Microarrays permit the analysis of gene expression, DNA sequence variation, protein levels, tissues, cells and other biological and chemical molecules in a massively parallel format. Robust microarray manufacture, hybridization, detection and data analysis technologies permit novice users to adapt this exciting technology readily, and more experienced users to push the boundaries of discovery.

Trends in microarray analysis

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Microarray analysis[1–5] is based on standard molecular biology, with a principal advantage being higher throughput and greater precision than traditional filter and blotting techniques[6,7]. Microarrays use high-density microscopic array elements, planar glass substrates, low reaction volumes, multicolor fluorescent labeling, high binding specificity, high-speed instrumentation for manufacture and detection, and sophisticated software for data analysis and modeling. The array elements react specifically with labeled mixtures, producing signals that reveal the identity and concentration of each labeled species in solution. These attributes provide miniature biological assays that allow the exploration of any organism on a genomic scale. Microarray analysis, similar to recombinant DNA[8] and the polymerase chain reaction (PCR)[9], is a foundational technology with broad applications in areas including genetic screening, proteomics, safety assessment and diagnostics.

Expression profiling, the original microarray assay

Expression (transcript) profiling allows researchers to generate quantitative gene expression information for many genes in many samples, using one- and two-color fluorescent schemes. In one-color analysis, used primarily in the context of chips made by photolithography[4,10,11], expression profiles for each sample are generated on a different chip using a single fluorescent label (for example, phycoerythrin) and then the different images are compared (Fig. 1*a*). In two-color formats[1–3,5,12], two RNA samples are labeled separately with different fluorescent tags (for example, cyanine 3 and cyanine 5 (Cy3, Cy5)), hybridized to a single microarray and scanned to generate fluorescent images from the two channels. A two-color graphical overlay can then be used to visualize genes that are activated or repressed (Fig. 1*b*). Both one- and two-color strategies allow comparisons of tissue types such as heart versus brain, normal versus diseased tissue samples, or time-course samplings of cell cultures subjected to different treatments or conditions, and both schemes yield high-quality gene expression data[13].

Technological underpinnings

Nucleic acid microarrays primarily use short oligonucleotides (15–25 nt), long oligonucleotides (50–120 nt) and PCR-amplified cDNAs (100–3,000 base pairs) as array elements. Genotyping applications, such as those designed to detect single-nucleotide polymorphisms (SNPs), require single-mismatch discrimination and use short oligonucleotides (oligos) to maximize the effect of chemical destabilization caused by mispairing. Short oligonucleotides and cDNAs also work well for expression analysis[1–5,10–13], although both types of elements have shortcomings. Short oligonucleotides sometimes lack the specificity required to ensure single-gene specificity in complex hybridizations, and for this reason a 'tiling approach' with multiple short oligos per gene is often used. Because of their extended length, PCR-amplified cDNAs produce strong signals and high specificity. The cDNA inserts are obtained readily from cDNA libraries, and are typically used for organisms for which there is limited genomic sequence available, although careful attention must be paid during sample preparation to avoid tracking errors.

Long oligonucleotides offer strong hybridization signals, good specificity, unambiguous sample identification and affordability[14,15]. They require the availability of genomic sequence information for each organism under study, but the copious amounts of sequence information now flowing from large-scale genome sequencing projects should help in this regard. Long oligonucleotides can be synthesized in 96- and 384-well formats, providing array elements optimized for binding specificity, melting temperature, concentration and purity. A large number of vendors (see Supplementary Table A online) provide high-quality long oligos for use in conjunction with microarray printing technologies. As an alternative, long oligonucleotides can also be synthesized *in situ* using ink-jets and micromirrors to control combinatorial synthesis directly on the microarray substrate (see Supplementary Note online).

Substrates for microarray analysis must provide stable attachment of array elements, low background signals, homogeneous surface chemistry and high-quality data. Glass (silicon dioxide) is the most common substrate material, and a plethora of glass slides and substrates are available, including those with atomically flat surfaces (see Supplementary Table B online). Typically, reactive amine, aldehyde or epoxide groups are attached covalently to silicon dioxide molecules on the glass surface using silane chemistry, and such surfaces allow stable attachment of oligonucleotides, cDNAs, proteins[16] and other molecules (see Supplementary Note online).

Microarray manufacturing technologies can be divided into two main categories: synthesis and delivery[17]. The synthesis approaches build DNA sequences directly on the microarray substrate by joining nucleotide building blocks (that is, A, G, C and T) in succession to produce the array elements. Photolithography, micromirrors, ink-jets and other technologies are used to deliver light, nucleotides and other reagents to the microarray surface for array element synthesis[18–20]. The delivery approaches use microarray elements prepared 'offline', depositing them onto the microarray substrate after synthesis using pins, ink-jets and other dispensers[1–3,21]. Synthesis technologies are generally used for commercial microarray manufacture, and delivery strategies tend to predominate in research laboratories where microarrays are manufactured 'in house' (see Supplementary Table C online). Contact and non-contact printing methods (see Supplementary Table D online) impart very high precision, with spot diameters varying by <5–10% from feature to feature[21,22].

