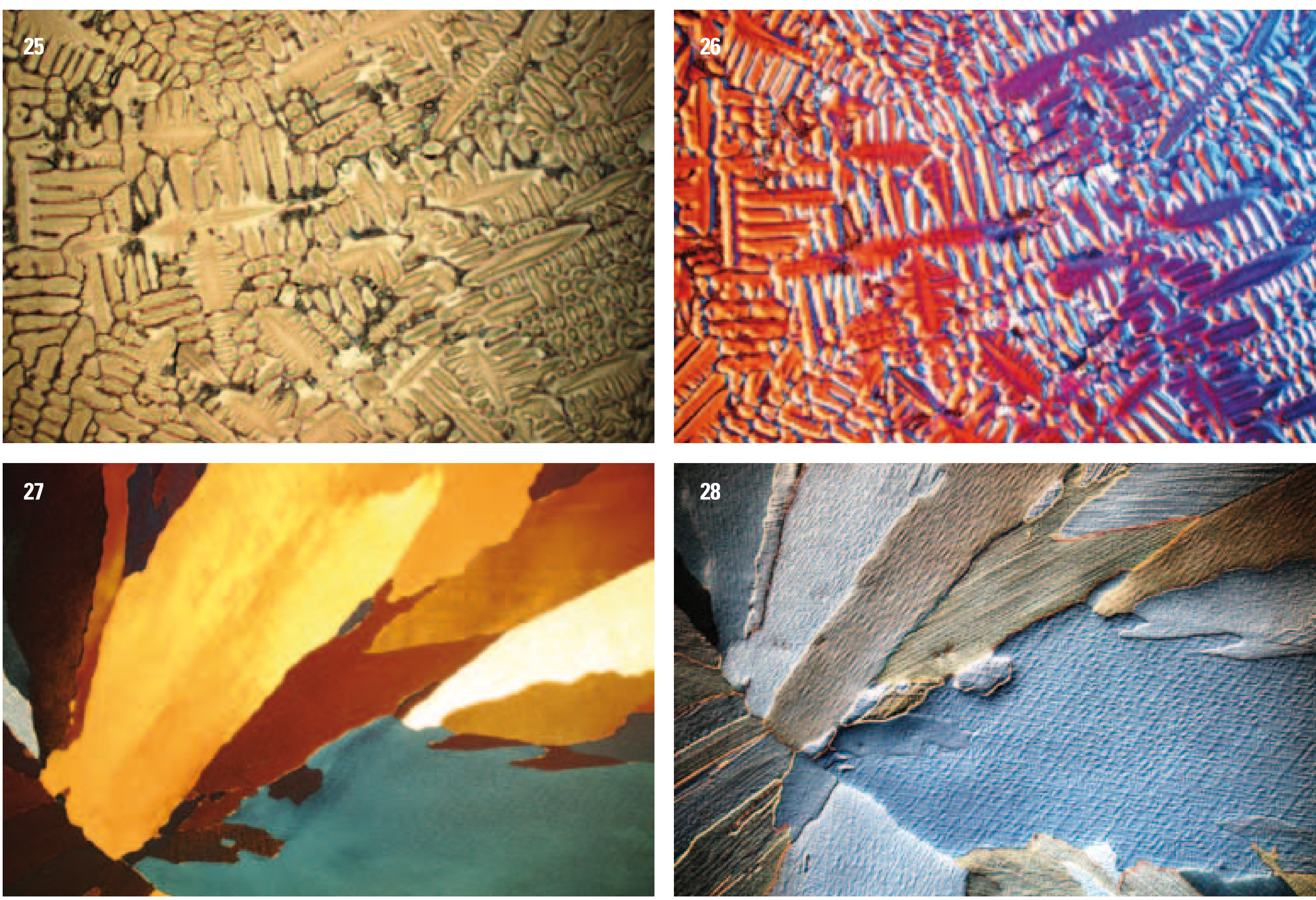




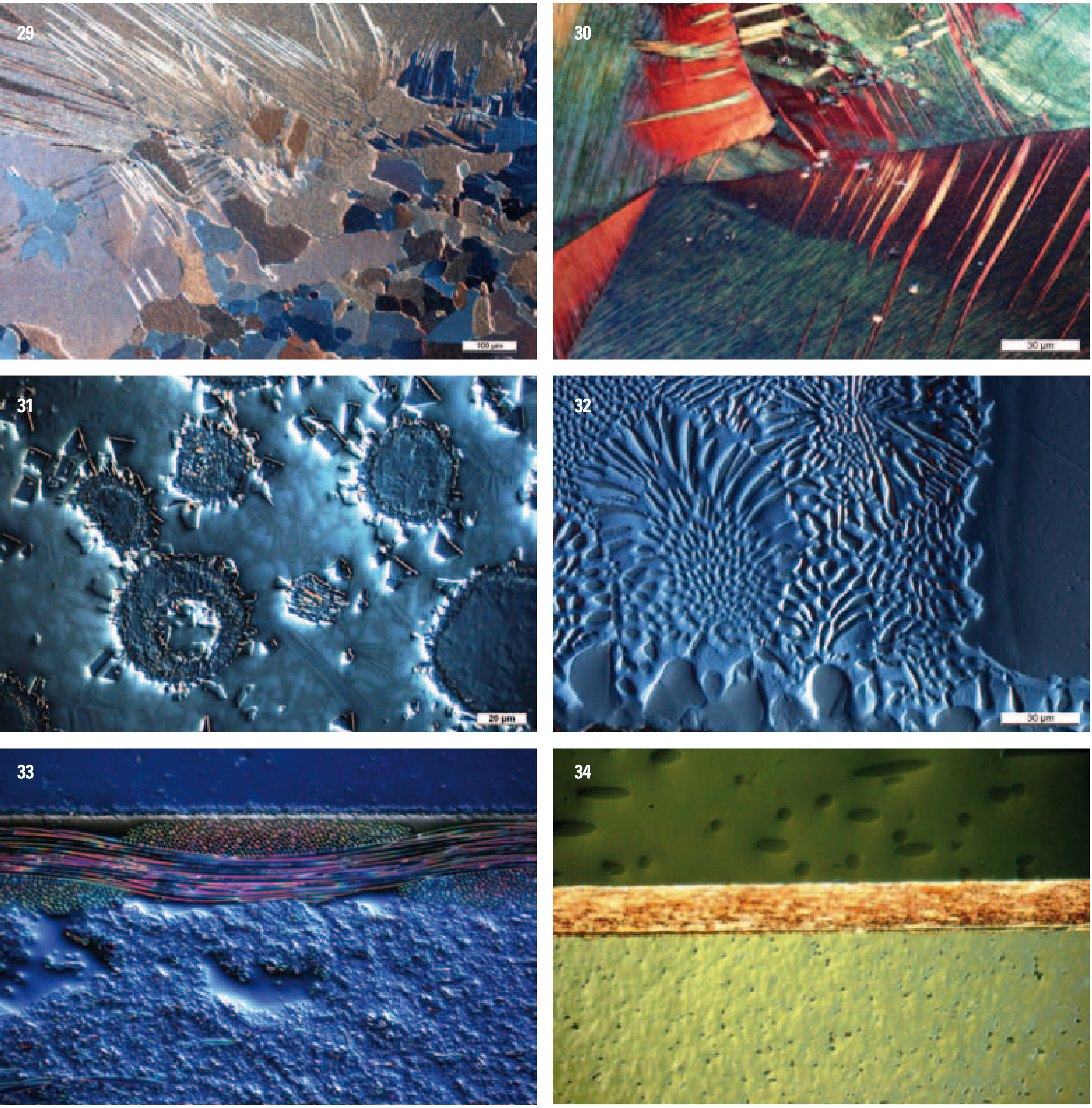
Figs. 19–21: Polarization with and without color etching. 19: 10 eurocent coin made of Nordic gold (K), 20: Structure of a cast tungsten carbide W_2C consisting of a needle structure, etch-polished with H_2O_2 and polarized, 21: Carbon fibers in a structural component, made of carbon fiber-reinforced plastic, unetched, polarized.



Figs. 22–24: Polarization with and without color etching. 22: Brass component with bonded glass fiber braid (K), 23: Capacitor with plastic – glass fiber core, copper-plated and soldered onto a bronze strip conductor (K), 24: Sintered wear protection coating containing bronze and graphite and ceramic particles, clearly visible deformation in the bronze through the calibration (K).



Figs. 25–28: Improved contrast using interference. 25: Brightfield image of a cast austenite structure caused by a laser melting process, 26: The same sample in interference contrast showing clear contrasting of the dendrites (B), 27: Center of a brass wire rod in brightfield, 28: The same sample in interference contrast with significantly better grain contrast and visualization of dendrites and their direction of solidification (K).



