Microfluidic Microwell and Microcapillary Biochips

Michael J. Minot\textsuperscript{a}, David W. Stowe
MinoTech Engineering Inc., PO Box 1539, Andover, MA 01810,

Michael A. Detarando\textsuperscript{b}, Joseph A. Krans, Jason L. Kass
Incom Inc., 294 Southbridge Road, Charlton, MA 01507

ABSTRACT

Utilizing nanotechnology, proprietary chemistry, and microfluidics, innovative firms are developing biochips and instrument systems that enable high-speed automated biomedical sequencing. Incom Inc. presents development results on five novel biochip technologies based on FiberOptic MicroSlide\textsuperscript{TM} and microcapillary technology. FiberOptic MicroSlides\textsuperscript{TM} are fiber optic interrogated (FOI) biochips made up of millions of fused optical fibers, and are uniquely suited as a platform for microarray applications. FiberOptic MicroSlides\textsuperscript{TM} (henceforth referred to as “MicroSlides” or “slides” in this paper) act as a ‘zero thickness substrate’ transmitting optical signals from top to bottom without spreading, so that fluorescent or luminescent activity on the surface or within a well can be directly coupled to a CCD device without additional optics. In contrast to bulk optics, the slides are compact and have excellent light-gathering power. They are an alternative to conventional microscopic slides for applications involving moderate-resolution bottom viewing (inverted microscopy). The surface of the MicroSlides can be etched or patterned with a permanent polymer to form microwell arrays, or microfluidic structures suitable for genomic and proteomic analysis, cell migration studies and other applications. Low-cost microcapillary array plates have also been developed. These plates act as microscopic test tubes, which enable picoliter reactions to be detected, counted and analyzed. Progress in developing large area (300 mm X 300 mm) arrays with up to 100 million capillaries, and diameter / length aspect ratios up to 10,000: 1 is presented. Results demonstrate negligible optical cross talk between capillaries, resulting in improved signal-to-noise ratios while minimizing false hits.

Key Words: Microcapillary, Microwell Array, Biochip, Fiber-Optic-Interrogated

1.0 BACKGROUND

The FiberOptical MicroSlide\textsuperscript{TM} has been developed as a substrate that overcomes performance limitations of conventional microscope slides, microarrays, or microtiter plates when optically interrogated through the thickness of the substrate. The MicroSlides\textsuperscript{TM} consist of many millions of minute optical fibers that have been fused together. When sliced and polished to form microscope slides, the fibers effectively transfer optical images from one surface of the MicroSlide to the other surface without additional focusing optics, regardless of the slide thickness. The finished MicroSlide is the optical equivalent of a zero-thickness window. The slides allow objects to be directly imaged on a CCD array without bulky or costly intervening focusing optics. Direct contact imaging provides an alternative to other techniques that are very sensitive to focus and vibration, and is therefore uniquely suited for robust (portable) applications. MicroSlides also provide other benefits that enable unique applications that will be discussed.

A FiberOptic MicroSlide\textsuperscript{TM} can substitute for a microscope slides or be used as a substrate for a microarray, microtiter plate, or other applications involving moderate-resolution bottom reading. When viewing an object through a MicroSlide, the nominal resolution is equal to the diameter of the constituent fibers. Presently, MicroSlides are available with a nominal 3\textmu m diameter, but smaller diameters may become available in the future.

In recent years, progress in biological sciences has been accelerated by the advent of microarray technology which allows high-throughput parallel experimentation and diagnostics. Various schemes are used for viewing or reading the microarrays including conventional microscopy, inverted microscopy, as well as dedicated reading or scanner instruments. Microarray readers are typically either ‘top’ readers or ‘bottom’ readers. Optical information can be directly imaged onto a CCD array (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

\textsuperscript{a} www.minotechengineering.com, e-mail: mjminton@minotechengineering.com, Phone : (978) 474-8034
\textsuperscript{b} www.incomusa.com, e-mail: mad@incomusa.com, phone : (508) 765-9151
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. An issue common to both configurations is that the base of the microarray and the focal plane of the optics have to coincide throughout the scan to produce an optimal signal. One way this can be ensured is by making the base of the substrate flat to a few microns over its entire area. An alternative is to incorporate an active focusing mechanism in the scanner, tracking the height of the scanning beam over the plate to compensate for undulations in the base, and to focus on the target, however the auto-focus optics adds considerably to the cost of the scanner instrument.

