

Microarrays: their origins and applications

Letter

It is disappointing that, in a review supposedly placing microarray-based assays into a wider historical and conceptual perspective, Schena *et al.*¹ so profoundly misrepresent the origins and development of this revolutionary technology.

DNA analysis methods have long been recognized as ligand assays, relying on the binding of target molecules to a specific 'recognition' reagent (e.g. antibody). Unsurprisingly, DNA analysis techniques thus frequently employ approaches previously exploited in an immunoassay context. For example, immunoassays have, since the 1960s, commonly relied on antibodies attached to solid supports, an analogous approach being later applied to DNA measurement².

The development of microarray-based assay methods conforms to this pattern of events. Such methods were first conceived of and developed by us in the mid- to late 1980s³⁻⁵, being described during that period in journals and meetings in Europe⁶ and later the USA (e.g. the 1991 Oak Ridge Conference⁷). We have since frequently discussed the principles on which they are based – and their likely impact on medicine – at international meetings^{9,10} and in refereed articles, including one in this journal⁸. Indeed, a historical overview of the ligand-assay field, culminating in the development of microarray methods, was the subject of the Ullman Award presentation at the 1998 Oak Ridge Conference¹¹.

In view of this background, it is astonishing that Schena *et al.* portray the technology as having originated in a method of constructing arrays devised (for other purposes) at the beginning of the 1990s and which is of limited applicability in the present context. This erroneous view underlies Schena *et al.*'s prediction that 'future studies will undoubtedly involve the parallel analysis of proteins, lipids, carbohydrates and small molecules' (reflecting the authors' somewhat belated realization that oligonucleotide microarrays merely exemplify chips that, more generally, 'can provide a quantitative measure of the molecules present in biochemical extracts'). In short, Schena

et al. appear to be oblivious to the fact that, prior to their use for DNA analysis, microarray-based methods had already been developed in areas they regard as the subject of future studies.

However, the technology was, from its earliest inception in the mid-1980s, perceived by us (and others) as potentially applicable to all forms of binding assay³ and has frequently been so described in meetings and journals. We nevertheless initially developed microarrays for immunodiagnostic purposes, not only because immunoassay constituted a methodology of unique biomedical importance in the 1980s but also because attainment of the high sensitivity demanded in this field presented the principal obstacle to the future ubiquitous use of microarray methods.

Thus, in collaboration with Boehringer Mannheim (which, although omitted from Table 1 of Schena *et al.*'s review, has played a pioneering role in the technology's development), we have not only developed oligonucleotide-based microarray methods for DNA and RNA analysis but also analogous antibody-based assays for protein and small-molecule analytes such as glycoprotein and steroid hormones, viral and allergen antibodies, where high sensitivity and specificity are key requirements¹².

Although Boehringer Mannheim has not widely publicized these achievements, its researchers have reported their activities at several international meetings, including the 1996 Oak Ridge Conference¹³, where project leader Hans Berger described the company's development of prototype machinery producing 5000 quality-controlled 'chips' per hour (since increased to 10 000), as well as typical applications of microarray technology.

Schena *et al.*'s review is also misleading in other respects. For example, the authors cite nine publications supposedly describing the use of confocal scanners in this context, omitting any reference to our original descriptions of such use^{3,4,6}, including those in past Oak Ridge presen-

tations^{7,14}. Ironically, Fodor *et al.*¹⁵ (authors of the earliest publication cited by Schena *et al.*) do not refer in their cited paper to confocal microscopy, nor is the Zeiss Axioskop 20 epifluorescence microscope they used of confocal type.

Similarly, we have often referred to our own microspotting techniques (these being obligatory when constructing antibody microarrays), although we have also (since 1991) drawn attention to the combinatorial methods devised by Fodor *et al.*¹⁵ to construct large polypeptide and polynucleotide arrays. Ink-jet spotting techniques have been relied on by Boehringer Mannheim, these representing an extension of our original methods, and enable the low-cost manufacture of antibody and oligonucleotide microarrays on an industrial scale.

