



Nanowell Arrays for High Throughput Functional Proteomics

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1 Post Genomic Challenge

- Can now determine & produce large #'s of gene products
- Critical next step -- ID function of these proteins
 - Determine protein biochemistry through exhaustive assays
 - simple assays on large #'s of proteins ($10^2 \dots 10^5$ proteins)
 - AND large #'s of these simple assays (# ?)

Bottlenecks ...

- chemical reaction must be allowed to take place
- reaction results must be assayed
- antibody tagging
- imaging via radioactive tagging or optical fluorescence

2 Nanowell Arrays

- High density format
- Proteins attached to each well
- Allows simple simultaneous batch processing and subsequent imaging

Designed to fit onto standard microscope slides

- compatibility with standard sample handling and imaging techniques

Cast in silicone elastomer (PDMS) from micromachined molds

- small feature sizes (1's mm ... 1's microns (or smaller))
- densities of greater than 10^4 wells per slide possible
- proteins attached to wells with crosslinker GPTS (Aldrich)
- 8×10^{-9} ug/ μm^2 may be attached to the surface in this way
- ($>> 8 \times 10^{-13}$ typical detection threshold for radio tagging)

3 Yeast Protein Kinase Assay

Used Nanowell Array technology to assay 119 (nearly all) of the protein kinases of *Saccharomyces cerevisiae* for ability to phosphorylate 17 different substrates

- simultaneous chemical reaction and assay of all proteins on single array
- included controls on chips

results published:
H. Zhu et al., *Nature Genetics*, vol. 26, Nov 2000, pp. 283-289.

Results

- 62 kinases exhibited preferential phosphorylation of 1 or 2 substrates
- a histidine protein kinase and phospholipid kinase phosphorylate protein substrates *in trans*
- 27 kinases readily phosphorylate poly (Tyr-Glu) (only 5 were previously known)
- many of these dual specificity kinases share common a.a. structures near catalytic region

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4 Conclusions

- Inexpensive, disposable microwell arrays were developed for high throughput screening of protein biochemical activity
- Microwell arrays are cast in silicone elastomer from micromachined molds
- Utility demonstrated through simultaneous analysis of 119 protein kinases from *Saccharomyces cerevisiae* assayed for phosphorylation of 17 different substrates
- Array densities may be readily increased several orders of magnitude

5 Immediate and Future Directions

- Continue working with present chips for simultaneous assays of 10^2 proteins
 - useful for functional subsets (e.g. protein kinases) of expressed proteins
- Demonstrate simultaneous assay of 10^3 proteins
 - e.g. *Saccharomyces cerevisiae* express a total of ~6300 proteins from genome (fully sequenced)
- Develop capability to increase density to 10^4 - 10^5 proteins (~100,000 proteins expressed by human genome)

• microwell array technology already scalable

• need to develop sample handling and appropriate measurement techniques