A wide variety of techniques have been employed to read or scan microarrays. These techniques differ considerably in terms of cost, resolution, sensitivity to focus or vibration and other product related factors. Laser readers often incorporate a fold mirror mounted on a rotating galvanometer where the deflected beam is focused onto the microarray by a telecentric lens that forms the objective.

One drawback is that telecentric lenses are relatively large, weighing hundreds of grams and cost approximately $2,000–$3,000 each. Alternative techniques depend on the fold mirror and the objective being moved back and forth over the smaller dimension of the array or scenarios in which the array is scanned in two dimensions. Top down inspection of microarray substrates is also commonly practiced. Often a laser beam is used to excite a fluorescent reaction, which is detected through a focusing objective lens. CCD cameras can also be used for top down scanning of microarray substrates. In another approach used for bottom scanning of microarray plates, a high-speed scanning galvonometer mirror is used to illuminate the cells situated on the top of a very thin microscope slide. In this scenario the light must pass through the thickness of the microscope slide substrate, and is subject to any distortion or optical effects that result. Another scenario employs multiple microlenses to focus through the bottom thickness of the microtiter (or microarray) plate. This approach is a very expensive approach since each well or microarray spot requires a dedicated miniature lens aligned to interrogate each well or spot. Because of the finite size of these lenses, the approach is not easily scalable to higher density microtiter (or microarray) plates.

### 1.1 Optical Characteristics of FiberOptical MicroSlides™

FiberOptical MicroSlides™ are manufactured using well established glass fabrication technology. The resulting MicroSlide product offers optical performance that is significantly differentiated from conventional (glass or plastic) substrates, microtiter plates, microarrays, microarray substrates, microscope slides, microarray plates or the like. FiberOptical MicroSlides™ are fabricated by bundling lengths of optical fiber and fusing them along their lengths. The fused bundle or block of fibers is then sliced into thin wafers such that opposing surfaces of each wafer consist of the proximal and distal ends of the optical fibers. Figure 1 depicts a FiberOptic MicroSlide substrate composed of multiple optical fibers that have been fused together.

Each fiber includes a central core glass region surrounded by clad glass.

Figure 2 is a pictorial representation of the manufacturing process used to produce the MicroSlides. The starting point is a core glass rod, sized to fit closely within a clad glass tube. Together they are loaded into a furnace where they are fused and drawn into long lengths of cane, typically about 2.5 mm in diameter. Long lengths of cane are assembled into billets, which are re-drawn forming the first ‘multi’. The process is repeated, with ‘multi’ assembled into a second billet, which is drawn again to form ‘multi–multi’ cane. During the “mold load” stage, “multi–multi’s” are cut to the desired block length and stacked into a pressing fixture (typically about the size of a loaf of bread). The assembled mold is placed into a pressing furnace. During ‘pressing’, the furnace heats and softens the fiber array under pressure, causing the structure to form a solid block. The block is then annealed and fabricated into finished product. For a FiberOptic MicroSlide, block material is cut into rectangular plates having the desired nominal thickness. Plates are ground and polished to target dimensions using glass finishing slurry and pad materials.

The functionality of the MicroSlide contrasts with that of a conventional microscope slide (Figure 3). The conventional microscope slide, having a thickness ‘T’ may have a sensor or detector in contact with the bottom as depicted in green below the slide. This sensor or detector could be a CCD array, or photosensitive film or other appropriate material. If a faint point light source (shown as a red dot) on the surface of the microscope slide radiates in all directions (360
degrees), half of the light radiates in the opposite direction of the slide, and is lost. The other half (θ = 180 degrees) radiates in the direction of the slide and is either transmitted or reflected back at the interface depending on the refractive index of the glass and surrounding medium. Light entering the slide propagates at all angles (θ) as shown in the figure. Depending on the thickness of the slide (typically 1–2 mm), the light emitted at large angles (70 degrees, for example) may spread as much as 5mm from the source by the time it propagates through to the other side. Therefore, to efficiently recover most of the emitted light from this point source, a focusing lens with about a 1 cm aperture would be required. For a nominal f2 lens, the focal plane will lie about 2 cm below the slide surface for this situation. The microscope/lens system may also be subject to focusing aberrations and chromatic aberrations when imaging the highly divergent light. These aberrations limit the resolution achievable with the system which may limit the ability to resolve faint fluorescent point sources in close proximity. Demands on the optics become much greater as the size of the array plate is increased.

