Fiber Optic Interrogated (FOI) Microwell Biochips

Michael J. Minot, Ph.D.
MinoTech Engineering Inc.†

Abstract: High density, all glass microarrays are produced by etching the distal ends of fused fiber optics. When used in conjunction with various encoding techniques, such as microspheres, the shortest assay times compared to other high-density analysis techniques have been demonstrated.

Introduction

Fiber Optic Interrogated (FOI) Microwells provide a platform for a variety of high density, reproducible and accurate biochip sensors. Reactions taking place inside etched wells at the ends of an array of optical fibers can be followed using a variety of chemical encoding techniques which are monitored by coupling the optical array to a CCD detection system.

A number of commercial firms (454 Life Sciences Corporation[1], Illumina Inc.[2], and others) and universities (Tufts University) are actively developing massively parallel, high speed genomic and proteomic analysis techniques that rely on a FOI Microwell platform. These analysis techniques are enabling a revolution that enhances scientific and technological understanding and has the potential to radically change the way medical problems are diagnosed and the way medical care is delivered.

These sensors combine the 'massively parallel' analysis achieved with millions of microwells, with speed achieved because of the very low sample volumes required, with the sensitivity of optical detection. In addition to applications in health related biotechnology, this platform will provide a basis for early detection of threats posed by bioterrorism. Innovative new products for environmental testing, such as the 'chemical nose' will be enabled by this platform.

First generation 'etched' FOI Microwells are already incorporated in the design of a number of commercial instruments being developed. These instruments are already revolutionizing our fundamental understanding of biotechnology issues.

Background

Sensors created by etching microwells at the distal end of individual fibers within a fiber optic array were initially developed by Professor David Walt and colleagues at Tufts University[3]. FOI Microwell Plates are produced by fusing multiple optical fibers to form a fiber optic array. Selective removal of core material leaves wells that serve as massively parallel, miniature 'test tubes'. 'Test tube bottoms' consist of high numerical aperture (NA) fiber optics that can be coupled to a CCD, to optically interrogate reactions occurring in the wells. Well dimensions can be custom fabricated and range in diameter from 3µ - 250µ or larger to suit various diagnostic requirements.

FOI Microwell Plates offer a variety of advantages as a platform for biosensors. The physical barrier provided by wells allows much higher sample density compared to alternative microarray techniques (ink jet, others) that depend on surface chemistry alone to separate one microdot from another. The ability to directly optically interrogate the reaction well provides a level of analytical sensitivity not achieved with other techniques. Use of EMA[4] fibers eliminates optical cross talk from well to well. When used in conjunction with various encoding techniques, such as microspheres, the shortest assay times compared to other high-density analysis techniques have been demonstrated[5].

FOI Microwell Plates are finding critical 'life sciences' applications as biosensors or 'biochips'. FOI Microwell Arrays have been used to detect small changes in DNA sequence and have been demonstrated as a useful tool for study of single-nucleotide polymorphisms (SNPs)[6]. FOI Microwell Plates can serve as a platform for polymerase chain reaction amplification (PCR). A single 25 mm X 75 mm plate was shown to function as 370,000 discrete reaction vessels achieving high yield amplification with a total reaction volume of only 15.3µL[7]. In other applications, FOI Microwells are used in conjunction with "BeadArray" technology, enabling accurate cost-effective high-throughput genotyping[8]. Similar bead based array platforms have also been shown to be effective for RNA profiling[9]. Other applications of FOI Microwells involve living cells, which can be grown and monitored 'in-situ'. Most cell types can be accommodated by matching fiber optic core size to the cell size requirements[10].

Manufacture of fiber optic arrays.

Incom Inc. (Incom) of Charlton, MA is the leading manufacturer of fiber optic arrays. Fiber optic arrays are used in a variety of products such as:

- Faceplates: A faceplate is the optical equivalent of a zero thickness window that can also be used for field-flattening, distortion correction and contrast enhancement.
- Image Conduit: Image conduit is a high-quality image transfer device that transmits an image from one end of the rigid fiber optic rod and displays it at the other end.
- Tapers: Fiber optic tapers magnify or reduce an image, in image transfer applications.
- Inverters: Inverters rotate an image through a predetermined angle, such as 180 degrees.
- Capillaries: Capillaries are a high-quality ordered array of small diameter hollow glass tubes.

Figure 1 and Figure 2 show optical fiber faceplates and tapers. These products are found in many commercial and military applications, such as cathode ray tubes, image intensifiers, and night vision systems.

Manufacturing practices for these and other fiber optic array products are well established. 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.

Figure 1 - Optical Faceplate

Figure 2 - Fiber Optic Tapers

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, while a load is applied. The block is then annealed and fabricated into finished product. For fiber optic array plates, block material is cut into rectangular plates having the desired nominal thickness. Plates are ground and polished to target dimensions using conventional glass finishing slurry and pad materials. Figure 3 is a pictorial representation of the manufacturing process. Additional details are available at the Incom web site.

