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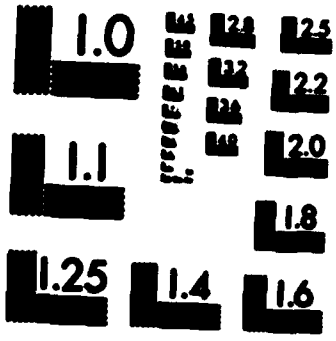

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Report USAFSAM-TR-84-4

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MICROPROBES FOR IN VIVO LIGHT INTENSITY MEASUREMENTS IN THE EYE

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Final Report for Period November 1979 - December 1980

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USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235

NOTICES

This final report was submitted by personnel of the Vulnerability Assessment Branch, Radiation Sciences Division, and the Fabrication Branch, Technical Services Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas, under job order 7757-02-53.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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MICROPROBES FOR IN VIVO LIGHT INTENSITY MEASUREMENTS IN THE EYE

INTRODUCTION

Many investigators have tried to measure in vivo light intensity profiles in the eye with microprobes. The retinal image is critical to estimates of both flashblindness and permanent injury from intense light. Stein and Elgin (4) first tried a specially constructed fiber optic bundle inserted through an incision near the limbus and placed in front of the macula of a rhesus (Macaca mulatta) monkey. Robson and Enroth-Cugell (3) used a fiber optic probe in anesthetized cats. The probe was contained in a stainless steel tube with a tip bent through 135°. The fiber optic diameter was 7 μm.

Forster (2) measured retinal images in rhesus monkeys with a probe inserted through the back of the eye. The probe was either a glass or quartz rod, pulled over heat to a minimum of 10 μm and vapor coated with 2000 Å of silver and 1 μm of electrodeposited copper for protection. His measurements were, unfortunately, variable, and somewhat larger than expected. The radical surgery undoubtedly created some changes in the physiological optics, but the integrity of the thin film coating after hours in tissue fluids was also suspect. Corrosion and microhole development, though hard to detect, could have significantly enlarged the effective diameter of the probe (and thus image size) during a long duration experiment of 18 hr or more.

Our purpose was to develop a microprobe for prolonged in vivo measurements in rhesus monkeys.

MATERIALS AND METHODS

To fabricate these probes, we pulled a quartz rod over heat to about 6 μm diameter and vacuum deposited a thin-film of chromium (Cr) around the substrate. Response as a function of the angle of incident light was measured for five microprobes.

Probe Fabrication

One-mm quartz rod was cut in 6-cm lengths and one piece placed in each of two chucks of an Industrial Science Associates Micro-Pipette Puller (Model 1). The two rod ends were sealed together with a Victor J-27 torch with natural gas and oxygen fit with a "00" size tip and adjusted to a pinpoint flame: The rod was pulled over heat by hand to a diameter of 100 μm. The puller solenoid then finished the pull, reducing the diameter to about 3 μm.

We cut the probes under a long focal length binocular microscope, using a probe micromanipulator and iris scissors secured to a stationary mount. The quartz probes, held in the probe micromanipulator, were positioned against the stationary blade of the scissors using the microscope. The probe tips were clipped leaving approximately 2 mm of thin shaft. Care was taken to score and bend the probes so as to obtain a break as smooth and perpendicular to the shaft as possible.

Probe Cleaning

The probes were carefully handled to prevent contamination by finger grease or foreign oils. Cotton gloves were worn at all times and the probes handled only with metal forceps. The probes were first soaked in hexane for 30 min to remove organic oils, then in carbon tetrachloride for 30 min to remove inorganic oils. The probes were then placed in a polyurethane holder and submerged in dichromic acid at 60-80°C for 30 min. The probes were rinsed with deionized water, placed in a Teflon holder, and gently boiled in Alconox detergent for 10 min. This process was repeated twice more with Alconox and deionized water and three more times with ethyl alcohol (200 proof) boiled on a hot plate.

After these rinses, the probes were removed and stored in a dry environment.

Immediately before deposition the probes were placed in an aluminum holder for degreasing. This holder was positioned in a large (2000 ml) beaker with the probe tips pointing upward. Ethyl alcohol was added to a depth of approximately 1 cm. The beaker was then covered with a piece of flat glass. The alcohol was slowly boiled away with a hot plate and the beaker promptly removed from the heat.

