Invited paper presented at the 11th Scintillation and Semi Conductor Symposium, Wash., D.C., Feb. 28-Mar. 1, 1968. (To be published in IEEE Trans. Nucl. Sci.)

REPORT NO. NYO-3406-7

CONF-680207--9

QUANTITATIVE STUDIES OF WEAK LIGHT SOURCES IN BIOLOGICAL SYSTEMS BY MEANS OF AN IMAGE INTENSIFIER*

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SUMMARY

Several image intensifier systems have been calibrated for gain and single electron detection efficiency. When these intensifiers are coupled to conventional microscopes the combined system provides sufficient light to allow photographic recording in short exposure times of certain biological systems that would ordinarily not be photographable or would require undesirably long exposure times. The photographs also provide quantitative information on the amount of light entering the microscope objective.

Experiments are described in which an image intensifier microscope system has been used to study bioluminescence. providing data on the temporal and spatial distribution of light output in several organisms. Other experiments are described in which the location of weak radioactive tracers has been possible in short exposure times, using thin overlying scintillators to provide the necessary light. System requirements are discussed for the extension of this technique to X-ray studies and fluorescence microscopy.

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*Supported by U. S. Atomic Energy Commission, Division of Biology and Medicine Contract AT(30-1)-3406.

I. INTRODUCTION

The purpose of this paper is to emphasize the quantitative information that can be obtained when a suitably calibrated image intensifier is applied to studies of weak light sources associated with certain biological systems. The plan will be to refer briefly to some work already completed, some studies currently underway, and indicate the sort of investigations planned for the immediate future. The justification for referring briefly to material already reported, or in press, is that the results of a particular effort of interest mainly to specialists appear in specialized journals. On the other hand the possibilities of the technique are of broader interest, and this interest is best served by presentation to an audience reflecting a broad spectrum of concerns.

II. GENERAL CONSIDERATIONS

Image intensifiers are available in several forms and have been described in numerous papers in previous symposia of this series. The device incorporates a photosensitive cathode of the same sort utilized in photomultipliers. This cathode converts incident photons to electrons with some characteristic efficiency. These electrons are kept in spatial focus by suitable electric and magnetic fields, accelerated and allowed to strike an output phosphor, usually after they have undergone some multiplication. The energy of the electrons is converted into light at the output phosphor, so that the image of photons incident on the cathode is reproduced with greater intensity at the output phosphor. The important parameters of the image intensifier are:

i) cathode efficiency, ϵ_c, defined as the ratio of the number of electrons emitted from the cathode to the number of photons incident. Typical values are $\sim$ 0.10 to 0.20.

ii) gain, G, defined as the ratio of the number of photons emitted at the output phosphor to the number of photons incident. Typical values are $\sim 10^4$ to 10^6.

iii) spatial resolution, expressed in terms of the number of line pairs per millimeter that can be resolved at the output phosphor. Typical values are $\sim$ 15-30 lp/mm.

iv) noise (or dark current), N_n, expressed in terms of the number of electrons leaving the cathode per second per square centimeter in the absence of an incident light signal. Unfortunately, typical values are 100-1000/cm^2 sec.

v) the average number of photons emitted from the output phosphor as the result of a single electron leaving the cathode. This is given by $N_e = \frac{G}{\epsilon_c}$.

Several methods are available for recording the signal on the output phosphor. For the work reported here the recording has been done simply by photographing the phosphor. For this method, it is important to know how many photons, N_g, must be incident on the recording film on the average in order for one grain to be developed. Typical values are N_g = 150 to 250. An associated parameter of interest is the efficiency, ϵ_e, with which a single electron that leaves the cathode can be detected on the recording film. This of course depends on the gain and spatial resolution of the tube. Typical values of this parameter for high gain tubes are 0.20-0.65.

All of the above parameters have been discussed in detail in the literature,[1,2] but the important point to note here is that they can all be measured. Thus, if a light source of interest is coupled to the cathode of an intensifier of gain G by an optical system that collects and transmits a fraction f_i of the light, and if the output of the intensifier is photographed with optics that collect and transmit a fraction f_o of the light to the film,

which in turn requires N_g photons per grain developed, then the number of photons, ν, leaving the source can be determined from the number of grains, η_g, developed from the relation:

$$\eta_g = \frac{\nu . f_i . G . f_o}{N_g} \qquad (1)$$

η_g has been determined in practice by actual grain count, and by densitometer measurements.

If the signal at the output appears in the form of isolated spots, corresponding to light signals so low in intensity that individual cathode electrons are separately observed, then the number of photons, ν leaving the source can be determined from the number of electron spots, η_e, seen on the film from the relation

$$\eta_e = \nu . f_i . \varepsilon_c . \varepsilon_e \qquad (2)$$

Each of these methods has been used in practice, and representative results will be described below.

The parameter of noise is very important and is usually the limiting factor in low light level detection. Whether or not a signal of given spatial-temporal intensity distribution can be detected with a given confidence can be shown to depend on the noise in a very straight forward way.[3] The important conclusion here is that one is always striving for noise levels that are very difficult to achieve in the manufacture of these tubes.

III. STUDIES OF BIOLUMINESCENCE

A. Noctiluca miliaris

An example of the application of image intensification to a quantitative study of bioluminescence has been published.[4] The purpose was to investigate quantitatively the subcellular origin of luminescence in Noctiluca miliaris. The arrangement used is typical of much of the work to be described and is shown in figure 1. The specimen, exaggerated in the figure (actual diameter

approximately 300 μ), was mounted on the stage of a phase contrast microscope and could be viewed directly through the ocular, photographed in conventional fashion by camera B, or viewed through the image intensifier tube and photographed by camera A. The microsources were found to be confined mainly to the perivacuolar cytoplasm and to be about 1 μ in extent (~0.5 mm. on the cathode of the image intensifier). Phase-contrast microscopy of this region showed many strongly phase-retarding bodies of the same size. By mapping the field of view of camera A in figure 1 with that of camera B results indicated in figure 2 were obtained. Thus, each of the microsources coincided closely with a phase-retarding body, but only about one tenth of all phase retarding bodies were in fact associated with microsources. Although individual microsources were very consistent in emitted intensity from flash to flash, there were marked variations among the sources. The mean number of photons per flash emitted by a microsource was determined in several ways. Using a calibrated photomultiplier exposed to a known number of microsources, a figure of the order of $\nu = 10^5$ photons per microflash was obtained.

A more direct determination of the number of photons per microflash can be made by applying the principles discussed in part II above. As mentioned above, the microflashes varied considerably in size and intensity. A typical microflash appeared on the film record of the image intensifier output as a region of the order of 0.5 mm. in extent. Within this region, a pattern of developed grains could be identified, corresponding to individual electrons leaving the cathode. By counting these "electron spots", the number of photons leaving the microsource could be determined according to equation (2). In this experiment:

$$f_i = 0.097$$

$$\varepsilon_c = 0.104 \pm 0.010$$

$$\varepsilon_e = 0.15 \pm 0.05 \quad (G = 8 \times 10^5)$$

The mean of 36 different microsources by this method gave:

$$\nu = (5.3 \pm 3.0) \times 10^4$$

The method of equation (1) was applied to 16 different microsources, with:

$$G = (8.0 \pm 1.0) \times 10^5$$

$$f_o = 0.050 \pm 0.005$$

$$N_g = 220 \pm 20$$

The result was:

$$\nu = (3.2 \pm 1.0) \times 10^4$$

The two methods are related in principle and must, of course, be consistent with each other. The average of both methods is

$$\nu = (4.5 \pm 1.5) \times 10^4$$

The image intensifier system can also be applied to the investigation of the time distribution of the photon output of the microsources. The photomultiplier trace of the macroflash shown in the inset of figure 1 leaves open the question of whether the time characteristic is due to a time distribution in the number of microsources giving out light at a given instant, or whether each microsource emits photons with nearly the same time distribution as that of the macroflash. The latter alternative is shown to be the case by results indicated by figure 3. To obtain such a picture, a mask containing a narrow vertical slit was placed over the anode of the image intensifier so that only 6 or 8 microsources were in the field of view. A flash was recorded to determine their positions. Then the film was moved rapidly in a horizontal direction to record a second flash in the form of horizontal streaks. A third flash with the film fixed served to determine whether the microsources in the original field of view had moved significantly during the sequence of flashes. It is interesting to note that similar techniques applied to a study of fire-fly luminescence indicate that the unit sources of photon emission bear a more complicated relation to the macroflash than that found in

Noctiluca. These results are being prepared for publication.

