Analytical Ancestry:
Evolution of the Array in Analysis

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BACKGROUND: Macro-, micro-, and nanosized arrays of test sites at various densities have emerged as important types of analytical devices in response to the need for high volume parallel analysis in both the research and the clinical laboratory.

CONTENT: This review explores the diversity of arrays of reaction vessels and arrays of reagents and of samples, with an emphasis on the earliest descriptions of the different variations. The scope of such arrays includes linear and 2-dimensional arrays of reaction vessels (e.g., microwell strips, microplates); linear and 2-dimensional arrays of reagents arrayed on pillars and posts; beads in wells; and reagents randomly arrayed (or dis-ordered) for use in next-generation sequencing. Micro- and nanofabrication technologies have been applied to the miniaturization of arrays to increase array density (e.g., DNA probe arrays) and produce arrays of analytical structures (e.g., cantilevers, nanoelectrospray nozzles).

SUMMARY: Arrays are now firmly established in many types of analytical devices, and this analytical format has gained widespread acceptance owing to the advantages of high-throughput automation and multiplex analysis. Ongoing “big biology” genomic and proteomic studies will ensure the continued dominance of array-based methods into the foreseeable future.

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Macro-, micro-, and nanosized arrays of test sites at various densities have emerged as important types of analytical devices. Early forms of analysis used apparatuses designed for a single analysis, such as a test tube or other type of single-test container. In both research and clinical laboratory settings high-volume parallel analysis is required, and that need is now met by devices that are based on arrays, for example, arrays of containers, arrays of spots (sample or reagent), and arrays of reagent-coated beads on a planar substrate.

The simple notion of creating arrays of features or structures on or in a surface or assembling an array of devices from identical subunits is of course not new, and parallels can be found in the natural world (e.g., a compound eye comprising thousands of photoreceptor units). Arraying has been motivated not only by the need for parallel processing (e.g., muffin-baking pan, cartridge loader) (1), but also by the need to store and transport multiple items (e.g., crates and cartons) (2–4) (Fig. 1A) (4) and to control equipment (e.g., punch cards to control weaving looms) (5, 6). Likewise, arranging objects into an array underpins the operation of display screens (7), dot-matrix printers (8), charge-coupled–device detectors (9), and televisions (mirror arrays) (10).

Much modern day analysis is now based on multiple analyses in devices that contain arrays of analytical test sites. This review traces the evolution and development of this type of analysis as recorded in the scientific and patent literature, with an emphasis on the use of multitest devices (e.g., carousels, microplates) and different types of microarrays.

Types of Arrays

Different combinations of characteristics possible for an array are listed in Table 1. The size of the individual reaction sites or vessels that make up the array can vary in size from macro to micro to nano, and can be in the form of a linear array or a 2-dimensional array. Arrays can be characterized based on density. For example, conventional 96-well microplates have a density of 1.1 wells/cm², whereas synthesized oligonucleotide arrays have much higher densities, e.g., $\approx 10 000$ different polypeptides per square millimeter (11).

Another possibility is a 3-dimensional array. Three-dimensional polymerized crystalline colloidal arrays coated with a boronic acid derivative have found applications in glucose sensing (12). However, a detailed analysis of the range and applications of 3-dimensional arrays is not within the scope of this article.

The following sections explore the diversity of 2-dimensional arrays of reaction vessels and arrays of
reagents or samples, with an emphasis on the earliest descriptions of the different variations.

**Arrays of Reaction Vessels**

It is easy to understand how convenience and efficiency motivated the transition from performing analysis in a single test tube to arranging the test tubes in an array in a test tube rack (13)(Fig. 1B). This idea then evolved into carousels (14), conveyors (15), spot plates (16), magazines (17), cassettes (18), strips of containers (19), and microwell plates (20). An early example is the test plate with an array of shallow depressions that served as test sites for spot testing, e.g., a microscope slide (21).

**LINEAR ARRAYS**

Linear arrays of reaction vessels are exemplified by microwell strips (22), cuvette strips (23), and magazines and cassettes of reaction vessels (24).

**TWO-DIMENSIONAL ARRAYS**

The conventional 96-well microplate exemplifies a macroarray of reaction vessels and can be traced back to the work of Takatsky in 1951, who created a prototype microplate by machining an array of 6 rows of 12 wells into a piece of plastic [Lucite (polymethyl methacrylate)] (20). In current microplates, the individual wells (approximately 7 mm in diameter × approximately 12 mm deep) are arranged in a format of 8
rows × 12 columns, and the plate measures approximately 127 mm × approximately 85 mm (25–26). The array of wells can be made continuous by fabrication of the wells on a continuous tape (27). Miniaturization of the microwells has spawned plates with smaller wells (e.g., 11.45-μL wells in Terasaki plates) (28) and with increasingly large numbers of wells, e.g., 384-well and 1536-well plates. Additional miniaturization has led to the production of plastic plates with up to 9600 wells in which the wells are in the nanoscale size range (29).