If two adjacent light sources are considered, the ability to resolve the two will depend on the extent to which their intensity profiles overlap in the image plane. Without intervening optics, a microscope slide is an ineffective means of imaging closely spaced sources onto a CCD array because the intensity profiles overlap nearly identically. If less-expensive intervening optics is used to capture the highly divergent rays, chromatic aberration or spherical aberration may become a problem. Alternatively, if the peripheral rays are eliminated with an aperture stop to reduce aberrations, the system collects light less efficiently and becomes less sensitive to faint light sources. Even with intervening optics, the microscope slide itself may contribute to chromatic aberration. For the example shown in Figure 4, a 1mm thick microscope slide can cause a differential displacement of 2.78µm between red and blue beams incident at 60 degrees.

1.2 Light Detection with FiberOptic MicroSlides™

Figure 5 depicts the optical performance of a FiberOptic MicroSlide™ and shows how it is differentiated from a conventional microscope slide. A MicroSlide™ is comprised of individual optical fibers that conduct light incident on one face to the opposing face. Each of the constituent fibers comprises a high–index glass core surrounded by a lower–index optical cladding, so that the resulting multimode fiber guides the light. The figure illustrates a parallel array of individual fibers, with the higher refractive index core glass, separated by the surrounding clad glass (black lines). A light source (shown as a red dot) is shown on the surface of the slide, contacting the proximal end of an individual fiber optic core. A sensor or detector in contact with the bottom of the slide is depicted (green) below the slide, in direct contact with the distal end of an individual fiber optic core. This could be a CCD array, photosensitive film, or other appropriate detector. The optical properties of the FiberOptic MicroSlide™ depend on the core and clad dimensions as well as the refractive index properties of the respective materials. Together, these parameters determine the numerical aperture (light gathering) and the modal properties (light guiding) of the MicroSlide. Using the ray approximation for meridional waves, the acceptance angle θ of the constituent fibers is given by:

Equation 1: \[ n_1 \sin(\theta) = \sqrt{n_{core}^2 - n_{cladding}^2} = NA \]

where θ is the acceptance angle of the faceplate, NA is the numerical aperture, and \( n_{core} \), \( n_{cladding} \), and \( n_1 \) are the respective refractive indices of the core, cladding, and the material immersing the input end of the fiber. For air, \( n_j = 1 \).

Figure 6 illustrates the capture of radiation from an isotropic point source for a fiber with NA < 1 (acceptance angle less than 90°). Only light radiated downward into the acceptance cone with vertex angle \( 2\theta \) is captured in guided modes of
the MicroSlide. (A small percentage of this light is also Fresnel reflected at the interface between air and glass.) The resolution achieved with the FiberOptic MicroSlide™ depends primarily on the dimensions of the individual fibers making up the slide and the resolution of the detector. In the present context, resolution is defined to be the distance between two nominal point sources at which they can first be distinguished as two discrete objects when viewed through a MicroSlide. Therefore, a smaller or finer resolution is better. The diameter of the fiber (\( \rho \)) as shown in the figure) is established during manufacturing. Most MicroSlide applications utilize standard fiber diameters of either 3 microns or 6 microns, well matched to the pixel size of a CCD detector, which range from 6–30 microns, but are commonly about 9–10 microns. Photographic film has a distribution of grain sizes; however the average is found to be between 0.8 – 3 microns. For many biological applications of a MicroSlide™, the purpose may not be to image an object, but to detect light emitted from a fluorescent or luminescent reaction. Figure 5 depicts a light source (object being viewed) that is considerably smaller than the diameter of the interrogating fiber. In that case, the light emitted by the light source would fill the fiber as shown by the arrows, and the ‘image’ of the light source would be the same size as the fiber itself. Since the fiber diameter can be 3 microns or less, and typical CCD pixel size is 9–10 microns, the MicroSlide preserves and does not detract from overall system resolution. In this case, the resolution is limited by the pixel size of the CCD.

Figure 6 depicts a scenario in which the light source (object being detected) is suspended in a medium (air, liquid etc) above the surface of the FiberOptic MicroSlide™. The emitted light might originate from a fluorescent or luminescent indicator used in an analytical or diagnostic technique. This light signal could then be detected by a sensor or detector in contact with the bottom of the MicroSlide™. Properly detected, this light signal would provide a definitive indication of the status of the reaction occurring within the droplet or well of interest. It is assumed that the light source is small compared to the size of the droplet or well and that the droplet or well is interrogated by 1 or more fibers, although such may not always be the case.