B. Gonyaulax polyedra

A further application of these quantitative techniques has led to some interesting results, previously unpublished, on the bioluminescence of Gonyaulax polyedra, in collaboration with J. W. Hastings of Harvard University and H. Sato of the University of Pennsylvania. Hastings et al.[5] have described the isolation of an active particulate bioluminescence system from this dinoflagellate and called these particles "scintillons". The particles are birefringent so that the number in a given sample can be counted when viewed through a polarization microscope. The sample can then be flashed (by lowering the pH of the buffer) and the number of photons emitted determined by the response of a calibrated photomultiplier. In this way it has been determined that the average number of photons per counted scintillon (i.e. birefringent body) is 410 ± 100. However, in the experiments above concerning Noctiluca it was found that only a small fraction of the "characteristic structures" in the cytroplasm give off light, and those that did emitted very large numbers of photons. It was therefore of interest to investigate whether all of the scintillon structures of Gonyaulax participate, whether some fraction like one tenth of them each emit ten times the average, or whether the photons originate in some previously undetected structure, unrelated to the scintillons. The intensifier techniques were applied to this question by preparing slides containing large numbers of scintillons, viewing these slides through a high magnification polarizing microscope, either directly for the purpose of counting the number of scintillons in the field of view, or through the image intensifier for recording the light output of the flash. A typical direct view through the microscope is shown in figure 4a, and an enlarged photograph of the flash of a large number of scintillons through the image intensifier is shown in figure 4b. There is some similarity in the two views. For the slides used, the direct count gave (5400 ± 260) scintillons

in the field of view of the image tube cathode (720 μ). The average number of spots counted in the photographs of the flashes was 755 ± 150. These numbers can be compared in several ways. Measurements showed that only 0.011 of the light was collected from the slide and transmitted to the cathode (.032 by the objective and transmission optics, 0.340 by the polarization analyzer). Thus, if in fact each scintillon is participating in the bioluminescence process and emitting on the average 410 photons per flash, then only 4.5 photons per scintillon will reach the cathode. For the tube used in this work, the cathode efficiency was measured to be 0.17, so that it is clear that the spot count on the film of the flash must be interpreted in terms of the single electron/$\frac{\text{detection}}{\text{efficiency}}$ of the system, which was separately determined to be 0.20 ± .06 at the gain used. Thus, under the assumption that there are 410 photons per scintillon, the number of scintillons contributing is indicated to be

$$\frac{(755 \pm 170)}{4.5 \times 0.17 \times (.20 \pm .06)} = 4900 \pm 1800$$

to be compared with the direct count of 5400 ± 260.

Another determination can be made by assuming that N photons are emitted per scintillon, of which 0.011 are incident on the cathode, and of these 0.17 emit electrons. Thus, each scintillon will result in

$$.011 \times 0.17 \times N = 1.85 \times 10^{-3} N \text{ cathode electrons.}$$

Since the detection efficiency for these electrons is 0.20 ± .06 we have an overall detection efficiency of

$$\phi_t = (0.37 \pm 0.10) \times 10^{-3} N$$

for detecting scintillons. From the results above

$$\phi_t = \frac{755 \pm 170}{5400 \pm 260} = 0.14 \pm 0.03$$

so that we have

$$N = \frac{.14 \pm .03}{(.37 \pm .10) \times 10^{-3}} = 380 \pm 150 \text{ per scintillon}$$

in good agreement with the result 410 ± 100 for the average number of photons per scintillon as obtained by the photomultiplier technique. The suggestion is thus very strong that the number of separate sources of light in this phenomenon is nearly equal to the number of birefringent particles identified as scintillons.

We are currently preparing to repeat this experiment with a much improved image intensifier, with single electron detection efficiency 0.65 instead of 0.20, and noise rate 20 electrons/sec cm^2 instead of the 100 electrons/sec cm^2 in the experiment described.

C. Localization of ATP in muscle cells

In collaboration with R. J. Podolsky of the National Institute of Health, and G. Calleja of Princeton University, efforts are underway to assay and localize ATP in a muscle cell by means of the light emitted when firefly enzyme (FLE) is added to the cell interior. The ultimate purpose will be to study ATP localization to the order of $2^1\mu$, which will require magnifications similar to this used in the study of Noctiluca.

Based on data in the literature[6] the amount of ATP in 100 μ length of frog muscle cell of diameter 100 μ is 10^{-9} grams. Results obtained using a mock-up system and a calibrated photomultiplier show that 10^{-9} gram of ATP emits $\sim 5 \times 10^6$ photons/sec in the initial stages of the process with optimum FLE dilution. For magnification 100X, these photons will be distributed over 1 cm^2 on the image intensifier cathode. The microscope optics accept and transmit approximately 3% of the light so that $\sim 1.5 \times 10^5$ photons/cm^2 sec arrive at the cathode (assuming a uniform distribution of ATP). For the intensifier used in this work the cathode efficiency is ~ 0.02 at the wave length of the bioluminescence so that $\sim 3 \times 10^3$ electrons/cm^2 sec leave the cathode. The electron detection efficiency has been measured to be .65 so that more than 10^3 electrons/cm^2 sec should be recorded. Since the noise

rate of this intensifier is only $\sim$ 20 electrons/cm^2 sec, detection should be possible. Any lack of uniformity of ATP distribution would make detection more favorable. In the initial phases of the work, crude cell preparations have been seen at 1X and 10X magnification, with relatively low numerical aperture optics.

In a related area, discussions involving Dr. J. Blinks of the Harvard Medical School and Dr. G. Calleja of Princeton University indicate that the system described will also be suitable for the detection of the Ca^{++} ion in muscle cells, using the enzyme aequorin.[7,8]

D. Studies of spatial structure of the luminescence in the early phases of ctenophore embryos.

This work has been initiated in collaboration with Dr. G. Freeman of La Jolla. Results obtained in the preliminary experiments show that the image intensifier is especially well suited to the detection of the luminescence in ctenophore embryos. Particular interest will center in the early stages (24 cells) where luminescence may already be associated with those cells that give rise to the ciliated plates. In experiments to date with embryos in more advanced stages of development, spatial structure in the luminescence was easily observed using an image intensifier even though efforts to photograph this structure by conventional means have always failed.

IV. RADIOACTIVE TRACER DETECTION

The general principles of radioactive tracer detection have been described.[3] The important point for present considerations is that a mounted biological specimen that has been tagged with a suitable radioactive tracer (say C^{14}) can be covered with a thin section of some conventional scintillator in such a way that the scintillator light from the decay electron can be focussed through a microscope on the cathode of the image intensifier. As has been shown[3] the number of signal electrons, n_s, at the cathode, per

square millimeter per second due to the radioactivity is given by

$$n_s = 2 \times 10^{14} \, R.S.f_i.\epsilon_c / \mu^2 M^2$$

where R is the specific activity in microcuries in a region of the specimen μ microns on a side,

S is the efficiency with which the scintillator converts the electron energy into light,

f_i is the fraction of light collected and transmitted to the cathode by the microscope optics of magnification M.

ϵ_c is the cathode efficiency.

