

# MICROSCOPICA ACTA

ZEITSCHRIFT FÜR WISSENSCHAFTLICHE MIKROSKOPIE  
UND MIKROSKOPISCHE TECHNIK

HERAUSGEBER - EDITORS

L. Jenny, Basel · W. Boguth, Basel · H. P. Rohr, Basel  
J. Grehn, Wetzlar

Band 75 · 1973/74 · Volume 75



S. HIRZEL VERLAG STUTTGART

Microsc. Acta

## A. Autoren der Beiträge — A. Authors of papers

- Albert, L.: Bericht über die gemeinsame Tagung der Belgischen, Deutschen und Niederländischen Gesellschaften für Elektronenmikroskopie in Lüttich, 3.—6. September 1973 . . . . . 364
- Bangerter, D., siehe Hunziker, O.
- Bánóczy, Joli, siehe Holmstrup, Palle
- Bereiter-Hahn, Jürgen, siehe Schliwa, Manfred
- Boguth, W.: Eine Einrichtung zum optischen Wechsel von Objekt- und Referenzmeßfeld beim Leitz-Durchlicht-Interferenzmikroskop (Mach-Zehnder-Typ), (Kurzmitteilung) . . . . . 361  
*A device for interchanging the object- and reference field in the Leitz transmission interference microscope (Mach-Zehnder type), (Short communication)*
- Bouchelet, Marcel, et Christian Moncel: Mise au point, pour observation microscopique prolongée de cellules vivantes, d'un système de thermostatisation et de perfusion continue pour microchambres de culture . . . . . 352  
*Microculture chamber for long term microscopic observation involving a continuous perfusion and thermoregulation device*
- Castenholz, Anton: Über eine neue Aufnahmetechnik — Streifen-Mikrophotographie — für fortlaufende Registrierungen an fixierten und bewegten Objekten der Mikroskopie . . . . . 309  
*Stripe-photomicrography, a new exposure technique for continuous registrations on fixed and on moving objects of microscopy*
- Cremer, Christoph, Christian Zorn and Thomas Cremer: An ultraviolet Laser microbeam for 257 nm . . . . . 331  
*Eine Laser-UV-Mikrobestrahlungsapparatur für 257 nm*
- Cremer, Thomas, siehe Cremer, Christoph
- Collan, Yrjö: Combining light and electron microscopic findings on individual cells: A theoretical and methodological study exemplified by combined electron microscopy and light microscope autoradiography . . . . . 48  
*Kombination von licht- und elektronenmikroskopischen Befunden an Einzelzellen: Eine theoretische und methodische Abhandlung, erläutert mittels der Kombination von Elektronenmikroskopie und lichtmikroskopischer Autoradiographie*
- Crefeld, Wolf: Die Bedeutung der Feinstkornentwicklung für den Informationsgehalt elektronenmikroskopischer Autoradiographien . . . . . 142  
*Grains of finer size in electron microscope autoradiographs: An assessment of their capacity to deliver pertinent information*

