Cellular and Molecular Biology™ **44** (5), 657-671
Printed in France.

0145-5680/97
1998 Cell. mol. Biol.™

INTRODUCTION OF THE GUEST-EDITORS WITH A SHORT REVIEW ON *HISTORY OF OPTICS IN JENA*

Karsten KÖNIG and Karl-Jürgen HALBHUBER

Institute of Anatomy II, Friedrich Schiller University, Teichgraben 7, D-07743 Jena, Germany

This special issue deals with modern laser microscopy in cellular and molecular biology. It reflects contributions of the main speakers of the International Practical Course on Laser Microscopy, held in the "Zeiss town" of Jena from 18-21 November, 1997. It was the year of the 150th anniversary of the foundation of the Zeiss enterprise. In November 1846, Carl Zeiss opened his first optical workshop in Jena (Fig. 1). Jena has a long tradition in optical engineering and in particular, in the development of microscopy techniques.

Jena was first mentioned as "Jani" in the records of the Hersfeld monastery in the ninth century. The charter is dated back to 1236. During the Reformation the town played an important role and Luther stayed incognito as a guest of the hotel "Schwarzer Bär". However, after the defeat in the Schmalkaldic War in 1547 the Elector lost an area including the town of Wittenberg with its university. After a suggestion from Melanchthon (1497-1560), Johann Friedrich the Magnanimous decided to establish a new university in Jena, which was officially opened on February 2, 1558. By the end of the 17th century it became one of the most famous German universities. Around 1800, Jena was the heart of classical German philosophy (e.g. Fichte, Feuerbach, Schelling, Hegel; later: Marx (dissertation), Nietzsche, Eucken), German literature (e.g. Schiller, Goethe, Hölderlin) and German romanticism (e.g. Schlegel Brothers, Schelling, Novalis, Brentano, Tieck). One of the outstanding persons of the romanticism circle in Jena was the young physicist **Johann Wilhelm Ritter** (1776-1810) (Fig. 2). At the age of 20 he worked as a critical reviewer of Alexander von Humboldt's publications, at 23 he published his own book on galvanism, and he supported Goethe in establishing a scientific laboratory. It was the time where the astrophysicist Sir Herschel discovered a radiation beyond the red spectral range, the infrared (IR) light. The 24 years old Ritter concluded that an unknown radiation should also exist on the opposite side of the visible spectrum beyond the blue range. Using silver chloride he discovered the ultraviolet radiation in 1801. In addition, he also became famous as the pioneer of scientific electrochemistry. However, Ritter did not get a permanent position at the university. Therefore, he left Jena and his family and moved to Munich in 1805 where he became a member of the Bavarian Academy of Sciences. Coming increasingly in the crossfire of critical opinions, Ritter lost his reputation as a genius of science. Disease and financial problems started to determine his life. He died at the young age of 33 (König, 1988; Richter, 1988).

Some years after Napoleon's successful battle in Jena and Auerstedt (1806), an optical engineer and young assistant of the famous professor Schleiden -pioneer of cell biology- would determine the name of Jena as "city of optics". His name was **Carl Zeiss** (1816-1888), the 5th child out of 12 of a turner family. With the support of Schleiden, who wrote a letter of recommendation to officials, Zeiss got the permission to open an optical workshop in Jena's downtown on November 19, 1846. One year later he manufactured his first simple microscope (Fig. 3). Buyer of microscope number 6 was Prof. Schleiden (Koch, 1946; Schomerus, 1952).

An important step into the welfare of the Carl Zeiss manufacture was the cooperation with **Ernst Abbe** (1840-1905), professor for mathematics and physics. Fig. 4, top, demonstrates the famous resolution formula of Abbe, the founder of the theory of microscope image formation. Manufacturing of microscopes "before Abbe" was based on experience and permanent trials. Urged by Zeiss in 1866, Abbe replaced the conventional manual methods by a scientific approach and introduced science in optics production. *Zeiss* microscopes now were equipped with well-calculated objectives and became known throughout the world. Carl Zeiss also started to produce instrumentation for analysis such as Abbe refractometers.

Besides the fruitful cooperation between Zeiss and Abbe, there was a third outstanding person involved in the success of the enterprise *Zeiss*, the chemist **Otto Schott** (1851-1935, Fig. 4). He established the modern glass research and is known as the "father of special glasses". With the foundation