If n_n is the number of noise electrons per second per square millimeter of cathode and if the desired level of confidence requires a signal $c\sqrt{n_n t}$, then the relation between signal and noise is given by

$$n_s t = c\sqrt{n_n t}$$

from which

$$R/\mu^2 = cM^2 \sqrt{\frac{\eta_n}{t}} \Big/ 2 \times 10^{14} \, S.f_i.\epsilon_c \qquad (3)$$

Some useful results have been obtained since those published in reference 3. A high gain 4 stage image intensifier tube (EMI type 9694) has been tested and found to have $n_n \approx .20$ electrons/mm.^{2} sec. Also, some thin ($0.003"^{+.000"}_{-.002"}$) crystals of CaF(Eu) mounted on microscope slides have been prepared by Harshaw Chemical Co. and found to have a scintillation efficiency of ~ 0.06. Since these crystals are clear, it is possible to focus the microscope through the scintillator material, an advantage CaF(Eu) shares with plastic scintillator over the translucent appearance of the ZnS preparations used in previous work.[3] A further advantage over ZnS is that the CaF(Eu) does not phosphoresce after exposure to ambient light. The relative performance of ZnS, CaF(Eu), and plastic scintillator is shown in figure 5. Studies of this sort, and observations made with known amounts of C^{14} activity confirm equation (3) and

indicate the following approximate scintillation efficiencies for the scintillators in the forms used:

Plastic scintillator: S = 0.02

CaF(Eu): S = 0.06

ZnS: S = 0.12

Thus, if CaF(Eu) is used in conjunction with the EMI tube ($\epsilon_c \approx 0.15$, $n_n = .20$) with microscope optics for which M = 50X and $f_i = 0.03$, and the information is required to have the high confidence provided by c = 3 (> 99%) then equation (3) becomes:

$$R/\mu^2 \approx 1.4 \times 10^{-7} \sqrt{\frac{n_n}{t}} \approx \frac{6 \times 10^{-8}}{\sqrt{t}}$$

This equation can be used to determine the time of exposure required if R/μ^2 is limited; or the specific activity required if the exposure time is limited.

A further development in the arrangement of the scintillator is underway in which thin sections of scintillator are mounted on fiber optics blanks. The microscope optics can then be focussed directly on the upper surface of the fiber optics so that high numerical apertures can be achieved using immersion optics without the danger of damaging the scintillator material. For applications at magnifications of 1X using image intensifiers with fiber optics input windows[3] the fiber backed scintillator can be mounted directly on the input window.

A. Application to studies of L-proline-U-C^{14} uptake in Schistosoma mansoni.

This work has been conducted in collaboration with Dr. A. W. Senft of the Marine Biological Laboratory and details will be found in other publications.[3,9] For the purpose of the present report it is useful to point out that the chief advantage of the image intensification technique over conventional autoradiography is in the ease of preparation of the samples and the short time exposures required for detection and localization of the radioactivity (seconds and minutes, instead of hours and days). The major

limitation of the technique is the spatial resolution limit of approximately 20 microns. Using the technique it has been possible to demonstrate a significant difference between male and female schistosomes in uptake and localization of proline. Males tend to accumulate proline anteriorly in and near the suckers, testes, the tegument, and within segments of the gut. Females exhibit a much lower overall uptake. The vitelline region is sparsely tagged; the ovary concentrates moderately.

B. Application to studies of hormone uptake in Coleus blumei Benth.

This work was done in collaboration with Professor W. P. Jacobs of Princeton University. Sections 5 mm. in length were cut from the internodes of Coleus blumei Benth, and indole-3-acetic acid (IAA), a plant growth hormone, was added to the upper end of the sections in a 1.5% agar gel. The IAA was labelled in the carboxyl group with C^{14} (specific activity 33 mC/mM). The concentration of IAA was 6 mg per liter. The IAA-agar was left on the upper cut surface for 4 hours, a period known from other experiments to be sufficient to allow hormonal amounts of IAA to be transported through 5 mm. stem sections. Some results are shown in figure 6. Transport is normally studied with liquid scintillation counter techniques, and spatial distribution by conventional autoradiographic methods. Although no new information has been added by the preliminary results shown in figure 6, it is clear from records that can be obtained in a few seconds that the transport occurs in the outer boundaries of the section. However, this is one application where the 20 μ resolution limit is a severe handicap, since for significant differentiation resolution of the order of one micron is required. The image intensification technique would be an advantage only for dynamic studies of the transport phenomena, and where the long periods of special handling (including freezing the specimen to prevent misleading dispersion of the active molecules) required by conventional radiography would be undesirable. The resolution limit remains, however, for the present.

V. APPLICATION TO HUMAN EYE EFFICIENCY DETERMINATIONS

As an example of the application of quantitative image intensification techniques to a biological problem different from those described above, brief mention will be made of an experiment recently completed, which will be reported in more detail in a later publication.

The quantum efficiency of the human eye has been measured over a period of years with contradictory results. The efficiency has usually been determined by comparing the performance of the human eye with that of an ideal device in which all of the light entering results in information processed for the performance of the prescribed task.[10-14] The quantum efficiency of the eye is then given by the ratio:

$$Q = \frac{\text{Quantity of light required by ideal device to perform task}}{\text{Quantity of light required by human eye to perform same task}}$$

The value of Q is found to depend upon the level of light to which the eye is adapted, and may also depend upon the task to be performed. A variety of tasks have been employed by investigators in the past, including threshold detection, "on-off" tests and brightness comparisons. Although the intuitive idea of quantum efficiency is very direct, a troublesome disagreement exists about the limitations to be accepted for the "ideal detector." The tendency has been to assume that the eye functions in a particular way and then to impose the equivalent limitation on the ideal device.

The method of the present determination was suggested by Rose[12] and is designed to remove the uncertainties associated with the ideal detector. The determination is made by comparing the performance of the eye with that of an image intensifier of known quantum efficiency. In some observations the task was to detect a pattern of light spots in a background of noise, while in others the pattern was reversed. The pattern was designed to prevent strain on the eye and to suppress problems associated with spatial

resolution. In the first part of each experiment, the eye was required to detect the pattern unaided, and in the second part was required to detect the same pattern at the output of the image intensifier. The ratio of the light levels required in the two parts provides a value for Q, according to the equation

$$Q = \frac{\text{light level aided}}{\text{light level unaided}} \cdot \epsilon_c$$

Since only one image intensifier was used in the second part, only one eye was used in the first part. The patterns used are shown in figure 7, and the experimental arrangement is indicated in figure 8.

The intensity of the light source, L, illuminating the pattern was regulated by means of a variac. Using the arrangement of figure 8c the intensity of light incident on the eye (or image intensifier) was measured for the relative values needed for the determination of Q. Since the photomultiplier was calibrated for absolute response, the light level of adaption of the eye was also determined.

It is very important to ensure that the filter F_λ has transmission properties appropriate to take care of the differences between the spectral response of the eye and that of the image intensifier cathode. In the experiments reported here, the cathode was type S.20, ($\epsilon_c = .09$), and the phosphor was type P.20. The filters* selected for F_λ were narrow band pass (~ 50 Å) centered at 5092 Å and 5469 Å, and were "blocked to infinity" such that the transmission for wavelengths from 6000 Å to 3 μ. was $\lesssim .005\%$ and that for wavelengths above 3 μ was $\lesssim .01\%$. These requirements were essential in view of the spectral output of the source and the difference between the spectral response of the S.20 cathode and the eye.

Experiments showed that the results were independent of the image tube gain over a wide plateau, and all measurements were taken on that plateau. Results obtained using the pattern of figure 7b are of particular

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*Spectrum Systems Inc.

interest since they provide values for the efficiency over a wide range of light levels to which the eye adapts. These results are shown in figure 9. Although the efficiency shows a systematic decrease with increasing light level, this decrease is not as much as reported by Barlow[14] and agrees with points that have been raised by Rose.[12] Other results still tentative indicate the applicability of this technique to studies of peripheral vs. straight view efficiencies, correlations with age, and effect of time on dark adaptation.

VI. APPLICATIONS TO STUDIES OF X-RAY DIFFRACTION PATTERNS

One particularly interesting application of image intensification techniques to the study of X-ray diffraction patterns is described by U. W. Arndt.[15] In preparation for a collaboration with Dr. G. F. Elliott, of King's College, London, for the study of low-angle X-ray diffraction patterns from living striated muscles during contraction[16] we have designed an approach based on the combination of the fiber optics backed scintillation detector described in part IV above with a 3 stage intensifier with fiber optics input window.* In this case the scintillation detector (i.e. X-ray sensitive cathode) is Cs I ($T\lambda$) $\sim$ 0.005" thick backed with thin beryllium. The detection efficiency is high for the X-rays of interest, but the resolution is restricted to $\lesssim$ 10 line pairs/mm. The advantage is that suitable patterns can be detected in exposure times much less than those required by conventional methods, thus reducing the problems associated with working on samples under constant tension.