Clean-room environments with low particle counts, regulated temperature and humidity levels, and controlled static

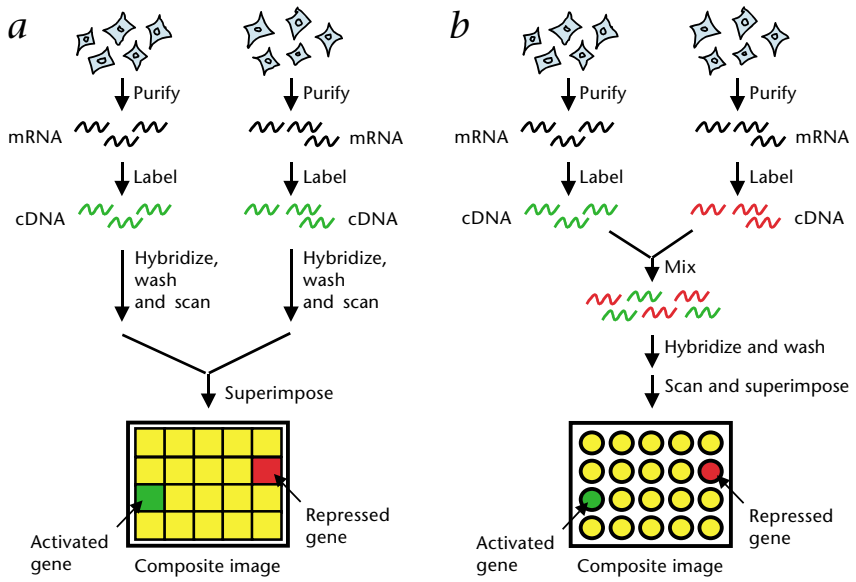

Fig. 1 Expression analysis by microarray. ***a***, One-color expression analysis uses a single fluorescent label (green wavy lines) and two chips to generate expression profiles for two or more cell samples. Activated and repressed genes (green and red squares, respectively) are obtained by superimposing images obtained from different chips. ***b***, Two-color expression analysis uses two different fluorescent labels (green and red wavy lines) and a single chip to generate expression profiles for two different cell samples. Activated and repressed genes (green and red spots, respectively) are obtained by superimposing images generated in different channels on a single microarray. Genes expressed equally in the two samples appear as yellow squares or spots in the two analyses.

electricity, organic vapors and biological contaminants can improve the quality of microarray manufacture in all settings, ranging from the smallest research laboratories to the largest commercial facilities (see Supplementary Note online).

Fluorescent probes for expression profiling are typically prepared from total RNA or messenger RNA (mRNA) by reverse transcription, although many different labeling strategies are available. Methods that use T7 RNA polymerase produce large amounts of amplified RNA and are widely used to generate probes from small amounts of sample. Because amplified RNA is produced by linear amplification with T7 polymerase, population skewing and the loss of quantitation are minimal. Control and experimental samples can be labeled separately with fluors that have non-overlapping emission spectra, including cyanine, Alexa, and other fluorescent derivatives. Two samples labeled with different fluors can be hybridized to a single chip to derive absolute and comparative expression information in the two samples.

Post-hybridization detection techniques, including intensely fluorescent dendrimer reagents[23], offer some major advantages over direct labeling procedures. Direct labeling generally reduces the efficiency of enzymatic synthesis and can introduce fluor-dependent labeling bias, mandating the use of larger amounts of RNA and 'dye-swapping' strategies to obtain strong signals and avoid experimental artifacts. Unincorporated dyes can also elevate fluorescent background if not removed before hybridization. Post-hybridization detection methods can reduce or eliminate these complications and provide cleaner, more reproducible results[23]. The use of 'less invasive' nucleotide analogs, such as aminoallyl derivatives, also obviates some of the complications seen in direct fluorescent labeling.

Microarray detection can be accomplished using a variety of fluorescence detection technologies including instruments that contain confocal optics, photomultiplier tubes (PMTs), and charge-coupled devices (CCD) (see Supplementary Table E online). Detection instruments produce graphical images in standard tagged-image file format (TIFF), which are two-dimensional, 16-bit numerical representations of microarray surfaces, with intensity values ranging from 0 to 65,536 (2^{16}). Numerical values are used to assign an expression value to each gene, and the data are typically saved in a tab-delimited format to allow their import into software programs for data mining and modeling[24].