Figs. 29–34: Examples of contrast enhancement using interference for samples with and without color etching. 29: Deformation twinning generated by abrupt reforming (K), 30: Contrasting of the slip bands in a deformed copper bronze sample (K), 31: A good image of the cast tungsten carbides in the nickel matrix is achieved with interference contrast, 32: Silver solder in copper/ ceramic compound (etch-polished + K), 33: Cross-section view of a circuit board, composite of different plastics, polished, 34: Cross-section view of an electronic component, ceramics, metal and glass-fiber reinforced plastic (B).

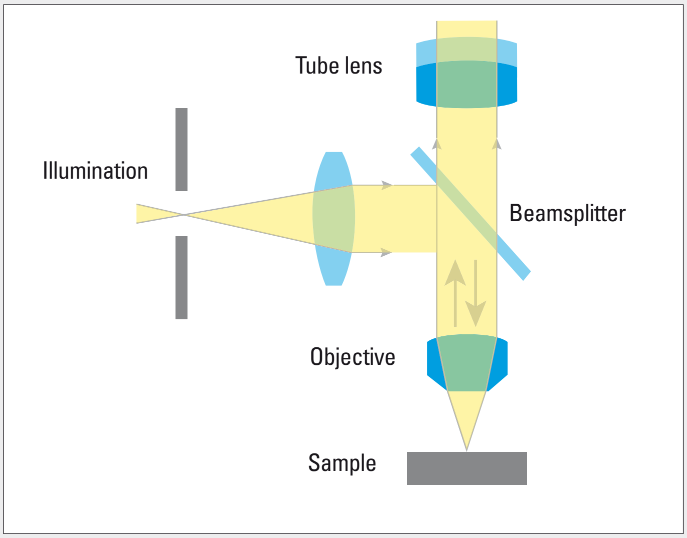
Contrasting Techniques in Incident Light

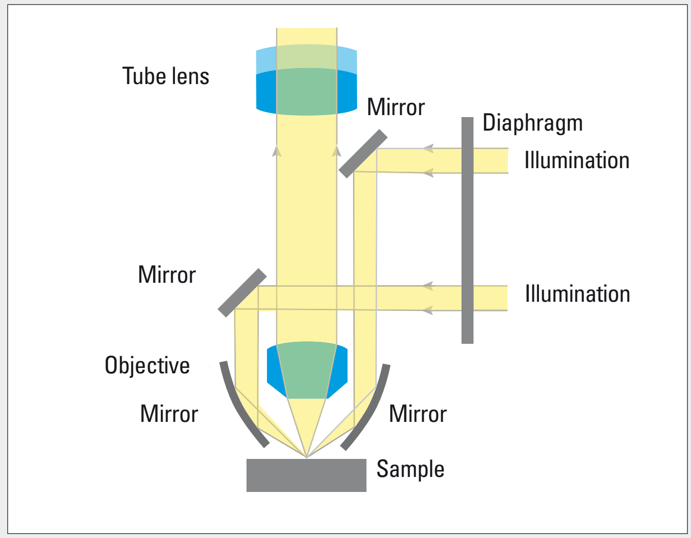
Brightfield

Only direct light falls on the sample surface, where it is absorbed or reflected. The quality parameters of the image are brightness, resolution, contrast and field depth.

Darkfield

Only refracted, diffracted or reflected light falls on the sample surface. Darkfield is suitable for all samples with structured surfaces and can also be used to visualize structures below the resolution limit. The surface structures appear bright on a dark background.



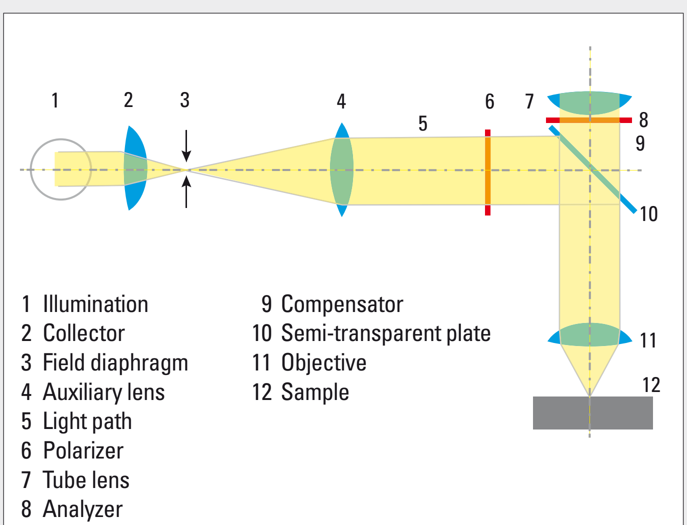


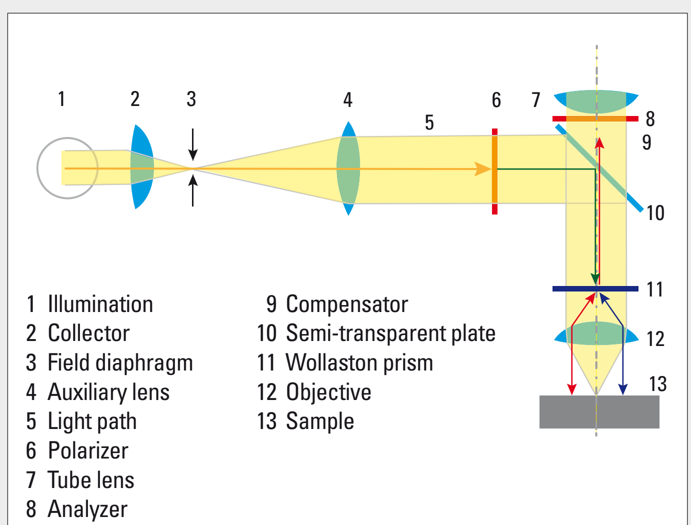
Polarized light

Natural light consists of light waves with any number of vibration directions. Polarization filters only let light waves through that vibrate parallel to the direction of transmission. Two polarizers crossed at 90° generate the maximum extinction (darkening). If the sample between the polarizers changes the vibration direction of the light, characteristic birefringence colors appear.

Differential Interference Contrast (DIC)

DIC visualizes height and phase differences. A Wollaston prism splits polarized light into an ordinary and an extraordinary wave. These waves vibrate at right angles to each other, propagate at different rates and are physically separate. This results in a 3D image of the sample surface, although no real topographical information can be derived from it.





Stereomicroscopy Exposes Counterfeiters

Genuine or Fake?

Anja Schué, Leica Microsystems

ID cards, driving licenses, birth certificates, A-level exams – the potential for individual perpetrators or gangs to gain advantages by forging documents is vast. And the more sophisticated the security standards, the better equipped experts have to be in order to clearly differentiate between genuine documents and fakes. In the forensic department of the Regional Council of Stuttgart, Germany, a high-end stereomicroscope from Leica Microsystems is helping to track counterfeiters. Every year, more than 1200 suspect documents are examined and assessed as a service for the regional police and public prosecutors.

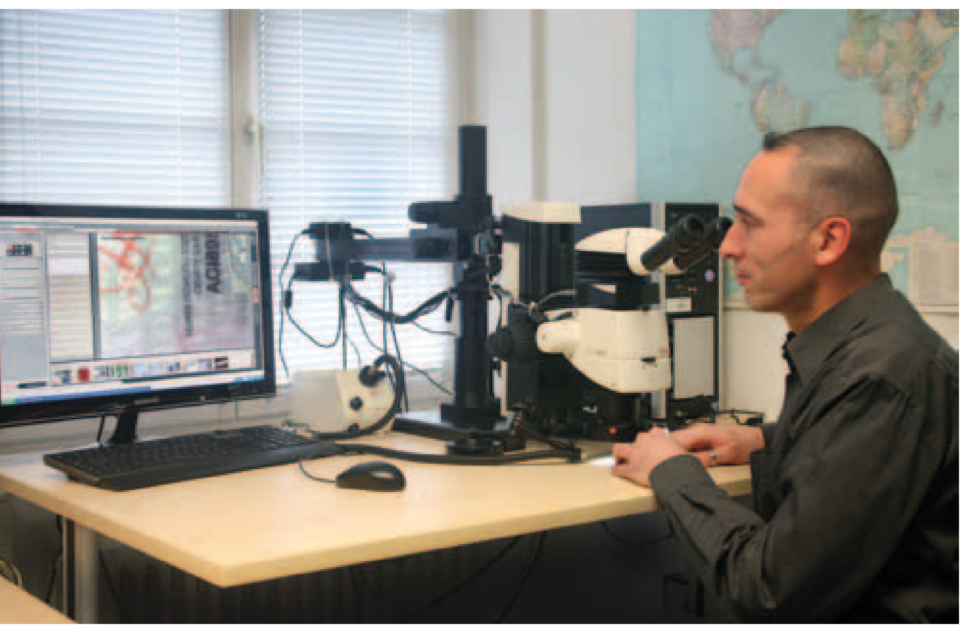


Fig. 1: For Martin Fischer, expert appraiser for document examinations at the District Council of Stuttgart, the Leica M165 C stereomicroscope with LED illumination, HD camera and full-HD monitor is an indispensable tool for examining the authenticity of suspicious ID cards, visas and all kinds of documents.

