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Choosing Components for a Microarray Scanner

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The Human Genome Project is producing a huge volume of data about the structure, organization and function of the estimated 50,000-100,000 genes within our DNA. Understanding their function, in particular, could improve ability to diagnose, treat and/or prevent disease.

Given the volume of "interesting" genes, the best way to study their function is with massively parallel analysis, e.g., gene expression microarrays.

Gene expression occurs when genetic information contained within DNA is transcripted into messenger RNA (mRNA) molecules that are then translated into the proteins that perform critical cell functions. Changes in the types and amounts of mRNA in a cell can indicate how the cell responds to environmental stimuli or other changes.

To study mRNA, researchers exploit the fact that it will bind specifically (hybridize) to the DNA template of its origin. By combining fluorescent markers with the mRNA, scientists can use photonics to quantify the amount of mRNA that binds to a specific DNA sample. By placing many DNA samples on one microarray, scientists can study, in parallel, the expression levels of hundreds or thousands of genes within a cell.

The usefulness and repeatability of this analysis depends on a large part on the photonic components and technologies – lasers, detectors and optics – used in reading the microarrays.

How microarrays work

A microarray is a small surface – for example, a microscope slide – onto which a researcher can place or synthesize many hundreds or thousands of tiny samples of DNA, cDNA, or oligonucleotides (fragments of single-stranded DNA).

After immobilizing the DNA target on the array surface, the researcher labels it with a fluorescent probe and allows it to hybridize for two to 12 hours. A gene scanner then detects the amount of labeled probe that hybridizes to the DNA; the intensity of the fluorescent light varies with the strength of the hybridization.

For some applications, such as genotyping, binary detection (fluorescence or no fluorescence) may be adequate to produce a result.

However, gene expression and single-nucleotide polymorphism (SNP) studies quantify differences in intensity. For this reason, sample preparation, microarray surface uniformity and gene scanner repeatability are critical.

Microarrays usually use probes labeled with two fluorophores, commonly cyanine 3 (Cy3, with peak absorption at 550 nm and emission at 570 nm) and cyanine 5 (Cy5, with peak absorption at 649 nm and emission at 670 nm).

To analyze the slide, a microarray reader uses a light source – a laser or lamp – to excite the fluorophore(s). A photomultiplier tube or CCD camera then detects the resulting fluorescence, and the system produces an image that shows the intensity ratio between the two fluorophores, generally reported as Cy5:Cy3. Conventionally, 'yellow' on a ratio image indicates a 1.0 (1:1) expression ratio (no difference in expression between samples). Red is higher expression levels of the Cy5 sample; green is higher levels in Cy3 sample. Black (dark) spots indicate that neither sample expressed.

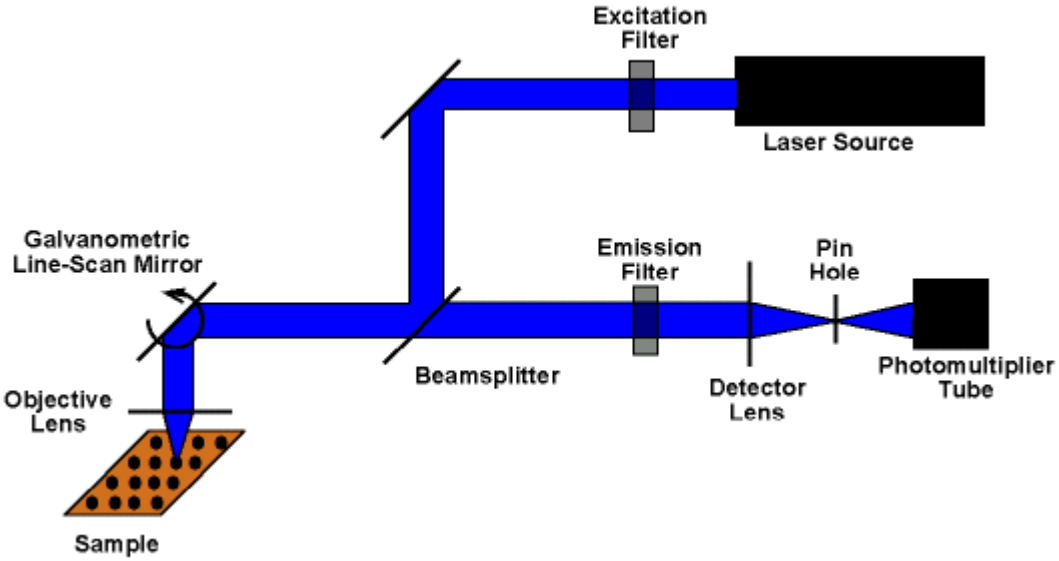


Diagram 1, Scanning Systems.