Transformed and normalized data are represented and modeled using a variety of software tools, including scatter plots, principal component analysis (PCA), cluster diagrams, self-organizing maps (SOMs), neural networks and other algorithms[25–29]. Although the mathematical and statistical basis of the computational tools is complex, each endeavors to provide functionally relevant relationships between genes and gene products, assign putative function to unknown sequences, identify potential disease markers, elucidate the biochemical basis of drug and hormone action, and so forth (see Supplementary Table F online). The experimental aspects of microarray analysis are linked to data extraction, analysis and modeling in the microarray workflow process (Fig. 2). Intranets and the Internet, together with relational database warehouses, figure centrally in generating, mining, storing and retrieving microarray data (Fig. 2). Downloadable software ('shareware') packages are available free of charge to microarray researchers worldwide (see Supplementary Note online). Forums on microarray data analysis, such as the Critical Assessment of Microarray Data Analysis (CAMDA), endeavor to provide much-needed bioinformatics standards (see Supplementary Note online).

Where is the technology headed?

As microarray platforms mature and expand into more research venues, including university core facilities, small research centers, public health laboratories and testing clinics, major trends are emerging in such areas as assay diversification, microarray density, genomic and focused arrays, proteomics and genetic screening.

The first microarray paper featured the small mustard plant *Arabidopsis thaliana*[1], but the technology quickly spread to studies of yeast[30], human[3] and mouse[4]. Assay diversification has continued at a brisk pace, with >100 organisms featured to date (see http://arrayit.com/e-library). Microarrays from one organism can be used to examine evolutionarily related species, permitting phylogenetic analysis on a genomic scale[31,32], and, coupled with DNA sequencing data, can be used for functional genomic analysis of any organism in the biosphere. Diversification of the microarray platform is also occurring at the level of array elements, which are quickly expanding beyond DNA to include cellular proteins[33], organic molecules[34], carbohydrates[35], peptides[36] and nanotube precur-

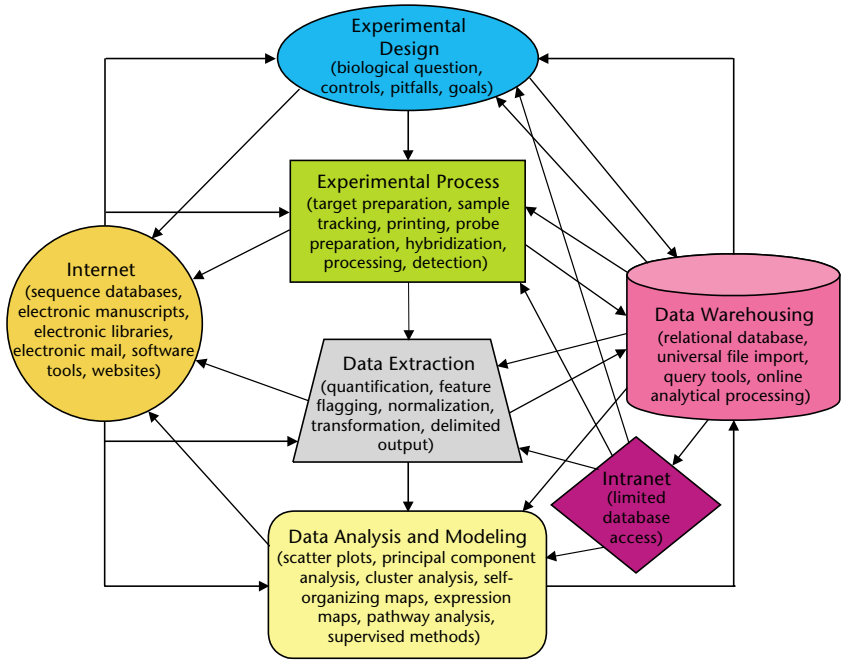

Fig. 2 Information workflow. Shown are the major phases of microarray analysis (colored icons) and their connectivity (arrows) in the microarray workflow process. Adherence to sound methodological principles ensures accurate data analysis, modeling and warehousing. Fully relational database designs and powerful query tools improve the usefulness of archived data. Two-way information flow via the Internet maximizes the benefits of electronic databases and resources, and Intranet designs allow limited access to the data warehouse. Facilitating tools and technologies from hundreds of sources superimpose readily over the experimental components of the process (see Supplementary Note online).

sors[37]. Microarray density, defined as the number of array elements per square centimeter of substrate, has also increased steadily over the years, permitting the manufacture of microarrays with tens of thousands of array elements and the analysis of entire genomes on single chips[38–40]. Concomitant with trends toward whole-genome analysis is the use of 'focused microarrays' containing only a few hundred elements. Focused microarrays complement whole-genome studies by providing a highly economical approach to detailed pathway analysis involving carefully selected gene subsets[41–43].

