# Design of an Optical Microprobe for In Situ Fluorescence[1]

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## Introduction

In situ biofluorescence measurements have in the past been restricted to surface fluorescence of tissue and organs. A rigid system of light beams have in general been employed to transport light between tissue and fluorometer. Recently, however, the technique has been developed into a more flexible method in which light guides have been substituted for the light beams (1, 4). This allows, for instance, for measurements of various changing metabolic states of cells in organs of an awake animal over a long period of time.

In this work we discuss the development of a light guide probe for measurement of fluorescence of cells not only on the surface of but also deep inside tissue and organs. It is clear that such a probe would allow studies not possible with a surface probe. For instance, the metabolic state of cells of different parts of a brain may and does vary considerably. A surface probe with a typical depth sensitivity of only a few hundred microns would not be capable of registering such a spatial variation. It is obvious that to avoid massive tissue damage in the use of such a probe it must necessarily be designed to have a needle-like structure with a small diameter. In this study probes having diameters in the range of 0.1 cm - 0.005 cm have been used and their light guide characteristics have been measured under various experimental conditions as described below.

## Some Design Parameters

A schematic block diagram of an in situ fluorometer in use at Indiana State University (2) is shown in (Fig. 1). Excitation light from a conventional incandescent filament source or a laser is selected in a Schoeffel minature monochromator and enters a Y-shaped light guide (a Schott uv transmission guide). The diameter of the shaft of this guide is 0.1 cm. As described below a glass (or quartz) rod shaped into a suitable microguide is adapted to the end part of the Y-shaped guide and transmits excitation light to the sample spot. Fluorescence light returns through the same microguide and part of this light is led through the second arm of the bifurcated guide to a second monochromator (Schoeffel double monochromator GM 200) through which fluorescence light is transmitted and recorded in a high sensitive photon counter (RCA 8850 photomultiplier).

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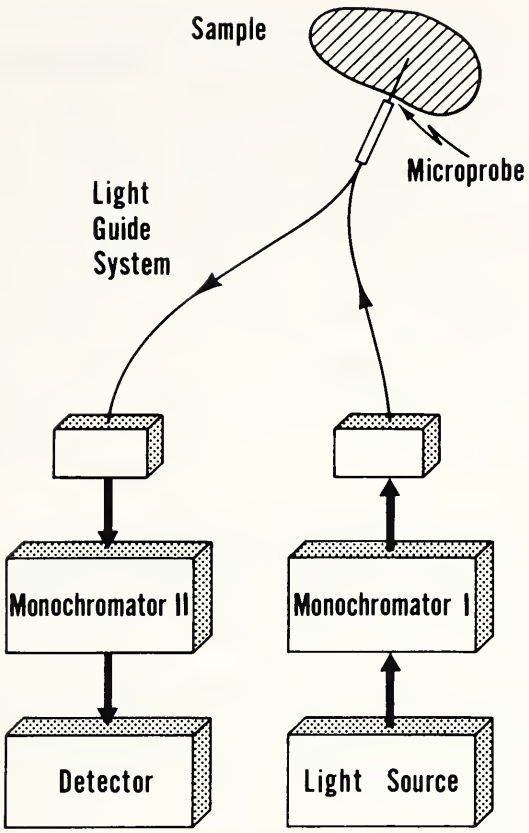


FIGURE 1. *Block diagram of in situ fluorometer.*

(Fig. 2) shows the principal design of the optical microprobe that has been found to be the most practical one in preliminary experiments. A pyrex glass (or quartz) rod is carefully heated and pulled by a micropipette puller to a straight tip forming the microprobe. This procedure allows for a tip size of less than 1 $\mu$m in diameter which is a standard size used in electrophysiological experiments. To prevent light from entering or leaving the rod its surface is covered by a thin layer of reflecting paint, leaving the tip the only unexposed surface. The layer also serves as a reflecting medium giving the rod light guide properties. The microprobe is in direct contact with the main light guide. A certain amount of reflection does occur in the interface but in general this can be tolerated.

It should be noted that due to the good solidity of the glass rod no special mechanical support of the microprobe is needed. Also, the positioning of the probe in tissue can follow a procedure much like the procedure used in conventional electrophysiological investigations.

Since the microprobe is a tapered light guide the entrance angle of light will not be the same as the exit angle. This means that in general a certain amount of backreflection will occur with corresponding loss of transmission. To estimate the severity of backreflection consider (Fig. 3). A ray entering the front surface with angle $\alpha_1$ is reflected inside the fiber and leaves the exit side surface at angle $\alpha_2$. If $d_1$ and $d_2$ are the diameters of the front and exit surfaces respectively and if only rays entering and leaving at the centers of the end surfaces are considered,

