A Simple UV-Microbeam for Partial Cell Irradiation

Many devices have been developed for irradiating small areas of cells with either UV- or ionizing radiation[1–7]. The purpose of the present communication is to describe a simple UV-microbeam which employs a reflex camera in the optical train.

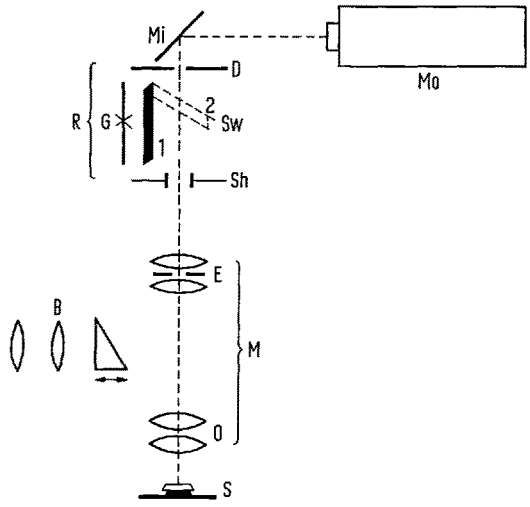
Method. As shown in the Figure, UV-light from a monochromator[8] is reflected by a first-surface aluminized mirror[9] onto a field diaphragm in the film plane of the reflex camera[10]. The image of the field diaphragm is focussed and demagnified through the microscope (equipped with an eyepiece and objective system which is 'achromatic' for UV- and visible light[11]) and projected through a quartz coverslip[12] onto the specimen.

In this system the image of the specimen is identical in size and position in the ground glass and in the film plane containing the field diaphragm. The UV-image to appear in the specimen plane is aimed and focussed by bringing a cross located in the center of the ground glass of the reflex camera into register with the cell structure to be irradiated. When desired, the exact shape and location of the aperture may be drawn on the ground glass in order to provide the operator with knowledge of precisely what area of the cell is irradiated. If desired, this instrument could be used for the irradiation of extended cellular areas while some portion of the cell was selectively shielded. Masks corresponding to the size and shape of the area to be shielded can be held in the film plane with fine wires or supported by a flat quartz plate. For any particular application the usefulness of such a UV-field with a 'micro-shadow' would be determined by the amount of edge diffraction and stray light in the system. These techniques will be especially useful when areas of highly irregular shapes are to be irradiated. For routine irradiations, the instrument could be aimed and focussed while observing a crosshair in a focussing eyepiece. If photographic records were desired, a separate side tube with either a partially aluminized mirror or a sliding prism would be required.

For convenience and to allow careful visual observation of the cells, the microscope is equipped with a trinocular tube employing a sliding prism that can be completely removed from the UV-optical-path. If the microscope body contains a glass lens for intermediary magnification in the UV-path, this lens must be removed or the UV-radiation will be absorbed. In the case of the standard brightfield 32 × Zeiss Ultrafluar objective, removal of this lens from the Leitz Labolux stand does not result in any gross deterioration in the quality of the visible light image. However, no information is yet available concerning the effects of this modification when objectives of higher magnification or phase contrast systems are to be used. The UV-exposure can be controlled either by removing the reflex camera mirror from the UV-optical-path or by the subsequent use of the shutter of the camera.

Results. This UV-microbeam has proved satisfactory for studies of mitotic delay in the sea urchin egg[13] and differentiation delay in the ciliate *Stentor*[14,15] and for classroom demonstrations of classical microbeam experiments such as loss of mitotic spindle structure and localized rupture of the cell membrane[1,2].

Discussion. The main advantages of this UV-microbeam are the simplicity of construction, the relative freedom from alignment problems, and the ease with which diaphragms or opaque masks of different sizes and shapes can be interchanged. The apertures commonly used in this system are large, ranging from 1 mm to as much as 2 cm in diameter. Systems employing microapertures require careful attention to the initial machinework and to the final alignment of the aperture[3]. Machining irregularly shaped microapertures or inserting opaque stops within



The UV-optical path of the reflex camera microbeam. When the swinging mirror (Sw) of the reflex camera (R) is in position 1, UV-radiation from the monochromator (Mo) is projected onto the specimen (S) which is covered with a quartz coverslip. The instrument is aimed and focussed with visible light from a substage condenser by moving the swinging mirror into position 2 so that an image of the specimen is projected on the ground glass screen (G) and brought into coincidence with the cross. First surface aluminized mirror (Mi); field diaphragm (D) with an aperture which defines the size of the microbeam; shutter (Sh); microscope (M); ultrafluar eyepiece (E); ultrafluar objective (O); binocular eyepieces with slide-in prism (B).