When Martin Fischer arrives at his desk in the Regional Council of Stuttgart early in the morning, there are usually several suspicious ID cards already waiting for his trained eye. They have been confiscated by the police in their night checks for Fischer to examine their authenticity. A year ago, the Chief Detective Superintendant qualified as a certified expert on certificate forgeries, fulfilling a professional aim that is perfect for his technical bent.

Competent in all techniques

"Anything serving as a document, whether printed, signed or sealed on paper, plastic or metal – a car registration plate, for instance – that constitutes an authorization or that can be forged to gain advantages, can land on my desk," says Fischer. "To detect professional forgeries and to furnish watertight evidence for my appraisals, I have to keep up with the latest technology." He knows all the document production techniques from traditional printing to the RFID chip and the new security criteria for ID cards.

From standard to strange

ID cards and driving licenses from all over the world account for roughly 80 per cent of his work, followed by birth certificates and proof of nationality. But there are plenty of strange and exotic cases, as well. "I recently had a German certificate from a foreign Goethe Institute to examine. Another unusual assignment was to check whether A-level math exam papers had been corrected afterwards to raise the quota," Fischer recounts.

"But my strangest case to date was a Senegalese single status certificate – a long, narrow strip of paper with a sort of writing I had never seen before. Before I can make any kind of statement, I am faced with the challenge of finding out what a document of that type is supposed to look like."

Although there is a personal story behind many certificate forgeries, or the verdict on a defendant may depend on Fischer's report, he always approaches cases with scientific rigor. "I don't read the circumstances of the case until I have provided the results, so that I can carry out the examinations in a neutral and unbiased way," stresses the expert.

Counterfeiters – creative and amateurish

Even experts like Fischer never fail to be amazed by the skilled and professional way counterfeiters operate today. ID cards and passports (as well as banknotes) are furnished with the highest security standards. Yet the more high-tech features an ID card has been given, the more the counterfeiters upgrade their equipment,

Fig. 2: A hologram inserted subsequently – the edges can be clearly seen under the microscope.

Fig. 3: The slightly impressed figures show that this is genuine relief printing.

and may even try to forge RFID chips, holograms and microtext or to imitate special printing techniques such as iris or intaglio printing or laser engraving. "Nevertheless," says Fischer, "counterfeiters never master all the security features equally well. They usually concentrate on a few particularly prominent features and put a lot of technology into attempts to perfectly reproduce them.

However, they then make amateurish mistakes with other features, and that makes it easier for me to catch them out. A common trick is to stick in holograms, chips or other components from genuine ID cards. These collages are not always easy to detect with the naked eye or by running a finger over them – depending on the skill of the counterfeiter."

The microscope – the most important tool

The first step in every new case is always to classify the document as authentic or fictitious. If Fischer does not recognize the type of document, he searches in databases for a reference sample. The second step is to check for obvious signs of manipulation. His trained eye and sense of touch detect major signs of manipulation immediately, for example if stamps have been rubbed out, numbers overwritten by hand or a new photo inserted in an amateurish way. This is followed by examination under a microscope. "I couldn't do this job without a good microscope. Most cases are decided during the microscopic examination," Fischer says. He works with the high-performance stereomicroscope Leica M165 C which has a 16.5:1 zoom and features LED right light, flexible LED fiber optic guide, a mobile swivel arm, a high-definition camera and a full HD monitor

3D image and LED illumination facilitate analysis

"The combination of good illumination and the 3D impression of the stereomicroscope are critically important for examining the finest details of the document's surface structure. The details of better forgeries only show up under the microscope – for instance, attempts to imitate laser engraving, gravure or relief printing elements, or good collages where the chip, hologram or serial number, etc. have been added afterwards. There are always discrepancies somewhere – at the edges, the transitions of fine linear printing patterns, or the orientation of the paper fibers," Fischer explains.

Looking for clues under the microscope

If only the photo has been exchanged, and this has been carefully done, typographic examinations or data readouts do not provide any useful information. The only way to find clues is under the microscope. Sometimes, Fischer bends the ID card over the edge of the table to look under the edge of the photo that has been stuck in. If he finds torn fibers on the side of the ID card that do not correspond to the back of the photo, the case is clear.

"A false photo is still the most common way of manipulating ID cards," says Fischer. "With good forgeries it's not always easy to find the clues. This is where I use the highest magnifications of the microscope. For basic examinations, the low zoom range between 20 and 30x is usually adequate."

Apart from the microscope, Fischer also uses other instruments, e.g. to examine certain security features, inks or stamp dyes under UV or IR light or to read out the personal data of the chip.

At the end of every examination, Fischer documents his results. His appraisal also includes the photos of the microscope images with thorough annotations. "I naturally take care to formulate the report in such a way that there are no outstanding issues," Fischer stresses. "I am rarely summoned to attend court proceedings to explain my reasons for an appraisal."

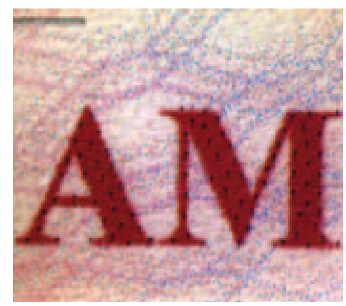


Fig. 4: This serial number was applied with an ordinary inkjet printer – not clearly detectable with the naked eye.

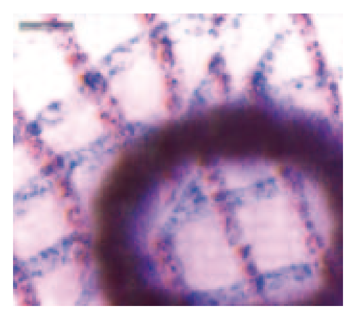


Fig. 5: This laser engraving is genuine. The laser actually burns the structure into the material. Laser engravings are applied to plastics used for modern ID cards, for example.

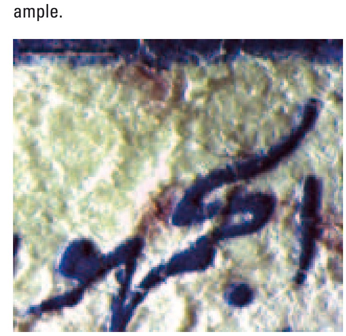
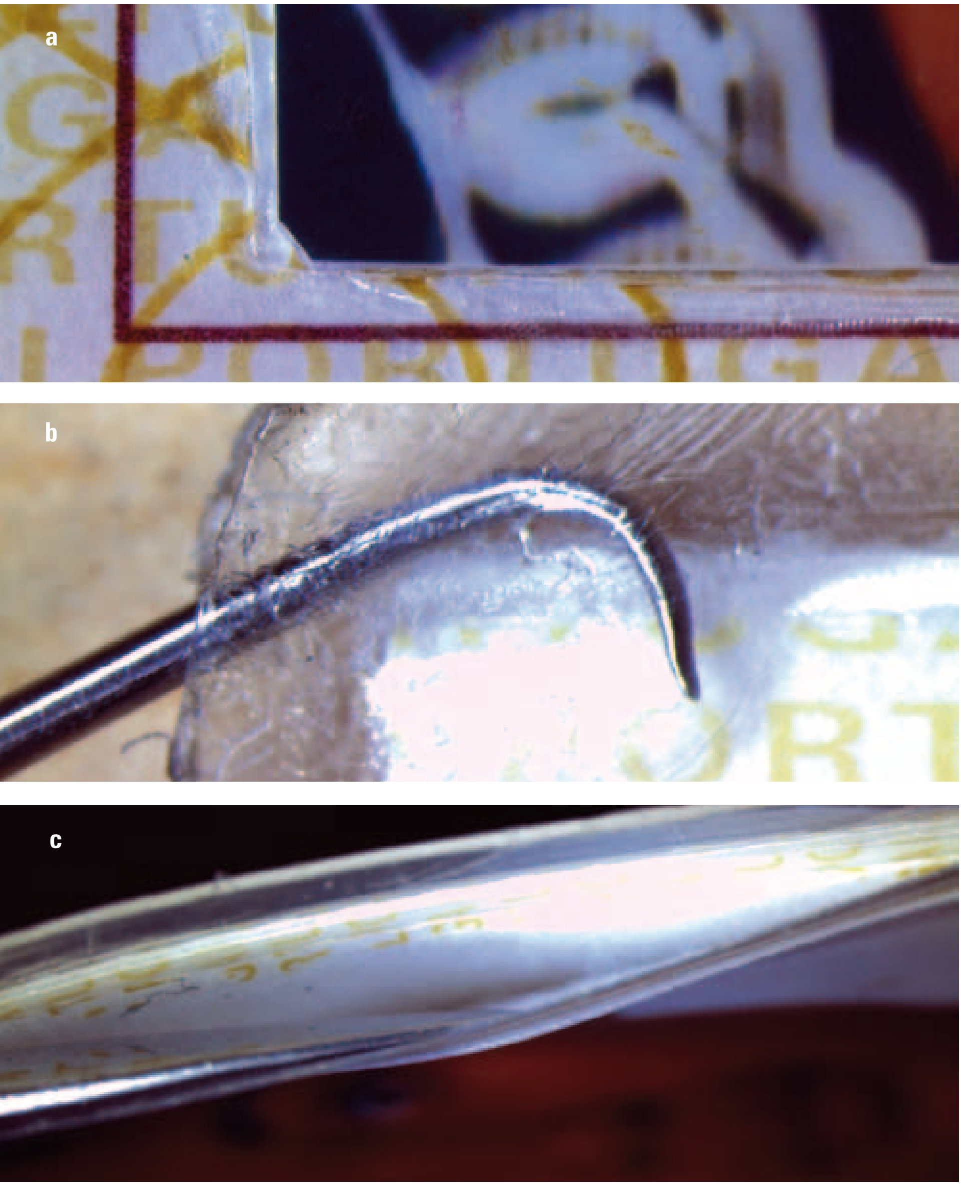