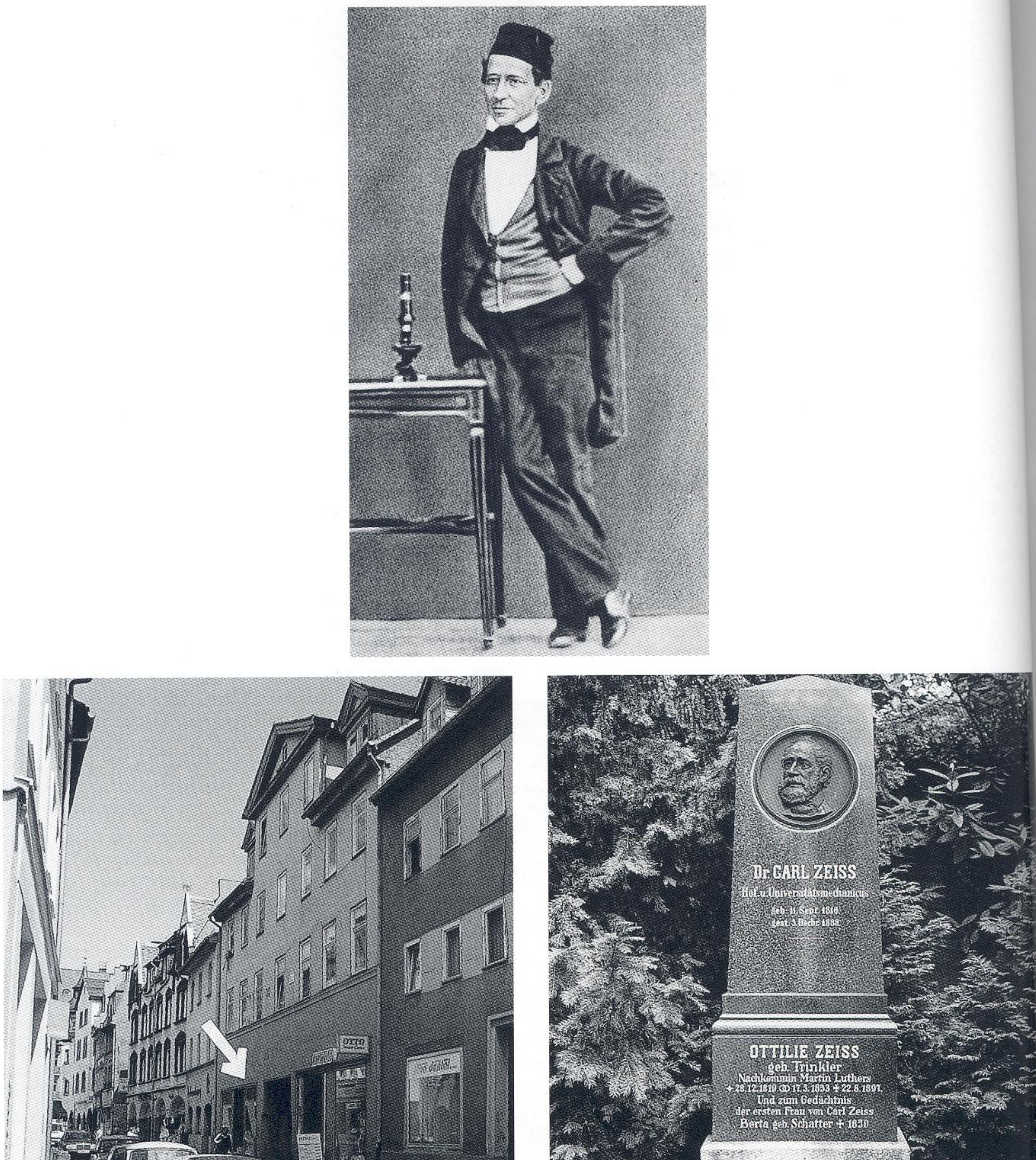

Fig. 1 *Carl Zeiss in 1861* (top, source: Zeiss Archive, number BI 8559), *his first workshop in Neugasse street* (bottom left), *and his grave stone in Jena* (bottom right).

Fig. 2 *The founder of UV radiation, Johann Wilhelm Ritter* (top, source: Museum *Romantikerhaus,* Jena) *and his apartment in Zwätzengasse street* (bottom).