Microfabrication techniques provide even larger numbers of wells, e.g., 99 856 cells on a 7.25-inch square silicon plate (30). Large numbers (1000 or more) of nanowells (118-nL volume) on a silicon chip have found utility for sample delivery in separation techniques such as capillary electrophoresis (31) and for sample delivery into mass spectrometers (e.g., array of nanoelectrospray nozzles on an Advion ESI Chip<sup>TM</sup> (32) (Fig. 2A). Microwells arrayed on a silicon chip, with each well an individual electronic channel with a lipid bilayer on top, can be created in situ and have found use in massively parallel nanopore sensing (33) (Fig. 2B). Microfabrication technology has also been used to produce arrays of reaction vessels designed for real-time PCR, such as the TaqMan<sup>®</sup> arrays that are in the form of preloaded 384-well microfluidic cards (34).

The possible geometric arrangement of 2-dimensional arrays includes radially disposed wells used in centrifugal analyzers (35), reaction carousels found in many modern clinical chemistry analyzers (36–38), and analyzers that use a compact disc format (39) (Fig. 2, C and D). An alternative way of defining an array of reaction zones is to print the desired pattern onto a surface, e.g., by using a hydrophobic material or a cell adhesive substance (40).

**Arrays of Reagents or Samples**

**LINEAR ARRAYS**

An early example of a linear array of macro-sized test sites is the simple multitest dipstick that comprises a series of pads impregnated with reagents attached to a plastic strip (41). Other examples include the linear array of allergen-coated threads in a MASTpette test chamber (42) and test sites on a membrane, e.g., Western blot (43, 44) and reagent-coated beads in a tube (45). The array can be placed onto a material that provides physical support or adhesion, or, as in the case of linear arrays of microfabricated cantilevers coated with a capture reagent, the support is part of a sensor mechanism (46).

**TWO-DIMENSIONAL ARRAYS ON A PLANAR SURFACE**

Two-dimensional arrays on a planar surface have found widespread use and, in the case of DNA arrays, have been highly controversial (47–49). Early arrays were produced by manual spotting of an array of materials (50, 51), for example by using the point of a dip pen on a glass slide for use in an immunofluorescence test (52), or printing (e.g., to keep 2 reagents apart) (53). Most work has focused on high density arrays, known as microarrays. Microarray manufacturing processes now include in situ synthesis (11, 54) (e.g., the Affymetrix chip) (Fig. 2E), spotting by using automated spotting devices (55), printing (including dip-pen methods at the nanoscale) (56), and microstamping (57). Multiple arrays can also be assembled to produce an array of arrays (Fig. 2F) (58). An interesting development has been the use of micromirrors to control in situ photochemical synthesis of microarrays—an example of an array controlling the fabrication of an array (59).

One application for oligonucleotide arrays has been in DNA sequencing-by-hybridization (SBH),<sup>3</sup> in which universal probes are designed to read all possible sequences in any DNA sample hybridized to an array of DNA probes (e.g., so-called zip-code array or universal array). In a later development, combinatorial SBH, 2 sets of universal short probes are used, in which one is attached to a solid support and the other is free in solution and labeled with a fluorophore. Unlabeled target DNA template mixed with DNA ligase and a solution-phase labeled probe set is hybridized to the support-bound probes. When both the array-bound and solution-phase labeled probes hybridize to the target DNA at contiguous complementary positions, they are covalently linked by DNA ligase, creating 1 long labeled probe attached to the array surface. The combinatorial process generates all possible probes that are complementary to the target (60, 61). Highly multiplexed methods for single-nucleotide polymorphism/mutation detection have also been developed by combining allele-specific discrimination or single nucleotide extension with read-out on zip-code arrays or universal arrays (62).

Two-dimensional arrays that combine electronic functions on a silicon chip are also being developed for nanopore-based sequencing These proprietary “planar lipid bilayer” chips have an array of microwells, each an individually addressable electronic channel (Fig. 2B). Lipid bilayers are formed at the top of the wells and nanopore constructs (a processive exonuclease enzyme combined with an α-hemolysin nanopore containing an adapter molecule, cyclodextrin) are added to the chip to sit within the lipid bilayers, forming a single hole in the bilayer. DNA bases are identified as they

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<sup>3</sup> Nonstandard abbreviations: SBH, sequencing-by-hybridization; NGS, next-generation sequencing.
Fig. 2. Examples of arrays of analytical structures, wells, beads, beads in wells, and reagents.