Fig. 6: The raised ink can be clearly seen in this genuine gravure printing.



Figs. 7a–c: a. On the left and underneath the photo, a manually applied cutting edge can be seen. Here, the authentic security foil has been cut open and the original photo removed. The counterfeiter then inserted a different photo inside these cutting edges and covered everything with laminated foil. b. A microtool is used to raise this "alien foil" at one corner of the ID card. c. The security pattern print under the photo is missing – in this case the yellowish lettering. Here the paper of the ID card was damaged when the original photo was removed.

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The Idea of Forging Documents…

… is no doubt nearly as old as the use of official documents and contracts. Even the history of Europe might have taken a different course had it not been for the famous Donation of Constantine, forged around the year 800, which gave the Church of Rome and the Pope authority over all other local churches and a secular claim to power equivalent to that of the emperor. The Vatican used this certificate to particular effect in the Middle Ages to defend itself against imperial paternalism. It was not until 600 years later that it was proved that this certificate, allegedly issued by Constantine I in the 4th century, could not be authentic. Nowadays, forgeries of this scale would be impossible. However, the temptation to gain advantages by false or manipulated documents is still strong enough to keep the police force and the courts extremely busy pursuing crimes of this kind.

Digital Microscope Sheds Light on Classical Sculptures

Ancient Feast of Color

Kerstin Pingel, Leica Microsystems

Everyone knows that antique marble sculptures were white. Or were they? Scientists of the Copenhagen Polychromy Network (CPN) help to show that the statues of the Greeks and Romans were decorated with extravagant ornaments and sumptuous colors. Using the Leica DVM3000 digital microscope, the conservators detect tiny traces of paint pigment that suggest a veritable feast of color in ancient times.

Minerals like blue azurite or green malachite were finely ground and mixed with binding agents such as egg or casein. The polychromy of the sculptures enhanced their three-dimensional impression and gave the viewer important clues on understanding the work of art. For instance, visitors to the Acropolis in ancient times could only recognize the figure of the "Persian horseman" as coming from the Orient by the typical diamond-shaped pattern on the trousers.

Interdisciplinary network for basic research

"We know that color was an integral part of all Greek and Roman sculptures," says Jan Stubbe Østergaard, Research Curator of ancient art in the Ny Carlsberg Glyptotek. "But we are far from really understanding this phenomenon." The aim of the Copenhagen Polychromy Network, an interdisciplinary research team consisting of various Danish institutes, is therefore to conduct research on sculptures in the Glyptotek and document traces of color. The matter is urgent because such traces are gradually disappearing.

Microscope images the only evidence

The microscope is the most important tool of conservator Maria Louise Sargent, as some of the remains of paint on the sculptures are so minimal that samples cannot be taken. "The only evidence of color is what I see through the microscope," explains the conservator. "So it's vital that I examine the sculpture systematically to enable the pigment traces to be found again at any time." Maria Louise Sargent uses a Leica M651 surgical microscope and a Leica DVM3000 digital microscope.

"The digital microscope offers great flexibility," says the conservator. "The statues are up to two meters tall and we have to scan every centimeter. What's more, the digital microscope can magnify up to 160x. The color pigments and residues of the original paint are no more than traces and we are now able to detect them more

Fig. 1: Sumptuous colors give life to this antique Greek statue of a lion (around 550 B.C.). Reconstruction of polychromy by V. Brinkmann and U. Koch-Brinkmann
Photo: Ny Carlsberg Glyptotek

Fig. 2: The limestone statue from Palmyra in Syria dates from 190–210 B.C. It was decorated with rich jewelry. Photo: Ny Carlsberg Glyptotek

easily and analyze them in detail. The digital technology allows us to record videos and images and show them on a monitor for discussion." After having examined the areas with paint residues on the monitor, digital images are recorded for documentation.

Traces of 2000 years revealed

Currently, Maria Louise Sargent is working on "The Beauty of Palmyra". The limestone statue from Palmyra in Syria dates from around 190–210 B.C. It was decorated with rich jewelry, which may have held a glass ball in its center. "The works of art are over 2000 years old, and they have quite a story to tell," says Sargent. "That is to say, I can see a lot of traces, including traces of pollution or aggressive cleaning agents. The difficult part is to identify the traces correctly." After all, the ancient painters used materials like red ochre as a colorant, which is an earth color. So what is paint, and what is just earth from the find spot? Charcoal was used for the black color, but modern air pollution is indicated by carbon particles, too.

Still a lot to do in polychromy research

Although the CPN has already delivered significant research results, polychromy research is still in its early stages. "Even though we can identify colors on antique sculptures, we don't know what they really looked like," says Østergaard. "We need to know a lot more about the refined details of technique and about the esthetic effects of the ancient polychrome works of art."

The new discoveries made examining the many sculptures of the Ny Carlsberg Glyptotek with the Leica M651 and the Leica DVM3000 digital microscope will be shown in a large exhibition in Copenhagen in 2012.

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Fig. 3: Traces of red, ochre-colored and black pigments can be detected looking through the digital microscope.
Photo: Ny Carlsberg Glyptotek

Fig. 4: With the help of a digital microscope, conservator Rikke Hoberg documents traces of color on a Syrian statue.
Photo: Ny Carlsberg Glyptotek

Fig. 5: Microscope image of the right eye of a Greek portrait. Eyelashes and other painted details are clearly visible.
Photo: Ny Carlsberg Glyptotek

Archeological Research on the Battle of the Teutoburg Forest

Where the Germanic Forces Beat the Romans

Kerstin Pingel, Leica Microsystems

"Germanic barbarians defeat super army!" That is the kind of news headline you might have seen in the year 9 AD about the victory of the Germanic tribes over three Roman legions under the command of Publius Quinctilius Varus. The Battle of the Teutoburg Forest is regarded as one of the most momentous battles of antiquity, and for a long time scientists have puzzled over where the fighting may have taken place. At this point, everything seems to indicate that the Romans met their demise on Kalkriese Hill north of Osnabrück, Germany. 2000 years later in the Kalkriese Museum, people are trying to reconstruct the events based on archeological finds and using a stereomicroscope from Leica Microsystems for restoring.

