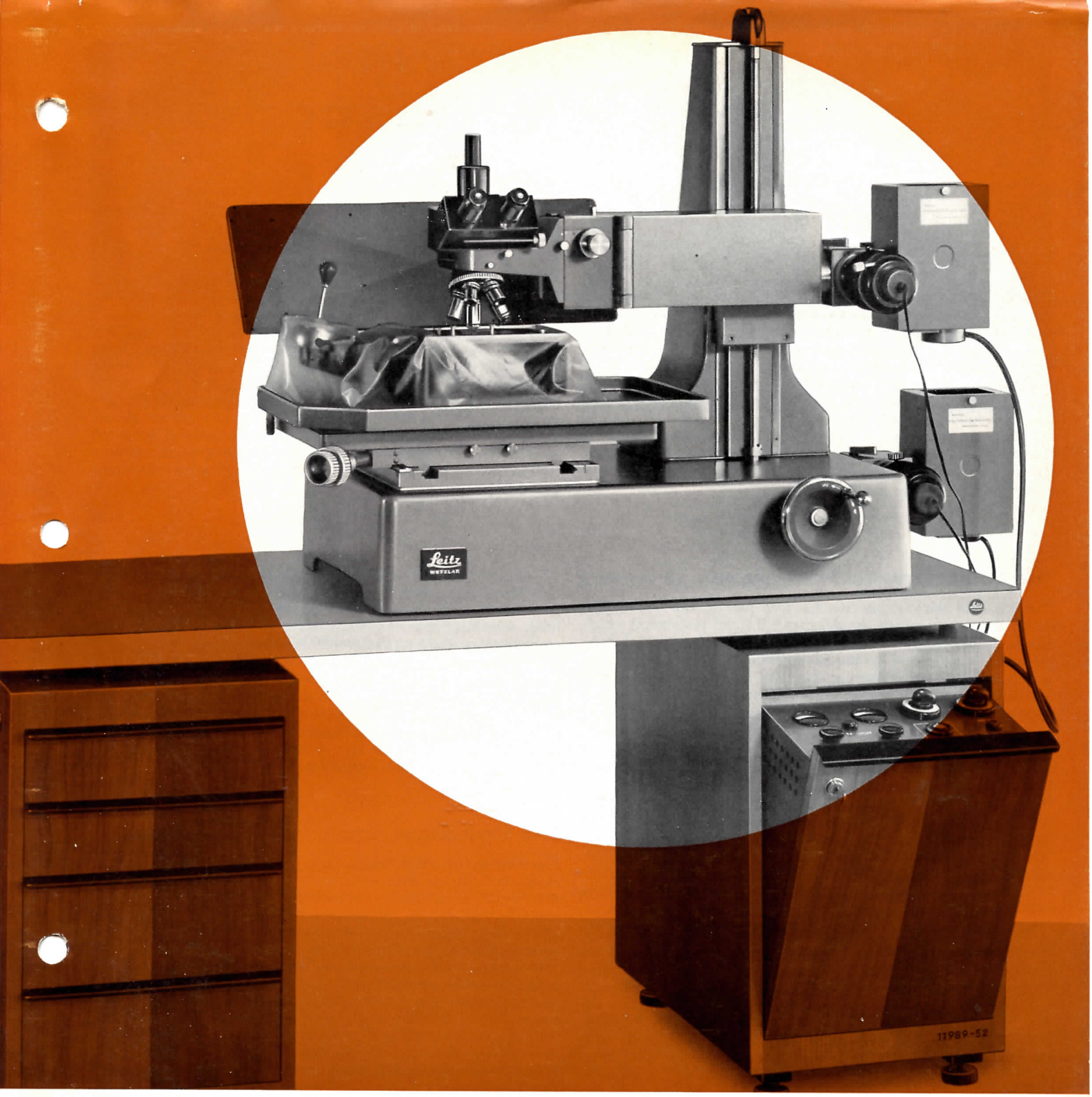

Leitz
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Intravital Microscopy

52 – 22 / Engl.