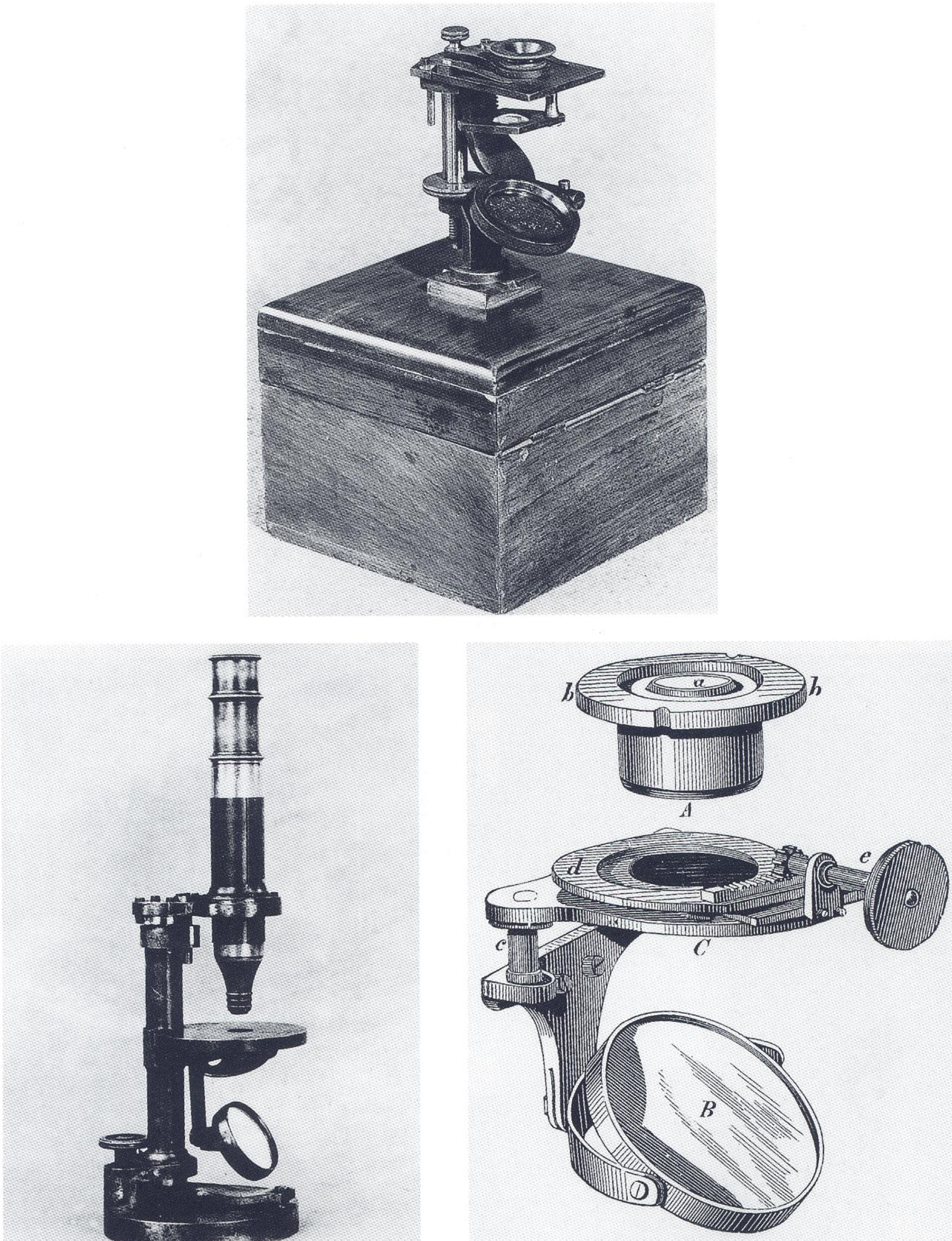

Fig. 3 *The first simple microscope from 1847* (top), *the first compound microscope from 1857* (bottom left, Zeiss introduced the movable mirror), *and an illumination device with a condenser and central aperture from Ernst Abbe in 1872* (bottom right) (source: Zeiss Archive, numbers BI 2753 (top), and 2019 (bottom right).

Fig. 4 *The Abbe formula of resolution in microscopy* (top). *Ernst Abbe and Otto Schott* (bottom) *looking at the novel chainless bike from Paul Rudolph* (source: Zeiss Archive, number BI1232).

of the glass-making factory *Schott und Genossen* in 1884, Jena finally became the birthplace of industrial optics. The founders were Otto Schott, Ernst Abbe, Carl Zeiss and his son Roderich Zeiss (1850-1919).

From that historical reflection, one can say that the success of the enterprise *Zeiss* was the point of view of the founder, Carl Zeiss, to recognize the importance of the natural sciences in industrial manufacturing. After the death of Zeiss, Ernst Abbe became the owner of the company.

The town of Jena gained from this fruitful union of industrial production and scientific research, in particular, as the Zeiss foundation was set up by Abbe in 1889. The foundation became the sole owner of the Zeiss works including a network of outstanding social benefits for the employees and huge financial support for the town of Jena. It provided extraordinary social duties such as a special old-age pension and the 8 hours-work day (since 1900), and founded e.g. institutes, the children's hospital, and the opticians' school (von Rohr, 1940).

The *Zeiss* enterprise was the place where the ultramicroscope was invented in 1903 by **Henry Siedentopf** (1872-1940) (Fig. 5) and **Richard S. Zsigmondy** (1865-1929, Nobel prize in 1925). In 1904, the first UV microscope was developed at *Zeiss* by **August Köhler** (1866-1948), who constructed the microscope and the detection system, and **Moritz von Rohr** (1868-1940), who developed the UV objectives (Fig. 6).