1.3 Imaging with the FiberOptical MicroSlides™

Certain applications require imaging rather than merely the detection of the light source. In the context of biological measurement, we use imaging to refer to situations where one wishes to measure the spatial relationship between a light source that spans many fibers. For instance, if one wishes to measure the separation or relative velocity of several point sources through a MicroSlide™, we consider this an imaging application. As previously discussed, if the light source object is in direct contact with the surface of the MicroSlide™, the obtainable resolution is approximately equal to the fiber diameter. If the light source object is located above the surface of the slide, the object can also be imaged but the resolution will deteriorate.

The effect of source height can be analyzed by considering a point light source located above the slide a distance \( d \) as shown in Figure 8. There will be a circle of illumination of radius \( R \) on the MicroSlide™ surface over which the light arrives within the acceptance angle \( \theta \) of the constituent fibers where \( R = d \tan(\theta) \).

The only light from the point source transmitted through the MicroSlide™ was incident within this acceptance circle of diameter \( 2R \) which is the resolution of the slide for a source height of “d”. However, as discussed later, the more useful parameter may be the minimum separation “s” between two light sources that can be resolved when viewed through the slide. In practice, the diameter of the circle of illumination increases in increments of fiber diameter \( \rho \) because the

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Figure 6 - All light incident within the acceptance cone of a MicroSlide fiber is captured by that fiber and propagated to the opposite surface (except for a small fraction of reflected and absorbed light).

Figure 7 - The light from a source (object being detected) suspended in a medium (air, liquid etc) close to the surface of the MicroSlide propagates to the opposite face without appreciable spreading.

Figure 8 - The FiberOptic Microslide collects and then transmits light emanating from a point source that falls within the acceptance circle of radius R.
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output side of the fiber is either illuminated across its entire aperture or is not illuminated at all. This ‘all or nothing effect’ results from the character of the modal propagation within the fiber. As long as the diameter of the circle of illumination on the input face is less than one fiber diameter, negligible degradation of the resolution occurs. (This approximation ignores the subtleties of whether the source is centered on the fiber or is located close to an edge.) It follows that the resolution eventually degrades as the point source is moved higher above the surface because the circle of illumination increases and more fibers are illuminated. The corresponding limitation on the height of the source d above the surface to confine the illumination circle to one fiber diameter and avoid degradation of the resolution beyond the intrinsic resolution $\rho$ of the MicroSlide is:

$$ R = d \tan(\theta) \leq \rho / 2 \Rightarrow d \leq \rho / 2 \tan(\theta) $$

For every additional increase in R by a length $\rho$, a new ring of fibers is illuminated, degrading the resolution and spreading the light from the single point source across more pixels of the CCD array. In Table 1 the maximum elevation of a source that avoids degradation of the intrinsic MicroSlide resolution is shown for several slide parameters. For some common slide structures, the calculated numerical aperture can be greater than unity, implying that the fiber can support modes internally at greater angles than can be excited by light incident from the outside. For these MicroSlides™, the effective NA is unity and the acceptance angle is $\theta=90^\circ$. For these slides (NA = 1) the point source must be in contact with the surface to maintain the resolution according to Equation 2.

Table 1 – Maximum source height for best resolution with exemplary MicroSlide designs.

<table>
<thead>
<tr>
<th>Fiber Diameter ($\rho$)</th>
<th>Numerical Aperture (NA)</th>
<th>Acceptance Angle (\theta) (in air)</th>
<th>Maximum source height (d) (Eqn. 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 $\mu$m</td>
<td>0.671</td>
<td>42.1°</td>
<td>1.7 $\mu$m</td>
</tr>
<tr>
<td>3 $\mu$m</td>
<td>1.010</td>
<td>90°</td>
<td>0 $\mu$m</td>
</tr>
<tr>
<td>6 $\mu$m</td>
<td>0.671</td>
<td>42.1°</td>
<td>3.3 $\mu$m</td>
</tr>
<tr>
<td>6 $\mu$m</td>
<td>1.143</td>
<td>90°</td>
<td>0 $\mu$m</td>
</tr>
</tbody>
</table>

For some imaging applications, the ability to resolve two point sources at height “d” when viewed through the MicroSlide may be the most important measure of performance. As shown in the following development, the performance is aided by the acceptance angle of the slide and also by the inverse square-law decrease of optical power with distance from the source.