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VII. APPLICATIONS TO FLUORESCENCE MICROSCOPY

As has been pointed out[17] image intensification techniques are very useful for the study of biological processes that change so rapidly, or are interfered with by excessive illumination to an extent that conventional recording techniques are not successful. By coupling the intensifier to a microscope for studies involving u.v. response of the specimen, or observing labels of fluorescent dyes, both exposure to radiation and danger of denaturation by excessive amounts of dye can be reduced significantly. The application is straightforward using considerations already discussed,[18] and could be extended by the use of narrow band pass filters between the microscope and the intensifier cathode.

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FIGURE CAPTIONS

Fig. 1 Experimental arrangement for intensification and recording of microsources of bioluminescence in Noctiluca miliaris through a phase contrast microscope. The flash was stimulated by an electrical pulse, synchronized with the shutter of camera "A". The photomultiplier signal, displayed on an oscilloscope, monitored the macroflash. The inset in the upper right shows the synchronization between the stimulus (upper trace), shutter opening of camera "A" (middle trace), and the macroflash as indicated by the photomultiplier (lower trace).

Fig. 2 (a) Photograph of image intensifier output of a microflash, field of view 40μ in diameter. Microsources are identifiable on a background of scattered light, from sources outside the field of view.

(b) Phase-contrast photograph of the same field as (a) taken several seconds before the flash. The dotted circles superposed on the phase contrast image represent the positions of the microsources in (a) after correction for the distortions introduced by the image tube. The spatial correlation of microsources and some of the phase-retarding bodies is evident.

Fig. 3 (a) Moving film record of a few microsources. A field of view 20μ wide was exposed, and microsources were stimulated to flash while the film was in motion. The distribution of the light intensity from the individual microsources is seen to agree with the photomultiplier record of the macroflash shown in the inset of Fig. 1.

(b) Position of the microsources revealed by a flash immediately after that of (a) above.

Fig. 4 (a) Direct photography through a polarizing microscope of birefringent particles extracted from Gonyaulax polyedra.

(b) Greatly enlarged photograph of the flash of a number of scintillons viewed through the image intensifier.

Fig. 5 (a) Specimen of Schistosoma mansoni labelled with L-proline-U-C^{14}. Scintillations from the C^{14} electron viewed at 1X magnification. The worm has assumed a ring shape during the mounting process, but this is not characteristic. The scintillator used was 0.003" thick Pilot B plastic. Exposure times were 5 sec. (left view) and 10 sec. (right view).

(b) Same as (a) above except that the scintillator was 0.003" CaF (Eu) supplied by Harshaw Chemical Co. and exposures were 1 sec. (left view) and 2 sec. (right view).

(c) Same as (a) above except that the scintillator was polycrystalline ZnS backed on mylar 0.003" thick supplied by Pilot Chemical Co. and exposures were 1 sec. (left view) and 2 sec. (right view).

Fig. 6 (a) Photograph of 100μ thick sections cut from Coleus blumei Benth stem. Section on right cut from portion adjacent to C^{14} tagged IAA , section on left cut from portion 5 mm distant from IAA. Artificial illumination provided to indicate shape of sections. 1X magnification.

(b) Scintillations from C^{14} in the sections shown in (a). ZnS scintillator, tube gain 8×10^5, exposure time 10 sec.

(c) Same as (b) except tube gain 5×10^4 and exposure time 2 minutes.

Fig. 7 Patterns used in eye efficiency measurements (furnished by Dr. A. Rose).

Fig. 8 (a) Viewing arrangement for unaided eye.

L: light source illuminating the pattern P

F_λ: filter to determine the spectral distribution transmitted

A: fixed aperture (3 mm diam.)

E: observers' eye

(b) Viewing arrangement, utilizing image intensifier.

F_n: neutral density filters

ℓ: lens to focus pattern on image intensifier cathode

IIT: image intensifier tube

T: telescope for (binocular) viewing of image intensifier output phosphor

(c) Arrangement for measuring illumination of pattern.

PM: calibrated photomultiplier

M: meter for recording photomultiplier output

Fig. 9 Values for the efficiency, Q, of the eyes of 2 observers determined using the pattern shown in fig. 7(b), plotted vs. the background light level P, to which the eyes were adapted, expressed in number of photons per sec per cm^2 of background entering the fixed aperture "A" of fig. 8. The light level 5×10^{-3} Ft.-lambert is indicated by an arrow on the ordinate.