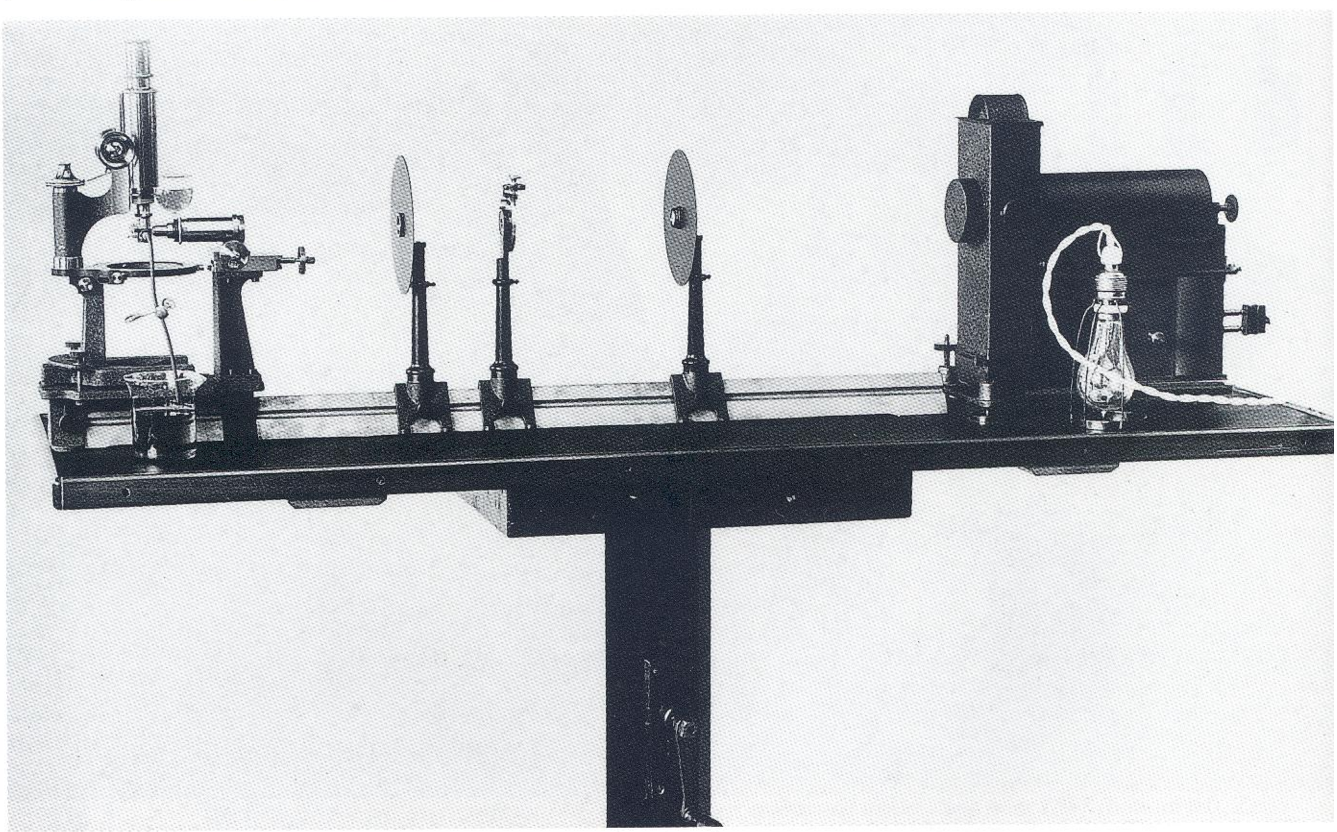

Fig. 5 *Henry Siedentopf and the slit-ultramicroscope* (source: Zeiss Archive, numbers BIII 1918 and BI 18083)

