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ABSTRACT

Over the last several years, a set of small biochip readers were designed at Argonne National Laboratory. All reader models accept the 1 x 3 inch standard microscope glass format. Each model was successfully evaluated by different institutions within the United States. Due to a patented enhanced illumination method and custom lens design, the parameters such as sensitivity, reproducibility, etc., are comparable with that of other biochip readers currently on the market, however, the ANL biochip readers are much smaller and faster in comparison. The ANL portable biochip readers can be easily customized for specific applications so that even the inexperienced user will be able to operate the reader correctly. Our most recent model, which is equipped with multicolor illumination and biochip thermo-control capability, will be discussed and experimental data will be presented.

The readers allow reading of 3D as well as 2D-planar biochips combined with a transparent reaction chamber or flow cell containing solution. Their compatibility with different biochip platforms were tested and will be discussed, along with the specific reader applications, during the poster session.

INTRODUCTION

Among the variety of biochips presently on the market, most are arrayed on a solid and transparent glass or plastic slide. The biochips can be briefly characterized as an array of specific molecular probes attached to a solid support. The probes are available for hybridization with a sample that contains target molecules (opposite scheme is the same in terms of the biochip reading). Fluorescence indicates results of specific interaction of the targets with the probes.

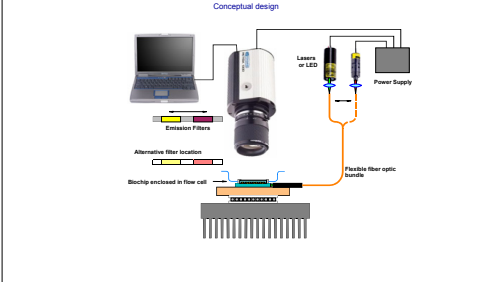
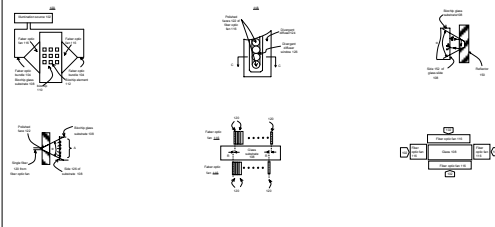
ANL 3D biochip platforms are oriented to use standard microscope glass slide as biochip support, fluorescence as a measure of hybridization, free space between any elements to read 3D and 2D biochips under "dry" or "wet" condition and can be integrated with a chamber. This, in many aspects, determined the concept of the Portable Biochip Readers developed at ANL.

The reader is typically an optical device that illuminates the biochip with light of a specific wavelength and detects the emitted fluorescent light from the biochip. As opposed to scanners which are well known at the market (initially created to read high density biochips in only a dry condition) ANL readers have large field of view that covers the entire biochip array. In order to create a biochip reader platform not only for the laboratory, but also for field application, all models were designed with respect to parameters such as small size, light weight, low energy consumption, durability, vibration-proof and shockproof.

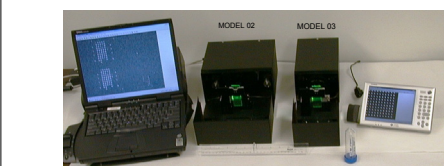
To design portable readers with a large field of view that is comparable with scanners in terms of sensitivity, reproducibility and reliability, a solution for proper illumination was required. A major problem in the quantitative analysis of biochips is the illumination of the array over the entire area of the array to be analyzed. Any non-uniformity in the illumination translates into differences in the intensity of the fluorescence signal and thus leads to erroneous results. A major problem for currently available commercial biochip analyzers that impairs the attempt at miniaturization is the use of raster scanning imaging techniques that require microscope based lens systems and "through the lens" illumination. These characteristics necessitate the use of precisely moving parts and high power lasers (known to cause damage of samples) to obtain sufficient detection sensitivity.

DESCRIPTION

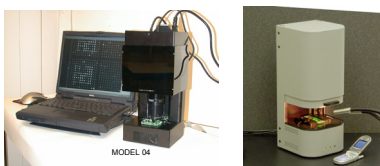
A method of biochip illumination, Patent US 6,620,623 B1 awarded to ANL, fit the task. Illumination is provided by a non-collimated laser source or a light emitting diode (LED). The light is directed to opposing sides of a glass substrate carrying a biochip by a pair of optical fiber bundles. Each of the optical fiber bundles are spread out to make a fan or more fibers thick and defining a line of optical fiber faces. This process randomizes any non-uniformity in illumination, creating a more uniform illumination source. A respective divergent diffuser engages each row of optical fiber faces coupling and diffusing the light evenly through the opposing sides of the glass substrate to illuminate the biochip supported by the glass substrate. The glass substrate functions as a secondary light guide. The divergent diffusers separate the optical fiber faces from the edges of the glass substrate, protecting the optical fibers from mechanical damage. A slide holder containing the fiber fan and diffuser supports the glass substrate carrying the biochip. The slide holder includes a set of positioning springs to engage the glass substrate and position said biochip in a focal plane. Light also can be directed to opposing ends of the glass substrate by a second pair of optical fiber bundles. Also a single optical fiber bundle can be used to direct light in one side of the glass substrate or three optical fiber bundles can be used to direct light into the glass substrate. This method of illumination provides a superior signal to noise ratio as compared with conventional illumination systems.