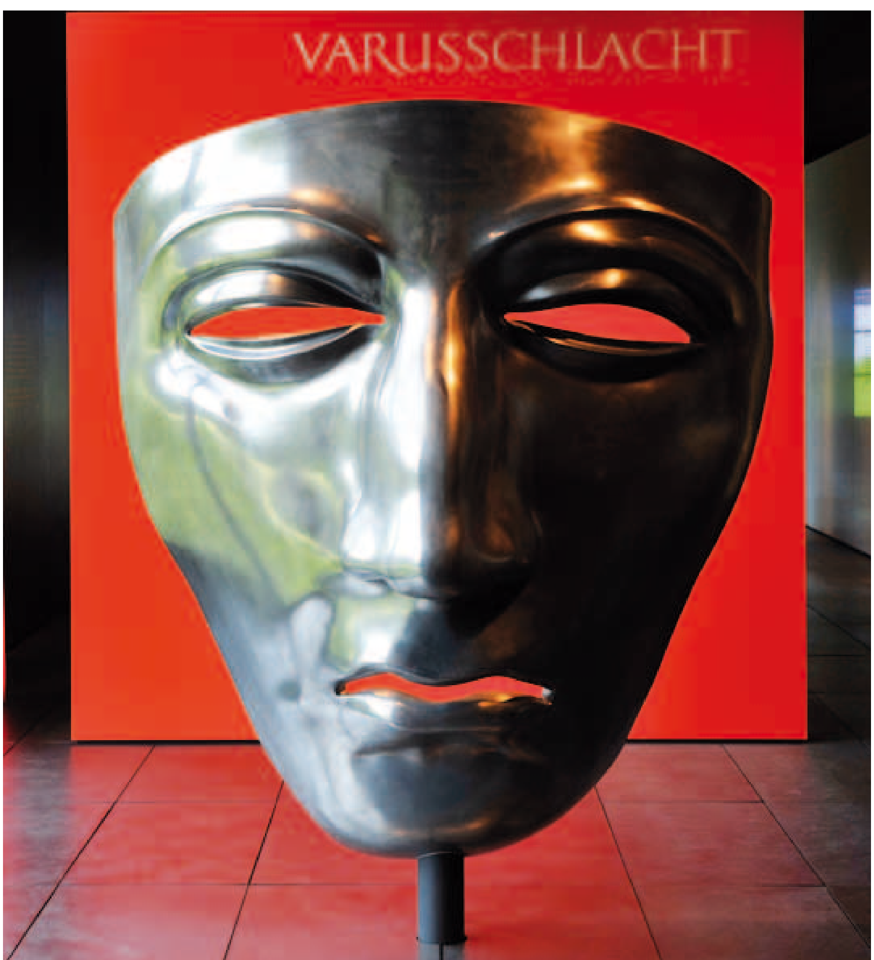

Fig. 1: An oversized copy of the Roman face mask serves as the upbeat to the permanent exhibition.
© VARUSSCHLACHT im Osnabrücker Land GmbH, photo: Hermann Pentermann

Returning to the Roman winter camp on the Rhine, Publius Quinctilius Varus, governor in Germania, had no idea of the impending danger when he received a message from his confidant Arminius, a born Cheruscan and leader of Germanic auxiliary troops in Roman service, asking him for support against rebellious Germanic tribes. Varus had his three legions turn around – and ran right into a catastrophe. Arminius had lured the Romans into an ambush that left the most powerful army of antiquity almost defenseless.

Surprise attack from the underbrush

The Germanic forces were far inferior to their opponents – both in terms of number and weaponry – and would have had no chance on an open battlefield. Therefore they turned to a kind of guerilla tactic. From the protection of the forest they repeatedly attacked the slow and totally surprised Roman platoon.

The Germanic forces had taken advantage of the peculiarity of the landscape. Between Kalkriese Hill and the Great Moor to the west, there was a natural narrow pass which prevented the about 20,000 men in the legions from keeping close together. There was no hope of getting into battle formation or reaching an agreement between the groups. Days of rain and heavy marching equipment also afflicted the Romans. By the third day the Romans suffered a devastating defeat and commander Varus took his own life. After that, in the following decades, the Romans retreated behind the Rhine border.

Fragments also tell a story

Since the first finds pointing to a battle between Romans and Germanic peoples were recovered in Kalkriese in 1987, approximately 6000 other pieces have surfaced. These include Roman weapons such as spearheads, fittings for weapon belts, sling ammo, and fragments of blades and sheathes. Among the most spectacular finds is the iron face mask of a Roman cavalry helmet. "This face mask was originally covered with silver. Since the Germanic forces hardly had any metal of their own, however, the silver was an especially welcome booty when plundering the battlefield," explains Gisela Söger, Press Spokesperson for Kalkriese Museum and Park.

Only metal has survived

Traces of the plundering can be found on almost all of the finds. Most pieces have survived only as fragments, because the Germanic forces forcefully tore off clasps and buckles to get to the desired metallic body armor. Unwieldy sheets of metal were folded or pressed together, but were then probably left sitting. Civilian equipment is also among the finds: Tent pegs, cookware, pruning knives, and axes for clearing woodland, even sewing needles. The remnants of transport crates, medical instruments, pens, and pieces of jewelry indicate that doctors, writers, merchants, and other civilians accompanied the train. That way the legions could be self-sufficient, as it were, in the enemy territory of Germania.

"The ground in Kalkriese is sandy, therefore only metal can survive over time here. Organic pieces decompose," explains Christiane Matz, a conservator. This means the transport crates remain identifiable only by their metal nails, which were found in a rectangular arrangement. The wood has decayed.

Penetrating into the past millimeter by millimeter

Since metal corrodes, the finds are concealed by thick layers of corrosion. By the color of the corrosion, however, Matz can already see what material she is dealing with. Iron has a rust-red layer of sand, while bronze has a compact, green-colored layer. Finally, silver finds have a black corrosion crust. "When uncovering an archeological find, I have to

Fig. 2: Millimeter by millimeter, conservator Christiane Matz uses a microscope to work toward the original surface of the 2000-year-old finds. Photo: Hermann Pentermann

Fig. 3: The Kalkriese finds enable the equipment of the Roman legionaries to be reconstructed.

Fig. 4: Ornamental iron studs, partly covered in silver. © VARUSSCHLACHT im Osnabrücker Land GmbH, photo: Christian Grovermann

Fig. 5: Witness of plundering: A breastplate torn off a Roman lorica segmentata
© VARUSSCHLACHT im Osnabrücker Land GmbH, photo: Christian Grovermann

Fig. 6: Contents of a Roman soldier's money bag. © VARUSSCHLACHT im Osnabrücker Land GmbH, photo: Christian Grovermann

apply careful mechanical precision to approach the original surface with its patterns and signs of usage," explains the expert. "The objects are not supposed to look like new. Rather the goal is to discover and work out details that can still provide us with information about the find after such a long time."

Millimeter by millimeter the restorer removes the corrosion. To do so, she has micro sand blasters, dental instruments, diamond cutting tools, and an ultrasound chisel at her disposal. Depending on an object's state of preservation and size, she may work on it for one or more days. "The most important instrument, however, is the microscope," says Matz. "That is the only way I can control the micrometer-small instruments and examine the results."

The Leica S6 E stereomicroscope has a particularly large working distance, which enables easy access to the object while it is being processed. "Since I work at the microscope for hours, the ergonomic viewing angle is very important to me," she adds.

Human and animal bone finds

Despite the particular ground conditions, two mule skeletons and a large number of unconnected human bones were also found in Kalkriese. "The mule skeletons were lying in an area where a defensive wall constructed by the Germanic peoples probably stood. When the wall collapsed, the mule was buried under the sod and thereby conserved by exclusion of oxygen," explains Gisela Söger. The human skeleton remains, on the other hand, were taken from the eight bone pits found to date.

Anthropological examinations show that they must have lain on the surface for several years before being buried. "From the sources we know that the Roman commander Germanicus visited the place six years after the battle and ordered the remains of the fallen to be buried," says Söger. "As the Romans were on enemy territory, they no doubt buried them in a hurry in bone pits."

New field of research: battlefield archeology

Kalkriese is the first battlefield of antiquity to be researched with modern scientific methods. That means that, apart from examining the finds themselves, their distribution is also studied. For the first time, fragments of finds are also playing a key role in the reconstruction of events.

International research scientists from a wide variety of disciplines are working together on the Kalkriese project. As well as archeologists, there are paleozoologists contributing knowledge on the animal bone finds, archeobotanists examining the traces of vegetation of over 2000 years ago and geologists showing how the ground was cultivated by Germanic tribes.

“The methodic basics elaborated in Kalkriese can now be compared with other find sites of military activity and further developed,” says Dr. Susanne Wilbers-Rost, Archeological Director in Kalkriese. Large areas in Kalkriese still await archeological investigation. Most of the find area, however, will be left to future generations, so that they can use improved methods to research this special place.

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Kalkriese Museum

Archeological examinations have been carried out in Kalkriese systematically since 1989. However, Kalkriese is not only a spectacular archeological excavation site, but also an archeological monument whose character and size pose unprecedented challenges for the research and monument conservation community. Apart from research, there is also a focus on communicating discoveries out to a wide public. The museum sees itself as a window to current science, harnessing an innovative overall concept to teach history and research. The museum has won several awards since it was opened in the year 2002.

www.kalkriese-varusschlacht.de

Fig. 7: Doctors traveled with the legions, too: bone elevator and handle of a scalpel.