Deposition

The Cr film was deposited on quartz probes with nominal tip diameters of 6 μm. The depositions were performed in a Veeco Vac 300 with a 12" bell jar pumped by an oil diffusion pump with a liquid nitrogen cold trap backed by a mechanical pump (Fig. 1). Deposition began when the system reached a pressure of 10^{-7} mm Hg. Powdered Cr (99.99% pure) was located 15.5 cm directly below the probe and supported in a tungsten boat. A manually operated shutter covered the source during outgassing. The probes were heated by radiation for about 20 min by moving the manual shutter while keeping the Cr below evaporation temperature. Then the Cr film was deposited to a thickness of 2000 to 3000 Å (monitored with a Veeco QM-311 thickness monitor) at a pressure of 1-5 x 10^{-5} mm Hg. The depositions were made rapidly at about 20 Å/sec. During deposition the probe holder rotated on an axis parallel to the probe shafts (Fig. 2). After deposition the probes were cooled for 20 min. Light and electron micrographs are shown in Figures 3 and 4 respectively.

During measurements in the eye, the probes were coupled to a fiber optic bundle with index-matching fluid, and bundle output was detected by a photomultiplier tube (Fig. 5). Figure 6 shows a light intensity profile measured with a probe in a rhesus monkey eye. Measurement was made with a 6-μm-diameter probe. The blurring effect of the probe has been removed. The curve has been hand-fit to data.

Acceptance Angle Measurement

To measure the acceptance angle, we used a simple procedure. We placed the probes at the focus of a collimated 647-nm laser beam passed through a 30-mm focal length lens. A photomultiplier tube measured the output from a fiber optic bundle coupled to the probe with index-matching fluid. First we mounted the probe at a vertical angle (about 30°) so that only the tip was illuminated by the laser beam. Then we made measurements at different angles on the horizontal axis while keeping the vertical angle constant.

DISCUSSION

The performance of the probe depended heavily upon several variables, such as angle of incidence, tip angle, effective diameter, and thin-film adhesion.

Acceptance Angle

Figure 7 shows the results of our measurements for dependence of light acceptance on incidence angle. On the average, the response dropped to 0.1 of its peak value at 23°. This is in interesting agreement with simple refraction theory which predicts an "acceptance cone" of 25° for an uncoated quartz probe in water. This emphasizes the importance of using the probe positioned as close to on-axis with the incident light as possible.

Tip Angle

The probe was not always cut so the tip was perpendicular to the probe axis. The more eccentric the angle of the tip, the more likely was the tip to receive some vapor-deposited Cr, diminishing its transmissivity. Light microscopy would allow only qualitative evaluation of the angle of the break, and probes were discarded or rebroken if the angle was greater than about 15°.

Effective Diameter

The effective diameter of the probe was undoubtedly smaller than the actual outer diameter. How much smaller depended upon angle of incidence, tip angle, and amount of tissue debris on the end of the probe. Based on light microscope examination of the probe tips, the effective diameters for our probes were probably 80% of the outer diameter.

Adhesion

Weaver and Hill (5) showed adhesion strength of Cr on glass increased with time, maximizing after about 100 hr (Fig. 8). According to Campbell (1), the best adhesion properties were observed if at least 400 Å of Cr was vapor deposited on glass. Every effort was made to insure good adhesion since the tissue fluids would readily attack a poorly adhered thin film and open undesirable apertures (Fig. 9).

Our rotisserie apparatus somewhat complicated our task of obtaining uniform deposition. Up to 8 probes were mounted tangentially around a 4.47-cm-diameter, 1.96-cm-thick aluminum cylinder. During deposition, the cylinder was rotated about an axis perpendicular to the direction of the source from the cylinder. As the probes were rotated, the distance of each probe from the source continuously changed. Therefore the Cr thin film varied in thickness around the circumference of the probe. The estimated variation in thickness of the thin film was 50%. Fortunately, this variation did not appear to affect the adhesion of the Cr film during the experiments.

Improvements

Future studies should avoid many of these problems by using a large probe capable of faithfully transmitting the entire image at once. One could then analyze the image outside the eye by expanding the image from the fiber and examining features of the image too small for previous study.