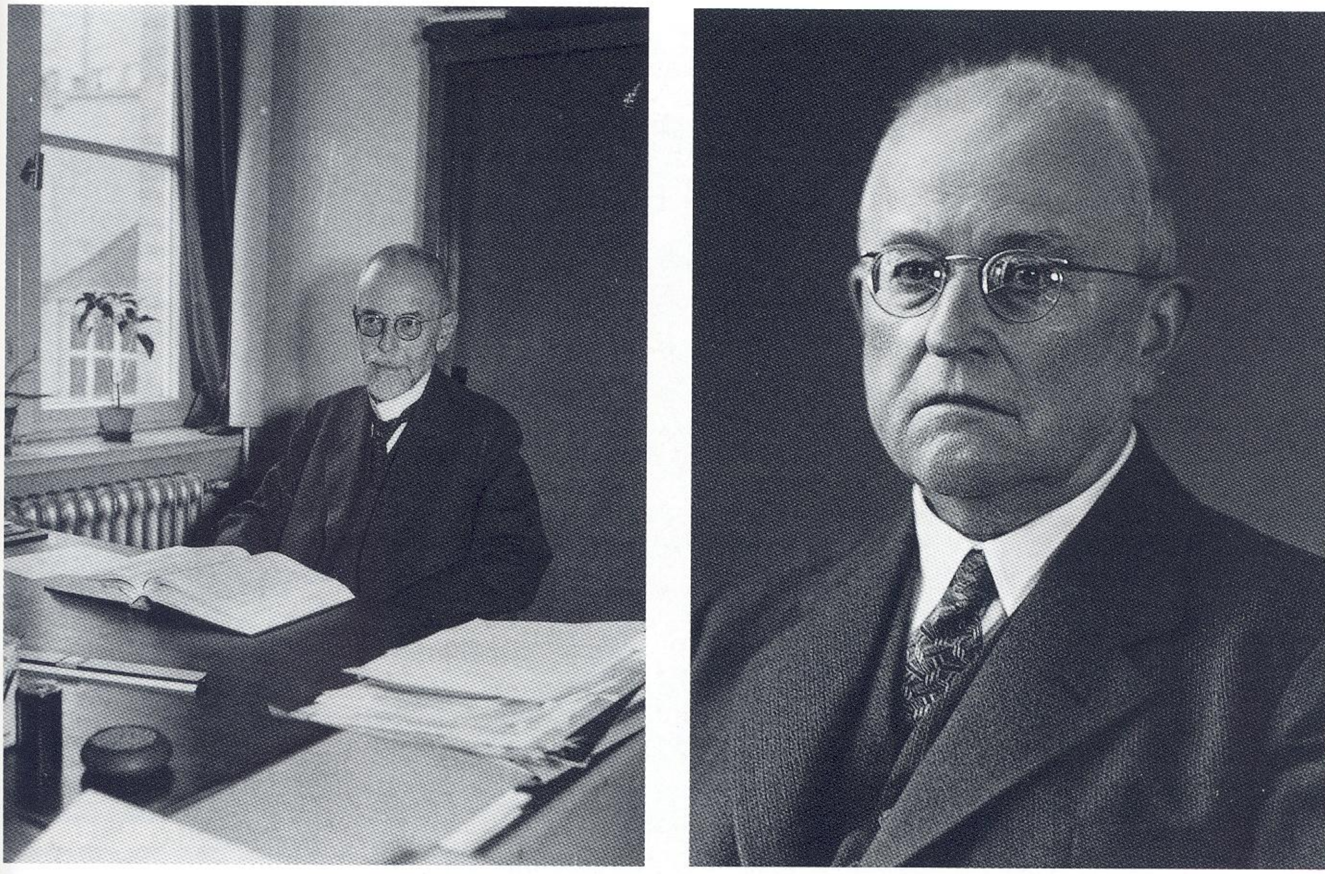

Fig. 6 *Ernst Köhler* (left) *and Max von Rohr* (right) - *the inventors of UV and fluorescence microscopy* (source: Zeiss Archive, numbers BIII 1791, BI 1310).

Köhler noticed fluorescence emissions with this setup. It was the beginning of fluorescence microscopy. Such a fluorescence microscope with 275 nm excitation source was shown during a microscopy workshop in Vienna in 1908. Using the novel fluorescence microscope, the physiologist **Bruno Johannes Stübel** (1885-1961) from the university in Jena became in 1911 the first person who performed (after a suggestion by *Zeiss* employees) the earliest microscopic observations of UV-excited cellular autofluorescence. He studied systematically a variety of tissues and microorganisms including organelle fluorescence (Stübel, 1911; Kasten, 1989). In order to get a pure UV excitation radiation he used a special 300 nm-400 nm broadband UV filter from H. Lehmann (*Zeiss*). Stübel found short-wavelength autofluorescence in nearly all cells, demonstrated that hemoglobin is non-fluorescent, and concluded that the high UV absorption of eye lenses (and of skin pigments) protect deeper laying tissues such as the retina from intense ultraviolet radiation. He also studied cell damage effects by UVA light and suggested the use of fluorescence as a diagnostic method in biology. Stübel wrote his dissertation (1908) and his habilitation (1910) in Jena and became a professor of physiology at the age of 29. In 1924 he moved to China and worked as professor at the Tungshi university until 1951. Later he tought at the Natural History Museum in Chicago and as a professor of ethnology at the university of Erlangen (Stübel, 1924; Giese and von Hagen, 1958).

The *Zeiss* company was also the birthplace of the first planetarium which was developed by **Walther Bauersfeld** (1879-1959). It was installed in the *German Museum* in Munich in 1923. The construction of the planetarium was based on the suggestion of the museum founder Oskar von Miller to build a "sky machine".

A highlight in 1936 in Jena was the setup of the prototype of the phase contrast microscope which was built in cooperation with the Dutch **Frits Zernike** (1888-1966, Nobel prize in 1953) from Groningen. Seven years later **Kurt Michel** (born in 1909) produced the first time-lapse film using a photomicroscope with automatic exposure control.

In 1945 the Zeiss factory was bombed and partly destroyed (Fig. 7). US troops occupied Jena and took 126 management staff and scientists ("we take the brain") into the Western part of Germany. They later founded an additional company *Zeiss* in Oberkochen near Ulm (1946: Opton Optische Werke GmbH, 1951: registration of Carl Zeiss company). After the Americans left Thuringia (they received parts of Russian-occupied Berlin) the Russians came to Jena. They took a part

Fig. 7 *Ruins of the main building of the Zeiss enterprise after Anglo-American bomb attacks on March 19, 1945* (source: Zeiss Archive, number BI 16032).

of the remaining staff (arrested and transported *Zeiss* and *Schott* families within 12 hours) and moved a lot of equipment to the Soviet Union (Fig. 8). In 1948 the *Zeiss* enterprise became state-owned and was named *VEB Carl Zeiss Jena*. Until 1991, two *Zeiss* companies existed in hard competition.

Some highlights at that time in Jena were the start of the industrial production of gas and solid state lasers in 1962, two years after Maiman's invention of the laser. In 1965, Carl Zeiss Jena presented a special laser microscope, the *laser microbeam analyzer* (LMA1, based on a ruby laser with µs pulse width which produced 2 µm spots). This analyzer was also used in cell biology. It was the first commercial microbeam apparatus (Fig. 9).

Based on a patent from the *Zeiss* scientist Gröbler (Gröbler, 1986), *Zeiss* Jena developed together with the physics department of the Friedrich Schiller university in 1988 a novel time-resolved fluorescence scanning microscope. It was based on an acusto-optical mode-locked argon ion laser with 100 ps pulse width at an 123 MHz repetition rate. The argon laser was also used as a pump source for a dye laser to provide picosecond pulses at higher wavelengths. The pulsed radiation was introduced into a scanning microscope. Fluorescence decay curves, imaging in a variety of simultaneous time windows with defined time-delay between excitation and detection, and lifetime determination was possible with a single photon counting unit. In combination with a computer for image analysis and with an polychromator and optical multi-

Fig. 8 *Removal of "brains" and material from the Zeiss factory during the occupation by American troops on June 9, 1945* (top), *and then by Russians in 1946* (bottom) (source: Zeiss Archive, numbers BIII 3634, 16546).