In summary, Schena *et al.*'s review presents a grossly misleading portrayal of the historical origins of microarray technology and of some of the techniques and concepts on which it relies. Most importantly, the authors misrepresent the ideas that led to the development of microarrays for analytical use, these being unrelated to 'early experiments on solid surfaces' dating from the early 1990s.

In reality, the key concept underlying these techniques' emergence was that high sensitivities are achievable using far smaller amounts of 'binding agent' (located at a high surface density on a solid support) than have, for decades, been regarded as obligatory. [It was widely believed in the early 1990s that, to achieve high sensitivity, it was necessary 'to bind the majority of the analyte present in a (test) sample'¹⁶. Few, if any, microarray methods conform to this concept.]

These methods in fact stemmed from our original recognition that, using high-specific-activity (e.g. fluorescent) labels, sufficient 'capture' agent could be accommodated on a 'microspot' a few μm in diameter to achieve ultrasensitive detection of a target analyte. This permitted the construction of microarrays, each microspot therein recognizing a different analyte. Thus, long before 1989, we had – using simple microspotting and confocal-scanning techniques – demonstrated, described and patented the construction and use of sensitive microarray-based assays,

these events long preceding the development of the particular method of array production that Schena *et al.* claim as having constituted the technology's genesis. Boehringer Mannheim has since gone much of the way towards industrializing the technology and making it available for use across a wide spectrum of diagnostic applications.

References

- 1 Schena, S., Heller, R. A., Theriault, T. P., Konrad, K., Lachenmeier, E. and Davis, R. W. (1998) *Trends Biotechnol.* 16, 301–306
- 2 Polsky-Cynkin, R., Parsons, G. H., Allerdt, L., Landes, G., Davis, G. and Rashtchian, A. (1985) *Clin. Chem.* 31, 1438–1443
- 3 Ekins, R. P. (1987) UK Patent Application 8 803 000
- 4 Ekins, R., Chu, F. and Micallef, J. (1989) *J. Biolumin. Chemilumin.* 4, 59–78
- 5 Ekins, R., Chu, F. and Biggart, E. (1989) *Anal. Chim. Acta* 227, 73–96
- 6 Ekins, R. (1988) *Ligand Q.* 7, 1–21
- 7 Ekins, R. P. and Chu, F. W. (1991) *Clin. Chem.* 37, 1956–1967
- 8 Ekins, R. P. and Chu, F. W. (1994) *Trends Biotechnol.* 12, 89–94
- 9 Ekins, R. P. and Chu, F. W. (1995) *Immunoanalysis of Agrochemicals: Emerging Technologies* (Nelson, J. O., Karu, A. E. and Wong, R. B., eds), pp. 153–174, American Chemical Society, Washington, DC, USA
- 10 Chu, F. W., Edwards, P. R., Ekins, R. P., Berger, H., Finckh, P. and Krause, F. (1997) *Immunochemical Technologies for Environmental Applications* (Aga, D. S. and Thurman, E. M., eds), pp. 171–184, American Chemical Society, Washington, DC, USA
- 11 Ekins, R. P. (1998) *Clin. Chem.* 44, 2015–2030
- 12 Ekins, R., Krause, F., Chu, F., Berger, H. and Edwards, P. (1996) *J. Clin. Ligand Assay* 19, 145–156
- 13 Rosen, S. (1996) *Clinica* 704, 18
- 14 Ekins, R. P. and Chu, F. W. (1992) *Ann. Biol. Clin.* 50, 337–353
- 15 Fodor, S. P. A., Read, J. L., Pirrung, M. C., Stryer, L., Tsai Lu, A. and Solas, D. (1991) *Science* 251, 767–773
- 16 Hay, I. D., Bayer, M. F., Kaplan, M. M., Klee, G. G., Larsen, P. R. and Spencer, C. A. (1991) *Clin. Chem.* 37, 2002–2008

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