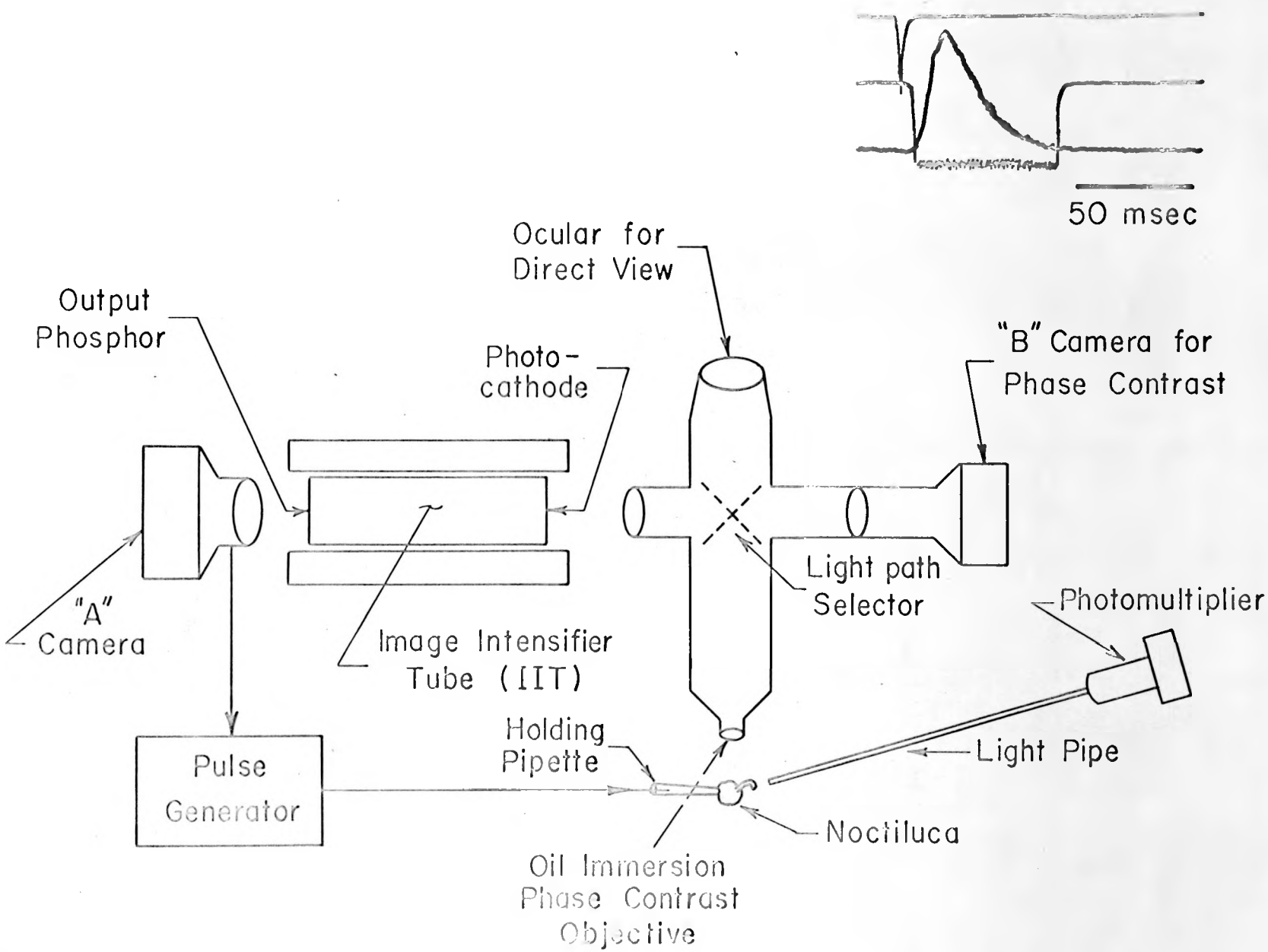
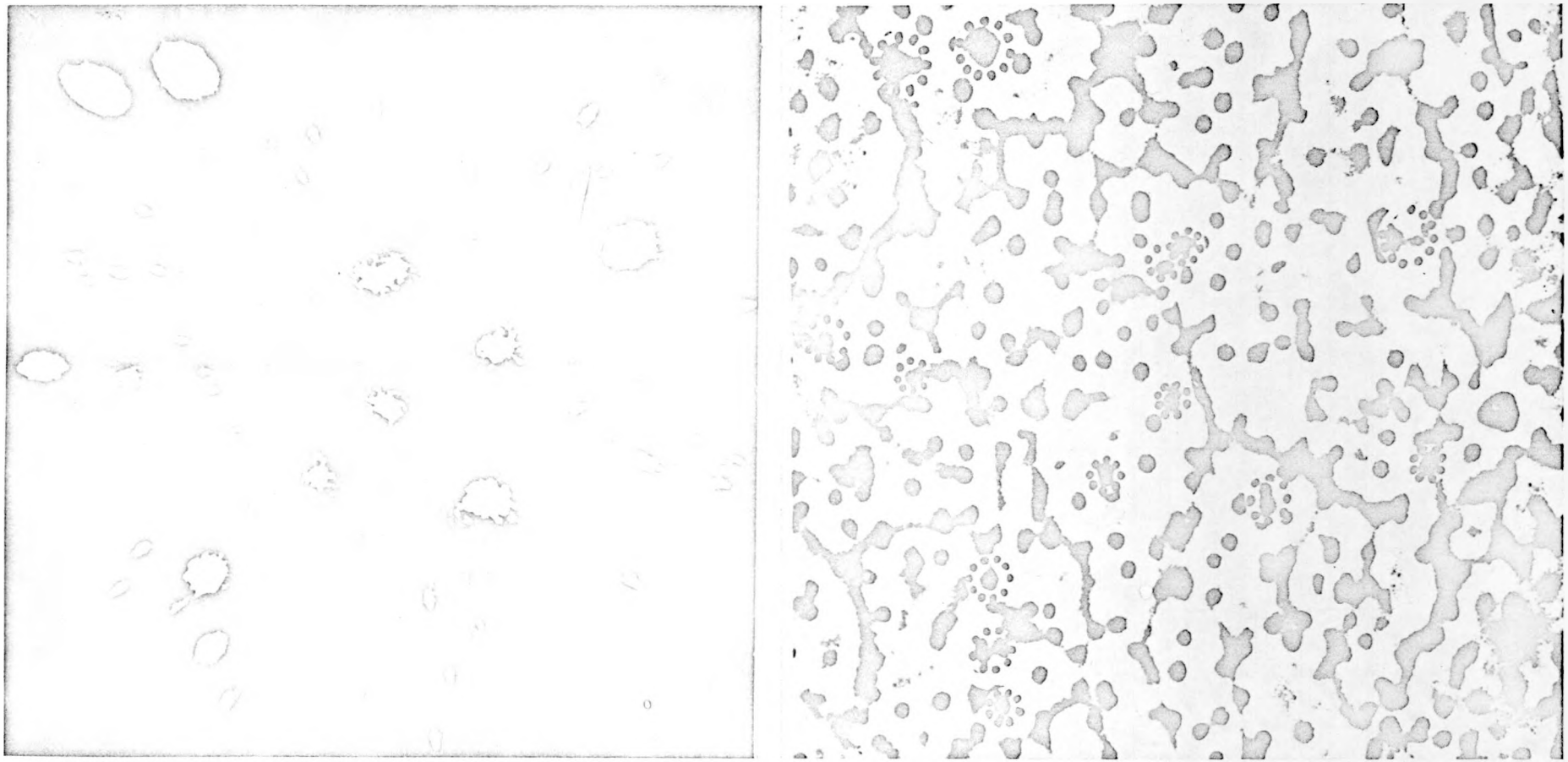
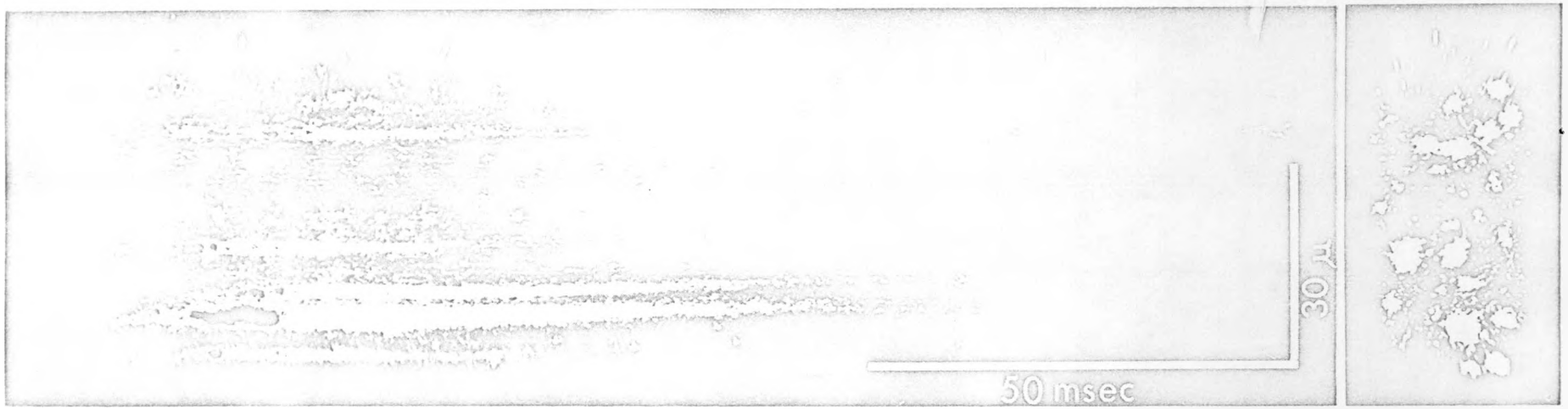

50 msec
Ocular for
Direct View
Output
Phosphor
Photo-
cathode
"B" Camera for
Phase Contrast
"A"
Camera
Image Intensifier
Tube (IIT)
Light path
Selector
Photomultiplier
Holding
Pipette
Light Pipe
Pulse
Generator
Noctiluca
Oil Immersion
Phase Contrast
Objective