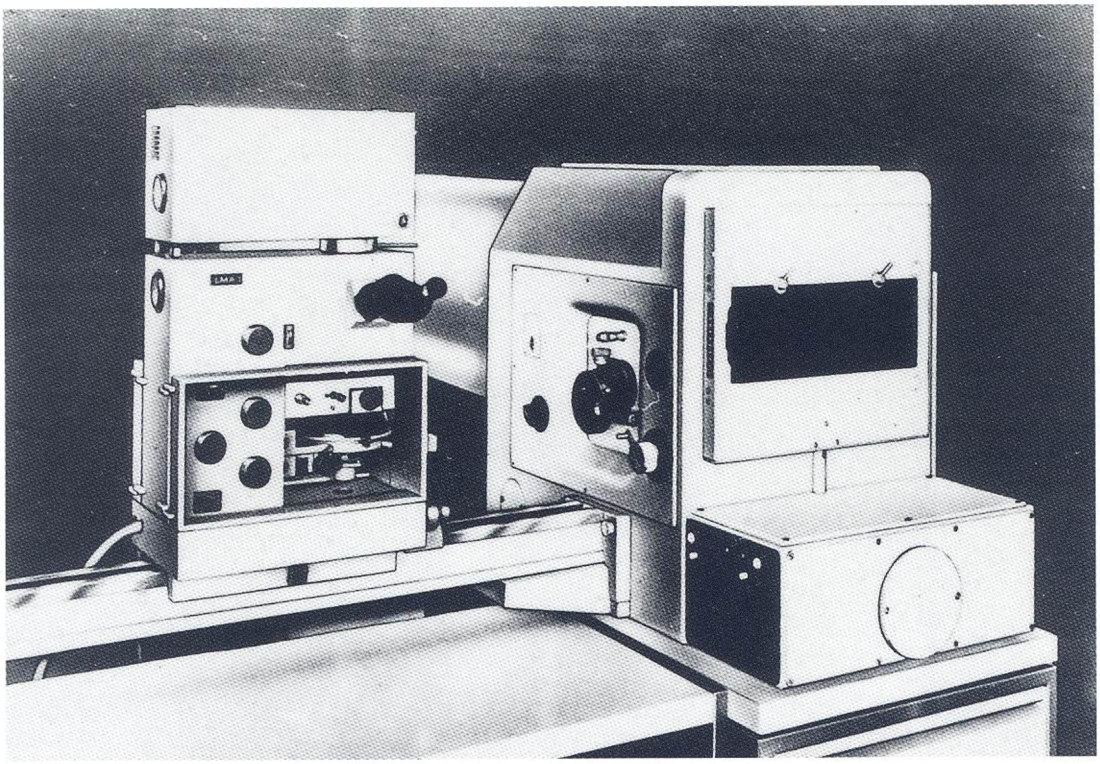

Fig. 9 *The first commercial laser microbeam apparatus LMA1* (source: Zeiss Archive, number Mess 2283).

channel analyzer for spectroscopy, fluorescence lifetime imaging (τ-mapping) with sub-nanosecond (300 ps) and submicron resolution (600 nm) as well as time-resolved fluorescence spectroscopy with 10 nm spectral resolution were performed. One of the first studies in 1988 with this novel time-gated laser scanning microscope was the investigation of intracellular porphyrin monomers and aggregates in tumor cells as well as in living tumor-bearing mice (Fig. 10) (Bugiel *et al.* 1989; König and Wabnitz, 1990). This first time-resolved picosecond laser scanning microscope opened a new field of research, however it was only used for one-photon excited fluorescence imaging. Although it was built in the division of *Nonlinear Physics* at the university under the guidance of Prof. B. Wilhelmi, unfortunately nobody at that time was thinking to look for nonlinear fluorescence excitation (just by replacing the long-pass filter against a short pass filter). The first two-photon excited fluorescence images of living cells taken with an ultrashort pulsed laser scanning microscope were published by an American group in 1990 (Denk *et al.*, 1990) who also filed a patent on nonlinear laser scanning microscopy. Two-photon excitation was predicted by Göppert-Meyer in 1931 in her Ph.D. Thesis and first realized with the availability of lasers by Kaiser and Garret in 1961. They detected the blue emission of $CaF_2:Eu^{2+}$ crystals with red light excitation of a 500 µs ruby laser. The nonlinear excitation requires extremely high concentrations of photons in space and time, e.g. high photon flux densities. Interestingly, such a high photon concentration can be achieved with microscopes in a tiny subfemtoliter focus volume when laser beams are focused to its diffraction limit in the submicron range with high numerical aperture objectives.

