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Bright Ideas in Fiberoptics

# Direct Contact Fiberoptic Plates for the Detection of Luminescent Cells

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October 30, 2007

## Abstract

Fiberoptic plates offer significant advantages when used for detection and readout of the optical signals from microarrays. The fiberoptic plates use fused optical fibers to transfer images coherently from the microarray to the detector sensor, thereby significantly enhancing system performance in certain direct contact imaging applications. An FOP system with NA of unity is shown to give optical signal-to-noise ratios of 18 provided the detector sensor is the same size as the microwell array. Applications of microarrays are reviewed, performance criteria and limitations of fiberoptic plates used for direct contact detection are discussed, and the signal-to-noise advantage is calculated. Suggestions for advances in components and instrumentation are given.


## Introduction

In recent years, progress in the biological sciences has been accelerated by the advent of microarray technology, which enables high throughput parallel experimentation and diagnostics. Microarrays are used for a wide variety of applications including biological investigations, genomic studies, development of new pharmaceuticals, and detection of biological agents. The arrays offer fast, accurate, efficient, and cost-effective results that heretofore would have required millions of independent experiments. In this paper the term microarray[1] is used to refer to any platforms used to conduct highly parallel biological or chemical testing such as microwell plates, picowell plates, microfluidic arrays, microcapillary arrays, fiberoptic plates, and biochips. Microarray plates offered by INCOM, Inc. include microwell plates containing many individual wells, microfluidic plates containing channels, microtiter plates, microcapillary plates formed with flow-through capillaries in various configurations, and fiberoptic plates for droplet tests on a flat surface. Although fiberoptic plates provide an excellent surface for droplet tests, the topic of this paper is the use of fiberoptic plates as very effective conduits for efficient readout of luminescent signals from any of the bioplates.

Various schemes have been employed for viewing, detecting, or reading luminescence from the bioactive materials contained in the microarrays. Detection schemes include conventional microscopy, inverted microscopy, cameras, and direct reading detector sensors, such as CCD or CMOS detectors.

Specific instruments designed to detect and read the information from a microarray are known collectively as microarray readers. These readers are typically either ‘top’ or ‘bottom’ readers.[2] Optical information can be directly imaged onto a detector sensor (with or without supplemental focusing optics) or can be detected using a laser scanner in conjunction with a photomultiplier detector. In either case, the reader must have clear optical access to the samples on the microarray. Viewing from the top surface allows access under all circumstances but is complicated by the depth of focus of the optics (many millimeters) and by challenges, in some cases, of interrogating the microarray through a droplet of liquid. By viewing the sample from beneath the plate, these shortcomings can often be negated.

In some cases, the use of a fiberoptic plate (FOP) affixed to the bottom of a microarray can provide excellent performance. The microarray is used for biological testing, and the intervening FOP is the optical conduit, faithfully transmitting the optical signal from the microarray to the detector sensor below it. Since the FOP is comprised of millions of fused optical fibers, it transmits the image with very little loss or distortion. Direct contact detection implies that the FOP is in direct contact with the detector sensor below it. In this role the FOP protects the detector sensor from the fluids in the microarray while efficiently transmitting the luminescent signal to the detector array. The fibers comprising the FOP prevent diffusion of the light as it propagates through the plate as would occur in a clear glass plate. Each FOP fiber confines the light to its core diameter, which is typically 3 or 6 microns for INCOM plates.

When used in the direct contact configuration, these plates can offer as much as a factor of 20 improvement in optical signal-to-noise ratio. In the remainder of this paper, the advantages and limitations of the FOP are discussed, and some opportunities for instrument developers are suggested. For convenience, a microwell plate will be used as the baseline platform for evaluating FOP performance.

## Background

In typical microwell applications, a mixture of biological materials is introduced into each well along with bioactive probe materials. Some of the wells will contain the specific bioactive probe for the biological material in the well, resulting in luminescence or fluorescence (when illuminated by light of the proper wavelength) from the well containing the sensitized biological material. (Luminescence[3] is the term that encompasses all forms of cold-body radiation and includes, among others, fluorescence and chemoluminescence.) Subsequent analysis of the bioactive wells allows the reactive materials to be identified. For bottom interrogation, the microwell array is assumed to have a thin transparent bottom that confines the liquid in the wells but allows light to pass out the bottom. As discussed below, the bottom can be a thin clear glass (or plastic) plate or it can be a fiberoptic plate.