**Figure 9** depicts two isotropic point sources, each of optical power $P_o$, located a height d above the MicroSlide surface and separated laterally by a distance L. The power received by an arbitrary fiber from a single source is:

**Equation 3:**

$$ P_f = I(s) \delta A_n = P_o \delta A_n / 4 \pi s^2 $$

where $P_f$ is the optical power received by the fiber, $P_o$ is the total optical power radiated by the isotropic source, s is the distance from source to the fiber, $I(s)$ is the optical intensity at the fiber, $\phi$ is the angular direction of the fiber relative to the normal to the source, and $\delta A_n$ is the projection of the cross sectional area of the fiber normal to vector s:

**Equation 4:**

$$ \delta A_n = \pi \rho^2 \cos(\phi) / 4 $$

From Figure 9 it follows that $d = s \cos(\phi)$, in which case, Equations 3 and 4 reduce to:

**Equation 5:**

$$ P_f(\phi) = P_o \rho^2 \cos^2(\phi) / 16 d^2 = \frac{P_o \rho^2 d}{16(R^2 + d^2)^{3/2}} $$

where $R$ can be expressed in terms of the rectangular coordinates in the plane of the slide: $R = \sqrt{x^2 + y^2}$. This formula only holds for $d \geq \rho$ because for lesser values of d, the angle $\phi$ changes significantly across the fiber aperture $\rho$ and must be properly integrated to give correct absolute power. Defining the normalized received power $P_N$ as the power
received by a fiber at angle $\phi$, it follows from Equation 5 that:

**Equation 6:**  
$$P_{r}(\phi) = P_{r}(0) \cos^{3}(\phi).$$

This relationship is graphed in Figure 10a. The graph shows that even for an isotropic source above a MicroSlide with NA = 1, the received optical power per fiber will have a maximum directly beneath the source that decreases as the angle of the fiber from the source increases as shown in equation 6. For two sources separated laterally by a distance “L” along the x axis, the analysis from Equation 5 can be extended to:

**Equation 7:**  
$$P_{tot} = \frac{\rho^3 d}{16} \left( \frac{1}{((x-L/2)^2 + y^2 + d^2)^{3/2}} + \frac{1}{((x+L/2)^2 + y^2 + d^2)^{3/2}} \right)$$

The total received power (Equation 7) is plotted in Figure 10a in which small heights of the source above the slide surface are expressed in multiples of the fiber diameter $\rho$. In Figure 10b, the optical power received through a MicroSlide is shown for two isotropic sources separated by 2.5 times their height above the slide surface ($L/d = 2.5$). It is evident from the figure that the received light can be readily resolved into two sources under these conditions. If the source height is doubled giving the ratio $L/d = 1.25$, the sources can no longer be resolved as shown in Figure 10c. In this case, the light source appears to be a single elongated blur. Figure 10c also shows the nominal square law decrease of peak power as the height of the sources above the slide surface is increased.

In summary, for point sources located above the top surface of the MicroSlide, the square–law decline of optical power and the projection of the fiber cross–sectional area allow the point sources to be laterally resolved if the source separation is about 2.5 times greater than the elevation above the microslide. From the image side, the point sources appear as a double–humped light distribution. The ability to resolve point sources through the Microslide can be used for counting the number of point sources, or for measuring velocities of the sources (cell mobility / migration studies) across the faceplate. The FiberOptical Microslide provides an optically efficient means of resolving and imaging sources and structures using direct contact detectors without the need for intervening focusing optics.

### 2.0 APPLICATIONS

#### 2.1 MicroWell Arrays, FiberOptical Bottom Plates and MicroFluidic Devices

FiberOptical MicroSlides can be used as an alternative to conventional microscope slides, or as ‘zero thickness’ substrates for Microwell arrays, MicroFluidic Devices and FiberOptical Bottom Plates. The Microslide substrate has the advantage of allowing direct imaging onto a CCD camera faceplate, minimizing the need for costly or bulky optics often associated with microscopes or microarray readers. CCD cameras with integral fiber optic bundle tapers can image over...
large area making it possible to directly and simultaneously interrogate FiberOptical MicroSlides™ that are the size of standard microscope slides or larger, or microtiter plates having FiberOptical MicroSlide glass bottoms. Direct contact imaging is inherently robust, making MicroSlide technology uniquely suitable for portable applications, including many relating to homeland security. The effectiveness of the FiberOptical MicroSlide™ for microarray applications can be further enhanced by various surface treatments and coatings including a) Passivated surfaces, b) Amine DNA coupling layer, c) Epoxy coupling layer, d) Aldehyde–group coupling layer, e) Permeable, 3D hydrogel coatings and f) other coatings, to alter hydrophobic or hydrophilic characteristics of the MicroSlide. They can be provided optically flat and chemically clean.