FIGURE 1



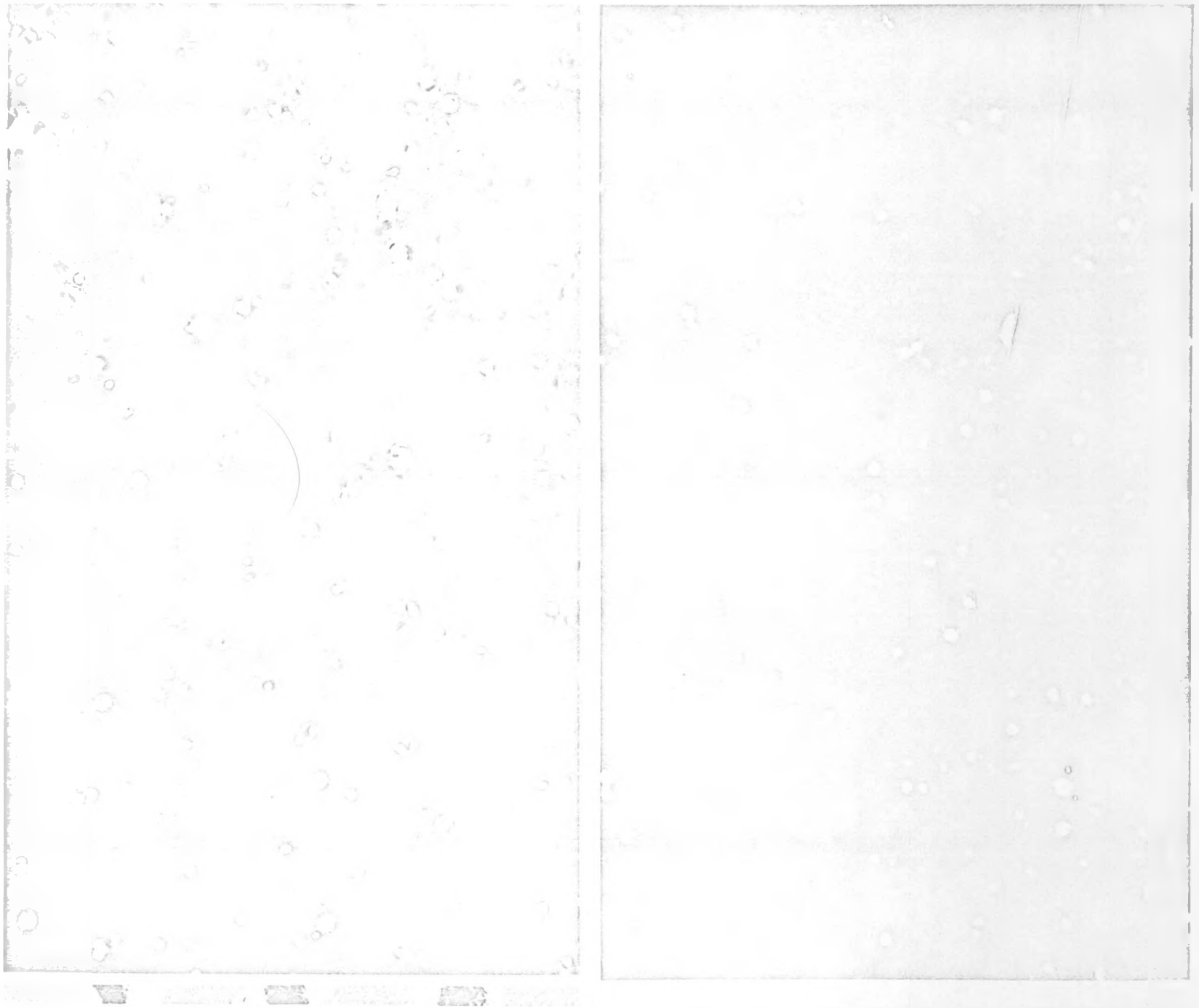
(a) (b)

FIGURE 2



(a) (b)

FIGURE 3



(a) (b)

FIGURE 4

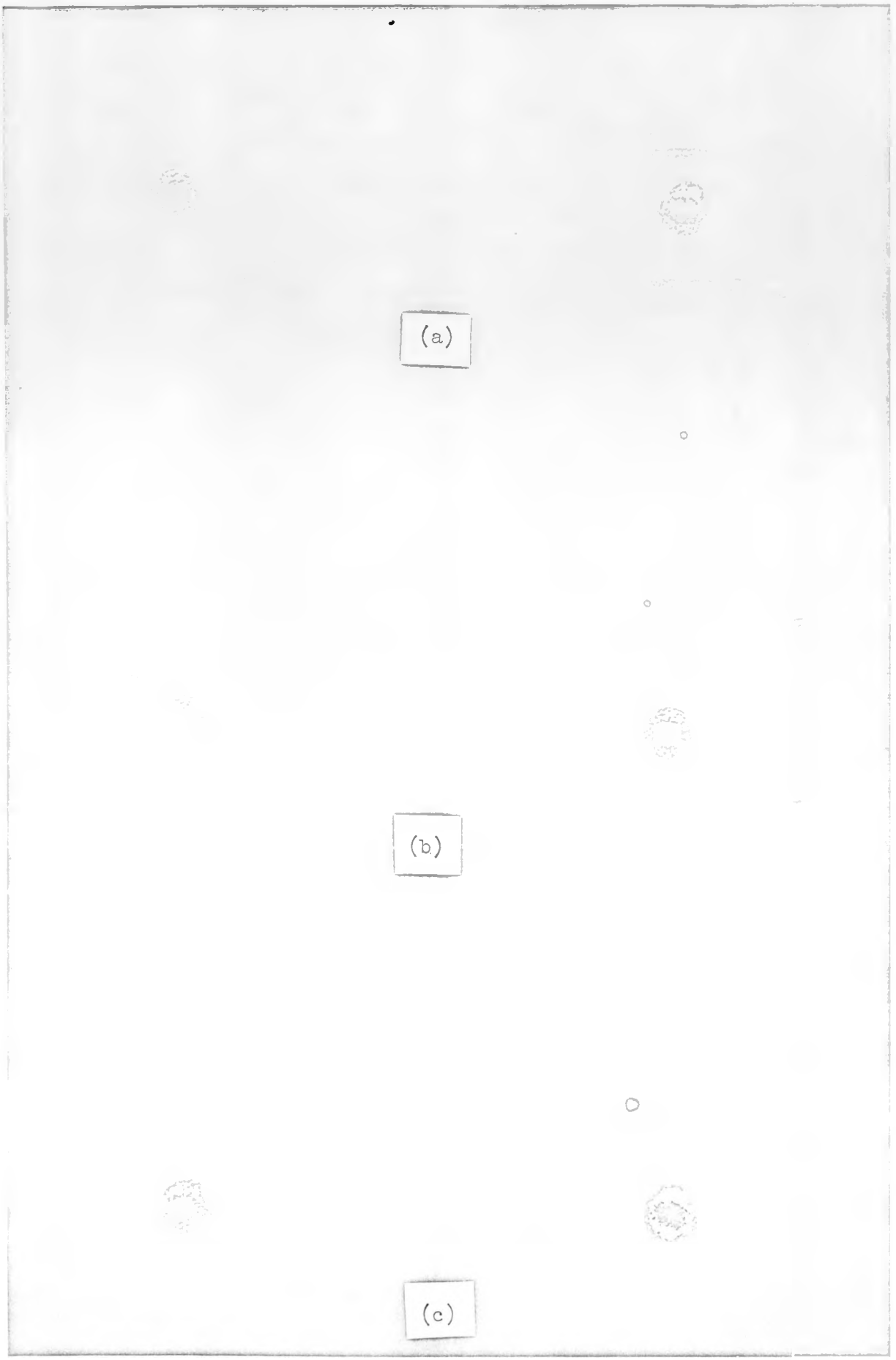
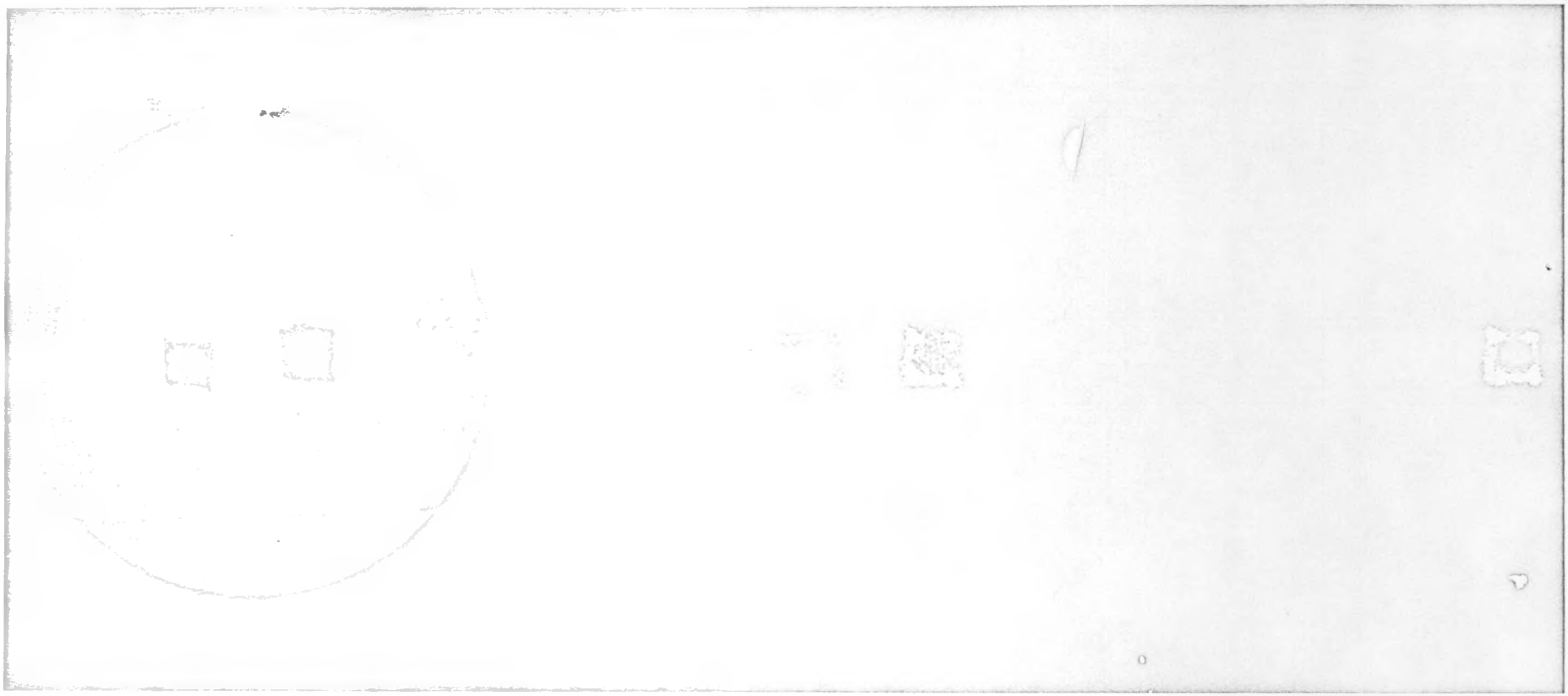
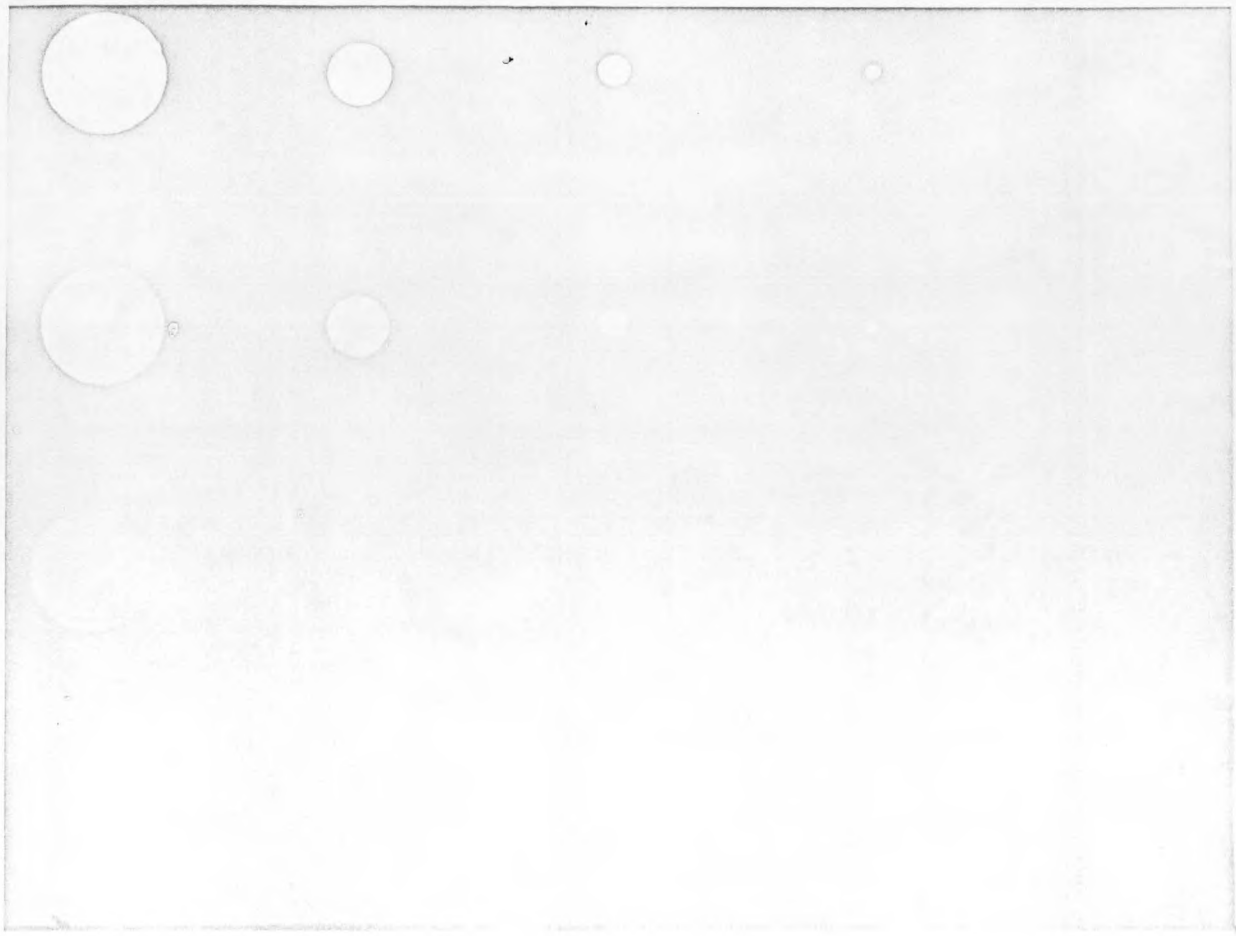