Daab, Peter, und Wilhelm Waidelich: Elektronenmikroskopische Untersuchungen der Photolyse von Silberhalogenidkörnern . . . . .	258
<i>Photolysis investigations of silver halides by electron microscopy</i>	
Dabelsteen, Erik, siehe Holmstrup, Palle	
Desser, Hans, und Ferdinand Ruzicka: Leukozytenhomogenisierung mit dem RF-1 Ribi Zellaufbrechgerät von Sorvall; eine elektronenoptische Studie . . . . .	130
<i>An electron optical study on homogenisation of leukocytes with a RF-1 Ribi Refrigerated Cell Fractionator (Sorvall)</i>	
Flórez-Cossio, T. J.: Die Anwendung des Färbeverfahrens Alcianblau-Kernechtrot bei Dickschnitten und Totalpräparaten in der Embryologie . . . . .	213
<i>The use of the alcian blue/nuclear-fast red staining method for thick sections and whole sections in embryology</i>	
Foh, E., H. Haug, M. König und A. Rast: Quantitative Bestimmung zum feineren Aufbau der Sehrinde der Katze, zugleich ein methodischer Beitrag zur Messung des Neuropils . . . . .	148
<i>Determination of quantitative parameters of the fine structure in the visual cortex of the cat, also a methodological contribution on measuring the neuropil</i>	
Grill, Dieter: Rasterelektronenmikroskopische Untersuchungen an Fichtennadel-Spaltöffnungen . . . . .	136
<i>Examinations on stomata of spruce-needles with the scanning electron microscope</i>	
Gruber, E.: Bericht vom Kolloquium über die Sichtbarmachung polarer Gruppen in der Cellulose, Graz, 29. – 31. März 1973 . . . . .	61
Gruber, E., K. John, H. Ulubay und J. Schurz: Topochemische Färbe- und Kontrastierungsreaktionen an festen Polymerphasen . . . . .	321
<i>Topochemical dyeing and staining reactions on solid polymer phases</i>	
Hantsche, H.: Die energiedispersive Röntgenanalyse am Rasterelektronenmikroskop. Anwendung für Biologie und Medizin (Übersichtsreferat, Teil 1) . . . . .	409
<i>The energy-dispersive X-ray microanalysis with the scanning electron microscope. Applications in biology and medicine (Review, Part 1)</i>	
Haug, H., siehe Foh, E.	
Heinzel, Werner: Modellversuch zur quantitativen Photometrie an Gewebeschnitten .	346
<i>Model experiments for quantitative photometry on tissue slices</i>	
Holmstrup, Palle, Erik Dabelsteen, and Joli Bánóczy: The limitations in the use of two films for quantitative autoradiography . . . . .	229
<i>Die Anwendungsgrenzen zweier Filme für quantitative Autoradiographie</i>	
Hunziker, O., D. Bangerter, W. Leimgruber, Ch. Schieweck, S. Vincenz and K.-H. Wiederhold: Preparation of serial sections from the deep-frozen cat brain with reference to stereotaxic planes . . . . .	452
<i>Herstellung von Serienschnitten am tiefgefrorenen Katzengehirn unter Berücksichtigung stereotaktischer Ebenen</i>	

- Johari, Om: Umfassende Material-Charakterisierung mit dem Raster-Elektronenmikroskop (übersetzt von B. und H. Pfefferkorn) . . . . . 1  
*Total materials characterization with the Scanning Electron Microscope (translated by B. and H. Pfefferkorn)*
- John, K., siehe Gruber, E.
- Kachel, Volker: Methodik und Ergebnisse optischer Formfaktoruntersuchungen bei der Zellvolumenmessung nach Coulter . . . . . 419  
*Methodology and results of optical form factors during the determination of cell volume according to Coulter*
- Koch, Karl-Friedrich, siehe Kraft, Winfried
- König, M., siehe Foh, E.
- Konitz, Hebert: Kohärenz und Auflösung in der Elektronenmikroskopie . . . . . 220  
*Coherence and resolution in electron microscopy*
- Kraft, Winfried, und Karl-Friedrich Koch: Ein neuer Mehrwellenlängen-Fluoreszenzilluminator für Forschung und Routine . . . . . 249  
*A new multi-wavelengths fluorescence illuminator for research and routine*
- Leibundgut, U., siehe Riede, U. N.
- Leimgruber, W., siehe Hunziker, O.
- Mehlhorn, Heinz, und Erich Scholtyssek: Die Parasit-Wirtsbeziehungen bei verschiedenen Gattungen der Sporozoen (Eimeria, Toxoplasma, Sarcocystis, Frenkelia, Hepatozoon, Plasmodium und Babesia) unter Anwendung spezieller Verfahren . . . 429  
*Cytological studies on host-parasite relationships in sporozoa (Eimeria, Toxoplasma, Sarcocystis, Frenkelia, Hepatozoon, Plasmodium, and Babesia) by means of different methods*
- Mihatsch, M., siehe Riede, U. N.
- Moncel, Christian, siehe Bouchelet, M.
- Muir, M. D.: Report on the Scanning Electron Microscope Symposium, Chicago, Ill., April 23–27, 1973 . . . . . 62
- Oehmichen, M.: Enzymatic activity of intravasal neutrophils and monocytes of rabbits at different age levels: First results by application of a combined cytochemical-autoradiographical method . . . . . 117  
*Enzymaktivität intravasaler neutrophiler Granulozyten und Monozyten von Kaninchen in verschiedenen Altersstufen: Erste Ergebnisse bei Anwendung der Kombination zytochemischer und autoradiographischer Methoden*
- Palladini, Guido, et Massimo Reitano: Une nouvelle méthode pour l'identification des groupes aminiques . . . . . 32  
*A new histochemical method for the identification of amino groups*
- Rast, A., siehe Foh, E.
- Reitano, Massimo, siehe Palladini, Guido