Traditionally, luminescence from the microwells has been detected using camera systems. The microwell array portion of this configuration is depicted in Figure 1a. A thin clear glass or plastic plate forms the bottom of the conventional microwell plate. Signal cells attach to the well bottoms and radiate light in all directions, some at large angles with respect to the normal to the plate as represented by the blue arrow. To capture this “high NA” light, the camera system must have a large numerical aperture, which requires a large camera lens located close to the plate. In these systems, the camera forms an image of the microwell array and focuses it onto the image plane of the camera where a detector sensor or film is located.

The camera increases the size and complexity of the detection system. The aperture, focal length, and object distance must be chosen to maximize system performance and sensitivity. In some cases, it may be necessary to scan the camera physically across the microwell plate to achieve the desired sensitivity and resolution, but scanning requires a

precise high-resolution mechanical system and may substantially increase the time required for measurements. Camera systems also require precise focusing on the bottom of the microwell plates and are subject to aberrations.

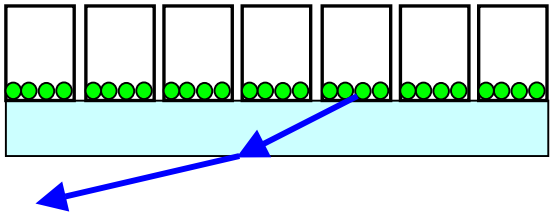

Figure 1a. Microwell array with clear bottom plate used in camera systems.

Fiberoptic plates (FOP) offer an alternative to the camera system. Luminescence from the microwells can be transmitted directly to a detector sensor without the complexity of the intervening camera system.

As depicted in Figure 1b, the FOP forms the bottom of the microwell array. For plates made with either 3- or 6-micron fibers, the diameters are sufficiently small that each microwell is typically interrogated by several fibers. For systems employing large detector sensors or relatively small (in overall dimensions) microwell arrays, the best performance is achieved when the FOP is coupled directly to the detector sensor. Since the fibers comprising the FOP intrinsically have a very high NA, the direct contact configuration provides an exceptionally large system NA with a correspondingly large signal-to-noise ratio.

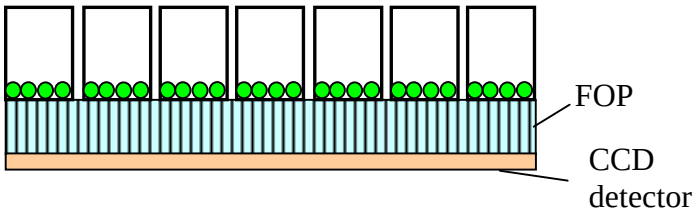


Figure 1b. Direct contact imaging of a microarray using an FOP bottom plate and CCD detector.

In this paper, several FOP designs are considered and the associated performance is evaluated. For the purpose of modeling the FOP system in biological applications, luminescent cells are assumed to be immersed in liquid in the wells of a microtiter plate or microwell plate. “Signal” cells are activated so that they attach themselves to the bottom of the well, whereas “noise” cells float in the body of the liquid. For analysis purposes, the noise cells are assumed to be floating at a height of 50 microns above the well bottom. The luminescence may be caused by chemical activation of certain biomaterials or it may result from the fluorescence of material radiated with the appropriate wavelength of ultraviolet light. Both the signal and noise cells are assumed to have a diameter of 20 microns and are assumed to radiate light of equal intensity. The diameter of fibers comprising the FOP will be assumed to have a diameter of 6 microns. A typical inside diameter for a well in a standard microtiter plates is about 0.67 cm, which will be assumed for these calculations; however, plates with much smaller well diameters are available and work just as well.

## Calculation of Signal-to-Noise Ratio

Regardless of the source of luminescence, the ability to distinguish the floating noise cells from the attached signal cells determines the optical signal-to-noise ratio of the system. The optical signal-to-noise ratio (SNR) is defined as the ratio of signal intensity to noise intensity. Since photodetectors and detector sensors produce an electrical voltage that is proportional to optical power, the conventional electrical signal-to-noise ratio is the square of the optical SNR. All of the SNRs discussed in this paper are optical signal-to-noise ratios.

The basis for the optical signal-to-noise performance of an FOP system can be understood by assuming that the fibers comprising the FOP have a numerical aperture (NA) of unity. For the portion of a signal cell in contact with an individual fiber, any light emitted with a downward angle from that portion of the cell is transmitted by that fiber directly to the detector sensor. In contrast, light emitted at large angles by a noise cell floating 50 microns above the bottom of the plate will not strike the fiber directly below the cell, but instead, it may travel laterally many fiber diameters before striking the FOP array. Consequently, the light from the signal cell will be restricted to the fibers in contact with the cell, whereas light from noise cells may be spread over an area on the FOP (and therefore on the detector sensor) many times the cell area. If the total light received across the entire detector sensor is summed, both cell types will contribute the same amount of energy; if a form of matched filtering is done wherein the light is summed over bright regions that are nominally the size of the cell diameter, then this localized response will be primarily from the signal cells. In contrast, the noise cells will contribute a faint background glow over a large area.

