Nanoarrays as an ultraminiaturized platform for novel screening applications

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Abstract

Sample volume and target protein abundance are frequent limitations in proteomic analyses. The elimination of those restrictions has the potential to enable new applications in biomarker screening and detection, disease progression profiling, and drug discovery. One logical solution to the problem is a further reduction in size beyond that currently achievable with microarray technology. The benefits to this approach include significant decreases in analyte volume and reagent costs, as well as increases in sensitivity and reaction speed due to the kinetic advantages of miniaturization.

BioForce Nanosciences has developed a dedicated instrument, the NanoArrayer™, for the construction of ultraminiaturized arrays of biomolecules on the sub-micron and micron scale. With droplet volumes in the attoliter to femtoliter range, these nanoarrays occupy a surface area thousands of times smaller than a standard microarray. The analyte volume required for analysis is drastically reduced, with typical assays consuming 1µl or less. This spatial size is ideal because it allows for the development of novel applications such as single cell proteomic analysis while permitting acquisition of statistically meaningful data by a number of methods. Nanoarrays may be analyzed using conventional techniques such as fluorescence microscopy, as well as forward-looking, label-free methods such as atomic force microscopy.

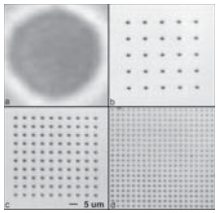


Figure 1.
(a) A state-of-the-art 50 µm microarray spot.
(b) A 5 x 5 nanoarray with 10 µm spot to spot pitch.
(c) A 10 x 10 nanoarray with 5 µm spot to spot pitch.
(d) A 20 x 20 nanoarray with 3 µm spot to spot pitch.

Methods

Arraying: All capture antibodies were diluted 1:1 in protein spotting buffer and arrayed at 5x5x10 µm spacing, with a minimum of 5 arrays/chip. Arraying was performed with a NanoArrayer (BioForce Nanosciences), which employs microfabricated surface patterning tools (SPTs) to actively transfer tiny droplets of fluid to the surface. Spot diameter is user controlled, and ranges from <1 µm to 20 µm.

All surfaces were Prolinker SAM on gold coated, alphanumeric indexed silicon chips (4mm x 4mm). For cross-reactivity studies 9 chips per capture antibody were arrayed. For the capture antibody sensitivity studies 10 chips per capture antibody (2 chips/dilution) were used. Antibody arrays constructed in this fashion were incubated overnight at 70% humidity.

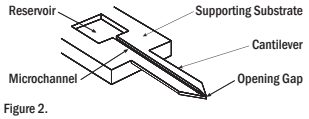


Figure 2.
Diagram of a quill-type surface patterning tool (SPT). Samples can be back-loaded into the reservoir or front-loaded by immersing the distal end in a microfluidic loading chip (not shown).

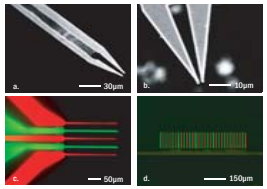


Figure 3.
(a) SEM image of a quill-type SPT.
(b) Close-up SEM image of the end gap (1µm).
(c) Fluorescence image of 5 lever SPT loaded with two fluorescently labeled proteins.
(d) Fluorescence image of 30 lever SPT loaded with two fluorescently labeled proteins.

Methods

Antibody binding: The arrayed surfaces were blocked with 500 µl of 1X casein solution (Vector Laboratories) per well of a 24 well Costar plate at room temp on a rocker. Surfaces were then incubated with 10 µl of the corresponding protein for the cross reactivity studies and 1 µl for the capture antibody sensitivity studies at room temp for 1 hr in a humidified environment. All incubations and washes were executed in a 24 well plate on a rocker. The chips were washed in PBST two times for 5 min each (500 µl per well) and tagged with the corresponding biotin labeled antibody (diluted 1:1000 in PBST) for 1 hr at room temp. The chips were then washed in PBST 2 times for 5 min each and tagged with Streptavidin Alexa Fluor 594 (diluted 1:1000 in PBST) plus Alexa Fluor 488 rabbit anti-rat IgG or Alexa Fluor 488 goat anti-rabbit IgG corresponding to the capture antibody arrayed (diluted 1:1000 in PBST).
In the absence of Alexa Fluor 594 signal, Alexa Fluor 488 was used to ensure that the capture antibody was arrayed on the surface.
The surfaces were then washed three times in PBST.
First wash was 5 min, second wash was 20 min, then the chip was transferred to a new well of PBST and observed by a fluorescence microscope.

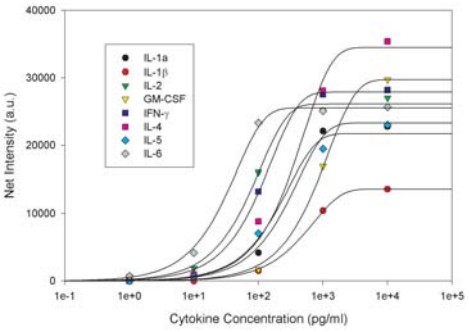
Imaging: A Nikon TE 2000U inverted microscope with 40X oil objective and filter sets for Alexa 488 and Alexa 594 dyes was used for fluorescence imaging. A cooled CCD digital camera with 1.3mp resolution was used to collect images. Metamorph was used for image capture and analysis. Array Pro Analyzer 4.5 and Microsoft Excel were used for data analysis and the results were plotted using Sigma Plot.

Figure 4.
The NanoArrayer system used to create these ultraminiaturized cytokine arrays.

Results

Cytokine Nanoarray Sensitivity

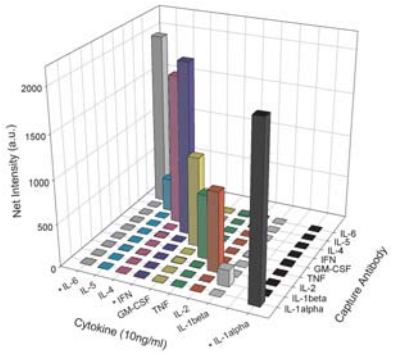
Figure 5.
Limits of detection for cytokine nanoarray assays using only 1 µl of analyte. IL-6 was detectable down to 1 pg/ml. At the sample volume of 1 µl, this equates to only 1 fg of protein (≈38 zmol). IL-1a, IL-2, IL-4, IL-5, and IFN-g were detectable at 10 pg/ml (10 fg protein). IL-1b and GM-CSF were detectable at 100 pg/ml (100 fg protein).



Results

Cytokine Nanoarray Specificity

Figure 6.
Cross-reactivity of antibody pairs used for the cytokine profiling nanoarrays. To enhance graphic representation of this data set, net intensities for IL-1a, IFN-g, and IL-6 were truncated to 2000 a.u. from 3152, 3668, and 3710 a.u. respectively. Antibody pairs were carefully selected from several vendors to ensure the high degree of selectivity shown here.



Conclusions

- The NanoArrayer is a useful tool for constructing ultraminiaturized protein arrays
- Nanoarrays can be used to assay protein samples as small as 1 µl
- Nanoarrays can detect concentrations matching or exceeding the sensitivity of ELISA techniques
- Nanoarrays can detect as little as 1 fg of protein (≈38 zmol), 50-fold less than ELISA techniques require
- With careful selection of antibody pairs, nanoarrays can offer extremely high selectivity

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