Selection of clad glass is based on insuring a good chemical and mechanical match to the core glass. Core glass composition is custom formulated to meet the unique optical requirements of the fiber array. In order to achieve the required quality, including optical homogeneity, melt 'campaigns' are conducted, typically involving approximately 100,000 lbs of glass, or more. Cost for fiber optic arrays greatly favors standard compositions that are used in high volume.

Figure 3– Incom, Inc. manufacturing process for fused fiber optic products, including fiber optic arrays.

Etching fiber optic arrays to form FOI Microwell Biochips

Incom has established dedicated facilities for etching fiber optic arrays and is now a leading supplier of FOI Microwell Plates to the growing biotechnology community. Fabricating FOI Microwell Plates takes advantage of the fact that the high refractive index glasses selected for the core material typically have much higher chemical solubility compared to the clad glass. A variety of different mineral acids can be used to selectively remove core glass. Solution chemistry and process details are typically optimized to produce uniformly etched wells, with no residual layer of etch byproducts remaining in the microwell array.

Finished FOI Microwell Plates must meet rigorous product specifications for dimensional tolerances, surface finish and quality, well diameter, depth and pitch (center to center distance between wells), as well as full product trace-ability. FOI Microwell Plates are typically inspected visually and microscopically, using an interference microscope to monitor well dimensions. Typical SEM and interference microscope images are shown in figure 4 and figure 5.

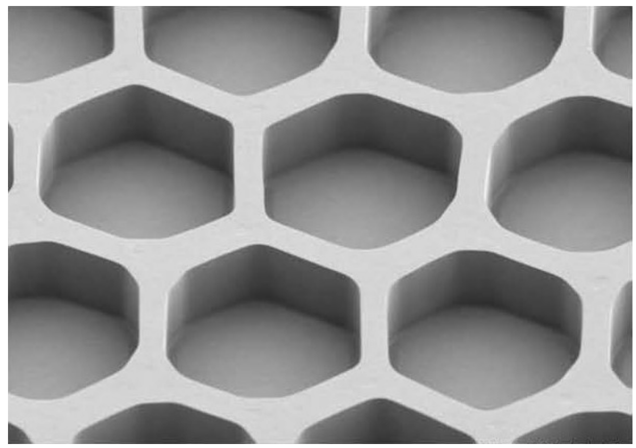

Figure 4 - SEM image showing fiber optic interrogated wells formed by etching a fiber optic array.

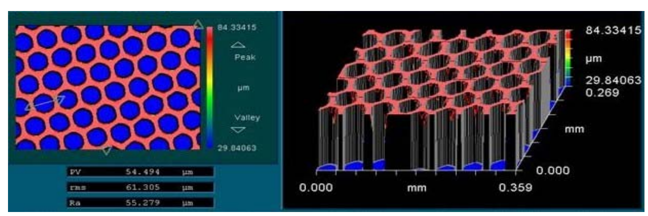

Figure 5 - Interference microscope image used to monitor the depth of fiber optic interrogated wells formed by etching a fiber optic array.

Genomic Analysis using the FOI Microwell Platform

454 Corporation of Branford, Connecticut has been a pioneer in developing high speed, massively parallel analytical techniques based on the FOI Microwell Plate. The diagram[11] shown in Figure 6 depicts the analysis scheme developed by 454 Corporation: 1) DNA fragments are isolated, PCR amplified, bound to beads and 2) deposited into minute wells on a fiber optic microarray (PTP)[12], 3) Reagents flow over the plate in a sequenced order. Light is released when a

nucleotide binds with its complement on each DNA fragment. 4) Each well is aligned with multiple pixels of a CCD camera which captures gathers and stores the light signal with data acquisition equipment. 5) Massive data analysis is used to assemble the DNA sequence.

The elegance of the 454 analysis scheme lies is the fact that the underlying methodology is inherently simple!

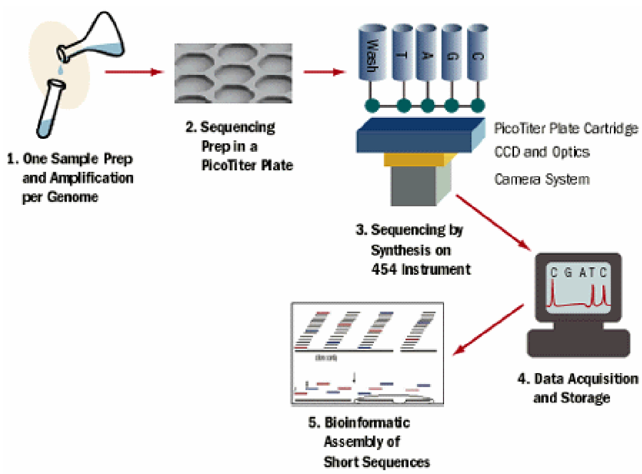

Figure 6 - 454 Corporation Analysis Scheme

Advantages of FOI Microwell Plates as a Platform for Biosensors:

FOI Microwell Plates offer a number of unique advantages as a platform for bio-analysis, compared to other techniques**:**

- **Highest Density -** The physical barrier provided by wells allows much higher sample density compared to alternative microarray techniques (ink jet, others) that depend on surface chemistry alone to separate one microdot from another.