The FOP approach offers several advantages for biological measurements. First, the direct coupling through the fiberoptic plate to the detector sensor results in a very fast and efficient readout of the signal. Each well is coupled directly through its own fiber or subset of fibers in the microwell plate, so there is little light loss. With properly designed plates, there is no significant cross talk between wells. Also, the FOP enables a very compact detection system. In contrast with a camera system, no additional space is required to allow for the focal length of the camera and all its supporting alignment and focusing fixturing. Furthermore, the exceptional numerical aperture of the fiberoptic plates can result in light-collection efficiency an order of magnitude greater than for some camera systems. In summary, the large numerical aperture of the fiberoptic plate results in differentiation between light emitted by cells affixed to the bottom of the microwell plate relative to light from cells floating above it, providing an improved signal-to-noise ratio relative to many camera systems.

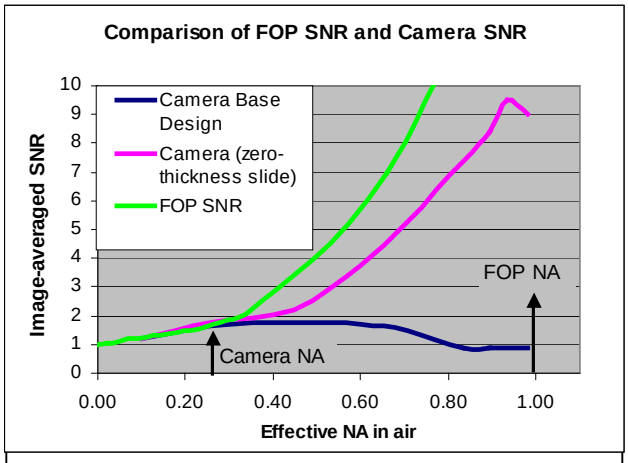


Figure 2. Dependence of SNR on NA.

The calculation of the SNR for camera systems involves some

additional considerations. In camera systems, the bottom of the microwell array would typically be a clear glass plate. Assuming a nominal thickness of 150 microns for the plate, the camera differentiates between the signal and noise cells by forming an image of them. The camera must be focused precisely through the glass plate and on the bottom of the well. In this configuration, the signal cells are in focus but the noise cells are out of focus so their light is spread across a larger area in the image plane. As in the FOP system, the larger NA allows more light to be received in a localized region from the signal cell. However, as the emission angle increases, the larger angle rays from the signal cell are defocused by the fluid in the well and the glass plate so that the image quality deteriorates. Consequently, SNR increases slowly with increasing camera NA. A good analogy for this effect is that objects observed off to the side in a fish tank (large-angle rays) are blurrier than objects directly in the center. For this reason, it is desirable to keep the clear bottom plate as thin as possible. The FOP configuration experiences none of these refraction issues because the light remains entirely within the water medium until it is captured by the FOP. Also, there is no restriction on the thickness of the FOP, because the fibers faithfully transmit the image through the plate, regardless of thickness. With the camera system, there is also an additional air-glass interface at the output of the microwell array that causes the rays to refract to an even larger angle in air (as depicted in Figure 1a), requiring a greater camera NA to achieve the same SNR. With the FOP, there is no air interface between the signal cell and the detector sensor so there are no deleterious refraction and defocusing effects.

The dependence of the SNR on the NA has been calculated for both the FOP and camera systems and is shown in Figure 2. The analysis of the camera system is an approximation based on ray tracing of both meridional and skew rays. For modeling purposes, the camera is assumed to be operating with a magnification[4] of negative 1 with an f/1 lens and a lens diameter of 25 mm. The accuracy of the calculation is not expected to match that which is achievable with quality optical analysis software, but it is sufficient to illustrate the dependence of SNR with NA. Increasing the numerical aperture dramatically increases the SNR of the FOP system because it increases power received from the signal cell but does not increase noise power. The FOP system is capable of giving SNRs greater than 20 for the baseline system when the full NA of the constituent fibers can be utilized. The gains of the camera system with increasing numerical aperture are less pronounced because of the deleterious effects of the thin plate on the bottom of the microwell array. For comparison, the performance of the camera system is shown if a zero-thickness microwell plate could be used. In this case, no defocusing effects would occur and the camera performance is similar to the FOP except for the correction for the refraction at the air interface, which does not occur, with the FOP. For numerical apertures less than 0.3 in air, the camera and FOP systems perform similarly. Given the degradation of the image quality caused by the clear plate in the camera system, it might be thought that substitution of an FOP in the camera system might be beneficial. However, the FOP only adds further to distortion of the image in the camera system and is only beneficial for direct contact detection.