Reading the slide

There are two basic types of microarray readers:

• **Scanning systems** use narrowband illumination (i.e., lasers) to excite the fluorophores, then capture the resulting fluorescence with photomultiplier tube (PMT) detectors. See Diagram 1, above.

The PMT converts incident photons into electrons via the photoelectric effect: a photon strikes the active surface of the PMT (the photocathode), generating an electron. The electron flows through a series of dynodes that multiply the electrons until they reach the anode. The resulting current from the anode is directly proportional to the incident light at the photocathode.

• **Staring systems** generally use wideband illumination, such as a xenon lamp, to excite the fluorophores, then capture the resulting fluorescence with an array detector, such as a charge-coupled device (CCD). See Diagram 2, right.

System design will determine which of these types of systems can analyze a given microchip more quickly. Typically, scanning systems deliver more excitation photons to the sample, resulting in generation and collection of more emission photons per pixel in a given amount of time. Although the staring system can captures a large portion of a microarray, the array detectors used are much less sensitive than PMTs, and the wideband excitation source is less efficient, so the camera must integrate for a longer period to capture the same amount of fluorescence signal as a scanning system.

A scanning system can excite/detect one color at a time (sequential scanning), or acquire both at once (simultaneous scanning). The latter is faster, but improperly designed systems can suffer from crosstalk between channels: emissions from (generally) the shorter wavelength fluorophore creeping into that of the longer wavelength and resulting in higher-than-

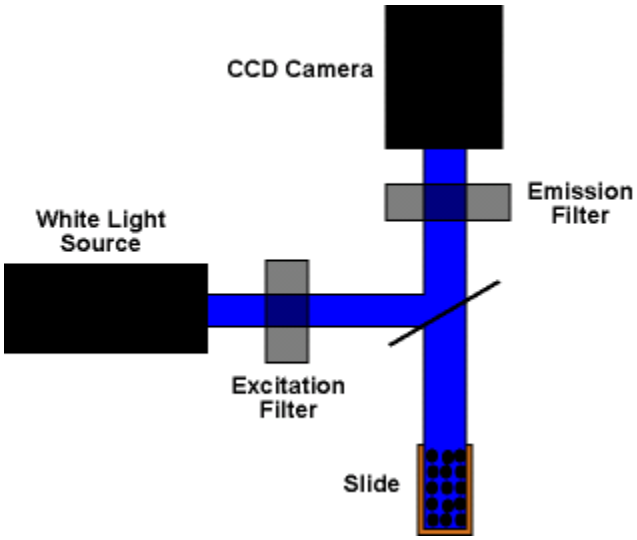


Diagram 2, Staring Systems.

real fluorescence reading in the longer-wavelength channel and a corresponding increase in the ratio.

To avoid crosstalk in a simultaneous scanning system, some system designers use lasers tuned to suboptimal excitation wavelengths that increase spectral separation between Cy3 and Cy5. Other designers have developed optical designs and filters to ensure that only photons at desired wavelengths reach the detection system.

Choosing components

System designers and users have determined some standard requirements for microarray reader technology, [1] but maximizing the signal-to-noise ratio (S/N) is the ultimate goal because this metric determines the confidence in the accuracy of a given signal measurement/the likelihood that a given fluorescence signal will be visible above the noise of the system.

System noise comprises several components:

• **Background noise**, which can come from intrinsic fluorescence from the glass microarray substrate, out-of-focus background signal (stray light and scattered light) and nonspecific hybridization.

• **Dark current**, which measures the number of electrons per second that a photon detector introduces from internal thermal emissions or leakage current from the dynodes of a PMT. To minimize dark current noise, choose a detector, such as the R6358, R4632, R6060 series, with very low dark current levels. Also, illuminate each pixel and integrate for as short a time as possible. For a CCD-based system, low-temperature operation is critical for longer integration times.

Electronic noise from poorly designed components can also increase dark current. Detector modules, such as the HC120 Series,, which include carefully engineered low-noise electronic circuits, can minimize this type of noise and reduce system design time.

• **Shot noise**, a random quantum effect. Because of its relationship with the signal photons, this type of noise increases with signal intensity, but only as the square root of the signal, so the signal-to-noise ratio increases with signal intensity. Optimal systems are shot noise-limited because quantum physics says shot noise will always be there. Shot noise will produce some difference in intensity between two "identical" spots, but printing and hybridization inhomogeneity will result in much more significant variations.