Microarray technology is also rapidly coming into use in the field of proteomics[44–48]. Small reaction volumes conserve expensive protein reagents and allow extremely rapid binding kinetics, and the parallel format permits the simultaneous measurement of binding constants, catalytic activities and other important parameters across all of the proteins in the cell, providing a comprehensive view of protein function. Advanced coupling chemistries allow efficient protein attachment in mild buffers at neutral pH, and fluorescent labels obviate the need for radioisotopes and improve assay safety. The high concentration of printed protein molecules stabilizes protein structure, and sophisticated printing buffers protect proteins against oxidation and permit ambient storage. A wide spectrum of biochemical processes can be studied by microarray, including protein–protein interactions (Fig. 3), protein-drug interactions, post-translational modifications and others. Protein–protein interactions that impart the activity of hormones, transcription factors, molecular motors and the like can be examined in the context of all of the proteins expressed in the cell, providing a detailed view of biochemical function. Pharmaceutical compounds that bind directly to cellular proteins and alter their function can be studied on a global scale, improving the efficiency of drug discovery. Glycosylation, phosphorylation, acylation, myristylation, prenylation and other post-translational modifications can also be studied by protein microarray, allowing a first-hand look at protein modification at the level of the proteome. It is anticipated that protein microarrays will increasingly replace many of the

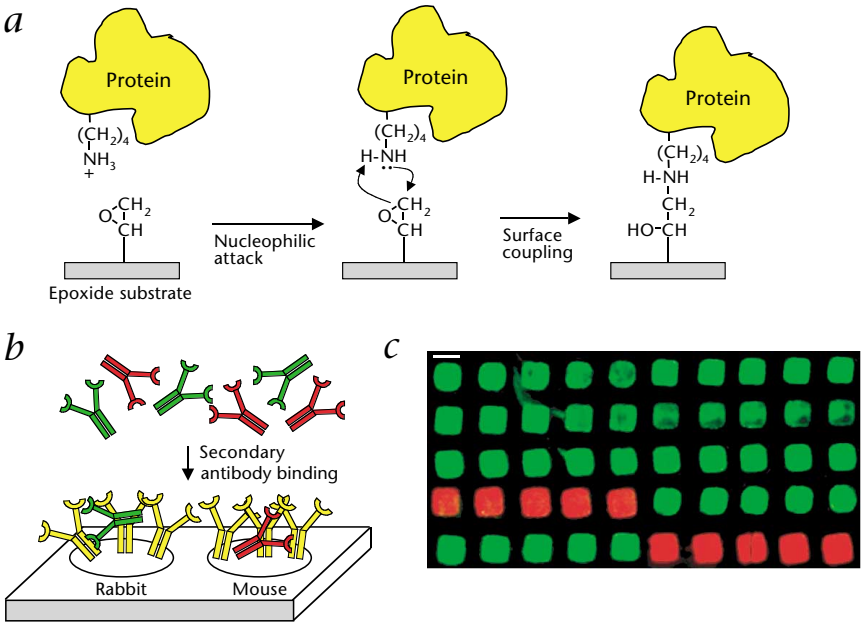

Fig. 3 Protein microarray technology. ***a***, Schematic illustration of a protein binding to an epoxide microarray substrate. Primary amines on the protein surface mediate nucleophilic attack and covalent coupling to the surface. ***b***, Schematic illustration of the primary and secondary antibody interactions shown in panel *c*. The yellow icons depict primary antibodies bound to the substrate, and the green and red antibody icons depict Cy3- and Cy5-labeled secondary antibodies, respectively. ***c***, Two-color protein microarray data in which monoclonal and polyclonal rabbit and mouse antibody samples were printed on an epoxy surface and stained with a 1:1 mixture of Cy3-goat anti-rabbit and Cy5-goat anti-mouse secondary antibodies. The Cy3 and Cy5 fluorescent data were compiled into a single, composite image. Bar represents 200 μm.

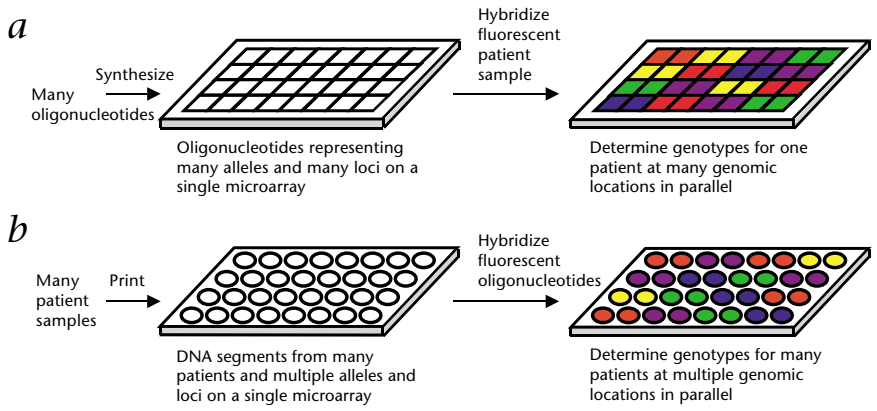

Fig. 4 Genetic screening by microarray. ***a***, Oligonucleotide microarrays. A large number of oligonucleotides representing many alleles and gene loci are synthesized at discrete locations on a solid surface and hybridized with a fluorescent patient sample, yielding genotyping information for one patient at many genomic locations in parallel. ***b***, Multipatient microarrays. A large number of patient samples representing multiple alleles and gene loci are printed at discrete locations on a solid surface and hybridized with fluorescent oligonucleotides, yielding genotyping information for many patients at multiple genomic locations in parallel.