Intravital Microscopy

Introduction

Direct microscopical observation of the course of biological processes within the living organism represents the ultimate goal of the research worker in the medical and biological fields. Although practical experiments in which all organs and tissues were investigated in living animals have been conducted for a long time, in clinical research it was necessary in the majority of cases to confine microscopical examination to structures which were directly accessible to the microscope. Direct observation of **intact tissue** inside the living body could not be carried out for lack of suitable instruments; as a result, intravital (or vital) microscopy was initially developed mainly for the study of the capillary bed (microcirculation). Further progress which, naturally, had a favourable effect on intravital microscopy of the capillary bed, too, became possible only with the development of new instruments allowing the observation of intact tissue or the intact cell in situ. It was essential that the manipulations necessary on the object should not produce pathological changes. Last, but not least, the designers had to bear in mind the need for a basic outfit of universal character for use with the already existing range of instruments. Only equipment allowing the research worker the greatest possible amount of freedom will permit the pursuit of broadly based research programmes with the aid of new methods of preparation and observation.

The LEITZ Intravital Microscope

The intravital microscope has become part of our production range after many years' development; it is an instrument which admirably meets the demands even of extensive research programmes. It is suitable for the observation of the structure, topography and function of the capillary bed as well as of other cell and tissue complexes in situ. Thus, effects of pathogenic factors such as temperature changes, drugs, etc. can be investigated in addition to the normal capillary bed. By means of specially developed chambers parenchymatous organs, or bone and bone marrow can be examined in the living state like histological preparations.
Interesting possibilities also open up in intravital fluorescence microscopy. Here, particularly instructive pictures of effective colour differentiation are obtained during the examination of resorption processes in the mucosa or the permeability of vessels with the aid of various fluorochromes or fluorescent drugs in order to study micro-circulation under normal or pathological conditions.

The Microscope Stand

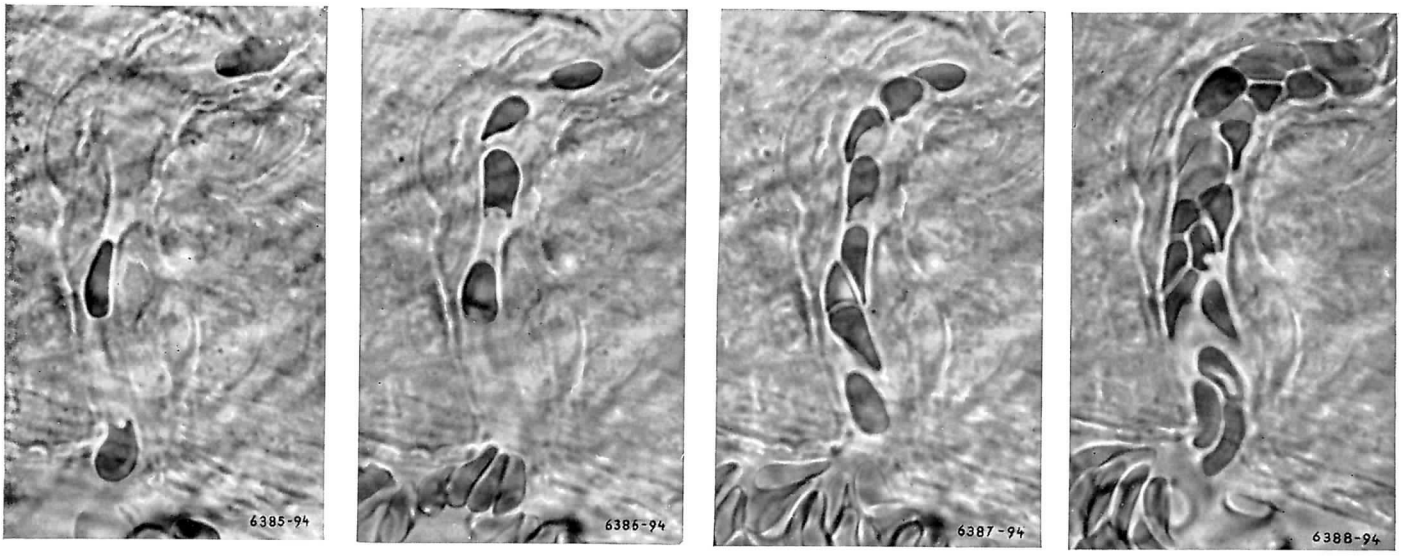
In order to meet the strict demands of rigidity and freedom from torsion the stand has been designed in compact form. It consists of the base, the vertical column, and the carrier arm supporting the focusing mechanism, tube, and incident light illuminator.
The tapering column for the carrier arm, and the mechanical stage with clamps for securing the animals are mounted on the extremely massive 32 x 20″ base, whose clear, smooth shape is interrupted on the front only by the handwheel for the vertical adjustment of the carrier arm. In addition, a bayonet changer for the transmitted light illumination is mounted on the right side of the base. In order to suppress vibration, the base rests on four elastic feet. Column and base are joined together by screws. The front of the column has a very wide dovetail guide appropriate for the long range of adustment (15″) of the carrier arm. The vertical adjustment of the carrier arm is actuated by a spindle whose pitch of 4 mm is especially suitable for operational requirements. It will thus often be possible to dispense with the coarse and fine adjustment of the tube during low- and medium power work, and to focus by means of the spindle alone. A counterweight inside the column ensures smooth, even movement. The carrier arm is aligned at right angles to the column, carrying the coarse- and fine focusing mechanism and the observation tube holder on the left. The incident-light source is attached on the right. Coarse- and fine adjustment actuate the tube, with coaxial drives; the movement is iimited only by two stops at the top and bottom of the 1¼″ (30 mm) adjustment range. Microscopists will welcome the convenience of such a large fine adjustment range, as it saves them the trouble of frequent changeovers between the coarse and fine adjustments. The tube is of the photo-binocular type for wide field eyepieces. It can be swung out of the field of operations through more than 90°, so that it does not obstruct the worker should the need for urgent manipulations on the object arise.