FIGURE 5

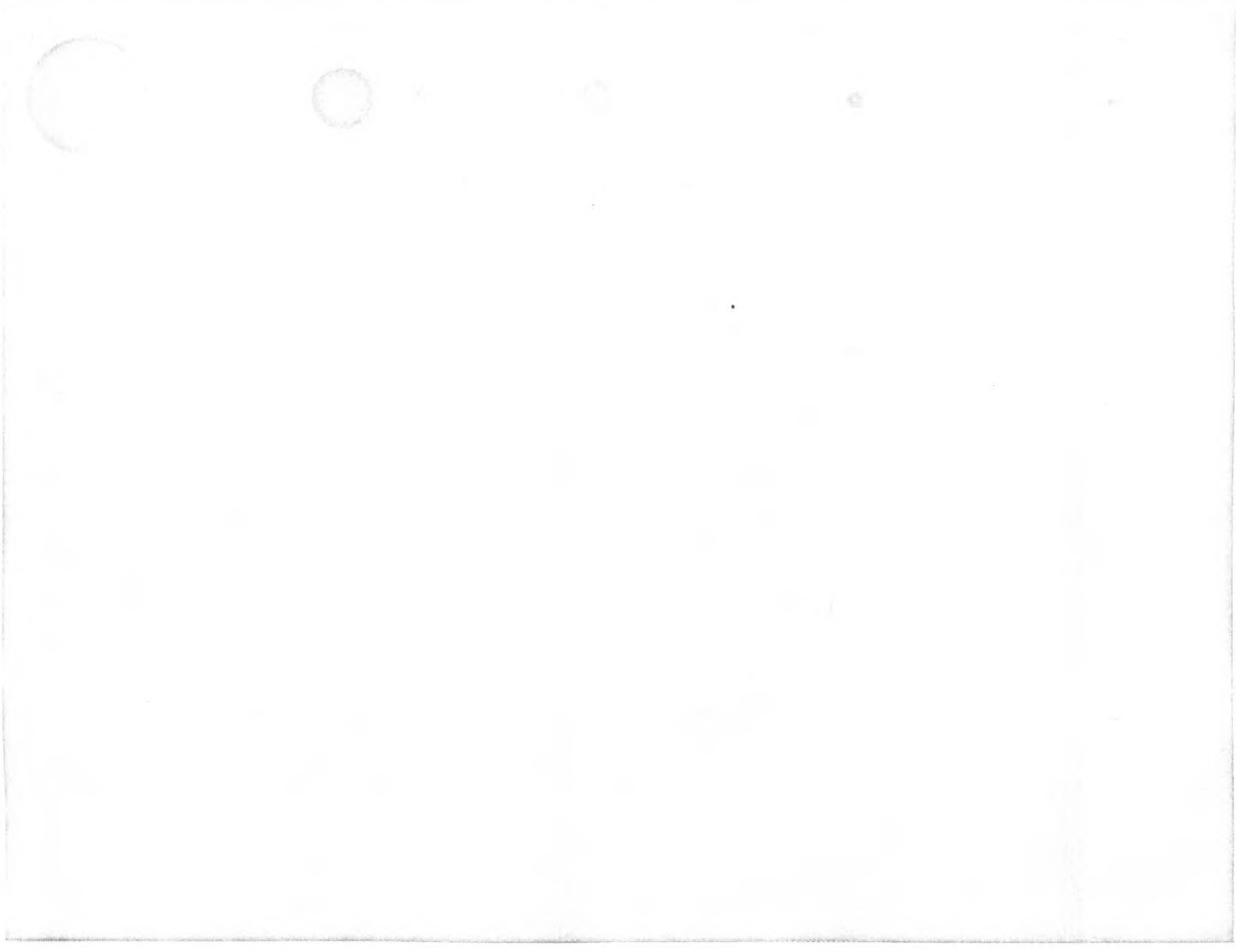


(a) (b) (c)