traditional macroscopic protein techniques, such as filter binding, column chromatography and gel-shift assays, in the coming years. The availability of gentle contact printing methods allows protein microarrays to be manufactured readily (see Supplemental note and Supplementary Tables B-D). Continued advances in surface chemistry, the preparation and purification of cellular proteins on a genomic scale, peptide synthesis, and fluorescent and colorimetric labeling and detection schemes will speed the proliferation of protein microarray assays. All of the basic principles established for DNA microarrays are extendable to protein microarrays, though the tools must be tailored to provide for the inherent differences between nucleic acids and proteins.

Another noteworthy trend is the expansion of labeling and detection techniques. One interesting non-fluorescent labeling and detection technology, based on resonance light scattering (RLS), uses gold and silver nanoparticles of defined diameter as molecular labels[49,50]. RLS particles emit intense monochromatic light in the presence of white excitation light, and the particles are extremely stable even when exposed to intense light sources. Another emerging labeling and detection scheme exploits conventional colorimetric labels, including enzyme reaction products, which exhibit a high degree of spatial resolution on microarrays printed on glass substrates coated with white membrane materials such as nitrocellulose and related polymers. Colorimetric labels pave the way for the conversion of numerous traditional enzyme-linked immunosorbent assay (ELISA) formats into microarray tests and the concomitant use of traditional flatbed scanners for signal detection[51–54]. The small scale of colorimetric microarrays as compared to standard ELISA assays provides much greater information content and considerable cost reductions.

Genetic screening is another bright horizon for microarray analysis. In genetic screening applications, DNA samples are examined to identify single-nucleotide changes, deletions and other minor sequence variants in genes that underlie genetic and infectious disease. Two main types of microarray formats have been developed for genetic screening and diagnostic applications. The first format uses oligonucleotide microarrays to detect sequence variants in a single-patient sample[55–57]. One advantage of 'single-patient' microarrays is that a large number of sequence variants can be screened in a single test (Fig. 4*a*), providing extremely affordable genotyping information on a genomic scale. A disadvantage is that the cost per patient is rather high, because a separate chip is required for each individual tested.

'Multipatient' microarrays offer a cost-effective alternative to oligonucleotide microarrays, particularly in applications in which a large number of patients must be screened for a relatively small number of loci. In the multipatient format, patient samples containing defined gene segments are printed into microarrays at distinct locations, and the microarrays are hybridized with fluorescent oligonucleotides complementary to the gene loci of interest (Fig. 4*b*). Normal, carrier and disease genotypes are scored easily because the printed patient samples hybridize to the fluorescent oligonucleotides with different efficiencies depending on the genotype of the patient. The main advantage of multipatient microarrays is the capacity to examine thousands or even tens of thousands of patients on one chip[58]. One disadvantage is that the patient samples must be printed into microarrays before genotyping, adding additional time to the assay as compared to preprinted oligonucleotide microarrays.

How good are the data?

Microarray analysis represents a classic 'precision in–precision out' technology. Experiments based on sound experimental design, optimized protocols, properly designed array elements, pure samples, robust manufacturing and surface chemistry, high-quality scanning, and correctly applied sample tracking, quantification and data mining tools yield highly valuable results. The current state of the art provides ≤5–10% variation in signal intensities among replicate array elements on the same microarray, and ~10–30% variation among corresponding array elements on different microarrays[59]. The variability introduced by the contributions of cell doubling time, differing biology of individual transgenic animals, clonal variation, environmental effects on gene expression and so forth almost always exceeds the technical components of microarray assays. These biological contributions need to be accounted for and dealt with in data analysis, through the use of replicates and downstream validation. Bioinformatics programs are beginning to encompass confidence measures of the data using analysis of replicate hybridizations. These advances will eventually allow microarray expression data to be used as stand alone metrics of biological function.

The main appeal of microarrays—and the reason the technology is proliferating over competing platforms such as beads, filters, gels, mass spectroscopy and PCR—derives from the inherent attributes of parallelism, miniaturization and automation, and the accuracy of the data[59] vis-à-vis the sophisticated instruments and software tools that are available to the re-

searcher. The capacity to conduct tens of thousands of gene determinations in a single reaction vastly improves the precision of the data and reduces the cost as compared to that of serial methods. A $500 microarray, yielding gene expression measurements for 10,000 genes, provides highly quantitative data for $0.05 per gene. The affordability of microarrays, the space-saving aspects of miniaturization and high throughput conferred by automation, and the widespread availability of commercial microarrays (see Supplementary Table G online) impart clear advantages over non-chip technologies.