[1] R. E. ZIRKLE, in *Advances in Biological and Medical Physics* (Ed. J. H. LAWRENCE and C. A. TOBIAS; Academic Press, New York 1957), vol. 5, p. 103.

[2] C. L. SMITH, in *International Review of Cytology* (Ed. G. H. BOURNE and J. F. DANIELLI; Academic Press, New York 1964), vol. 16, p. 133.

[3] R. B. URETZ and R. P. PERRY, Rev. Sci. Instr. *28*, 861 (1957).

[4] D. S. HATHAWAY and G. G. SELMAN, J. Embryol. exp. Morph. *9*, 310 (1961).

[5] H. GLUBRECHT, Biophys. *1*, 78 (1963).

[6] S. INOUÉ, in *Primitive Motile Systems in Cell Biology* (Ed. R. D. ALLEN and N. KAMIYA; Academic Press, New York 1964), p. 549.

[7] A. FORER, Expl. Cell Res. *43*, 688 (1966).

[8] Bausch and Lomb High Intensity Grating Monochromator equipped with a 200 W Super Pressure Mercury Source, stabilized with a 1000 VA. Sola constant voltage transformer. A simple UV-lamp may be substituted for the monochromator if heterochromatic UV-radiation is satisfactory.

[9] Edmund Scientific Corp., Barrington, N. J.

[10] Ernst Leitz Aristophot Camera-Microscope.

[11] Carl Zeiss Ultrafluar. The system focusses 2600 Å UV and 5550 Å visible light images within 1–2 μ of each other.

[12] Quartz coverslips (0.35 mm thick) are obtainable from A.D. Jones Optical Works, Inc., Cambridge, Mass. or Carl Zeiss, Inc. Thinner coverslips (e.g. 0.17 mm) may also be used with the glycerine immersion objectives.

[13] R. C. RUSTAD, Photochem. Photobiol. *3*, 529 (1964).

[14] B. R. BURCHILL, Effects of metabolic inhibitors and radiations on regeneration in the ciliate *Stentor coeruleus*, Ph.D. Dissertation, Western Reserve University (1966).

[15] B. R. BURCHILL and R. C. RUSTAD, Radiat. Res. *31*, 648 (1967).

apertures less than 0.1 mm in diameter would be technically difficult. Since the UV-transmitting objective is mounted in a rotating nosepiece and the choice of condensers is optional, there is no necessity of moving the specimen to another microscope for observation with other types of optics, and the instrument can be used for routine purposes when partial cell irradiation experiments are not in progress[16].

Résumé. On peut irradier les régions sous-cellulaires si la radiation UV d'un monochromateur est limitée par les ouvertures interchangeables de champ dans le plan de la pellicule photographique d'un appareil reflex, monté sur un microscope. L'image UV est démagnifiée par un oculaire et un objectif achromatique aussi bien à la lumière UV qu'à la lumière visible.

R. C. Rustad

Departments of Radiology and Biology,
Case Western Reserve University,
Cleveland (Ohio 44106, USA), 28 February 1968.

[16] Acknowledgments. This work was supported by contracts No. AT-(40-1)-2730 and No. W-31-109-ENG-78, Report No. COO-78-172, with the U.S. Atomic Energy Commission. The first instrument was assembled in 1962 while the author was affiliated with the Department of Biological Sciences, Florida State University.

Elektrische Eigenschaften von Glasmikroelektroden nach Membranpotentialmessungen an Zellkulturen

Glasmikroelektroden nach Ling und Gerard[1] zur intrazellulären Membranpotentialmessung haben die unerwünschte Eigenschaft, an ihrer Spitze elektrische Potentiale, sogenannte «tip-potentials» zu bilden. Adrian[2] und Küchler[3] messen am isolierten Froschskelettmuskel um so kleinere Membranruhepotentiale, je negativer das «tip-potential» der Elektrode ist. Über spezifisch verursachte Änderungen des Elektrodenwiderstandes und des «tip-potentials», wie sie bei Potentialmessungen an Zellkulturen nach unseren Beobachtungen eintreten können, soll hier berichtet werden.