In the FOP approach, the NA of the constituent optical fibers is critical. The numerical

aperture is defined in Equation 1.

1) $NA = \sqrt{n_{core}^2 - n_{cladding}^2} = n\sin(\theta)$

The maximum acceptance angle of the fiber is $\theta$, the index of refraction of the material surrounding the output of the fiber is n; $n_{core}$ and $n_{cladding}$ are the fiber core and cladding indices respectively. For some glass combinations, the radicand (the expression beneath the square root sign) can be greater than unity, giving a complex value for $sin(\theta)$. This merely means that the fiber is capable of supporting more modes internally than can be excited by light incident in air ($n$=1).

If the faceplate is immersed in water, the acceptance angle is modified because the index $n$ = 1.33, and it is possible to excite additional modes in the fiber. Faceplates with an air numerical aperture >1 exhibit the best light-gathering power when used in water.

The NAs available in some of INCOM's FOPs are shown in Table 1 along with the SNR that would be achieved with the FOP coupled directly to a detector sensor. The various NAs quoted in the table are achieved by a suitable choice of glass combinations for the core and cladding of the constituent fibers. One of INCOM's standard FOPs has an NA in air of 1.0, which has been selected for the baseline system configuration and provides a SNR of 18.

**Table 1. Comparison of signal-to-noise ratios for floating cell height = 0.050mm**

| System Configuration | 0.35 NA FOP | 0.66 NA FOP | 1.0 NA FOP | 1.1 NA FOP |
|---|---|---|---|---|
| SNR Averaged Over Image | 2.2 | 7.0 | 18.0 | 23.1 |

*Dependence of SNR on Floating-Cell Height*

The height of the floating noise cell above the well bottom also affects the signal-to-noise ratio as shown in Figure 3. As the height increases, both the FOP and camera systems are better able to distinguish between the two cells, but once again, the FOP approach is significantly better because it does not experience the defocusing introduced by the clear bottom plate in the camera system. The SNR improves with the height of the noise cell because its image is spread over more fibers in the FOP as the height increases or over a larger image area in the camera system. The SNR is measured by averaging the optical intensity over a localized region equal to the signal cell size. The SNR increases because the relative intensity of signal cells to noise cells increases as the noise cell gets further away.

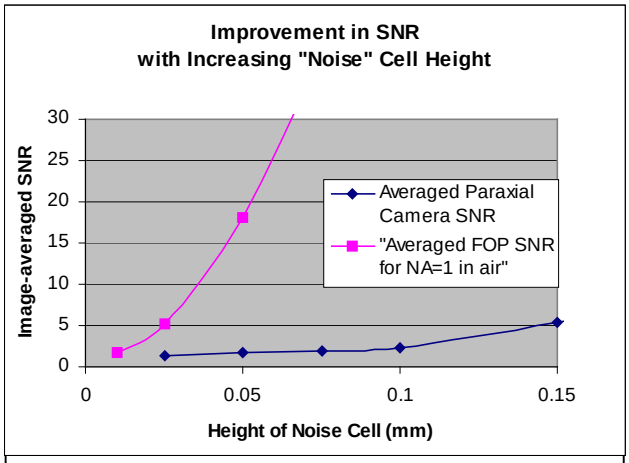


Figure 3. Dependence of SNR on floating-cell height.

*System Magnification to Match Detector Array Size*

The prior examples show the substantial benefit of using direct contact FOP microwell plates with detector arrays to obtain very large signal-to-noise ratios and good discrimination between signal and noise cells. All of the prior examples assume that the detector sensor is physically as large as the microwell plate being interrogated. Currently, commercial CCD arrays are available in rectangular or square formats with linear dimensions ranging from 10 mm to 60 mm.[5,6] Also, the advent of the digital camera has dramatically reduced cost and improved performance of detector arrays.

There is an increasing interest in miniaturized diagnostic equipment. Also, interest in smaller microwell arrays, known as picowell plates, is increasing.[7,8] The use of these smaller bioplates with available sensor arrays will often allow direct contact imaging to be performed with the full benefit of the associated signal-to-noise ratio.