Model 04 Design Basis:
The laboratory Model 04 is a multicolor, thermally-controlled reader designed to image biochips that are fabricated on a standard (25 mm x 75 mm) glass microscope slide. Model 04 is 6" W x 5.80" H 12.6" H has 20x20mm field of view, focusing and optical zoom capabilities, a 0.78" x 3" 1"x3" glass slide observation area and it weighs 10 pounds. The Model 4 consists of a CCD camera, power supply for the camera, two laser sources, a thermally controlled table, and a temperature control interface. A desktop or laptop computer can be used to interface to the camera. The thermally controlled table, camera, temperature controller and laser sources are contained within a black anodized aluminum case to avoid spreading the sample to smelt light during acquisition. Two lasers illuminate the edges of the slide via light guides that are attached to the table. The light guides are attached to each laser through a cylindrical cap, and the circular fiber bundles are routed to disperse at each side of the biochip where they are transformed from a circular to a linear cross section. This process randomizes any non-uniformity in the illumination and provides uniform illumination over the entire area of the biochip. The laser light illuminates the chip from inside the glass. To acquire an image, a biochip is placed in slide holder that is mounted on a temperature controlled unit. The slide is illuminated from a set of selected biochips area in a set of view camera case cover and the laser light illuminates the slide. The CCD camera detects the fluorescent pattern and the video signal is transmitted to the computer where the pattern can be visually displayed, analyzed, and logged by custom-written software. The laboratory model is able to maintain a user-defined temperature on the table for conducting dynamic, thermal experiments and analyses. The ability to control temperature and read 3D biochips is particularly well suited for protein biochip analysis which require a need for a solution phase environment for proper protein interactions and stability.



Model 02: is 5.2"x11.5"Dx 5"H, Mass 3.5 lbs.
Model 03: 12.2Wx10"Dx 5"H, Mass 4.5 lbs.
Both models have: green illumination, focusing, its control and zoom capability from 0.01 sec to several minutes of exposure time, 11x9 mm field of view, 2.25"x 0.5" observation of the "1"x3" glass slide.



Aurora Photonics commercial product developed on the basis of ANL reader Model 04

MEASUREMENTS

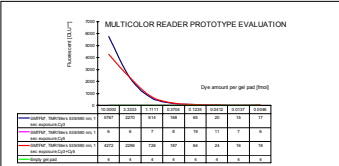
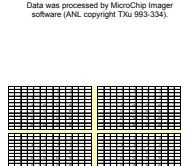


Fig. 1 Detecting by SMTFM of the arrays #2212 and #2213. Fluorescent signal was averaged from 16 array elements. Temperature 20°C, exposure time 1 second. Arrays were loaded with fluorescent dyes Cy3 and Cy5. Arrays illuminated by filtered red light 535 nm. Light source is Mercury arc lamp, 100w.



SMTFM - Stationary Multicolor Thermo-controlled Fluorescent Microscope

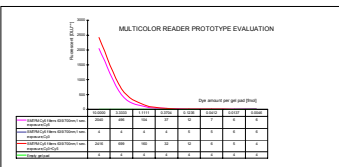
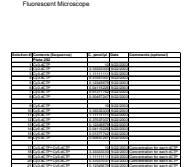


Fig. 2 Detecting by SMTFM of the arrays #212 and #213. Fluorescent signal was averaged from 16 array elements. Temperature 20°C, exposure time 1 second. Arrays loaded with fluorescent dyes Cy3 and Cy5. Arrays illuminated by filtered red light 635 nm. Light source is Mercury arc lamp, 100w.



SMTFM - Stationary Multicolor Thermo-controlled Fluorescent Microscope

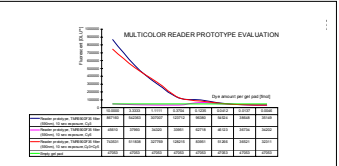


Fig. 3 Detection by Reader of the arrays #2212 and #2213. Temperature 20°C. Fluorescent signal was averaged from 12 array elements. Arrays illuminated by green light. Light source is green laser diode 532-4-50m, 50mw. Arrays were loaded with fluorescent dyes Cy3 and Cy5.

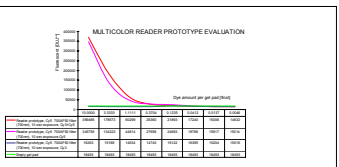
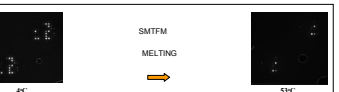


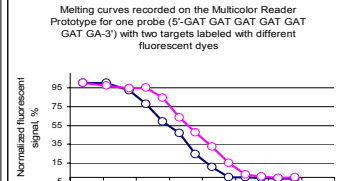
Fig. 4 Detection by Reader of the arrays #2212 and #2213. Temperature 20°C. Fluorescent signal was averaged from 12 array elements. Arrays illuminated by red light. Light source is red laser diode 635-640 nm, 5mw. Arrays loaded with fluorescent dyes Cy3 and Cy5.