- Rieb, Jean-Pierre: Une installation de microcinématographie pour oeufs de Téléostéens, avec prises de vues à intervalles de temps déterminés . . . . . 338  
*An installation for time lapse microcinematography of Teleost eggs with variable frame cycle*
- Riede, U. N., U. Leibundgut und M. Mihatsch: Automatisierte Strukturanalyse am Beispiel der Orotsäure-induzierten Spongiosaveränderungen . . . . . 243  
*Automatical analysis of histological structures in the case of orotic acid induced alterations of spongiosa*
- Ruzicka, Ferdinand, siehe Desser, Hans
- Schieweck, Ch., siehe Hunziker, O.
- Scholtzseck, Erich, siehe Mehlhorn, Heinz
- Schurz, J., siehe Gruber, E.
- Schliwa, Manfred, und Jürgen Bereiter-Hahn: Morphologische und physiologische Grundlagen der Pigmentbewegung in Fisch-Melanophoren; I. Methodik der Bewegungsanalyse . . . . . 235  
*Pigment movements in fish melanophores; morphological and physiological studies; I. Methods in motion analysis*
- Snipes, Robert L.: Fixation and embedding of cell monolayers in Leighton culture tubes for electron microscopy . . . . . 43  
*Fixierung und Einbettung von Monolayer-Zellen in Leighton-Kulturröhrchen für die Elektronenmikroskopie*
- Söngen, Helmut: Probleme der wirklichkeitsgetreuen Beschreibung biologischer Objekte in der Lichtmikroskopie . . . . . 20  
*Problems of the realistic description of biological objects in light microscopy*
- Ulubay, H., siehe Gruber, E.
- Vinzenz, S., siehe Hunziker, O.
- Waidelich, Wilhelm, siehe Daab, Peter
- Wiederhold, K.-H., siehe Hunziker, O.
- Zorn, Christian, siehe Cremer, Christoph

## **An ultraviolet Laser microbeam for 257 nm \*)**

### **Eine Laser-UV-Mikrobestrahlungsapparatur für 257 nm**

CRISTOPH CREMER <sup>1)</sup> <sup>2)</sup>, CHRISTIAN ZORN <sup>2)</sup> und THOMAS CREMER

Institut für Humangenetik und Anthropologie der Universität Freiburg i. Br.

Eingegangen am 7. August 1973

#### **Summary**

A laser-uv-microbeam is described for the wavelength 257 nm which allows microirradiation of preselected sites of living cells with an effective spot size of approx.  $0.5 \mu\text{m}$  in diameter, as measured by fluorescence experiments and by uv-induced lesions in stained cell specimens and in unstained living cells. The maximum irradiance power density is approx.  $10^2 \text{ erg}/(\text{s} \cdot \mu\text{m}^2)$ . The ultraviolet light is produced by frequency-doubling of the 514.5 nm line of a continuous wave argon-ion-laser. The optical arrangement in the irradiation microscope is similar to that used in a fluorescence incident light microscope. Focusing and observation are done by means of the same quartz-objective ( $\times 100$ ).

#### **Zusammenfassung**

Eine Laser-UV-Mikrobestrahlungsapparatur wird beschrieben für die Wellenlänge 257 nm. Sie gestattet eine gezielte Mikrobestrahlung lebender Zellen mit einem effektiven Fokusbereich von ca.  $0,5 \mu\text{m}$ , bestimmt aus Fluoreszenzexperimenten und aus UV-induzierten Läsionen in gefärbten Zellpräparaten und in ungefärbten lebenden Zellen, bei einer maximalen Bestrahlungsstärke von ca.  $10^2 \text{ erg}/(\text{s} \cdot \mu\text{m}^2)$ .