One method for producing MicroWell arrays takes advantage of the different properties of the fiber core glass versus the clad glass. The core glass can be selectively removed based on its different chemical properties, leaving an array of ‘test tubes’ having walls formed by the fiber cladding with bottoms consisting of fiber optic core glass. ‘Test tube bottoms’ can be coupled directly to a CCD to optically interrogate light-emitting reactions occurring in the wells. Well dimensions can be customized during manufacture by choosing appropriate core diameters and wall thicknesses. Well diameters can be selected from 3µm - 250µm diameter, depending on the application.

When a light-producing (luminescent or fluorescent) reaction occurs on the surface of the MicroSlide, the light travels through the fibers of the MicroSlide to the opposite surface which can be butted directly to the outer surface of the CCD camera faceplate. Light travels from the surface of the MicroSlide through the camera faceplate and impinges directly onto the CCD sensor.

**Figure 11** shows an SEM view of a microwell array used commercially for high-speed genomic analysis. The wells are about 44µm in diameter, enabling a typical 1” X 3” MicroWell MicoSlide Array to contain 300,000 wells. More than 1,000,000 wells are available on larger size plates. Each well is interrogated by a single optical fiber. During genomic analysis, DNA fragments or other biological materials are isolated, PCR amplified, and bound to beads and loaded into the wells. Special fixtures hold the plate in the sequencing instrument and facilitate flow of reagents over the bead-loaded wells. Reagents flow over the plate in a sequenced order. Light is released when a nucleotide binds with its complement on each DNA fragment.

**Figure 12** depicts the direct contact viewing of the MicroWell MicroSlide array, which is placed in direct contact with the faceplate of the CCD camera. The faceplate of the CCD camera is made from the same fiber optic construction that is used to produce the MicroSlide. The MicroSlide can serve as a ‘sample carrier’ that is removable, interchangeable, and disposable. Each well is aligned with multiple pixels of a CCD camera, which captures, gathers and stores the light signal with data acquisition equipment. Massive data analysis is used to assemble the DNA sequence. Using these techniques, scalable, highly parallel sequencing has been demonstrated with significantly higher throughput compared to state-of-the-art capillary electrophoresis instruments.

Microwell arrays and microfluidic devices can also be formed by applying surface coatings onto a MicroSlide substrate. Proven photolithographic techniques are used to apply photocurable epoxy-based photoresists to form permanent, well adhered microwells or microfluidic structures on the surface of MicroSlide substrates. These manufacturing techniques offer several benefits including significant cost reductions, a choice of several glass substrate compositions, optimization of the optical properties of the substrate (numerical aperture (NA), optical resolution) and a wide range of microwell dimensions. Photolithographic techniques facilitate fabrication of perfectly ordered arrays of wells, with tightly controlled well-to-well spacing. Standardization of the fiber optic array substrate will enable just-in-time, lean manufacturing strategies, while reducing hazardous waste and the associated environmental impacts characteristic of chemical etching manufacturing strategies. **Figure 13** depicts a fiber optic interrogated microwell array formed by depositing a photolithographically patterned polymer on the surface of a
FiberOptical MicroSlide. **Figure 14** shows an actual low-magnification view of the polymer walls of wells formed onto the surface of a FiberOptical MicroSlide. **Figure 15** shows a higher magnification view focused onto the well bottom showing the 3μm diameter optical fiber structure of the well bottom. These figures demonstrate an important feature of the microwells formed using this technique: each well can be optically interrogated by multiple fibers, enhancing resolution within the well. In the array shown in **Figure 15**, each 120μm diameter well is interrogated by 1,323 X 3μm diameter fibers.

**Figure 16** - Microfluidic Biochip formed by applying a permanent photolithographically patterned polymer structure onto the surface of a MicroSlide. Reactions occurring in the analysis zone are optically interrogated through fused fiber optics that make up the substrate.

**Figure 17** - Incom’s FiberOptical Bottom Plate incorporates a MicroSlide glass bottom bonded to a 96 or 384 well plastic upper.

**Figure 18** - Optical isolation between adjacent fibers allows closely spaced microarray reactions to be ‘spotted’ onto the bottoms of each FiberOptical Bottom Plate well.