Detector choices

To maximize S/N, the first step is to choose a detector with high sensitivity in the emission wavelength region of the chosen fluorophores. [R7400,R3896,H7422] Also, look for reliability in performance: linearity of output over a wide variety of incident light intensities.

For a scanning system that uses a PMT, the PMT's amplification depends on the number of dynodes in the PMT and the voltage applied. Gains of 10^7 are possible.

For optimal performance, the user will want to set the PMT gain so that the brightest signals use most of the system's dynamic range. Generally, increasing PMT voltage beyond this optimal setting does not improve S/N because at high gain levels noise increases more than signal. Dropping the PMT voltage below an optimal range to reduce gain (for example, if the fluorescence intensity of the sample is saturating the detector) also does not improve S/N because the PMT's photon-to-electron conversion process is not as efficient at low gain levels. Instead, reduce the laser power.

For a CCD, spatial resolution will be a significant consideration. To capture a microscope slide (25 × 76 mm) at 10-µm resolution, a CCD would need 2500 × 7000 pixels, or two frames at 1600 × 1200, stitched together. Acquiring and electronically stitching together multiple images could impact total analysis time.

Optical considerations

A standard microscope objective has a high numerical aperture (light collection efficiency), but its field of view is limited and not typically very uniform. This type of lens collects more light in the center than at the

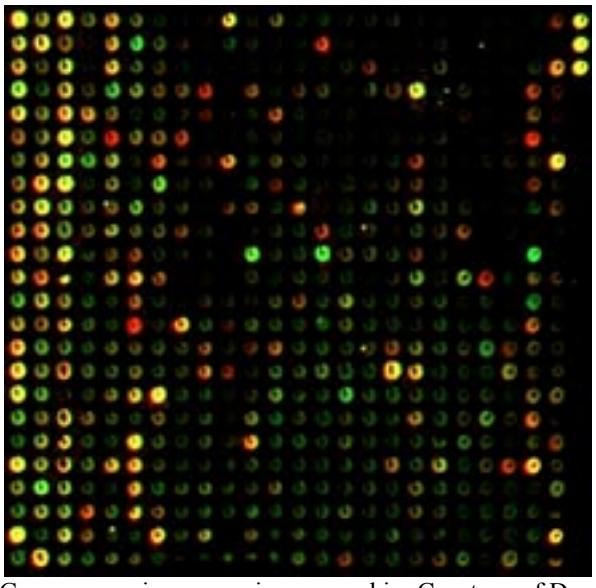
edges. Thus, most microarray analysis systems use only the center of the optical system, moving either the sample or the lens to keep it centered on an element.

Scanner-based systems typically use a form of confocal optical system with two optical paths, one from the laser to the sample (the excitation path) and one from the sample to the detector (the emission path). The excitation path has a relatively low numerical aperture (typically around 0.1); the emission path needs to maximize energy collection and so should have as high a numerical aperture as possible. The resulting tradeoff is that it will have a correspondingly smaller depth of field.

Reducing the depth of field too far will result in the system being unable to accommodate imperfections in slide surface uniformity: Even high-end slides are not uniform in flatness and thickness to the micron level.

Optical filters are also critical for producing good results. Common fluorophores have a very small Stokes shift (the difference between excitation and emission peaks), so separating excitation light from the emission light requires precise optical filtering. Lasers emit at only well-defined excitation wavelengths, somewhat simplifying the design of filters for scanning systems. Simultaneous scanning systems may use lasers at less-than-optimal excitation wavelengths to increase the separation between the two fluorophores. Photodiode-based feedback controls are critical for stabilizing the laser in this type of application, however; the laser's wavelength and power will vary with temperature.

To avoid photobleaching of the sample, it is also best to match the laser spot to the system resolution, e.g., 5- to 10-µm diameter for a 10-µm-resolution system.



Gene expression on a microarray chip. Courtesy of Dr. Leming Shi, Chipscreen Biosciences Ltd.

References

1. M.L. Mace Jr. et al. (March 2000) "Novel Microarray Printing and Detection Technologies," Table 4, p. 56, in Microarray Biochip Technology, Mark Schena, Ed., Eaton Publishing Co.

Related Links

Overviews of Microarray Analysis Technologies and Techniques

- DNA Microarray (Genome Chip)
- Anatomy of a Comparative Gene Expression Study
- DNA Microarray Animation
- Rockefeller University Gene Array FAQ

Microarray Printing

- Build Your Own Microarrayer
- Printing Arrays
- Troubleshooting Guide to Microarray Printing

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