Antimicrobial Coating for Educational Microscopes

A Contribution to Laboratory Hygiene

Janika Wiesner, Leica Microsystems

Bacteria are part of our world. There are countless numbers of them in the human body and they are completely harmless. But in people with a weak immune system or at the wrong place they can cause serious illness. Educational microscopes that pass through many hands are potential breeding grounds for germs. To solve this problem, Leica Microsystems and SANITIZED AG in Burgdorf, Switzerland have designed AgTreat™ – an antimicrobial coating technique using the active substance silver for the Leica educational microscopes. Christoph Fankhauser, Customer Support SANITIZED AG, is responsible for the initialization and coordination of the antimicrobial inspection of customer samples. He reports on the benefits of AgTreat™.

Christoph Fankhauser,
Customer Support, SANITIZED AG

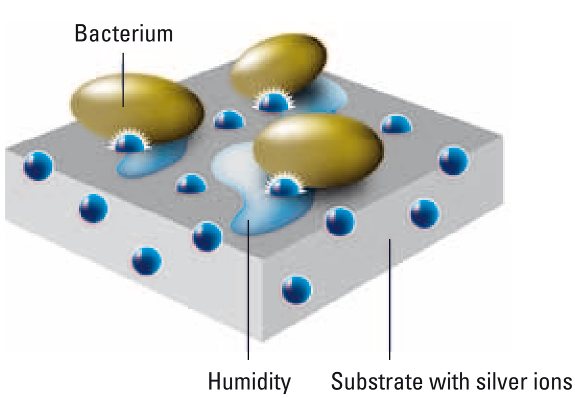
Why are plastic surfaces like those of microscopes a particularly good breeding ground for germs and fungus?

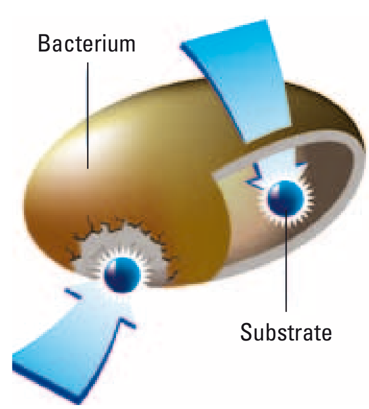
To be able to spread, germs need a carbon source to feed on. Many plastics provide such a source. Another major factor, though, are the people working at the microscope, who contaminate the surfaces with an ultrathin film of dead skin flakes, saliva or perspiration. Particles of dust that settle on microscope surfaces can also help bacteria to spread. Regular disinfection is important. If a cleaning cycle cannot be carried out occasionally, or between two cleaning cycles, an antimicrobial coating with AgTreat™ helps keep germ growth in check.

Why are silver ions so effective against microbial surface pollution?

Silver has long been known as an active substance. Even the Romans were hygiene pioneers in that they used silver cutlery to prevent germ growth. Silver tablets are used to disinfect groundwater wells in Africa, for example. Silver is highly active, particularly in relation to germs, unicellular organisms and microbes, although this property also has undesirable effects such as staining. The crucial point is: How can this reaction be controlled? To do this, we have incorporated silver particles in a glass ceramic container. This releases the silver particles exactly when they are needed – i.e. at body temperature and high humidity, which are ideal conditions for microbial growth. The positively charged silver then reacts with the negatively charged bacteria. This

The silver ion finds the bacterium with the help of humidity and inactivates it.





This is how silver ions act on bacteria:

1. Destabilization of cell membrane
2. Blocking of respiration
3. Inhibition of ingestion
4. Inhibition of cell division

destabilizes the cell membrane of the unicellular organism, preventing cell division and thus inhibiting bacterial growth.

What harmful bacteria does AgTreat™ protect against?

The AgTreat™ coating protects users from a large number of bacteria types. We examine the particularly relevant germs in our laboratory. Some of them are hospital germs like the methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to certain antibiotics and is a particular risk for antibiotic patients whose own immune system is incapacitated. A temperature of about 37 °C is ideal for MRSA to multiply and eventually paralyze the complete organism. However, we're also talking about food-relevant germs such as *Escherichia coli* bacteria which mainly populate the intestinal tract but can lead to dangerous infections elsewhere. Food can also often contain salmonella, which cause serious diarrhoeal diseases.

Fig. 2: SANITIZED carries out around 20,000 microbiological tests every year in accordance with standardized methods.

How do you test the efficacy of the AgTreat™ coating on Leica microscopes?

The surface we are testing is seeded, i.e. brought into contact with, a liquid containing bacteria. The liquid is covered with a film to create an evenly distributed layer. This is then incubated for a day under ideal growth conditions for bacteria at 37 °C storage temperature and at least 90 per cent relative humidity. After 24 hours, the counting procedure is begun to see how many of the original number of about 100,000 germs have survived this test.

What result did you get for the AgTreat™?

On an untreated sample there are about a million germs after a 24-hour incubation period. On a treated sample, we ideally only find a fraction of the germs that were put there. In the case of the Leica DM500 und DM750 with the AgTreat™ coating, we found that germs had been reduced by between 90 and 99.9 per cent.

Fig. 4: The set of dilution is stroked out with the micro pipette for determining the exact germ count.

How high do you rate the importance of the antimicrobial function of AgTreat™ for school and university microscopes?

Being young and active, students are usually in contact with a large number of people – not only at university, but also in their free time. This means that any germs they encounter could multiply very rapidly. The danger is when germs are passed on to people whose immune system is temporarily or permanently weakened in some way. Large groups of people with different physical conditions and hygienic requirements are always a potential risk in this respect.

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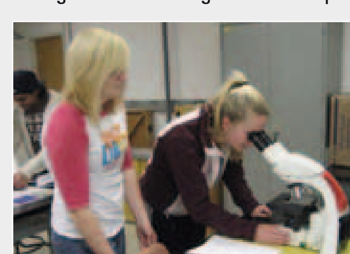
Eileen Sylves supervises the General and Developmental Biology student laboratories at the University of Buffalo, NY. There, the students examine various types of cells under the microscope. She explains why the faculty deliberately chose the Leica DM750: "Besides their excellent optics and illumination, we liked the fact that the educational microscopes of Leica Microsystems are easy to pack and to move from one laboratory to another. However, the key deciding factor was the AgTreat™ coating. We have up to twelve different people using each microscope. The silver ion coating protects our students from the germs they would otherwise be exposed to, particularly in the winter months."



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3D Visualization of Surface Structures

Vertical Resolution – Small Steps, Big Effect

Daniel Göggel and Georg Schlaffer, Leica Microsystems

One of the main features of a digital microscope is the speed and ease with which it enables surface models to be created of macroscopic and microscopic structures. In a qualitative evaluation, these provide a better understanding and a more detailed documentation of the specimen. In addition, quantification of the surface provides valuable information about the composition of the surface and its wear. Which specimens are suitable for use with a Leica digital microscope, and what are the limitations of the method used?

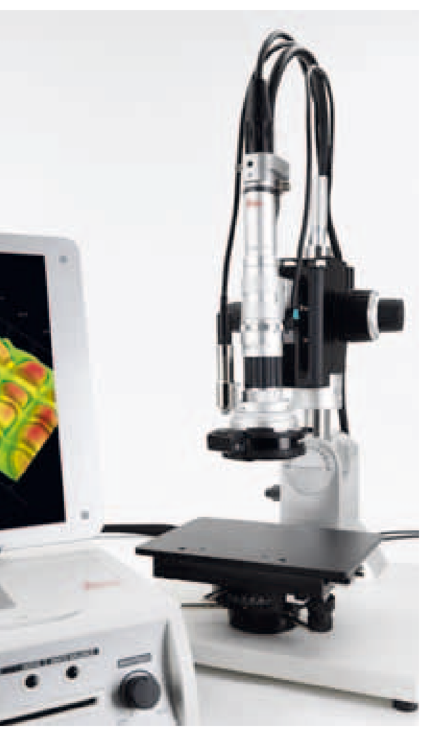


Fig. 1: The Leica DVM5000 digital microscope with flexible tilting stand and rotary xy stage allows reliable inspection and analysis of the sides of samples or inclined surfaces.

The three-dimensional imaging of the Leica DVM2000 – 5000 digital microscopes is based on the principle of focus variation. The limited depth of field of the optics is utilized to determine depth information for the specimen. Vertical movement of the specimen relative to the objective determines the focus information along with the distance to the optics. For each vertical position, the area of the image that is in sharp focus is separated from the blurry area, and both are processed by the software to create a surface model. One of the advantages of this method is that in addition to the height information, the texture of the specimen is also documented. Which influencing factors are determinative for successful creation of a 3D surface model and how do these variables influence lateral and vertical resolution?