The manufacturing process described above can be extended to produce various photolithographically patterned microfluidic devices onto the surface of an FOI MicroSlide. Incom’s FOI Microfluidic Device, shown in **Figure 16** incorporates microfluidic inlets and outlets, as well electrical contacts with an analysis zone that is optically interrogated by thousands of minute optical fibers that enable real-time monitoring of fluorescence or luminescence based reactions; cell counting, or spectroscopic analysis. The device is custom patterned on a MicroSlide substrate designed to enhance optical resolution for bottom reading applications. It is well suited for CCD counting applications or monitoring with inverted microscopy, and can be used for various applications such as flow cytometry or biochips for biohazard detection. MicroTiter Plates are also fabricated utilizing MicroSlide glass bottoms as shown in **Figure 17**. With over 4 million optical fibers interrogating each well, Incom’s FiberOptical™ Bottom Plates offer high resolution and excellent optical collection efficiency when compared to microtiter plates with conventional glass or polystyrene bottoms. Optical isolation between adjacent fibers allows closely spaced microarray reactions to be ‘spotted’ onto the bottoms of each well as shown in **Figure 18**.
2.2 MicroCapillary Array Plates

The manufacturing techniques illustrated in Figure 2 can also be used to fabricate related structures known as microcapillary array plates. These plates are the equivalent of FiberOptical MicroSlides™, but without the central glass core. The microcapillary array is comprised of uniform-diameter hollow capillaries, numbering from 10,000 to 10,000,000 in a single standard plate, as illustrated in Figure 19. The array plates do not provide the efficient optical guiding properties of the FiberOptical MicroSlide™ because there is no optical core. However, there is sufficient direct radiation from the end of the tubes and from partial reflections from the interior walls to conduct fluorescent experiments within the capillaries. Typically, the capillaries are used as independent test tubes or reagent vessels for biochemical experiments. The flow-through capability of the capillaries provides a convenient means of introducing selected biomaterials and reagents into the bioplate. When a suitable bioactive probe or target material is introduced into a capillary, fluorescent light emission occurs. A portion of the light from the reaction propagates out the end of the capillary where it is captured and analyzed to provide information about the reaction. For some investigations, the optical activity within a capillary is used to identify which capillaries contain materials that must be removed for further analysis. The flow-through nature of the plate allows easy removal of these materials. In a recent paper, Gazenko recites some of the many advantages of microcapillary arrays for rapid inexpensive large-scale diagnostics. The flow-through design combined with strong capillary surface tension forces enables introduction of materials that would be impossible with closed-end tubes of the same diameter. By concentrating bioactive fluorescent material into precise picoliter volumes, it is possible to detect fluorescent signals from a single cell. Gazenko also discusses the use of microcapillaries to grow microcolonies of cells that stack vertically within the confining capillary walls as illustrated in Figure 20. By confining the cells in this fashion, light from the microcolony can be detected from an extremely small number of cells, that can also be colored by light absorbent or fluorescent markers, leading to reduction in incubation times from days to a few hours relative to traditional Petri plates. This application of microcapillary technology may lead to the achievement of pre-symptomatic detection of extremely small concentrations of antigens (from viruses, toxins, bacteria, etc) and other advances in rapid diagnostics.

Microcapillary array plates are fabricated with a layer of highly absorbing glass surrounding each capillary so negligible cross talk occurs between capillaries, thereby assuring that the light emanating from the end of the capillary has only originated from reactions occurring within that particular capillary. The capillaries are available in diameters that interface well with CCD arrays, enabling recording of activity in each capillary simultaneously without the need for costly intervening optics. Alternatively, the capillaries can be individually scanned for activity, but this approach increases the data acquisition time appreciably. In summary, some of the key attributes of the MicroCapillary Array Plate are:

- Excellent platform for massively parallel biochemical studies in flow-through format.
- Capillary diameters available from 5 microns to 250 microns.
- Optical cross talk between capillaries eliminated by incorporation of absorbing glass into structure.
- Plate thicknesses available from 0.5mm to 600mm.
- $10^4$ to $10^7$ capillaries in a standard $3.4'' \times 5''$ plate.
- Sufficient light received at the capillary outputs to facilitate fluorescent measurements.
- With innovative fabrications techniques, plates are available with independent choice of capillary diameter and plate thickness.
- Capillaries diameters interface well with CCD arrays, eliminating costly intervening optics or time-consuming scans.
3.0 CONCLUSIONS

Successful completion of the Human Genome Project in 2003 laid the foundation for ongoing development and commercialization of novel technology and instrument systems to enable rapid sequencing of genomes. Utilizing nanotechnology, proprietary chemistry, and novel microfluidic biochips, innovative firms are racing to develop methods and instrument systems that enable diagnostic analysis (sequencing) hundreds of times faster than conventional techniques. FiberOptical MicroSlides are one contribution to this growing market that facilitates these high-speed analytical techniques. The markets for MicroSlides include pharmaceutical, biotechnology, and agricultural companies as well as universities and research institutions. Applications include: drug discovery, life science research, in vitro diagnostics, disease management, forensic medicine, drug–abuse testing and diagnostics for homeland defense.