CONCLUSION

We fabricated 6-μm-diameter microprobes for measuring in vivo intensity profiles in the rhesus monkey eye. The Cr coating remained intact through 18-hr experiments. Images were successfully measured with a resolution limited by, at most, the probe outer diameter, but more likely, by the even smaller effective diameter. Future measurements should use large optical fibers to transmit the entire image out of the eye for higher resolution analysis.

ACKNOWLEDGEMENT

We thank Dr. Taboada for his consultation on vacuum deposition and suggestions for technique improvements, and SSgt Rhur, Mr. Bower, Mr. Wise and Mr. McDougall for their technical assistance.

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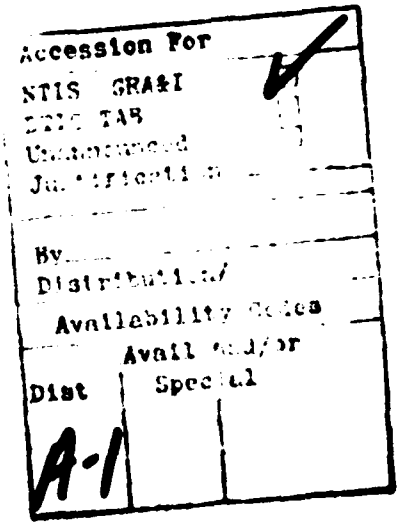

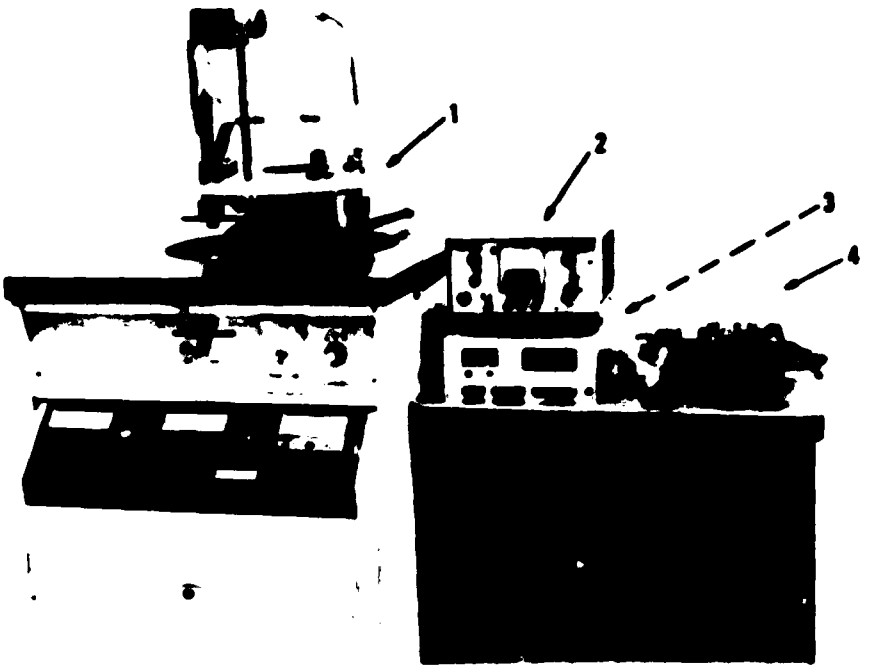

Figure 1. Vacuum deposition apparatus: 1. Veeco vacuum chamber; 2. D.C. power supply for rotisserie; 3. Veeco thickness monitor; 4. Crystal head cooler.

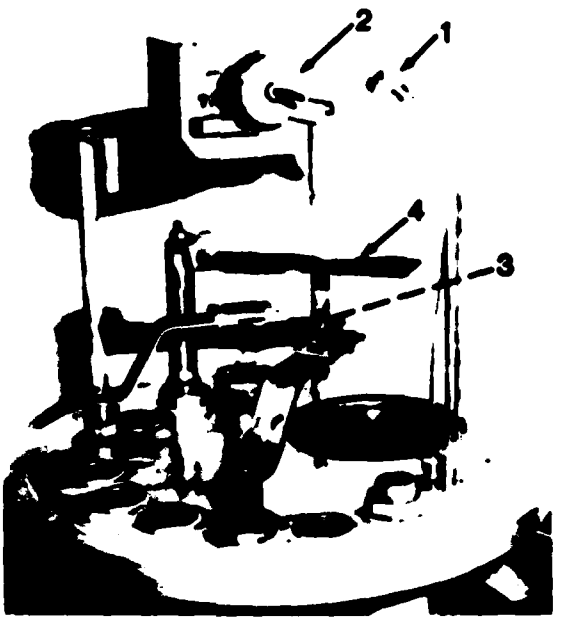

Figure 2. Vacuum chamber: 1. Thickness monitor crystal head; 2. Rotisserie; 3. Chromium in boat; 4. Shutter.