Das ultraviolette Licht wird erzeugt durch Frequenzverdoppelung der 514,5 nm-Linie eines kontinuierlichen Argon-Ionen-Lasers. Die optische Anordnung im Bestrahlungsmikroskop ist ähnlich derjenigen in einem Fluoreszenzauflichtmikroskop. Fokussierung und Beobachtung erfolgen durch dasselbe Quarz-Objektiv (100 : 1).

### **1. Introduction**

UV-microbeams for partial cell irradiation have been used for more than 60 years [11]. The large effective spot size ( $\geq 2 \mu\text{m}$ ) and the low irradiance power density ( $10^{-2} \text{ erg}/(\text{s} \cdot \mu\text{m}^2)$ ) obtainable with conventional uv-sources [8], however, have limited their applicability in producing small defined lesions on cell organelles mov-

---

<sup>1)</sup> Dipl.-Phys. CHRISTOPH CREMER, Institut für Humangenetik und Anthropologie der Universität Freiburg i. Br., Albertstraße 11, D-7800 Freiburg i. Br.

<sup>2)</sup> Parts of this investigation will be presented in doctoral dissertation submitted to the faculty of Biology, University of Freiburg i. Br.

\*) This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 46).

ing relatively fast: The irradiation damage is widespread and the required irradiation times range between several seconds and a few minutes.

Recently, pulsed laser sources have been used to avoid these disadvantages [2, 5, 9]. So far, no device for partial cell irradiation has been described, which makes use of a continuous laser-uv-source with a wavelength in the absorption maximum of the DNA in connection with a focusing objective of high aperture (n.a.  $\approx 1$ ).

In this report, a cw-laser-uv-microbeam is described for the wavelength 257 nm which allows microirradiation of organelles of living cells with an effective spot size of 0.5  $\mu\text{m}$  in diameter and an irradiation power density of  $10^2 \text{ ergs}/(\text{s} \cdot \mu\text{m}^2)$ . Our group has set up this device to study possibilities of producing defined lesions on chromosomes in mammalian cells. Experiments were undertaken to obtain information on whether by using a laser-uv-microbeam focal lesions of the DNA could be produced in nuclei of Chinese Hamster cells without preventing further cell proliferation [4].

## 2. General description

The basic design of the device is represented diagrammatically in figure 1:

A continuous wave (cw) laser beam with a wavelength of 514.5 nm, emitted by an argon-ion-laser (1), is transmitted through an ammonium dihydrogen phosphate (ADP) crystal (2). Thus, due to nonlinear optical effects in the ADP-crystal, frequency doubling of the 514.5 nm laser light occurs and coherent uv-light with a wavelength  $\lambda = 257.3 \text{ nm}$  is emitted, being collinear with the exciting laser beam. The uv-beam is separated from the exciting green laser beam by a prism (3) and reflected by two mirrors (4) to a beam splitter (5) which diverts a small fraction of the uv-light to a detector system (6) for power measurement. The duration of irradiation is controlled by a photographic shutter (7). By means of a selecting mirror (8), reflecting the uv-light while transmitting the red light, a red pilot beam ( $\lambda = 632.8 \text{ nm}$ ) emitted by a low power He-Ne-laser (9) is aligned collinearly to the uv-beam. A mirror (11) reflects the beams into the irradiation microscope (12 – 19). To facilitate sterile conditions, the irradiation microscope is located in an adjoining room, and both beams are transmitted there through a quartz window. The optical arrangement in the irradiation microscope is similar to that used in a fluorescence incident light microscope: uv-beam and pilot beam pass a dispersing lens (12) with a negative focal length of approx. 5 cm. After reflection by a selecting mirror reflecting the uv-light to approx. 80% and visible light to 10%, the beams are focused into the object plane (14) by an immersion objective of high magnification and aperture (15). To measure the uv-power falling into the aperture of the objective, it can be replaced by a photodiode (15 a). Simultaneously, the focusing objective serves for observation of the specimen by either an ocular system (16) or a television monitoring system (17). Photographs of the specimen are made with the attached camera system (18). The specimen can be observed also during irradiation because the selecting mirror (13) transmits the light produced by the illumination system (19) to approx. 90% in the whole visible spectrum.