The FiberOptical MicroSlide represents a radical departure from the traditional design of plain microscope slides. The MicroSlide efficiently collects incident light, including light arriving at large incident angles. This light is guided to the bottom surface without further divergence, creating an image of the incident illumination pattern. If the luminous source is within several fiber diameters of the surface, the location and characteristics of the source are conveyed to the opposing surface of the array without further distortion. This image can then be coupled directly to a CCD array without intervening optics facilitating microarray reader designs that are high speed, compact, robust and potentially portable. Since the fiber size and CCD pixel size are similar, the CCD array is able to simultaneously sample the output of each fiber independently. In contrast, a lens system designed to capture light from a plane 3” X 3” glass slide, that addresses issues relating to aberrations, vignetting, and focal length would likely be expensive, bulky and sensitive to vibration. A simpler camera could be used to image a portion of the plate but would require scanning to capture the entire plate. For many massively parallel biological experiments, the decrease in data rate associated with scanning is not acceptable.

FiberOptical MicroSlides can replace conventional microscope slides in a number of applications, including use as a microscope slide for inverted microscopy, as a substrate for a microarray, and in glass-bottomed microtiter plates. FOI biochips using FiberOptical MicroSlides are being developed 1) as a stand alone replacement for conventional microscope slides for certain bottom reading applications, 2) as a substrate for photo-lithographically patterned micowell arrays and microfluidic biochips, and 3) as the bottoms for glass-bottomed microtiter plates. Many other applications are expected to evolve to take advantage of this substrate material. In these direct contact applications, FiberOptical MicroSlides offer the following advantages:

1. Images can then be coupled directly to a CCD array without intervening optics facilitating economical microarray reader designs that are high speed, compact, robust and potentially portable.
2. Provide image-plane transfer eliminating problems relating to substrate thicknesses normally associated with bottom viewing of conventional microscope slides or microarrays.
3. Offer significantly improved resolution compared to a conventional microscope slide, microarray substrate or microtiter plate.
4. Offer far greater (10,000 X) light collection efficiency than conventional microscope slides, microarray substrates or microtiter plates.
5. Significantly reduce the effects of chromatic dispersion compared to conventional microscope slides, microarray substrates or microtiter plates.
6. Eliminates problems associated with focusing inverted microscopes through the thickness of conventional microscope slides.
7. Provide sufficient spatial resolution for monitoring spot reactions. (Magnifying optics can provide higher resolution, but with added complexity.)
8. Are ground & polished to insure tight tolerances for bottom flatness, planarity, and reduced variability across the surface.
9. Incorporate embedded optically absorbing glass which eliminates optical cross talk between fibers.
10. Are available with a full range of functional coating chemistries for DNA and protein microarraying or other specialty applications including super-clean, optically flat, passivated surface – SiO2, Amine DNA coupling layer, Epoxy DNA coupling layer.

Microcapillary arrays are a related non-imaging product that also has the potential to dramatically enhance biological testing. Major advantages of the microcapillary array plates are:

1. Serves as a high-density flow-through platform for massively parallel testing with easy introduction of
2. Offers dramatic reduction in test incubation times with unique capillary geometry.
3. Prevents optical cross talk between capillaries to assure data integrity.
4. Available with independent choice of diameter and plate thickness to meet specific experiment requirements.
5. Durable, dimensionally stable, and easily cleaned because of all glass construction.

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8 Image courtesy of 454 Life Sciences, Branford, CT
11 A biochip, also referred to as a DNA micro-array, is a glass or silicon wafer that is designed for the purpose of accelerating genetic research. It may also be able to rapidly detect chemical agents used in biological warfare so that defensive measures could be taken. (http://www.markets.duke.edu/student_it/mms190_fall2001_webteams/team3/defining.html)
12 Plain microscope slides are the familiar clear rectangular homogeneous glass plates used to hold specimens for examination under a microscope and cover glasses are the smaller, thinner glass plates used on the microscope slides to cover specimens for protection during examination.