If it is still necessary to use a microwell plate that is larger than current practical detector sensors, several other options are available. These include scanning, conventional camera optics, and the use of fiberoptic taper technology. In some cases, it may be possible to scan the detector sensor or microwell plate relative to each other to gather all the information from the plate. However, scanning will inherently increase the measurement time.

Alternatively, in a camera system, the microarray image can be demagnified. Demagnification is achieved by increasing the object distance of the lens, thereby creating a smaller image of the microwell array that matches the size of the detector

array. The f-number of the camera system is related to the f-number of the lens and the magnification[9] according to Equation 2.

$$2) \quad f\#_{\text{system}} \equiv \frac{1}{2NA_{system}} \approx (1-1/m)\, f\#_{lens}$$

where *m* is the magnification and the *f#* of the lens is F/D where F is the focal length and D is the lens diameter. For the prior calculations, a magnification of negative 1 was assumed, which gives an inverted image equal in size to the object. If the image size must be demagnified to match the size of the detector array, there is nominally a proportional decrease in NA and a reduction in the signal-to-noise ratio according to Figure 2.

In the case of a large microarray with an FOP, the output of the FOP can be matched to the detector array by attaching a fiberoptic taper to the detector sensor. The taper is selected so that the large end matches the size of the FOP and the small end matches the dimensions of the detector array. All the light from the FOP enters the large end of the taper, including the high NA rays. However, in the tapered region, the taper angle causes the large-angle rays to exceed the local NA of the fiber. These rays are absorbed by the taper and do not propagate to the smaller end. The reduction in NA is approximately equal to the factor by which the linear dimension must be reduced to match the detector array to the size of the microwell plate. The examples in Table 2 show that signal-to-noise ratios greater than unity are common with an FOP even when some demagnification is required.

**Table 2. Signal-to-Noise Ratios vs. NA and Magnification**

| Product | Intrinsic numerical aperture (in air) | Reduction/ Magnification | SNR |
|---|---|---|---|
| INCOM FOP | 1.1 | 1/1 | 23.1 |
| INCOM FOP | 1.1 | 2/1 | 4.8 |
| INCOM FOP | 1.1 | 3/1 | 2.4 |
| INCOM FOP | 1.1 | 4/1 | 1.9 |
| INCOM FOP | 1.0 | 1/1 | 18.0 |
| INCOM FOP | 0.66 | 1/1 | 7.0 |
| INCOM FOP | 0.35 | 1/1 | 2.2 |
| Camera | 0.26 | 1/1 | 1.6 |
| Camera | 0.26 | 4/1 | 1.0 |

*Other Considerations*

Both approaches have other factors that may affect their performance. For efficient detection, transmission losses through the FOP should be minimized. For fluorescent applications, FOPs must be utilized that do not exhibit significant fluorescence. INCOM can provide FOPs that are customized to minimize or eliminate fluorescence at wavelengths of interest.

For the remote camera system, the camera must be focused precisely on the signal cells to achieve the desired SNR. If the cover slide varies in thickness or is not perfectly perpendicular to the camera axis, the image of the signal cell is defocused, reducing the SNR. Also, aberrations in the lens system may blur or distort the cell images, reducing the SNR, particularly for large-angle skew rays.

## Summary

The desire to perform large-scale biological experiments with great sensitivity and small volumes of bio-reagents has led to strong interest in effective detection techniques for monitoring reactions in microwell and picowell plates. Direct contact imaging of a microwell through a fiberoptic bottom plate to a detector array has been evaluated for biological systems in which noise cells float above signal cells. The signal cells are activated so they attach to the FOP at the bottom of the wells. The high numerical aperture of the FOP allows most of the light from the signal cell to be localized in a small area on the detector array whereas noise-cell light spreads across many pixels. An FOP system with NA of unity is shown to give optical signal-to-noise ratios of 18 provided the detector array is the same size as the microwell array. Signal-to-noise ratios increase even further if the noise cell floats more than 50 microns above the well bottom (the 50 micron value was chosen for the baseline calculations). In general, the direct contact detection system enabled by the FOP provides better noise discrimination than camera systems because it eliminates distortion and aberrations that occur at large numerical apertures in camera systems. If the microwell plate is larger than the detector array, other options are available such as scanning, demagnification of the camera image, or the use of fiberoptic tapers for the FOP system.

In conclusion, direct contact imaging through an FOP to a detector array provides excellent signal-to-noise performance for biological measurements in microwell plates properly matched to the detector array. As detector arrays become available in larger sizes, the FOP will increasingly become the preferred configuration for detection systems.

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[4] Magnification - Wikipedia, the free encyclopedia

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