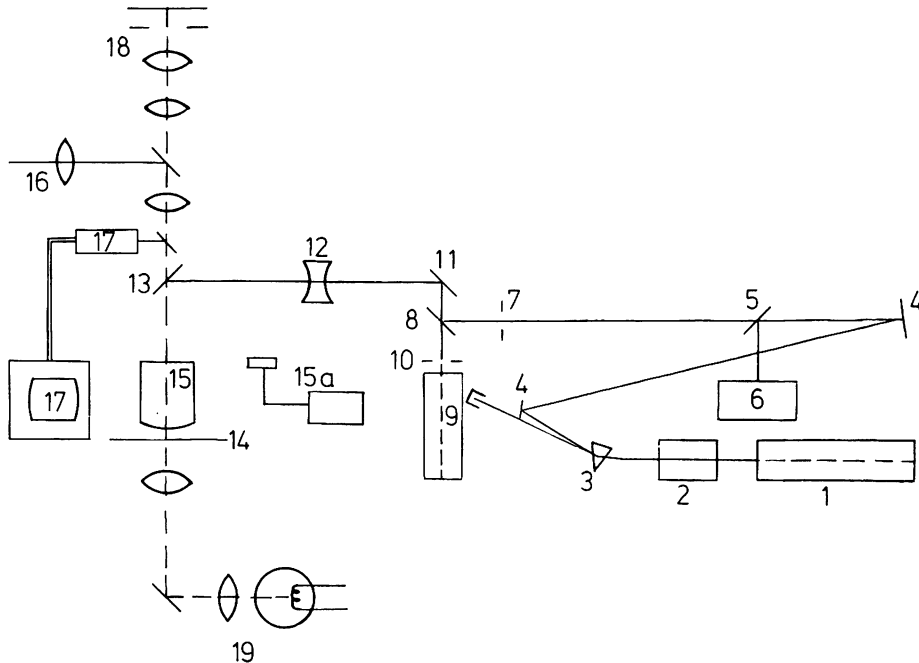


Fig. 1: Diagram of laser-uv-microbeam:

- |                        |  |
|------------------------|--|
| 1 = argon-ion-laser    | 13 = selecting uv-mirror (Balzers)   |
| 2 = uv-generator       | 14 = object plane  |
| 3 = separating prism   | 15 = microscope objective<br>(either Zeiss Ultrafluor<br>×100/0.85 Glyc Ph or<br>×100/1.25 Glyc) |
| 4 = mirrors            | 15 a = photodiode  |
| 5 = beam splitter      | 16 = ocular system   |
| 6 = uv-detector system | 17 = TV-system   |
| 7 = shutter            | 18 = camera system   |
| 8 = selecting mirror   | 19 = illumination system   |
| 9 = He-Ne-laser        |  |
| 10 = shutter           |  |
| 11 = adjustable mirror |  |
| 12 = dispersing lens   |  |

To microirradiate living cells, a special tissue chamber (Fa. Tecnomara) designed by P. HÖSLI is used, which allows slight squeezing of the cells. It is placed on a fine adjustable thermoregulated microscope stage. The area selected for microirradiation can be marked by two different procedures:

- The selected region is placed into the axis of the pilotbeam focus, which is visible as a red spot through the ocular or on the television screen. UV-microirradiation then occurs at the selected region marked by the spot.
- The selected region is marked with a cross-hair located in the image plane of the objective. With help of the dispersing lens (12, Fig. 1), the uv-beam is adjusted in such a way that the fluorescing spot produced by the focussed uv-light on a test specimen in the object plane is congruent with the intersection of the cross-hair.



### 3. Details of construction

The argon-ion-laser is a model 52 Be-A from Coherent Radiation Lab., with a prism wavelength selector, which selects the 514.5 nm line in TEM<sub>00</sub> mode (beam diameter 1.4 mm, beam divergence 0.8 mrad). The cw-output power (max. 1.4 W) is regulated by varying tube current and measured by a calibrated internal power meter. The frequency doubling is performed with a uv-generator model 440 from the same company. Prisms, mirrors, optical benches, mirror mounts etc. are standard ones. The selecting mirror (13, Fig. 1) in the irradiation microscope was developed by Balzers AG/Liechtenstein. All other elements of the irradiation microscope are from Zeiss/Oberkochen. The focusing microscope objectives used were Ultrafluar 100/0.85 Glyc. Ph and Ultrafluar 100/1.25 Glyc. The laser-uv-microbeam apparatus stands on heavy tables to protect it against vibrations. Due to separation of the microscope table from the laser table, working with the irradiation microscope (12–19, Fig. 1) does not disturb the optical arrangement for uv-generation and measurement (1–11, Fig. 1).