Pushing the envelope

What will microarray assays look like ten years from now? Although nobody has a crystal ball to look into the future, many exciting applications can be envisioned as the technology progresses. Microarrays will continue to transform basic research, both technologically and methodologically. The technical transformation will see the replacement of radioisotopes and other low-tech methods with fluorescent microarray assays and computational approaches to biology. Methodologically, there will be a continued shift in the direction of discovery-based research at the expense of hypothesis-driven studies.

Recent successes in benchmarking microarrays by the US Food and Drug Administration[60–65] suggest broad applications for assessing the safety of food, drugs, vaccines, medical devices and other products of consumer interest. Identification of food-borne bacterial pathogens by microarray would reduce the incidence of food poisoning, illness and death associated with bacterial contamination of meat, seafood, dairy products and other foods. A related use of 'bacto-chips' would be in clinical settings, to establish the identity of organisms in patients admitted to hospitals with systemic bacterial infections. The capacity to type unambiguously all the common bacteria on a single chip within a few hours of sampling would allow high-speed testing in agricultural, manufacturing and clinical settings.

The efficacy and safety of pharmaceuticals is also amenable to microarray analysis, and both genotyping and gene expression assays can be envisioned in clinical trials. Genotyping microarrays could be used to parse the clinical trials population into responders and non-responders, enhancing the accuracy of drug-testing results, and allowing drugs to be tailored to specific subsets of the population according to clearly identifiable markers in the patient population. Gene expression microarrays could be used to examine the physiological effects of drug administration, allowing the analysis of pathways and the identification of side reactions in which drugs bind promiscuously to cellular proteins, producing toxic side effects. Promising lead compounds could be optimized at the chemical level to minimize side reactions, using gene expression information as the functional readout. Expression arrays could also be used to profile and test medical devices that emit γ-rays, X-rays, microwaves, radio waves and other forms of electromagnetic radiation to explore interactions between biological tissues and various forms of radiation.

Population-based screening also holds great promise for identifying disease carriers, thereby reducing the incidence of genetic disease. Particularly in the case of common genetic disorders that exhibit carrier frequencies of ≥1%, affordable microarray-based genotyping assays could be used to identify disease-causing alleles in the population and reduce the chances of passing along inherited disorders. Both oligonucleotide and multipatient microarrays can be used for patient screening, and the availability of this technology may promote the availability of home testing kits for breast cancer, cystic fibrosis, sickle cell anemia and other common genetic diseases. In one model, consumers would obtain testing kits from a public health clinic or local pharmacy, self-sample a droplet of blood that contains their genetic material, send the sample to a central microarray testing facility, and enter a private access code to obtain the confidential testing results through the Internet. Armed with accurate genotyping information, patients could then work closely and confidentially with their family physician or health care professional to obtain accurate and up-to-date information on lifestyle issues, drug therapies and family counseling advice to ensure the highest-quality health care vis-à-vis their genetics.