Optics

In microscopy, depth of field is in many cases an empirically understood metric. In practice, the correlations between the numerical aperture, resolution and magnification determine this parameter. With their adjustment options, today's microscopes create a balance between depth of field and resolution that is optimal for the visual impression – two parameters that in theory are inversely correlated.

In DIN/ISO standards, the specimen-side depth of field is defined as the "axial depth of the space on both sides of the specimen plane in which the specimen can be moved without detectable loss of sharpness in the image focus, while the positions of the image plane and objective are maintained." However, the standard does not give any clues on how to measure the detection threshold of the deterioration of focus. Particularly at low magnifications, the depth of field can be significantly increased by stopping down, i.e. reducing the numerical aperture. This is usually done using the aperture diaphragm or a diaphragm that is on a conjugated plane to the aperture diaphragm. However, the smaller the numerical aperture, the lower the lateral resolution. Thus it is a matter of finding the optimum balance of resolution and depth of field depending on the structure of the specimen.

Texture of the specimen

The texture of the specimen surface encompasses all of its features and characteristics. These include color and brightness characteristics of the surface. As described above, the principle of focus variation is based on the methodical approach. The better the specimen can be divided into sharp and out-of-focus areas, the better the results of the surface model will be. This method is particularly well suited to textures that have a good contrast. As in many application areas of microscopy, the illumination is given an especially important status, as it frequently determines success or failure. Selecting a suitable illumination makes it possible to document even a specimen with little texture. For example, you can select an oblique incident illumination that makes even hidden structures visible.

Mechanical resolution in the vertical direction

The third influencing factor in this equation is the mechanical resolution in the vertical direction. This term means the smallest possible steps in the z-direction of the focusing drive, which is usually motorized. To make full use of the performance capacity of the optics, the smallest possible step must be smaller than the currently used depth of field, as otherwise image data are lost. A motorized focus drive with a resolution of 10 µm, for example, is suitable at a depth of field of 15 µm.

The lateral and vertical resolutions that are possible with a Leica DVM system depend on various influencing factors, such as the surface structure or illuminator, and thus must be determined depending on the application. Interpolation attains a vertical resolution of one-half of the applied depth of field. The lateral resolution is determined by the numerical aperture of the magnification used.

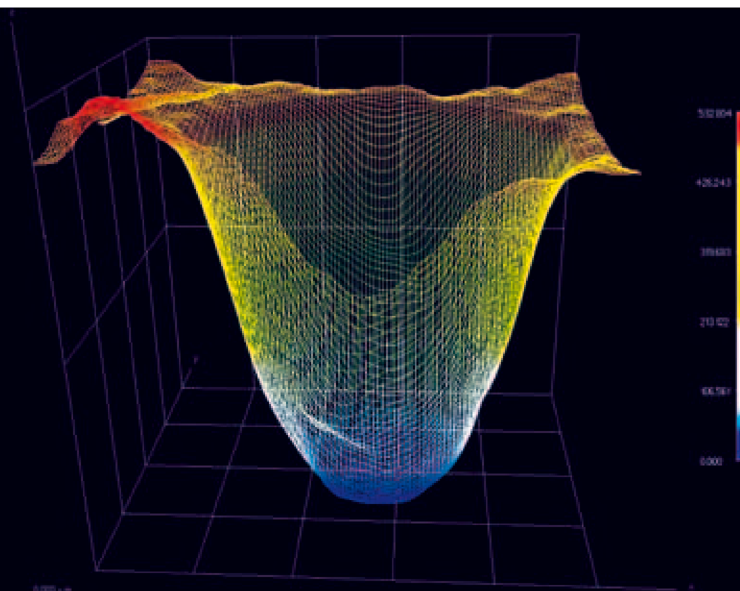
Depth of field – Berek's formula

The author of the first publication on the subject of visually perceived depth of field was Max Berek, who published the results of his extensive experiments as early as 1927. Berek's formula gives practical values for visual depth of field and is therefore still used today. In simplified form, it is as follows:

$$T_{VIS} = n \cdot \left[\frac{\lambda}{2 \cdot NA^2} + \frac{340\ \mu m}{NA \cdot M_{TOT\ VIS}} \right]$$

T_{VIS}: Visually perceived depth of field
n: Refractive index of the medium in which the specimen is situated. If the specimen is moved, the refractive index of the medium that forms the changing working distance is entered in the equation.
λ: Wavelength of the light used; for white light, lambda = 0.55 µm
NA: Specimen-side numerical aperture
$M_{TOT\ VIS}$: Visual total magnification of the microscope

If in the equation above, we replace the visual total magnification with the relationship of the useful magnification ($M_{TOT\ VIS}$ = 500 to 1000·NA), it becomes clear that in a first approximation, the depth of field is inversely proportional to the square of the numerical aperture.



Maximum vertical resolutions of Leica DVM systems

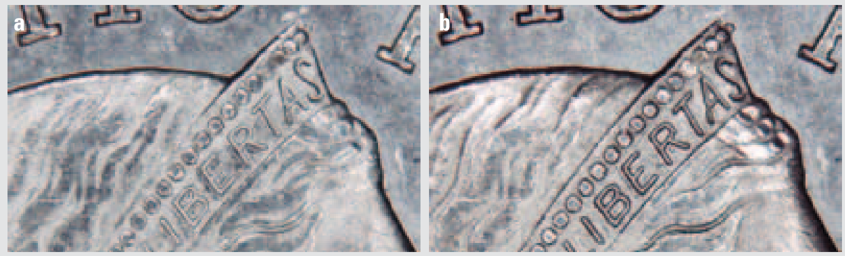
Zoom	Depth of field at V_{max}	Vertical resolution
Leica VZ75 C @ 160x	250 µm	125 µm
Leica VZ80 C / Leica VZ80 RC @ 400x	80 µm	40 µm
Leica VZ100 @ 350x (10450392)	420 µm	210 µm
Leica VZ100 @ 700x (10450393)	110 µm	55 µm
Leica VZ100 @ 1400x (10450394)	4 µm	2 µm
Leica VZ100 @ 1400x (10450395)	3 µm	1.5 µm
Leica VZ100 @ 3500x (10450411)	1 µm	500 nm
Leica VZ100 @ 7000x (10450412)	700 nm	350 nm
Leica VZ700 C @ 2500x (10450531)	1.6 µm	800 nm

Illumination

Selecting the suitable illumination is critical to the success of the examination. The modular design of the Leica Digital Microscopes enables you to combine the selected optics with the optimal illumination for the application. There are the following methods to choose from:

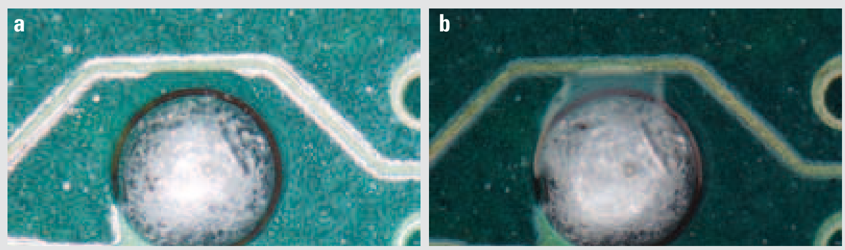
Variable oblique incident illumination:
This method changes the illumination direction from vertical to lateral. This approach is particularly suitable for visualizing scratches or small recesses.

a Coin in incident light
b Coin in oblique incident light



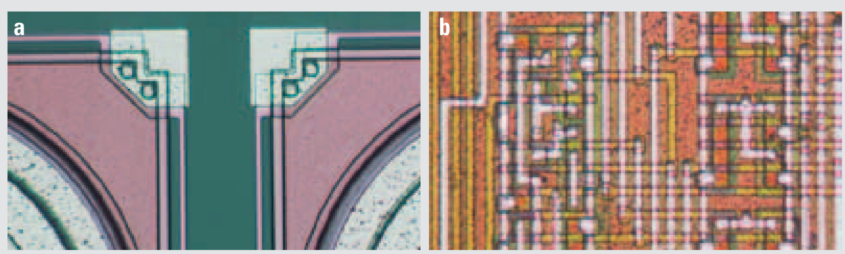
Diffuser:
For shiny surfaces, the dynamic range of the camera is insufficient in many cases and many areas of the specimen are overexposed. A diffuser provides reliable reduction of the overexposed area.