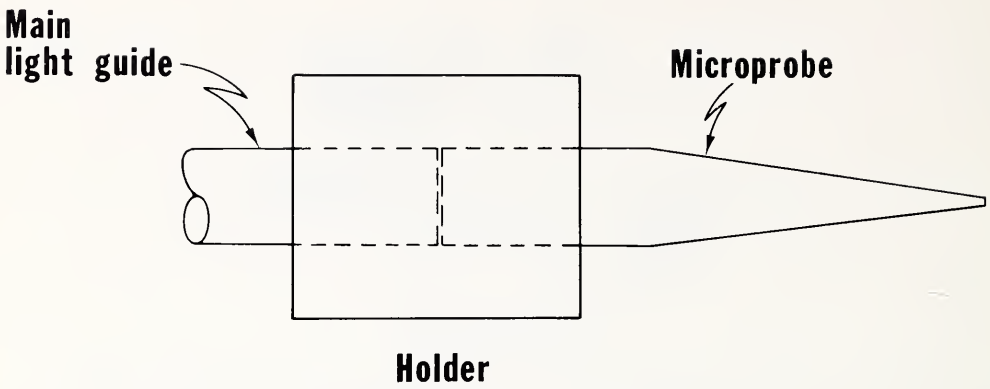


FIGURE 2. *Optical microprobe.*

elementary considerations show that the following relation holds for $d_1$, $d_2$, $\alpha_1$, and $\alpha_2$

$$d_1 \sin \alpha_1 = d_2 \sin \alpha_2 \qquad [1]$$

Choosing $\alpha_2 = 90°$ we obtain the condition on $\alpha_1$ for backreflection

$$\sin \alpha_1 = \frac{d_2}{d_1} \qquad [2]$$

For instance, if $d_1 = 0.1$ cm and $d_2 = 0.01$ cm we find that $\alpha_1 = 5.7°$. This means that for an entrance angle $\alpha_1$ larger than 5.7° light transmitted along the fiber will be reflected back or leaving it before reaching the exit surface.

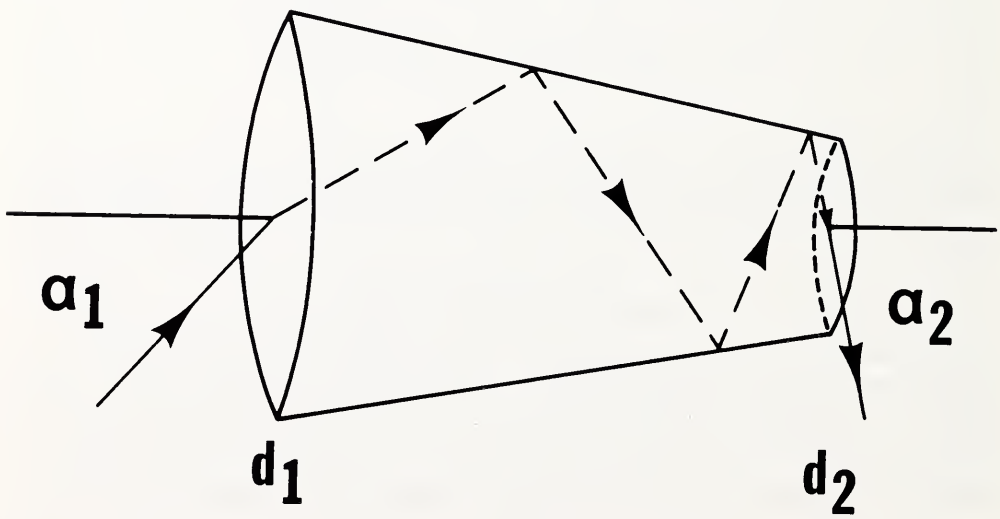


FIGURE 3. *Ray diagram of tapered light guide.*

If the light source of the microprobe system is of a conventional incandescent filament type, a spread of several degrees in beam divergence is usually present. A substantial backreflection and corresponding loss of transmission may therefore occur according to the discussion given above. On the other hand if the tapered light guide probe is fed light from a laser which has in general a very low spread in beam divergence ($\lesssim 0.1°$) a low backreflection effect is expected. Choosing $\alpha_1 = 0.1°$ and the $d_1/d_2$ ratio as stated previously ($d_1/d_2 = 10$) relation [1] indicates that the exit angle $\alpha_2$ becomes approximately 1°, which still represents a small beam divergence. The angle amplification in the

tapered light guide will obviously not cause any significant problem until the diameter ratio $d_1/d_2$ approaches a factor of one hundred.

It should be noted that in the actual fluorometer (Fig. 1) the main light guide is bridging the path between the microprobe and the light source. However, the discussion given above about angle amplification in the tapered probe is still correct, since the main light guide is not tapered and conserves therefore the exit and entrance angles. Also, it should be noted that as far as angle amplification and transmission is concerned it is only the trip in the direction from main light guide to sample that is critical in the microprobe. Fluorescence light traveling in the opposite direction exhibits an angle demagnification and therefore does not give rise to any transmission losses through backreflections.

## Results and Discussion

The two most important parameters to be determined experimentally for a microprobe are the cross-sectional profile of the light beam leaving the exit surface of the probe and the transmission of light in the probe.

