

Microarray Measurements in Routine Applications

Developers of multiplexed assays and tests based on microarrays can now address a wider application base thanks to lower cost, robust instrumentation. A key and enabling factor is the optical detection, a crucial but often neglected part of microarray technology.

TEXT

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Microarray technology is increasingly used in routine applications in many fields – ranging from diagnostics to food analysis to homeland security. These assays rely on optical detection, which mostly involves fluorescence measurement. Detection of microarrays demand a new generation of “microarray readers”, and is currently performed using large and expensive laser scanner-based readers. Their high cost, complexity of handling and large footprint which consumes valuable lab space, however, limit their use to

labs with full analytical infra-structure. This conflicts with the requirements of small and miniaturized biochip applications intended for de-centralized use in labs away from hospitals, in doctors’ offices or at the point-of-care – at the patient’s bedside. To overcome this barrier, an innovative, small, rugged and cost-efficient detection platform was developed for use in mobile and easy-to-use analytical instruments. This platform supports commercialization of routine microarray applications and, in the end, offer decisive benefits to users and their customers.

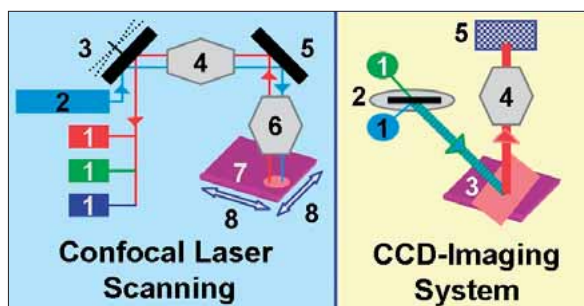
The role of optical detection in microarray experiments

Most microarrays require an optical detection system, because the chemistry is based on fluorescent dyes. Thus any fluorescent microarray detection system has to excite these dyes of the sample and collect the emitted light in a quantitative fashion. Furthermore, because the size of a microarray spot typically is in the μm range, fluorescence images of the microarrays must be acquired with an appropriate magnification and high resolution. Along with efficient chemistry, the robustness of the analysis is dependent on high quality optical instruments for generating, archiving and reproducing reliable images. Technically speaking, fluorescence images are generated from a microarray with two techniques: Confocal Laser Scanning and CCD Imaging.

Confocal Laser Scanning uses a Photomultiplier Tube (PMT) as a sensor, and can only detect one signal at a time. Therefore the excitation of the sample must be done sequentially by a scanner. Here a single-wavelength laser beam is scanned back and forth (raster scanned) across the sample, exciting an area representing a single pixel at a time. The emitted light travels back through the objective lens and is collected by the PMT, which finally gives one single digital value which is proportional to the intensity of the emitted light. All PMT values combined together result in a 2-dimensional image of the sample.

The CCD Imaging System can produce a 2-dimensional image of the sample from one single exposure. For excitation a filtered LED-light source is used for homogeneously illuminating the entire sample area. The emitted light from the entire field of view (i.e. microarray) then is captured through corresponding emission filters by a stationary CCD array. Thereafter, the signal intensity of each pixel on the CCD array is converted to a digital image.

One of the main differences between a Confocal Laser Scanning instrument and a CCD-based analytical system is the overall technical complexity of the former device. A PMT-based scanner requires expensive components such as laser sources and delicate scanning mechanics. A CCD-instrument operates with less moving parts, smaller components, and no light sources that may be hazardous to health.



Measurement principle of a confocal PMT-based scanning- and a CCD based System: *Laser Scanner for Research*: 1- PMT; 2-Laser; 3-Scanner; 4-Scan Lens; 5-Mirror; 6-Objective Lens; 7-Sample; 8-X-Y Sample Movement. *CCD-System for Routine*: 1-LED; 2-LED Optics; 3-Sample; 4-Microscope Lens; 5 CCD based Measurement.