Note: Supplementary information can be found on the Nature Medicine *website.*

1. Schena, M., Shalon, D., Davis, R.W. & Brown, P.O. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467–470 (1995).
2. Schena, M. Genome analysis with gene expression microarrays. *BioEssays* **18**, 427–431 (1996).
3. Schena, M. *et al.* Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proc. Natl. Acad. Sci. USA* **93**, 10614–10619 (1996).
4. Lockhart, D.J. *et al.* Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nature Biotechnol.* **13**, 1675–1680 (1996).
5. DeRisi, J. *et al.* Use of a cDNA microarray to analyse gene expression patterns in human cancer. *Nature Genet.* **14**, 457–460 (1996).
6. Grunstein, M. & Hogness, D.S. Colony hybridization: a method for the isolation of cloned DNAs that contain a specific gene. *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965 (1975).
7. Southern, E.M. Blotting at 25. *Trends Biochem. Sci.* **25**, 585–588 (2000).
8. Jackson, D.A., Symons, R.H. & Berg, P. Biochemical method for inserting new genetic information into DNA of Simian Virus 40: circular SV40 DNA molecules containing lambda phage genes and the galactose operon of *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **69**, 2904–2409 (1972).
9. Mullis, K.B. & Faloona, F.A. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol.* **155**, 335–350 (1987).
10. Wodicka, L., Dong, H., Mittmann, M., Ho, M.H. & Lockhart, D.J. Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nature Biotechnol.* **15**, 1359–1367 (1997).
11. Cho, R.J. *et al.* Parallel analysis of genetic selections using whole genome oligonucleotide arrays. *Proc. Natl. Acad. Sci. USA* **95**, 3752–3757 (1998).
12. Lashkari, D.A. *et al.* Yeast microarrays for genome wide parallel genetic and gene expression analysis. *Proc. Natl. Acad. Sci. USA* **94**, 13057–13062 (1997).
13. Yuen, T., Wurmbach, E., Pfeffer, R.L., Ebersole, B.J. & Sealfon, S.C. Accuracy and calibration of commercial oligonucleotide and custom cDNA microarrays. *Nucleic Acids Res.* **30**, e48 (2002).
14. Kane, M.D. *et al.* Assessment of the sensitivity and specificity of oligonucleotide (50mer) microarrays. *Nucleic Acids Res.* **28**, 4552–4557 (2000).
15. Hughes, T.R. *et al.* Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nature Biotechnol.* **19**, 342–347 (2001).
16. Lesaicherre, M.L., Lue, R.Y., Chen, G.Y., Zhu, Q. & Yao, S.Q. Intein-mediated biotinylation of proteins and its application in a protein microarray. *J. Am. Chem. Soc.* **124**, 8768–8769 (2002).
17. Schena, M. *et al.* Microarrays: biotechnology's discovery platform for functional genomics. *Trends Biotechnol.* **16**, 301–306 (1998).
18. Fodor, S.P. *et al.* Light-directed, spatially addressable parallel chemical synthesis. *Science* **251**, 767–773 (1991).
19. Singh-Gasson, S. *et al.* Maskless fabrication of light-directed oligonucleotide microarrays using a digital micromirror array. *Nature Biotechnol.* **17**, 974–978 (1999).
20. Theriault, T.P., Winder, S.C. & Gamble, R.C. Application of ink-jet printing technology to the manufacture of molecular arrays. in *DNA Microarrays: A Practical Approach* 2nd edn. (ed. Schena, M.) 101–120 (Oxford University Press, Oxford, 2000).
21. Rose, D. Microfluidic technologies and instrumentation for printing DNA microarrays. in *Microarray Biochip Technology* 5th edn. (ed. Schena, M.) 19–38 (Eaton Publishing, Natick, MA, 2000).
22. Hegde, P. *et al.* A concise guide to cDNA microarray analysis. *Biotechniques* **29**, 548–550 (2000).
23. Stears, R.L., Getts, R.C. & Gullans, S.R. A novel, sensitive detection system for high-density microarrays using dendrimer technology. *Physiol. Genomics* **3**, 93–99 (2000).
24. Bellenson, J.L. Expression data and the bioinformatics challenges. in *DNA*