The Mechanical Stage

The mechanical stage is of sturdy construction, moves on ball races, and has an area of 20¼ x 11¼ (505 x 280 mm). A trough with object base and fine adjustment can be attached to it by means of two screws. In this case the normal 100 x 150 mm travel of the stage must be limited to 40 x 50 mm with the aid of stop screws. The stage is operated by a coaxial drive on the left. For incident light investigations without the trough and the substage condenser holder the aperture in the mechanical stage can be covered with a metal or glass plate.

Object base with fine adjustment

Microscopy on the living object calls for continuous adjustment of the object within the field of view because of its unavoidable movement. The normal adjustments of a microscope stage would be too coarse for this purpose. However, the single-lever operation adopted in our micromanipulator (List Micromanipulator 52-8) has proved its superb efficiency also in the intravital microscope. This design makes it possible to control the horizontal movements of the object base and hence the object with extreme accuracy by means of a single lever. All the movements of the control lever are truly transmitted to the object. The range of movement can be adjusted continuously from fractions of a millimetre to 4.5 mm, so that the object remains within the field of view of the microscope even under high magnifications. Condenser and fine adjustment mechanism are protected by means of a plastic foil against liquids such as physiological saline solution, etc., which are collected in the trough and drawn off through an outlet.



Figs. 1a–d: Various functional aspects of the same capillary (rabbit ear chamber), interval between exposures approx. 5 secs., exposure time $^{1}/_{10,000}$ sec. Objective UO 100, periplanatic eyepiece 6x. The changes in the shape of the red blood corpuscles and the endothelial cell nuclei are clearly visible. Photographs by P.-I. Brånemark, M. D., Anatomical Institute, Gothenburg, Sweden.

Securing the Animal

It is a special feature of this universal intravital microscope that it allows the examination not only of the small animals used normally, but also of larger mammals up to medium-sized dogs on a special "animal receptacle". The holder for the living objects is attached to the back of the trough and adjustable in all directions. The detachable carrier plate supports the animal and the necessary accessories such as OP lamp, anaesthetic supply ducts, etc. The carrier plate itself does not form part of our equipment; experimental objects and additional elements vary so much that it must be left to the user to satisfy his individual needs.

Types of lighting equipment

Living tissue is sensitive to heat, or to photo-dynamical effects depending on the spectral characteristics of the light; the research worker is therefore faced with often entirely new problems of illumination. To add to these difficulties most tissues are not transparent enough to be examined without preparatory treatment. Thus, only organs with thin edges can be introduced directly between microscope condenser and objective for immediate observation. Thicker organs are sometimes transilluminated with light conductors. Frequently, however, completely new methods have to be developed by the research worker. Invariably the microscopist will find the Universal Lamp Housing 250 with which the microscope is equipped for both incident and transmitted light suitable for all his requirements. The Lamp Housing 250 has been designed for all air-cooled high-pressure or special lamps of up to 250 W, and accommodates the CS 150 W high-pressure mercury lamp for A.C. for fluorescence microscopy, or the XBO 150 W Xenon lamp for photomicrography.