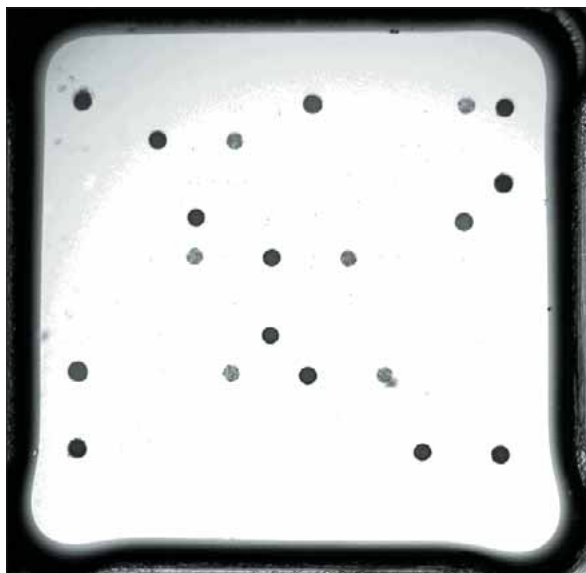


Image obtained from a DNA Microarray for HPV Genotyping taken with Sensovation's LumiSens® Reader.

Although Confocal Laser Scanners may offer high flexibility for well equipped research labs, the CCD Imaging systems are more suited for robust, smaller, portable and lower cost applications where ease of use is important.

Routine microarray detection instruments

Current prices for Microarray readers range between 20,000 Euro up to several 100,000 Euro. There is no low-cost microarray detection instrumentation on the market for Point-of-Care diagnostic assays. This situation represents a barrier to the market penetration of many new microarray based assays, targeting affordable and routine diagnostic applications.

To address the increasing demand for routine microarray readers Sensovation has introduced LumiSens®, a fluorescence detection module. LumiSens® has full fluorescence microscope functionality, with a considerable smaller footprint and lower cost. LumiSens® is a module which is optimized for routine microarray detection and enables the use of Microarrays on a much broader base than before.

The design of the module became possible, because of the advances in CCD technology and in LED technology. Both technologies were driven by totally different mar-

kets. The advances in CCD technology were driven by digital photography. Over the past few years the pixel resolution and the overall quality of CCD sensors increased dramatically while in parallel the prices continuously dropped. The advance in LED technology was driven by the automotive and lighting industries which demand brighter and more powerful LED's with more colors.

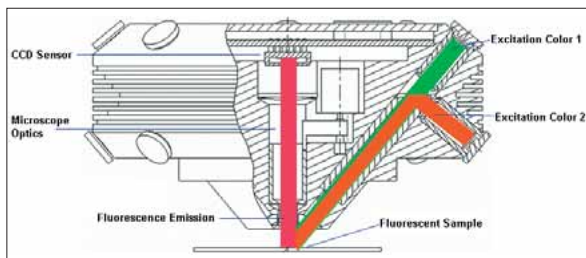
These two technological advances, together with an increasing demand for miniaturized fluorescence detection systems, contributed to the development of the innovative LumiSens® detection module, which incorporates 10 high power LED's and a high resolution, 8.3 MPixel CCD.

The LEDs are used as the sources of fluorescence excitation. A modular design allows measurement of 2 fluorescent dyes. The respective emitted light is captured with a microscope, including optimized emission filters. The 3.4x magnification allows visualisation of most common spot- and microarray sizes. Finally the image is captured by an 8.3 MPixel CCD sensor, equipped with microlenses for increased quantum efficiency.

LumiSens® is used for imaging all sorts of microarrays, biochips and even microarrays in 96-well-plates (Multiplexing). LumiSens® permits operation in mobile, easy to use analytical instruments - in routine diagnostic testing, Point-of-Care and biotechnology. Due to its small size, its ruggedness (no moving parts), its compactness and cost effectiveness LumiSens® enables assay developers to enter new applications and markets in routine diagnostic testing, Point-of-Care testing and biochemical analysis. □



LumiSens® miniaturized fluorescence microscope for Biochip detection



LumiSens® light path schematics.

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