Material und Methoden. K.B.-Krebszellen wachsen als monolayer in der Deckglaskultur. Die Messungen des Membranpotentials erfolgen in einer feuchten, thermostatisierten Messkammer[4]. Eine Kombination aus Mikroskop (Phasenkontrast) und Mikromanipulator (beides Fa. Carl Zeiss Jena) erlaubt eine schonende Punktion der Einzelzelle unter direkter mikroskopischer Kontrolle. Die Glasmikroelektroden (äusserer Spitzendurchmesser $\leqslant 0{,}5\ \mu$, «Tip-Potential» $\leqslant 5$ mV, 20–40 MΩ) werden durch Kochen im Vakuum mit 3 mol KCl gefüllt (s. Levine[5]). Die Potentialmessung erfolgt symmetrisch über KCl-Agarbrücken und Kalomelelektroden mit dem Negativ-Capazitance-Elektrometer der Fa. Disa Elektronik Kopenhagen (Eingangswiderstand $> 10^{10}\Omega$, Gitterstrom $< 10^{12}$ A, Eingangskapazität < 1 pF). Anzeige und Registrierung erfolgt mittels Oszillograph und Kamera[6]. Widerstand und «Tip-Potential» werden unmittelbar in der Messkammer und im Nährmedium der Zellen (siehe [7]) vor Beginn der Messungen und nach jeweils 50 Zellpunktionen gemessen. Als indifferente Elektrode taucht ein dünnes, mit 3 mol KCl-Agar gefülltes Glasröhrchen in das Nährmedium.

Ergebnisse und Diskussion. Das Membranpotential in der K.B.-Zellkultur unterliegt etwa einer Normalverteilung[7], das mittlere Membranpotential beträgt 11–13 mV (das Zellinnere negativ). Die Punktion der Zellen erfolgt normalerweise bei unseren Messungen so, dass die Elektrodenspitze das Deckglas nicht berührt. Auf diese Weise kann man mehrere hundert Zellen punktieren, ohne dass sich Widerstand R_{EI} oder «Tip-Potential» TP_{EI} der Mikroelektrode wesentlich verändern. Sticht man dagegen durch die Zellen hindurch und drückt man die Elektrodenspitze gegen das Deckglas, so verändert die Mikroelektrode ihre elektrischen Eigenschaften (siehe Figuren 1 und 2). Mit zunehmender Zahl von Zellpunktionen wächst der Elektrodenwiderstand, die Elektrodenspitze wird negativer (Figur 1). Offenbar scheint es sich um einen «Verschmutzungs»-Effekt zu handeln, Zellmaterial wird in die Öffnung der Mikroelektrode hineingedrückt. Dies führt zu charakteristischen Veränderungen der elektrischen Eigenschaften der Meßsonde.

Die Figur 2 zeigt, wie sich mit zunehmendem Elektrodenwiderstand das «Tip-Potential» erhöht. Küchler[3] und

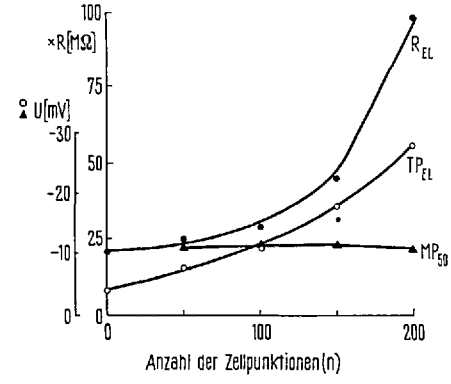


Fig. 1. Die Veränderungen von Widerstand R_{EI} und «Tip-Potential» TP_{EI} einer Glasmikroelektrode in Abhängigkeit von der Zahl n der punktierten K.B.-Krebszellen. MP_{50}, Mittelwert des Membranpotentials aus jeweils 50 Zellen.

[1] G. Ling und R. W. Gerard, J. cell. comp. Physiol. *34*, 383 (1949).
[2] R. H. Adrian, J. Physiol. *133*, 631 (1956).
[3] G. Küchler, Pflügers Arch. Ges. Physiol. *280*, 210 (1964).
[4] K. Redmann und D. Moschcau, Acta biol. med. germ. *20*, 255 (1968).
[5] L. Levine, Experientia *22*, 559 (1966).
[6] K. Redmann, Ch. Stolte und D. Lüders, Naturwissenschaften *54*, 255 (1967).
[7] K. Redmann, Biophysik *4*, 92 (1967).