Figure 3. Chromium-coated probe. Calibration bar: 100 μm

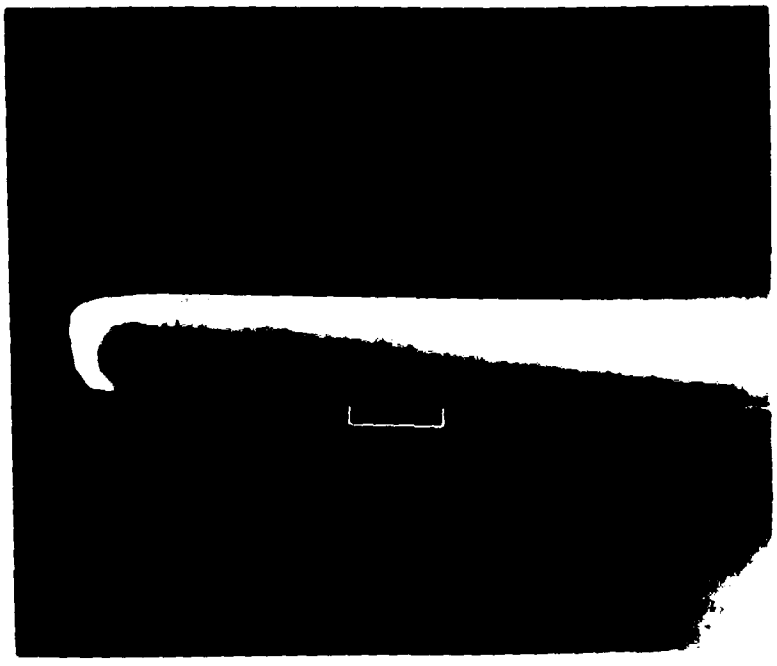

Figure 4. Chromium-coated probe. Calibration bar: 10 μm

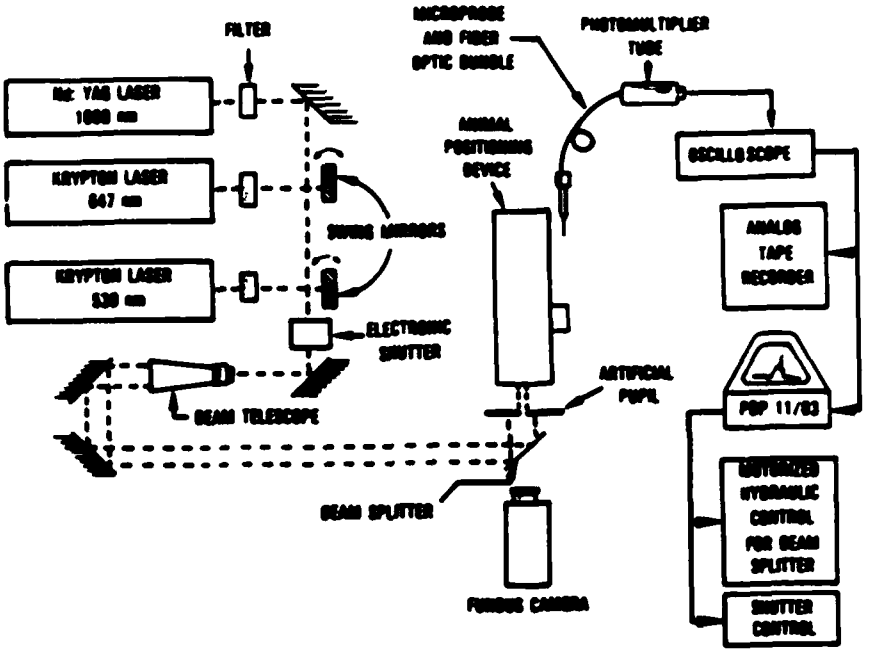

Figure 5. Experimental apparatus to measure light intensity profiles in the monkey eye in vivo.