FIGURE 6

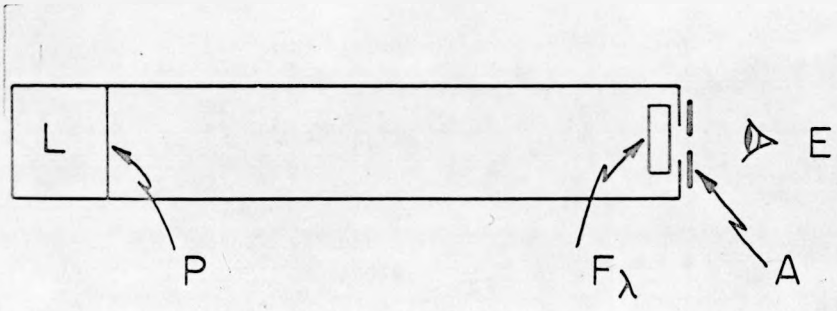


(a)

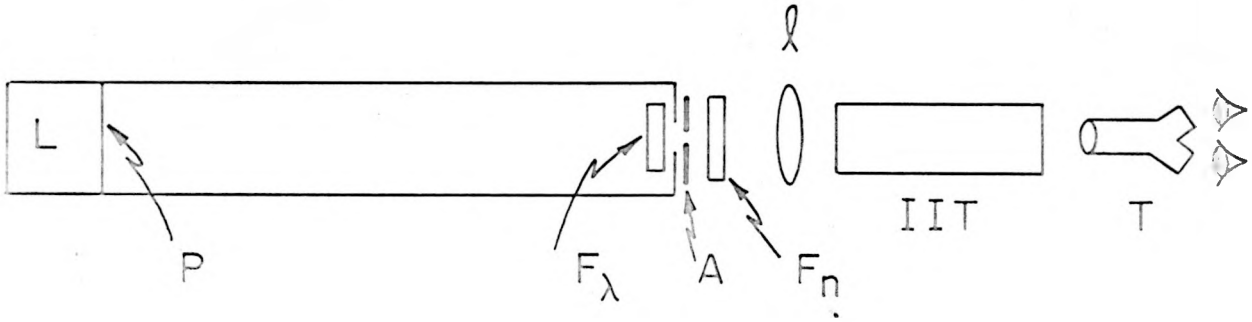


(b)

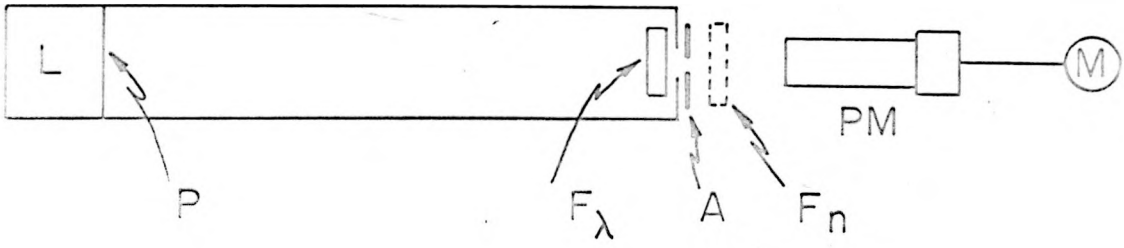
FIGURE 7



(a)



(b)



(c)

FIGURE 8

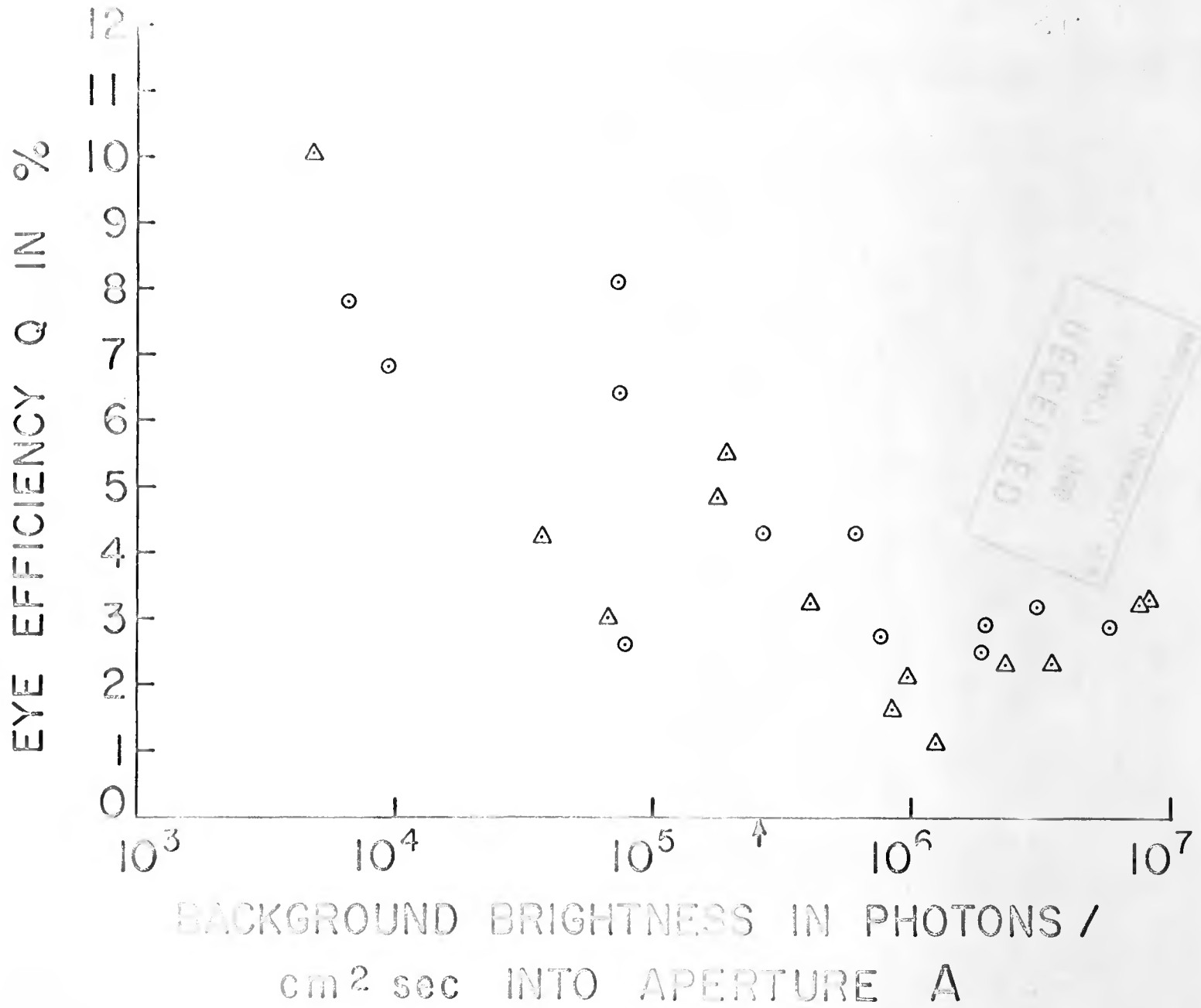

EYE EFFICIENCY Q IN %
0
1
2
3
4
5
6
7
8
9
10
11
12
10^3
10^4
10^5
10^6
10^7
BACKGROUND BRIGHTNESS IN PHOTONS /
cm^2 sec INTO APERTURE A

FIGURE 9