### 4. Measurements

Absolute uv-powers were measured with a calibrated spectroradiometer system (Model 585, EG + G). After careful adjustment of position and temperature of the ADP-crystal, a maximum uv-output of 1.1 mW was measured behind the separating prism (3, Fig. 1), the power of the exciting beam being 1450 mW. The uv-output is regulated by varying the power of the exciting laser beam. Due to losses in lenses, objectives and mirrors the irradiation power in the focus is reduced to 1 to 10% of the uv-power measured immediately behind the prism. To measure the irradiation power in the object plane, an image of the focus was transferred onto the diffusing disk of the spectroradiometersystem by means of a second ultrafluar-objective, the front lenses of the two objectives being optically connected by glycerine. To determine the smallest effective spot size in the object plane, (14, Fig. 1), three procedures were used:

- a) A quartz object micrometer, whose lines consist of an organic compound, was placed into the object plane. By directing the uv-microbeam on a line, the organic compound was excited to fluorescence, and the fluorescent area was microphotographed. Measurement of the spot sizes thus produced gave a diameter of the fluorescent area of 0.4  $\mu\text{m}$  (Fig. 2). Variation of the exposure time between 15 and 120 s did not significantly alter the spot size.
- b) In homogeneously stained nuclei paling was induced by uv-microirradiation, the irradiation times being approx.  $10^{-1}$  s. The resulting spot diameters obtained from photomicrographs were approx. 0.5  $\mu\text{m}$ .
- c) In unstained cell organelles (nucleoli) of living cells, lesions were produced with a phase contrast objective (Ultrafluar 100/0.85 Glyc Ph), they became immediately visible after microirradiation as darkening spots, the smallest diameter of the spots being approx. 0.5  $\mu\text{m}$  (Fig. 3), irradiation time 1 s. From our experience it can be said that an effective spot size with a diameter smaller than 1  $\mu\text{m}$  can be reproduced easily in routine experiments.



Fig. 2: Production of a fluorescing spot in the object plane by Laser-uv-micro-irradiation. The objective is a Zeiss Ultrafluar 100/1.25 Glyc, and the specimen a Zeiss quartz object micrometer, on which the fluorescing spot is produced. The distance between two lines matches  $10\ \mu\text{m}$ .

## 5. Discussion

### 5.1. uv-output

The power of the uv-beam generated in the ADP-crystal is proportional to the square of the power of the exciting laser beam [7]. The proportionality factor between the uv-power and the square of the laser power is specified by the manufacturer to be  $4 \times 10^{-3}/\text{Watt}$ . The experimentally found values were approx.  $1 \times 10^{-3}/\text{Watt}$ .

### 5.2. Focusing

The f-number of the focusing objective being very small, theoretical analysis of focusing has to use electromagnetic vector theory of diffraction to get an exact solution of the focusing problem [1]. For qualitative consideration use of scalar wave theory should be sufficient [3]. On this basis, calculations for laser beams are available [6]. Comparing the experimental results with these calculations, we conclude that 80 to 95% of the total energy delivered into the focus plane should fall into an area within the spot diameter observed. Furthermore, due to the strong convergence of the focused beam, high irradiation power density is achieved only in a region the extension of which along the optical axis is comparable with the spot diameter [6]. Thus it can be expected that the effects of uv-microirradiation outside the focus can be kept relatively small.