All controls for the adjustment of the lamp condenser, the vertical and lateral movement of the burner, and the centration of the reflector can be conveniently reached from outside. The filter changer takes up to 4 filters for visual observation, photomicrography, or fluorescence investigation in any desired combination. In addition, an interchangeable heat filter has been introduced into the optical path.

The 6 V 5 A. low-voltage lamp is attached to the outside of the lamp housing. It is available as a centring or a pre-centred model. Our lists 51₃-72 Lamp Housing 250 and 52-30 Fluorescence Microscopy contain further details.

Optical Equipment

Condensers

The two-diaphragm condenser after Berek with swing-out front lens, N.A. 0.95, is suitable for bright field examinations. The use of other condensers, e.g. for phase contrast observation, is also possible.

Revolving Nosepiece

The quintuple nosepiece with permanent intermediate optics fits into a horizontal dovetail guide and is easily interchanged against other optical equipment such as the Ultropak illuminator for incident light. The slide for fluorescence suppression filters and polarizing filters is fitted above the nosepiece.

Objectives for Transmitted Light

All objectives, including the highest powers, can be used provided the object and the experimental conditions are suitable. Where parts of the body are difficult to reach we recommend the use of our ULTROPAK objectives because of their concial shape. If details of tissues, which generally lack contrast, are difficult to identify, contrast can be increased under certain conditions, as with the chamber technique and phase contrast objectives. See also our list 51₃-36a.

Objectives for Incident Light

Incident light examinations are carried out with the ULTROPAK® incident light illuminator, which for the intravital microscope has been equipped with built-in intermediate optics and a changing device. However, since the majority of investigations in intravital microscopy take place in transmitted light, the ULTROPAK for investigations exclusively in incident light is of less interest. It is more important for examinations in combined incident and transmitted light. Our List 51₃-36 contains a detailed description of the design and function of the ULTROPAK illuminator.

Eyepieces

The demand of the intravital microscopist for the largest possible field of view is met by our periplanatic wide-field eyepieces. Depending on requirements our GW or GF periplanatic eyepieces are recommended.

Photographic Records

The techniques of preparation and observation in intravital microscopy are so difficult that they claim the microscopist's full attention. The research worker will therefore greatly appreciate the availability of a fully automatic camera, producing perfect results even of rapid picture sequences, to meet his need for photomicrographic records. Our fully automatic ORTHOMAT® microscope camera is eminently suitable for this purpose. Simply pressing the release button sets in motion all the functions needed to produce a photomicrograph, leaving the microscopist free to concentrate entirely on his observation. Since the exposure determination can be individually adjusted to allow for the characteristics of the preparation, even small object details such as single capillaries can be accurately exposed. Any changes in brightness during the actual exposure are automatically compensated.

The shortest exposure time possible with the ORTHOMAT is $^1/_{100}$ sec. In connection with the modern high-pressure lamps it can be realized in many cases and will generally be adequate for the photography of living objects. However, if even shorter exposure times are necessary, e.g. for very rapid movements, the use of our Mikroblitz 300 is indicated; the light flux cannot be varied with the ORTHOMAT microscope camera, since the flash is merely synchronized with the shutter movement; any light reduction necessary must be achieved by means of a neutral grey filter.

The simplest form of photomicrography is possible with the 9x12 cm camera attachment or with the LEICA® body, which is used with either the mirror reflex attachment or the MIKAS micro-attachment. Both the 9x12 cm camera attachment and the micro-attachment include a flash-synchronized time- and instantaneous shutter, so that they can also be used with our microflash. For further details please consult our lists ORTHOMAT and Camera Attachments.

For cinemicrographic work the cine-camera should be attached to a wall completely separate from the microscope.