a Solder point without a diffuser
b Solder point with a diffuser



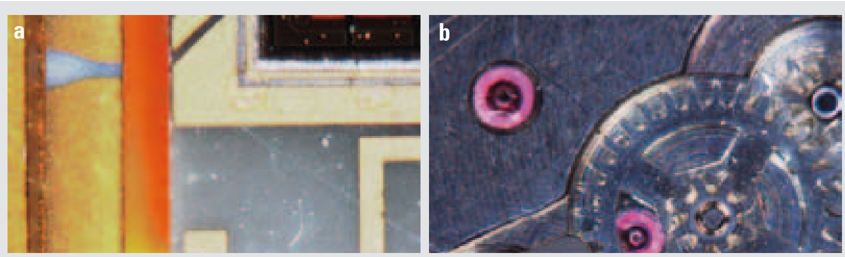
Coaxial illuminator:
A coaxial illuminator is used for very shiny or reflective surfaces, such as wafers or metal sections.

a, b Semiconductor structures with coaxial illumination



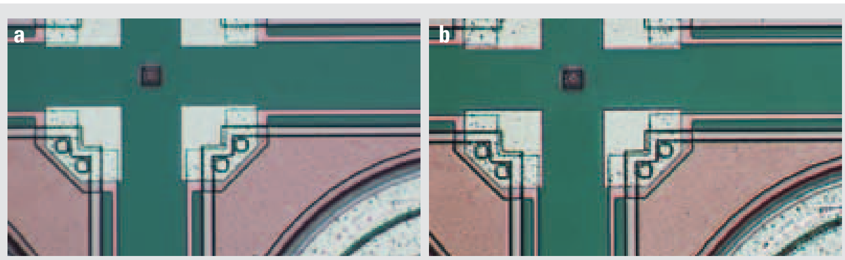
Polarized light:
Polarized light is used to suppress reflections or for documentation of plastic materials.

a Plastic with polarized light
b Clockwork with polarized light



Coaxial illuminator with directed light:
Here, the directed light creates a three-dimensional impression of the specimen. This is helpful in many cases for determining the surface with greater accuracy.

a Semiconductor structures with coaxial illumination
b Semiconductor structures with directed coaxial illumination



New Inspection Microscopes Leica DM8000 M and DM12000 M

Detect Faster!

Stefan Motyka, Leica Microsystems

Inspection, process control and defect analysis of wafers or LCDs and TFTs has to be fast, accurate and ergonomical. With the Leica DM8000 M and the Leica DM12000 M Leica Microsystems launches a new line of products for the inspection of 8 and 12 inch wafers. The new optical functions can ensure higher resolution and throughput.

To detect macro defects, the inspection microscopes have a micro/macro mode for rapid scanning of large components. The macro magnification captures an object field of approx. 40 mm – that's almost four times more than with conventional scanning objectives.

With the Leica macro mode you detect defects which are invisible for conventional light microscopes such as an insufficient development at the edge or within the center of a wafer. If you want to take a closer look, just press a single key to switch from macro to micro mode and inspect the defect in brightfield, darkfield or DIC. Press another key to switch to the UV mode for even higher resolution or the oblique illumination (OUV) mode.

Oblique UV mode

The OUV mode combines oblique illumination and UV light, enabling you to view your sample in top resolution from any angle. This is especially helpful for edge inspection in wafer production, the inspection of pyramid shaped parts on solar panels, or of surface structures on microelectronic components, as information on the topography of the sample often helps to better understand the structure. The accuracy of the inspection results can be enhanced within a minimum of time.

Integrated LED illumination

The illumination is based on the latest LED technology and is fully integrated into the microscope. Due to the low heat radiation and the absence of a lamphousing, the impact of the microscope on the cleanroom environment is minimized. For the first time, LED illumination is also used in UV mode, so that conventional ozone-producing high-pressure lamps can be banned from the cleanroom. The power LEDs have a long life time and a very low power consumption. There is no need for bulb change or downtimes due to maintenance.

Ergonomy means quality

It has been proved that ergonomically designed workplaces help to increase productivity and enhance work quality. The Leica DM8000 M and DM12000 M are optimally adaptable to any user with their individually adjustable Ergo tube and height adjustable focus knobs. This prevents hand, arm, and shoulder tension and ensures a comfortable, fatigue-free grip – without additional arm supports. All the controls are easy to reach, so that users don't have to take their eyes and hands away from the microscope to switch to a different contrasting technique.

Bringing High Definition Imaging Into the Classroom

Discovering the World in HD

Urs Schmid and Vince Vaccarelli, Leica Microsystems

Viewing, capturing, annotating and archiving microscope images has become indispensable for the education of university-level microscopy classes. Leica High Definition Imaging Systems provide a complete solution for efficient learning in the microscopy classroom.

The High Definition signal has over twice the sharpness and clarity of analog video signals together with a far superior color resolution. The specimens appear clearer and more lifelike. As the live stream image is directly created inside the camera, the live image speed rate is much higher.

Working independently at the HD microscope workstation

Eileen Sylves is the lab supervisor of the State University of New York at Buffalo. Her lab is equipped with various microscopes. Additionally she has set up a station with a Leica DM750, a Leica ICC50 HD microscope camera and a laptop computer. The students of the Developmental Biology Course, an advanced course for senior students who are planning a career in Life Sciences, have to monitor the development of zebra fish embryos over time, including photos. “The Leica ICC50 HD microscope camera delivers excellent color images with a high resolution. And it is very easy to operate: It encourages students to take ownership of their images,” says Sylves.

The more time an instructor has to actively teach, the more students can learn. Therefore, the Leica DM750 educational microscope with integrated camera provides outstanding high resolution and fast live images. This allows instructors and students to look at the live monitor image together to discuss specimen details.

Even when the student is viewing the sample through the eyepieces, the instructor is able to watch the work on screen. Besides the possibility of connecting a now

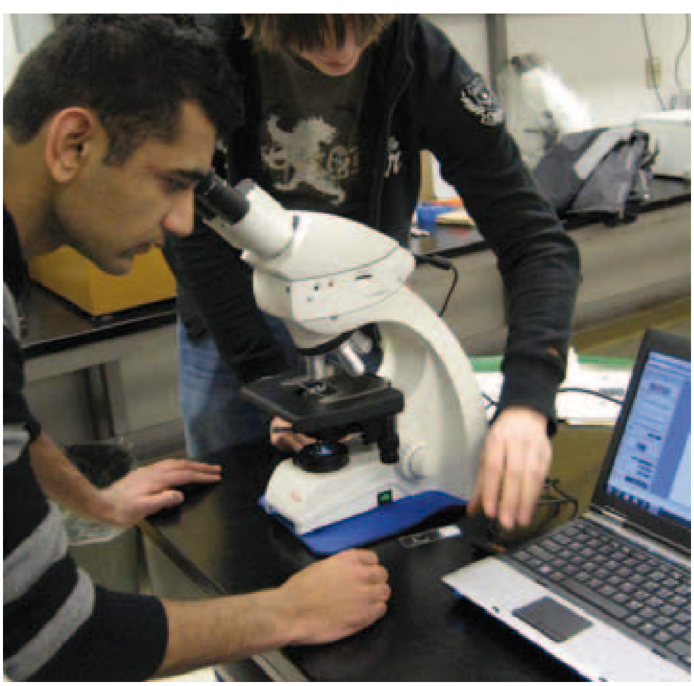


Fig. 1: Working in real time thanks to ultra high resolution and high-speed live imaging: The Leica ICC50 HD microscope camera is an indispensable tool for the students at the State University of New York.

Fig. 2: The workstation is equipped with a Leica DM750, a Leica ICC50 HD microscope camera and a laptop computer.

more easily affordable HD monitor, the system also allows the use of computers and even special educational networks.

Perfect integration: high-resolution camera module

The Leica ICC50 HD is a fast, high-resolution camera module that seamlessly fits between the viewing tube and the microscope body of the Leica DM series. It delivers up to full HD resolution (1920 x 1080 pixel). The user can either display the images on a HD display or save the images on a SD card.

Most functions can be controlled on the camera itself or the optional IR remote control. Pushing one of the two buttons on the camera quickly switches camera modes, performs white balancing or saves the image on an SD card.

Camera software with many extra features

Leica LAS EZ software is an easy-to-use camera software that is provided free with every Leica microscope camera. It enables the student to control and capture images, annotate, take measurements and documents on his or her project. With the new version 2.0 freehand annotation is available on the live and saved image.

Basic program for Apple users

Leica Acquire is a basic software for Mac OS operating systems. It provides an ideal common, easy-to-use, consistent platform for basic education, industry and life science applications. Leica Acquire guides the user through the camera settings for high-quality imaging and allows easy fine-tuning of the image to obtain the best quality.

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