(A), Advion ESI chip with an array of 400 nanoelectrospray nozzles. Image of the ESI Chip® was provided by Advion BioSystems, Ithaca, NY. (B), Part of Oxford Nanopore’s planar bilayer array sequencing chip. Each microwell is an individually addressable electronic channel from which measurements of current through a nanopore may be recorded. The Oxford Nanopore company is scaling its arrays through hundreds to thousands of parallel channels. Image reproduced with permission from Oxford Nanopore Technologies, Oxford, UK. (C), Gyrolab Bioaffy compact disc for nanoliter-scale immunoassays; and (D) close-up of the array of analytical microstructures around the circumference of the compact disc. Images reproduced with permission from Gyros AB, Uppsala Science Park, Uppsala, Sweden. (E), Affymetrix GeneChip® DNA probe array. Image courtesy of Affymetrix, Santa Clara, CA.

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travel through the pore. “Solid-state” arrays of nanopores, fabricated from synthetic materials, are also under development (63).

In another array variation, one location on an array can serve as a sample reservoir that delivers sample to other array locations. For example, a laminated test card has been designed with 7 radially disposed test chambers containing stained red cells for drug testing that are located around a central sample application well (64).

**ARRAYS OF PILLARS OR POSTS**

More complex surface architectures are possible, as in the case of arrays of pillars. The pillars (150 μm high × 50 μm wide) are created photolithographically on a silicon wafer chip and are arranged in groups of 200 in microchannels on the chip. Immunoassay reactions are performed on the top surface of the pillars (65).

**ARRAYS OF BEADS IN WELLS**

An array of beads in wells in a plate or assay tray is an “array in an array” type of design. Early examples of this design are immunoassays based on polystyrene beads or balls coated with a reagent and held in the 5 × 4 array of wells in a tray (66) (Fig. 2G). Other versions of this type of assay employed a reagent-coated insertable carrier that locates onto a pin within each well of an array of wells in a 5 × 4–well tray (67).

**RANDOM (OR DISORDERED) ARRAYS**

An array of oligonucleotide-coated beads can be assembled in a random order and then the identity and location of each bead can be determined in a subsequent decoding step (achieved by sequential rounds of DNA hybridization), as in the randomly ordered BeadArray technology used by Illumina (Fig. 2H). An array of wells is patterned into an optical imaging fiber bundle. Individual pieces of fiber optic cable can be assembled into a 1.4-mm diameter array comprising approximately 50 000 individual fibers with the well at the end of each fiber containing a bead. These are fused together into a hexagonally packed array. The arrays are formatted into an array of arrays, in a pattern that matches the wells of standard microtiter plate to further increase throughput (68, 69).

In recent years there has been significant academic and commercial investment in alternative technologies for DNA sequence analysis, and recently a few so-called next-generation sequencing (NGS) platforms have become available (70). NGS systems, such as those from Roche/454, Illumina/Solexa, Life Technologies/AB SOLiD 4, and Azco Biotech differ in the details of their technology, but all have the common characteristic of generating data by imaging emissions over time from a large random array. Examples of NGS arrays include random arrays of beads captured in an array of picoliter...
reaction chambers (PicoTiterPlate), as in the Roche/454; random arrays of adapter-ligated genomic DNA fragments bound to immobilized primers on a surface, as in the Illumina/Solexa; and random arrays of beads bound on a surface, as in the Life Technologies/AB SOLiD (e.g., the SOLiD 4 can hold 400,000,000 beads) (Fig. 2I). NGS systems also include random monolayer arrays of beads, as in the Azco Biotech Polonator. In addition, single-molecule sequencing-by-synthesis technology such as that from Helicos Biosciences uses poly(dT)-oligonucleotides that are anchored to the surface of an array of 25 channels in a flow cell (approximately 100 million strands/cm²). A library of poly(dA)oligos-tailed templates hybridize to the poly(dT) oligonucleotides to produce a random array, and the position of each hybrid is then determined by camera imaging in successive rounds of the sequencing reaction (71). The array format is also exploited in the Pacific BioSciences PacBio RS sequencer, in which the sequencing reactions are performed on templates randomly distributed in 80,000 zeromode waveguide wells, each holding 20 zL (72–74). In another array-based method, sequencing is performed on 300-nm spots photolithographically patterned as a grid on a silicon chip of 25 × 75 mm. The sequencing substrate, present in coils of single-stranded DNA (nanoballs), self-assembles by adsorption and then binds randomly to the activated spots to produce a random nanoarray on the silicon chip, and the chip is then imaged (75).

Conclusions

The array format is now firmly established in many types of analytical devices, and the scope of arrayed substances encompasses the spectrum of complexity and size from molecules to cells and tissues to whole organisms (76, 77). Widespread acceptance of this format is attributable to the advantages of high-throughput automation and multiplex analysis. Ongoing “big biology” genomic and proteomic studies will ensure the continued dominance of array-based methods into the foreseeable future.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

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