Microarrays: A Practical Approach 2nd edn. (ed. Schena, M.) 139–165 (Oxford University Press, Oxford, 2000).
25. Carr, D.B., Somogyi, R. & Michaels, G. Templates for looking at gene expression clustering. *Statistical Computing and Graphics Newsletter* **8**, 20–29 (1997).
26. Michaels, G.S. *et al.* Cluster analysis and data visualization of large-scale gene expression data. *Pac. Symp. Biocomput.*, 42–53 (1998).
27. Eisen, M.B., Spellman, P.T., Brown, P.O. & Botstein, D. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* **95**, 14863–14868 (1998).
28. Evertsz, E., Gupta, R., Starink, P. & Watson, D. Technology and applications of gene expression microarrays. in *Microarray Biochip Technology* 5th edn. (ed. Schena, M.) 149–166 (Eaton Publishing, Natick, MA, 2000).
29. Zhou, Y-X, Kalocsai, P., Chen, J-Y. & Shams, S. Information processing issues and solutions associated with microarray technology. in *Microarray Biochip Technology* 5th edn. (ed. Schena, M.) 167–200 (Eaton Publishing, Natick, MA, 2000).
30. Shalon, D., Smith, S.J. & Brown, P.O. A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. *Genome Res.* **6**, 639–645 (1996).
31. Sniegowski, P. The genomics of adaptation in yeast. *Curr. Biol.* **9**, R897–R898 (1999).
32. Bjorkholm, B. *et al.* Comparison of genetic divergence and fitness between two subclones of *Helicobacter pylori*. *Infect. Immun.* **69**, 7832–7838 (2001).
33. MacBeath, G. & Schreiber, S.L. Printing proteins as microarrays for high-throughput function determination. *Science* **289**, 1760–1763 (2000).
34. Gao, X. *et al.* A flexible light-directed DNA chip synthesis gated by deprotection using solution photogenerated acids. *Nucleic Acids Res.* **29**, 4744–4750 (2001).
35. Wang, D., Liu, S., Trummer, B.J., Deng, C. & Wang, A. Carbohydrate microarrays for the recognition of cross-reactive molecular markers of microbes and host cells. *Nature Biotechnol.* **20**, 275–281 (2002).
36. Robinson, W.H. *et al.* Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nature Med.* **8**, 295–301 (2002).
37. Chen, B., Parker, G. II, Han, J., Meyyappan, M. & Cassell, A.M. Heterogeneous single-walled carbon nanotube catalyst discovery and optimization. *Chem. Mater.* **14**, 1891–1896 (2002).
38. DeRisi, J.L., Iyer, V.R. & Brown, P.O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680–686 (1997).
39. Hughes, T.R. *et al.* Widespread aneuploidy revealed by DNA microarray expression profiling. *Nature Genet.* **25**, 333–337 (2000).
40. Sudarsanam, P., Iyer, V.R., Brown, P.O. & Winston, F. Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **97**, 3364–3369 (2000).
41. Heller, R.A. *et al.* Discovery and analysis of inflammatory disease-related genes using cDNA microarrays. *Proc. Natl. Acad. Sci. USA* **94**, 2150–2155 (1997).
42. Xiong, M., Li, W., Zhao, J., Jin, L. & Boerwinkle, E. Feature (gene) selection in gene expression-based tumor classification. *Mol. Genet. Metab.* **73**, 239–247 (2001).
43. Selaru, F.M. *et al.* Artificial neural networks distinguish among subtypes of neoplastic colorectal lesions. *Gastroenterology* **122**, 606–613 (2002).
44. MacBeath, G. & Schreiber, S.L. Printing proteins as microarrays for high-throughput function determination. *Science* **289**, 1760–1763 (2000).
45. Zhu, H. *et al.* Global analysis of protein activities using proteome chips. *Science* **293**, 2101–2105 (2001).
46. Haab, B.B., Dunham, M.J. & Brown, P.O. Protein microarrays for highly parallel detection and quantitation of specific proteins and antibodies in complex solutions. *Genome Biol.* **2**, 1–13 (2001).
47. Wilson, D.S. & Nock, S. Functional protein microarrays. *Curr. Opin. Chem. Biol.* **6**, 81–85 (2002).
48. Eickhoff, H. *et al.* Protein array technology: the tool to bridge genomics and proteomics. *Adv. Biochem. Eng. Biotechnol.* **77**, 103–112 (2002).
49. Yguerabide, J. & Yguerabide, E.E. Resonance light scattering particles as ultrasensitive labels for detection of analytes in a wide range of applications. *J. Cell Biochem.* Suppl. **37**, 71–81 (2001).
50. Bao, P. *et al.* High-sensitivity detection of DNA hybridization on microarrays using resonance light scattering. *Anal. Chem.* **74**, 1792–1797 (2002).
51. Taton, T.A., Mirkin, C.A. & Letsinger, R.L. Scanometric DNA array detection with nanoparticle probes. *Science* **289**, 1757–1760 (2000).
52. Hong, T.M. *et al.* Profiling the downstream genes of tumor suppressor PTEN in lung cancer cells by complementary DNA microarray. *Am. J. Respir. Cell Mol. Biol.* **23**, 355–363, 2000.
53. Cao, Y.C., Jin, R. & Mirkin, C.A. Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. *Science* **297**, 1536–1540 (2002).
54. Lau, W.K., Chiu, S.K., Ma, J.T. & Tzeng, C.M. Linear amplification of catalyzed reporter deposition technology on nylon membrane microarray. *Biotechniques* **33**, 566–570 (2002).
55. Hacia, J.G. *et al.* Determination of ancestral alleles for human single-nucleotide polymorphisms using high-density oligonucleotide arrays. *Nature Genet.* **22**, 164–167 (1999).
56. Syvanen, A.C. From gels to chips: 'minisequencing' primer extension for analysis of point mutations and single nucleotide polymorphisms. *Hum. Mutat.* **13**, 1–10 (1999).
57. Tõnisson, N., Kurg, A., Lõhmussaar, E. & Metspalu, A. Arrayed primer extension on the DNA chip—method and applications. in *Microarray Biochip Technology* 5th edn. (ed. Schena, M.) 247–263 (Eaton Publishing, Natick, MA, 2000).
58. Schena, M. Genetic screening and diagnostics. in *Microarray Analysis* 1st edn. (ed. Schena, M.) 387–403 (Wiley-Liss, Hoboken, NJ, 2002).
59. Yue, H. *et al.* An evaluation of the performance of cDNA microarrays for detecting changes in global mRNA expression. *Nucleic Acids Res.* **29**, E41 (2001).
60. Chizhikov, V., Rasooly, A., Chumakov, K. & Levy, D.D. Microarray analysis of microbial virulence factors. *Appl. Environ. Microbiol.* **67**, 3258–3263 (2001).
61. Beaucage, S.L. Strategies in the preparation of DNA oligonucleotide arrays for diagnostic applications. *Curr. Med. Chem.* **8**, 1213–1244 (2001).
62. Paweletz, C.P. *et al.* Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene* **20**, 1981–1989 (2001).
63. Soini, H. & Musser, J.M. Molecular diagnosis of mycobacteria. *Clin. Chem.* **47**, 809–814 (2001).
64. Al-Khaldi, S.F., Martin, S.A., Rasooly, A. & Evans, J.D. DNA microarray technology used for studying foodborne pathogens and microbial habitats: minireview. *J. AOAC Int.* **85**, 906–910 (2002).
65. Kipps, T.J. Advances in classification and therapy of indolent B-cell malignancies. *Semin. Oncol.* **29**, 98–104 (2002).

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