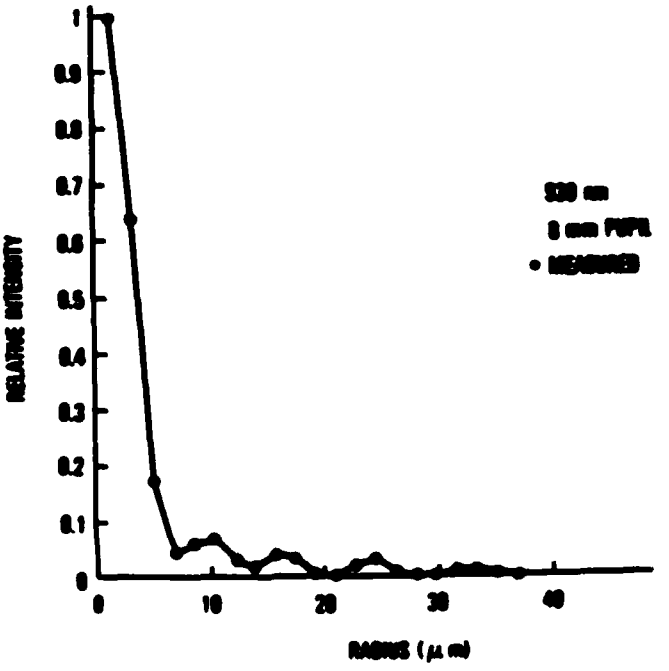

Figure 6. Typical measured image intensity profile for 530-nm laser light.

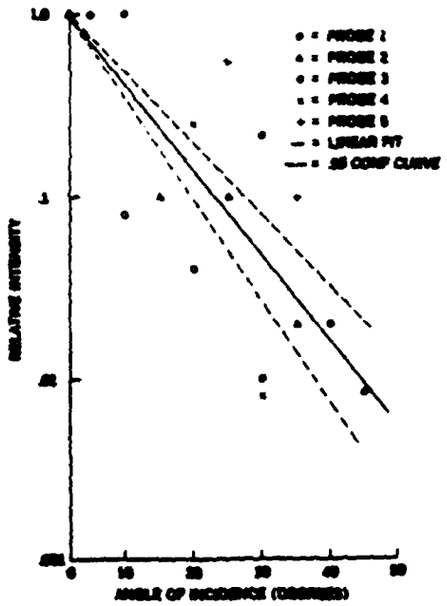

Figure 7. Probe response as a function of angle of incident light for five probes. The line represents a least-squares fit. Confidence intervals are shown.

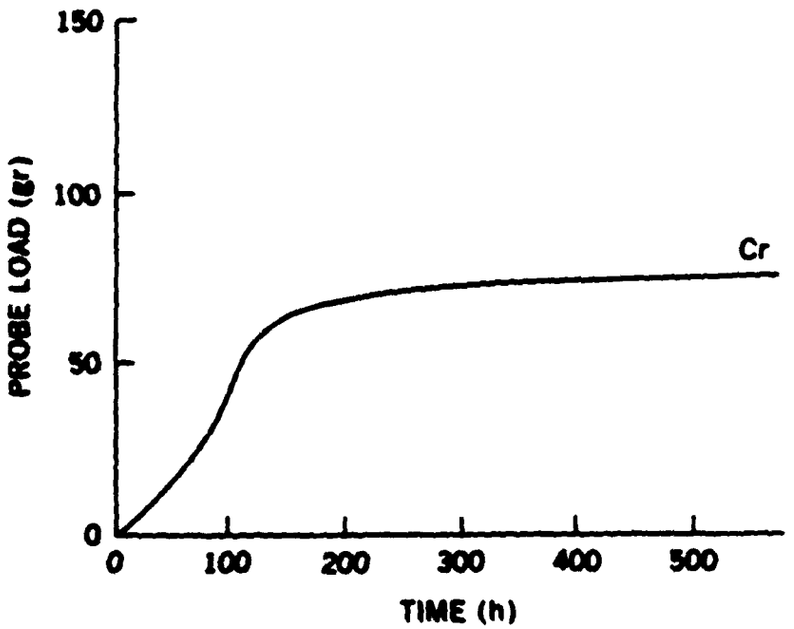

Figure 8. Adhesion as a function of time for chromium on glass at room temperature (Cr 150 Å thick). (5)

Figure 9. Hole in chromium thin film. Calibration bar: 5 μm

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