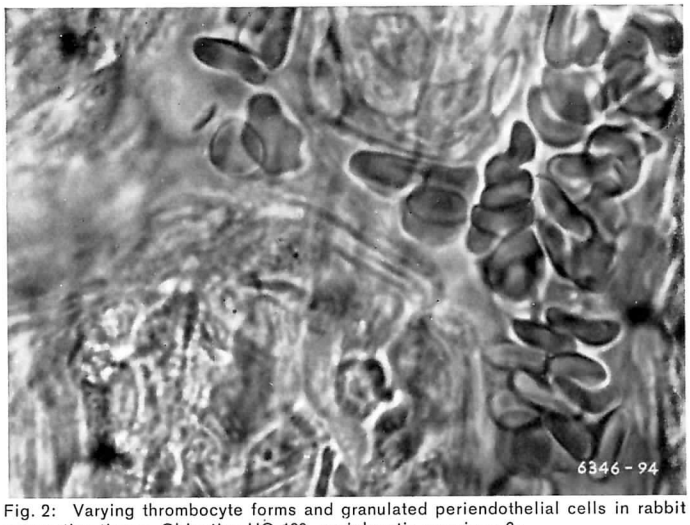


Fig. 2: Varying thrombocyte forms and granulated periendothelial cells in rabbit connective tissue. Objective UO 100, periplanatic eyepiece 6x.

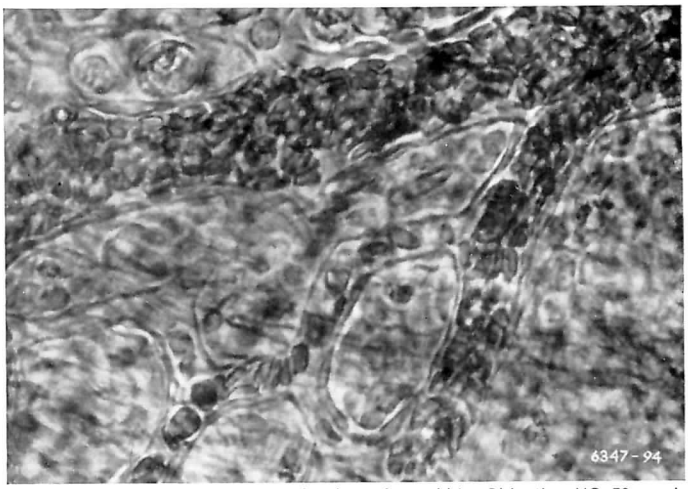


Fig. 3: Capillary bed in the ear chamber of a rabbit. Objective UO 50, periplanatic eyepiece 10x.

Work bench

The microscope is supplied with its own work bench. A 60x25" Resopal-covered table top rests on the sturdy lower parts of the work bench. The left-hand cupboard contains four drawers for the accessories; the fold-away control desk is housed in the right-hand cupboard. The controls are conveniently arranged on the panel and readily accessible. The electrical equipment includes two transformers for the low-voltage lamps and the starter units necessary for the CS 150 W and XBO 150 W burners. Warning lights indicate the correct function of the illumination.

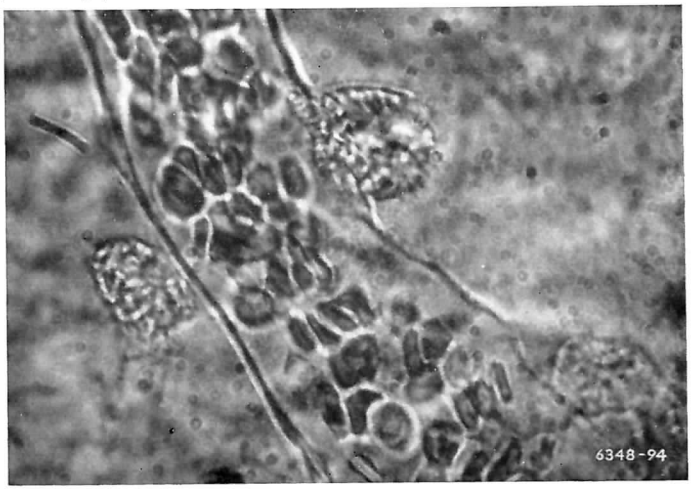


Fig. 4: Histiocytes near endothelial cells. Objective UO 75, periplanatic eyepiece 10x. All photographs by P.-I. Brånemark.

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The information given below is merely an outline of some already published possibilities of intravital microscopy and does not remotely claim to be a complete bibliography of intravital microscopy.

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