### 5.3. Comparison with other uv-microbeams

The smallest effective spot diameter applied in biological microirradiation experiments on living cells with the purpose of producing defined lesions in unstained cell organelles, using conventional sources, was reported to be approx.  $2\ \mu\text{m}$  [8]. Irradiation times varied in these cases from 5 s to 480 s, the maximum irradiation power densities being approx.  $10^{-2}\ \text{erg}/(\text{s} \cdot \mu\text{m}^2)$ . A comparison with the data of the laser-

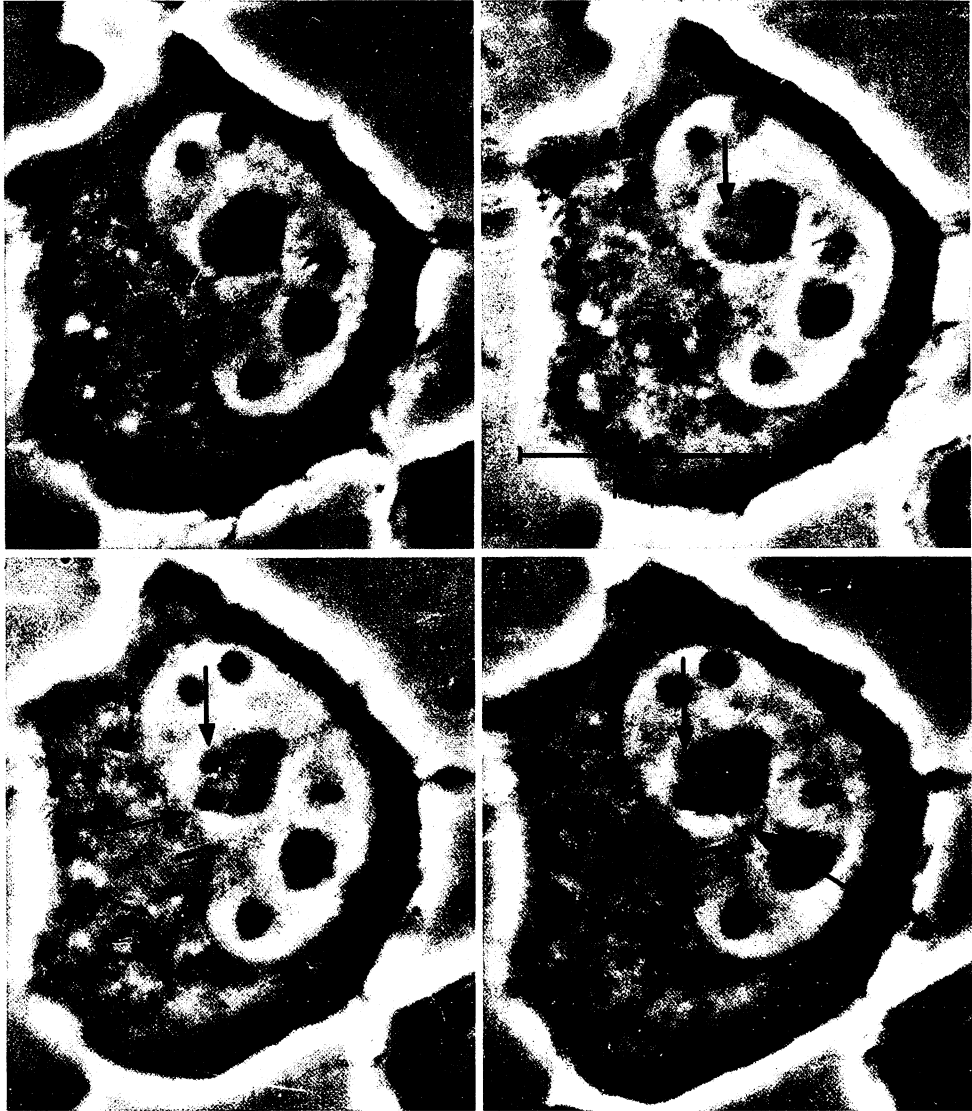


Fig. 3: Laser-UV-Microirradiation of an unstained living Chinese Hamster cell. — a) Prior to irradiation. — b)–d) Subsequent microirradiations of nucleolus, visible as dark spots; time between irradiations: 2 min. Phase contrast, objective Ultrafluor 100/0.85 Ph Glyc (used for irradiation and observation). Magnification 3200 : 1.