1991, two years after the Wall came down, was the year of reunification of *Zeiss* Oberkochen and *Zeiss* Jena. However, inspite of all positive aspects, this unification was combined with a deep cut in employment. Compared to the situation of 1989 with 25,000 Zeiss employees in Jena (about 100.000 inhabitants), the *Carl Zeiss Jena GmbH* has now a workforce of about 1,500. The other "daughter" of the former VEB Carl Zeiss *Jenoptik AG* (head: Dr. Späth) employs also about 1,500 in Jena (Fig. 11). The largest employer in town is now the university with 6,459 jobs (12,114 students), and in fact, it is the largest employer in the state Thuringia.

In 1994 the microscopy division of the enterprise Zeiss took its headquarter in Jena. One of the highlights in Jena's microscope developments nowadays is the prototype of a compact femtosecond laser based multiphoton microscope (LSM560). The novel field of multiphoton microscopy is also topic of a common "Bioregio" project between Zeiss and the university, in particular with our institute. A femtosecond and picosecond laser scanning microscope for non-resonant two- and three-photon fluorescence excitation was also part of the "hardware" of the International Practical Course. The 80 workshop participants from 13 countries had the possibility to get hands-on experience and to image visible fluorescence of their microscopic samples with the near infrared (NIR) ultrashort pulsed laser radiation. Other workstations with various

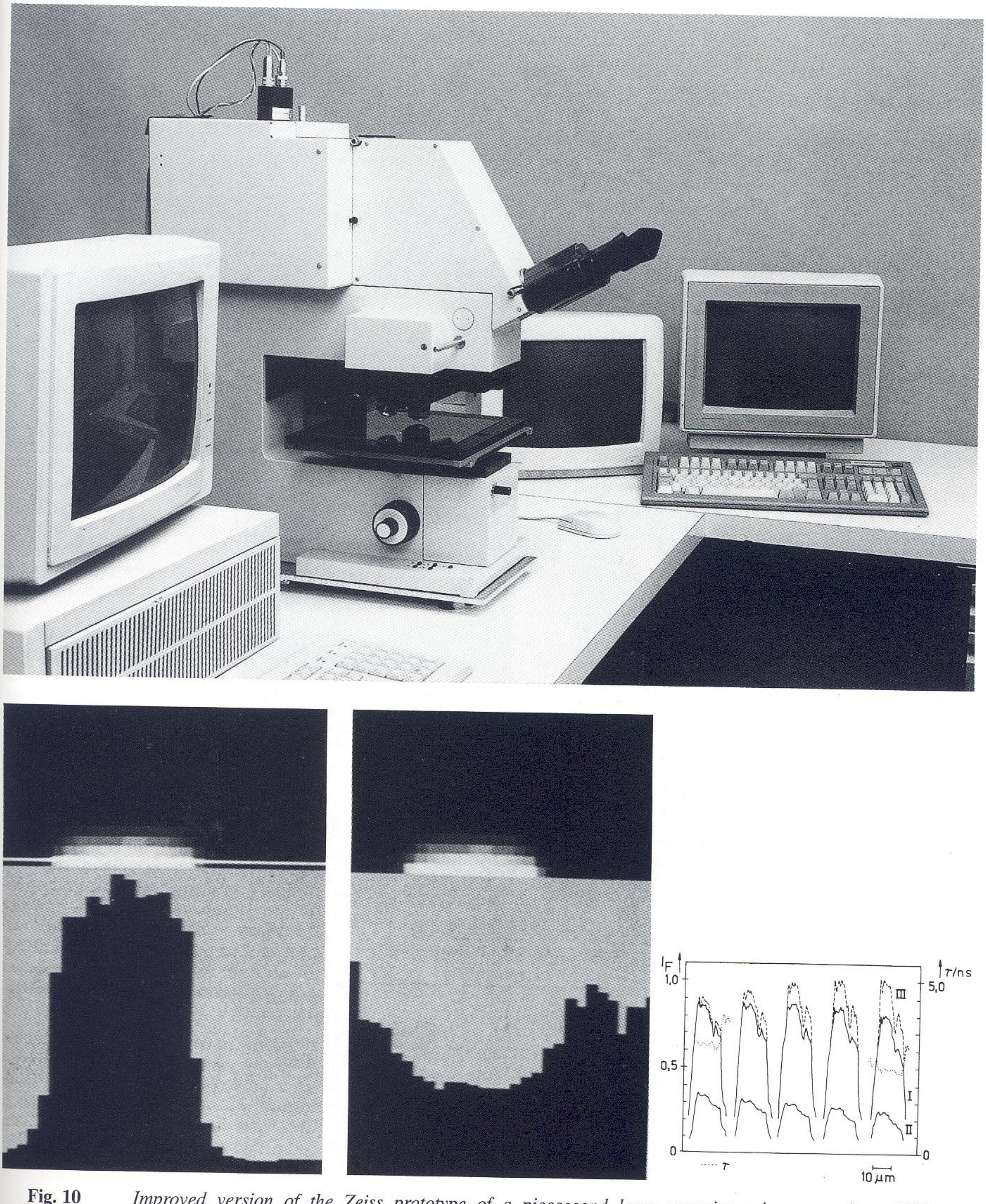

Fig. 10 *Improved version of the Zeiss prototype of a picosecond laser scanning microscope from 1988* (top, source: Zeiss Archive, number: G7809), *fluorescence lifetime imaging of a porphyrin-incubated cancer cell* (bottom left), *and time-resolved fluorescence detection of porphyrin photoproduct formation* (bottom right) (I: 0.7-3.6 ns; II: 3.6-6.5 ns; III: 0-7 ns).