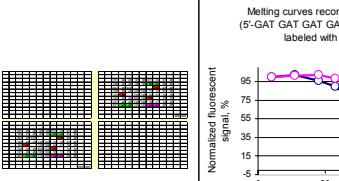
SMTFM - Stationary Multicolor Thermo-controlled Fluorescent Microscope



SMTFM - Stationary Multicolor Thermo-controlled Fluorescent Microscope



Melting curves recorded on the Multicolor Reader Prototype for one probe (5'-GAT GAT GAT GAT GA-3') with two targets labeled with different fluorescent dyes



Melting curves recorded on the SMTFM for one probe (5'-GAT GAT GAT GAT GA-3') with two targets labeled with different fluorescent dyes

ACKNOWLEDGMENTS
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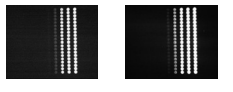
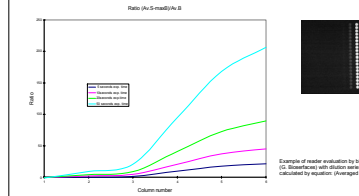
Consider the relative standard deviation (RSD), defined as the standard deviation divided by the average value, is a measure of the uniformity of illumination. The RSD is the simplest criteria that reflect the quality of illumination. The uniformity of illumination is better and the error is less when the RSD is less.

	Average value	Standard deviation	RSD
Direct illumination	12.9	3.0	0.23
Indirect illumination	7.3	0.47	0.06

On basis of data analysis the indirect scheme of illumination provides 3.6 times better uniformity and correspondingly 3.6 times less error than the first conventional illumination scheme.

Biochips containing a set of probes were produced by using slides 3D-Link, Superadsorbent, and Hydrogel. Probes Immobilization was carried out according to procedures recommended by the manufacturers. After hybridizations with a mix of Texas Red labeled target oligonucleotides (5 fmoles) biochips were dried. Hybridization signals from the biochips were recorded on the biochip reader 400 and on a commercially available scanner, Model Bio-Chip Imager, Part No.: 602-3013001 manufactured by Packard Instrument Company, Inc., now it is Packard Bioscience.

Slide name	CORREL for all elements	CORREL for groups
3D-Link	0.87	0.89
Superadsorbent	0.81	0.82
Hydrogel	0.81	0.82



Examples of reader evaluation to biochips from different companies. Fluorescence and green channel (Cy5-SMTFM) with diffusion barrier (bottom); measured under different exposure time. Data sets (ColorWavelength, AverageFluorescenceSignal, AverageBackground)

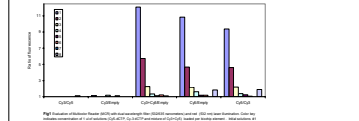


Fig. 3 Biochip probes layout

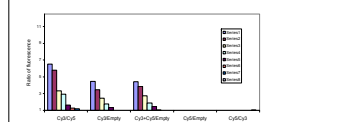


Fig. 4 Biochip probes layout

SUMMARY

Accepts the standard (25 mm x 75 mm) microscope slide format in use by the majority of biochip manufacturers. The laboratory prototype has been successfully evaluated by different institutions within the United States. Due to the patented enhanced illumination method and custom lens design, no moving parts are required and parameters such as sensitivity and reproducibility are comparable with that of other biochip readers/scanners currently on the market. The optical systems captures the entire array image simultaneously field of view is 20x20 mm (using a CCD camera, allowing times of measurement from 3-10 seconds for most commercially available arrays, substantially faster than the 5 to 10 minutes required for scanner optics in most applications).

The ability to image the entire array at once allows the user to accumulate fluorescent signal over any desired time period, which provides measurements in a wide dynamic range of fluorescent intensity.

Open source operator friendly image analysis software. This software can be configured to accommodate a range of algorithms for data analysis from very detailed research purposes to very simple interfaces such as the push button get answer that ANL made for DARPA's specific applications.

Multicolor illumination and a biochip thermal table capability allowing unprecedented control of temperature dependent probe/target interactions during array processing and imaging.

Reading of 3D as well as 2D-planar biochips combined with a transparent reaction chamber or flow cell containing solution.

The ability to control temperature and read 3D biochips is particularly well suited for protein biochip analysis due to the need for a solution phase environment for proper protein interactions and stability.

The compatibility of the laboratory model with different biochip platforms has been tested. Aurora Photonics, Inc. and Aurora Biosystem have licensed the rights from Argonne National Laboratory to patented biochip illumination technology, and custom optics allowing for the miniaturization of biochip analyzers.