The profile of the light beam has been measured in an experimental set-up consisting of a light detector and a small screen with a sharp edge which was movable, horizontally, across the opening slit of the detector. The microprobe was placed close to the detector slit with its center line perpendicular to the slit area and in such a position that the screen could block the light path to the detector. By recording the light intensity change in the detector as a function of the position of the screen, a measure of the integrated beam profile was obtained. (Fig. 4) gives two representative measurements of the horizontal beam profiles. The diameter of the entrance surface of the probes was in both cases equal to 0.1 cm while the diameters of the exit surface were measured in a microscope to be approximately 0.02 cm and 0.04 cm respectively. The light was supplied by a laser (TRW 83A Argon laser; beam divergence: 0.1°). The wavelength of light used was 480 nm. The distance between the probe and the detector slit was approximately 0.1 cm.

From a practical point of view the diameter of the cross-section of the beam as seen by the detector can be defined as the screen movement needed to change the detector intensity from 5% to 95% of maximum intensity. Using this definition, the curves in (Fig. 4) show that the beam diameter is about equal to the diameter of the exit surface of the microprobe. The light leaving the microprobe therefore forms a beam that is approximately parallel.

These measurements are in accordance with the theoretical results discussed in the previous section and show that by the use of a high quality light beam, the effect of angle amplification taking place in the tapered light guide can be neglected at least for tip diameters larger than 0.005 cm. As also indicated previously, this should not be true, however, if an incandescent filament source is used. Measurements with such a source performed according to the same scheme as those presented in (Fig. 4) show that a considerable beam divergence appears from the probe with corresponding transmission losses. The transmission of light for laser-fed microprobes is approximately 20% for an exit

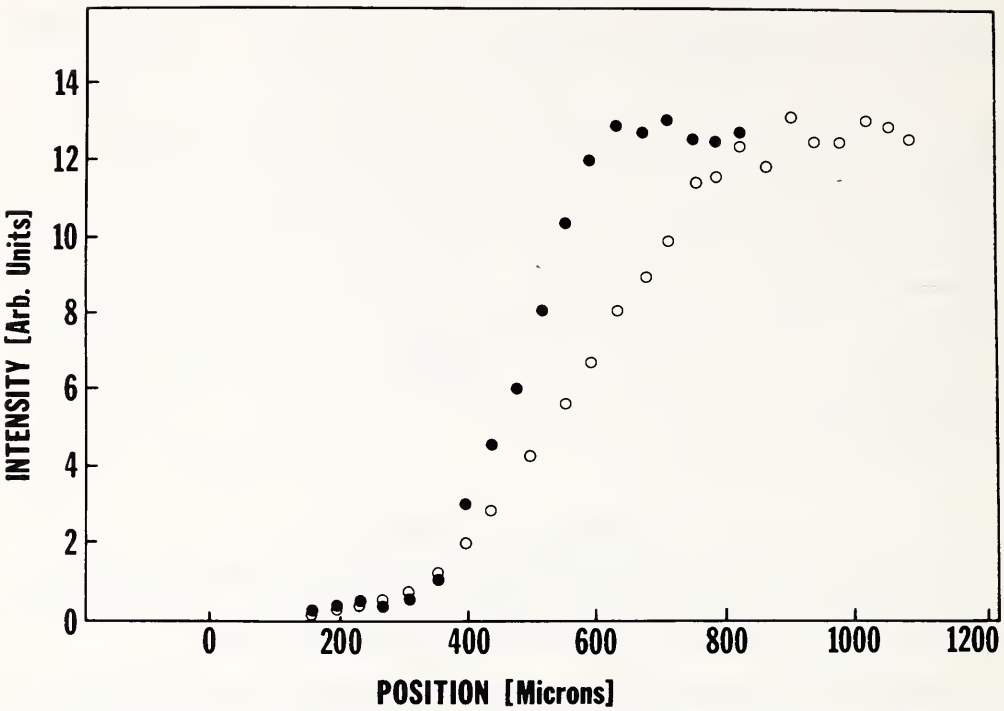


FIGURE 4. *Integrated intensity profile of light beam from microprobe for two probes with tip diameters of approximately 0.02 cm and 0.04 cm respectively.*

diameter of 0.005 cm; the main factor causing the transmission loss is attributed to absorption of light by the covering paint. The transmission of light in the microprobe when a filament source is used is at least a factor of five smaller than in the case of the laser indicating the presence of a large backreflection effect.

The main conclusion from these preliminary studies of microprobe design is that reasonably high quality light characteristics of the probe can be obtained if it is used in combination with a laser beam. The parallelity of the exit beam of the probe achieved in this case indicates that in biological material the volume of the sampling being seen by the probe will be approximately $\ell d^2$. Here d is the diameter of the probe tip and $\ell$ is the effective distance of absorption of light in biological material which is usually a few hundred microns for visible light but may be considerable shorter for uv light (3). In a typical situation, therefore, with $\ell$ = 0.01 cm and d = 0.01 cm the probe will measure fluorescence from a well-localized region with a volume of approximately $10^{-6}cm^3$.

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