uv-microbeam described above shows the improvement available by using a coherent uv-source: The laser-uv-microbeam has an effective spot diameter four times smaller ( $\approx 0.5 \mu\text{m}$ ), and the irradiation power density of max.  $10^2 \text{ erg}/(\text{s} \cdot \mu\text{m}^2)$  is several orders of magnitude higher than in the conventional device. MORENO using a conventional uv-source with a wavelength of  $\lambda = 275 \text{ nm}$  observed focal lesions in KB cell

nuclei irradiated with an energy density of  $10^{-1}$  erg/ $\mu\text{m}^2$  [10]. With our apparatus it is possible to apply such an uv-energy density within a few milliseconds. This short irradiation time facilitates an irradiation of defined segments of moving cell organelles. Our results indicate that using the small uv-laser focus a considerably higher energy density can be delivered to cell nuclei without preventing further proliferation [4].

Laser-microbeams for biological research using ultraviolet light emitted by pulsed laser sources (20 ns to 50  $\mu\text{s}$ ) have been reported for 351 nm, mixed with green and blue laser light [2], for 347 nm [5], and for 265 nm [9].

Contrary to pulsed laser sources, a continuous wave laser source offers the possibility to vary irradiation power density and irradiation time independently from each other. Since  $\lambda = 257$  nm is in the absorption maximum of DNA, a continuous wave laser uv-microbeam as described in this paper may be especially useful for microirradiation of chromosomes in living metaphase cells.

#### *Acknowledgement*

We wish to thank Drs. W. KRONE, U. WOLF and L. SCHOELLER for discussion and suggestions; we are grateful for valuable advice to Drs. K. WEBER, W. SCHMIDT (Fa. Zeiss/Oberkochen) and Dr. P. HÖSLI, Amsterdam.

The selecting mirror in the irradiation microscope was developed by Fa. Balzers AG/Liechtenstein (Drs. H. PULKER and TH. RITTER).

#### **References**

- [1] BARAKAT, R.: Vector Diffraction Theories. In: (E. Wolf, Edit.): Progress in Optics, Vol. I, p. 99–104. North-Holland Publ. Company, Amsterdam (1965).
- [2] BERNS, M. W.: A simple and versatile argon laser microbeam. Exp. Cell Res. **65**, 470–473 (1971).
- [3] BESSIS, M., et G. NOMARSKI: Irradiation Ultra-Violette des Organites Cellulaires Avec Observation Continué En Contraste De Phase. The J. Bioph. Bioch.. Cytol. **8**, 777–789 (1960).
- [4] CREMER, C., T. CREMER, C. ZORN, W. KRONE, I. NIENHOLD and L. SCHOELLER: Effects of CW-Laser-UV-Microirradiation ( $\lambda = 257$  nm) of Nucleus and Cytoplasm on Survival and Cell Growth of Chinese Hamster Cells. In preparation (1973).
- [5] HILLENKAMP, F., R. KAUFMANN, E. REMY: Der Laser als Instrument der Zellforschung im Mikro- und Submikrobereich. Laser **1**, 40–42 (1971).
- [6] INNES, J. D., A. L. BLOOM: Design of Optical Systems for Use with Laser Beams. Spectra Physics Laser Technical Bulletin **5**, 1–10 (1966).
- [7] KLEINMAN, D. A.: Theory of Second Harmonic Generation of Light. Phys. Rev. **128**, 1761–1775 (1962).
- [8] MORENO, G.: Partial Cell Irradiation by Ultraviolet and Visible Light: Conventional and Laser Sources. Int. Rev. exp. Pathol. **7**, 99–137 (1969).
- [9] MORENO, G., C. SALET et M. BESSIS: Micro-irradiation de noyaux cellulaires par rayonnement laser ultraviolet. C.R. Acad. Sc. Paris **269**, Série D, 781–782 (1969).
- [10] MORENO, G.: Effects of Ultraviolet Micro-Irradiation on Different Parts of the Cell. II. Cytological Observations and Unscheduled DNA Synthesis after Partial Nuclear Irradiation. Exp. Cell Res. **65**, 129–139 (1971).
- [11] TSCHACHOTIN, S.: Die mikroskopische Strahlenstichmethode, eine Zelloperationsmethode. Biol. Zbl. **1**, **32**, 623–630 (1912).