Fig. 11 *The Carl Zeiss Jena GmbH* (top) *and the Jenoptik AG* (bottom) *emerged from the former VEB Carl Zeiss Jena.* Note in front of the bottom image the ruins of the "anatomy tower" where Goethe visited lectures on anatomy. In 1784, Johann Wolfgang von Goethe found in Jena the intermaxillary bone in human beings.

Zeiss microscopes included time-gated fluorescence microscopes in the picosecond range, laser tweezers, ultraviolet microscissors, confocal laser scanning microscopes, a fluorescence correlation spectroscopy (FCS) set-up, a fluorescence *in situ* hybridization (FISH) station for multigene detection, and the prototype of a scanning near field microscope.

A major topic of the international workshop was the application of near infrared laser microscopy in cellular and molecular biology. Two important novel types of NIR microscopes are used in life sciences. There are the laser tweezers (optical traps) for optical micromanipulation, optical picoNewton force determination, and their use as novel diagnostic tools. Laser tweezers, founded by Art Ashkin from AT&T Bell Labs (Ashkin 1970; Ashkin *et al.* 1987), are based on highly focused continuous wave (cw) NIR radiation. The other type is the femtosecond/picosecond scanning NIR laser microscope for pinhole-free, intracellular 3D multiphoton fluorescence imaging as well as for nonlinear 3D photochemistry. The large application scale of these NIR microscopes reflect their use not only as novel diagnostic tools, but also as tools of micromanipulation (e.g. as therapeutic tools in laser-assisted *in vitro* fertilization). The other major topic was high-resolution laser microscopy using near field scanning microscopes with <100 nm resolution, 4π- and theta microscopy (two-objective arrangements) with $\leq$100 nm resolution and fluorescence correlation spectroscopy for single molecule detection.

We hope that the *International Workshop on Lasermicroscopy* (colour front page) also provided a contribution to demonstrate the permanent potential of the city of Jena as a top center of optics and sciences. This special issue with major talks from the workshop should give an overview of the very new developments and applications of laser microscopy in cellular and molecular biology.

Acknowledgments – We wish to thank Dr. W. Wimmer (Zeiss Jena GmbH), Dr. P. Fischer (Institute of Anatomy II, University Jena), M. Szabo (Institute of Anatomy I, University Jena) and M. Schmidt (Museum Romantikerhaus).

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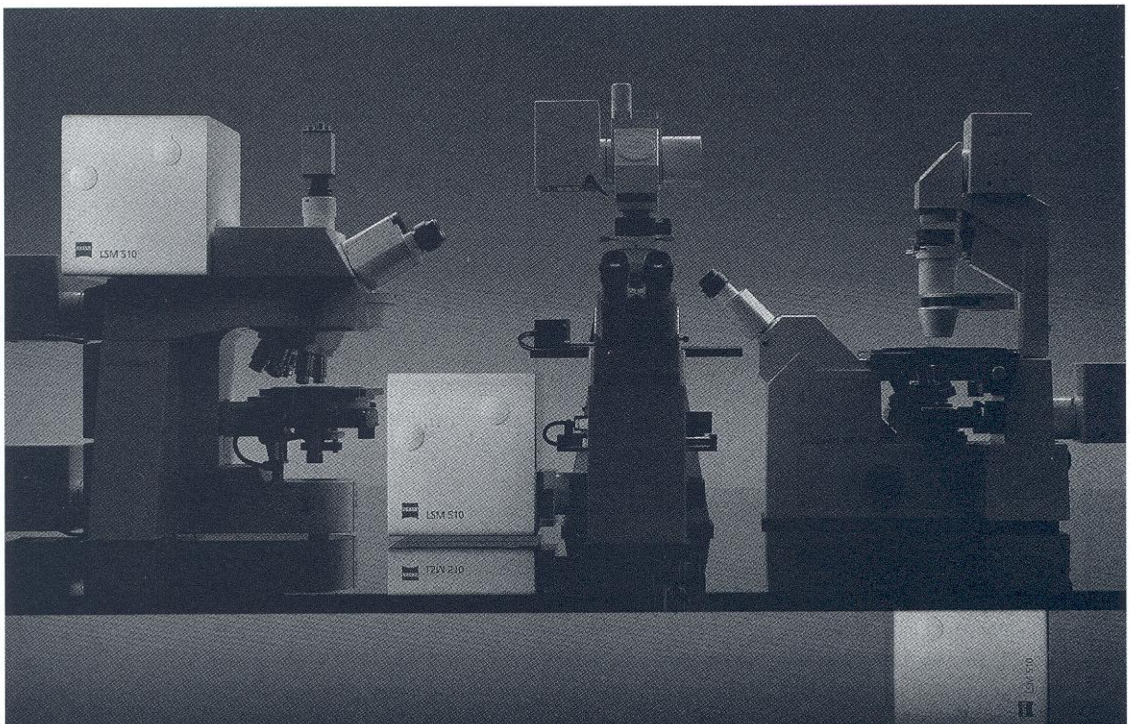
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