- **Direct Optical Interrogation -** The high numerical aperture (NA) designed into the fiber optic core provides direct optical linkage to the reaction wells, which can then be interrogated by a variety of sensors, such as a CCD.

- **No Cross Talk -** Use of EMA fibers eliminates optical cross talk from well to well.

- **Shortest Assay Times -** When used in conjunction with microspheres, and by coupling the fiber optic array to a CCD detection system, the shortest assay time compared to other high-density analysis techniques have been demonstrated

Custom Configurations, Surface Treatments & Coatings

FOI Microwell Plates can be custom fabricated to meet the requirements of a number of different applications. Incom's proprietary manufacturing technique makes it possible to specify and carefully control well diameter, well depths, spacing and layout. A variety of different configurations have been developed to meet various diagnostic requirements:

Bacteria microscope slides: with 4µ diameter wells, 4µ deep.

Mammalian Cell microscope slides: with 21µ diameter wells, 10µ deep.

Genomic & Proteomic Analysis: with 42µ diameter wells, 50µ deep.

100µ (or larger) Diameter Wells: produced to your specifications suitable for cell growth or crystal growth applications.

All standard sizes: 1"X3" (microscope slide), 2"X3" (double slide), 3 ⅜" x 5" (micro-titer plate), or custom to meet you requirements.

In addition, these all glass biochips can be surface treated to enhance coupling.

Custom Surface Treatments & Coatings

- Super clean, optically flat
- Passivating surface – SiO_2
- Amine DNA coupling layer
- Epoxy DNA Coupling layer
- Epoxy Protein coupling layer

About Incom, Inc.

Incom, Inc., was founded in 1971, as a direct descendent of American Optical Laboratories. Early innovations include: the first to use statistical EMA; the development of high voltage low scintillation faceplates for CRT's; the longest fused fiber optic faceplate (19" x 2"); colored gemstone material; 12"x 12" faceplate; G-12 compatible NA 1.0; and the only company to achieve the quality standards and sizes required by the phototypesetting industry.

In 1984, Incom introduced the patented Iris Gen III Input Window and then began work on the patented MEGAdraw process that eventually led to achieving 60mm diameter MEGAdraw. Later developments included many more firsts: the first 135mm diameter taper, the first twister/taper and the first 165mm diameter taper. Incom continually strives to increase the diameter and performance of their product line. In 1994, Incom acquired the fused fiber optic product line developed by Galileo Electro-Optics, opening new markets and providing access to existing markets with imaging requirements beyond the current state-of-the-art.

Recent development activities have resulted in the development of etched micro-well faceplates for use in the biotechnology field and the introduction of a 200 mm fiber optic imaging taper, the world's largest.

For further information, contact Incom Inc. at: 508-765-9151, sales@incomusa.com, or on the Internet at www.incomusa.com .

---

†MinoTech Engineering provides technical consulting services to Incom Inc. Dr. Michael J. Minot is President and a consultant with MinoTech Engineering. He can be reached at 978-474-8034, on the Internet at www.minotechengineering.com or at customerservice@minotechengineering.com

References

1 454 Life Sciences Corporation, 20 Commercial Street Branford, CT 06405.

2 Illumina, Inc. 9885 Towne Centre Drive, San Diego, CA 92121-1975

3 Pantano, P., Walt, D.R., Chem. Mat. 8, 2832-2834, 1996.

4 EMA - Extramural Absorbing: darkly colored glass specifically included in the construction to absorb and eliminate stray light.

5 Fergusson, Jane A., Steemers, Frank J., and Walt, David R., "High-Density Fiber-Optic DNA Random Microsphere Array" , Analytical Chemistry, Vol. 72, No. 22, November 15, 2000.

6 Fan Jianbing, Che Diping, Zhao Chanfeng, Zhou Lixin & Feng Wenyi, "High-density fiber optic array technology and its applications in functional genomic studies", Chinese Science Bulletin Vol. 48 No. 18 1903-1905, 2003.

7 John H. Leamon, William L. Lee, Karrie R. Tartaro, Janna R. Lanza, Gary J. Sarkis, Alex D. deWinter, Jan Berka, Kenton L. Lohman "A massively parallel PicoTiterPlate™ based platform for discrete picoliter-scale polymerase chain reactions", Electrophoresis, 24 No. 21, 3769-3777, 2003.

8 Oliphant, Arnold, Barker, David L., Stuelpnagel, John R., and Chee, Mark S., BeadArray Technology: Enabling an Accurate, Cost-Effective Approach to High Throughput Genotyping.

9 Dickinson, Todd., Craumer, William, "RNA Profiing with bead-Based Array Platforms", Genetic Engineering News, Volume 23, Number 20, November 15, 2003.

10 Walt, David R., Taylor, Laura C., "Biosensor Array Comprising Cell Populations Confined to Microcavities", US Patent No. 6,377,721 B1, April 23, 2002.

11 Diagram courtesy: Bio IT World, 10/10/03.

12 PTP - Pico Titer Plate