

# **Microarrays**

**New England Bioterrorism Preparedness Workshop**

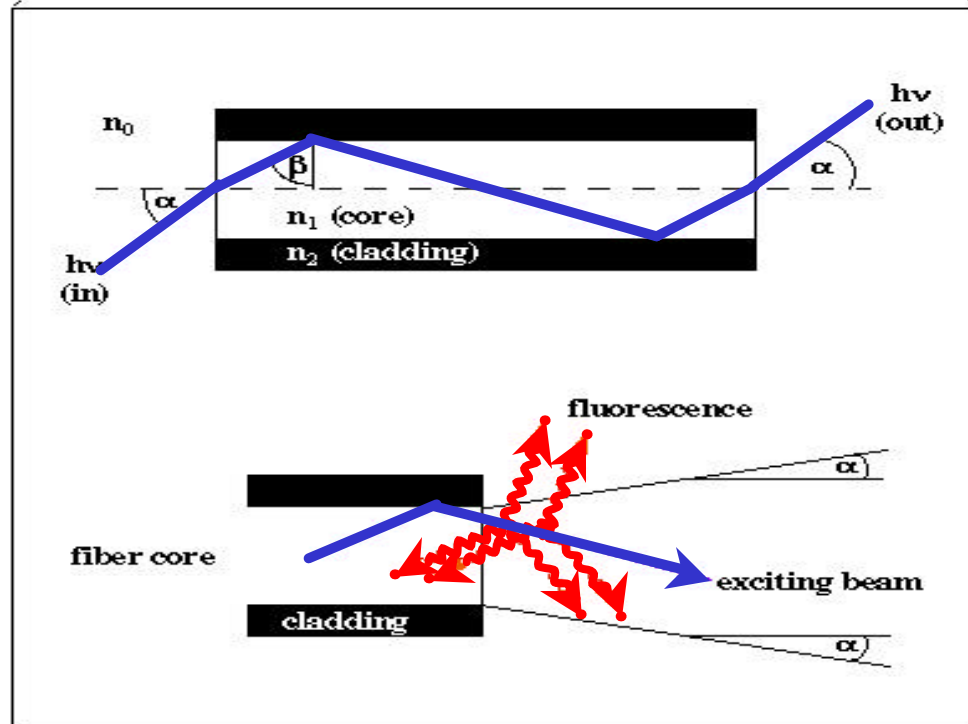
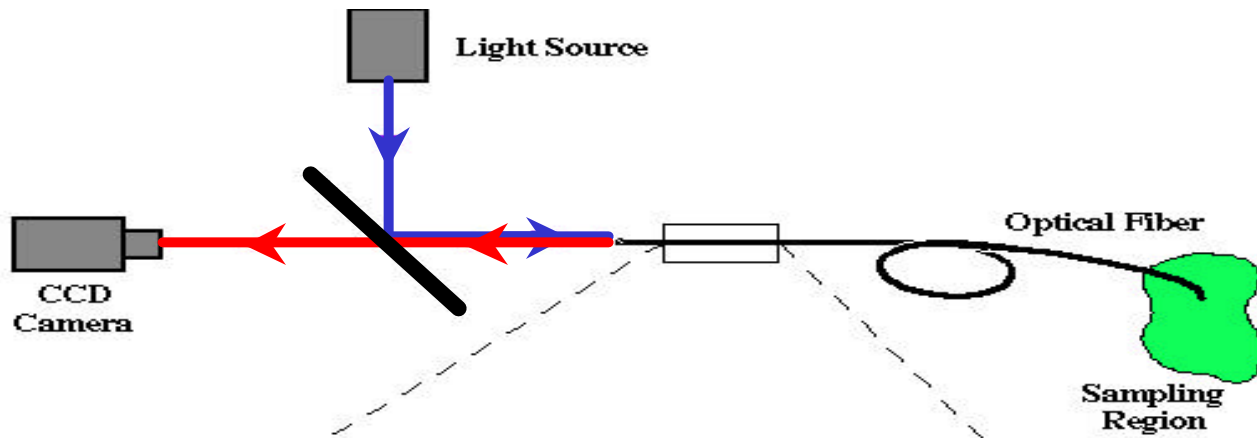
**Dr. David Walt**

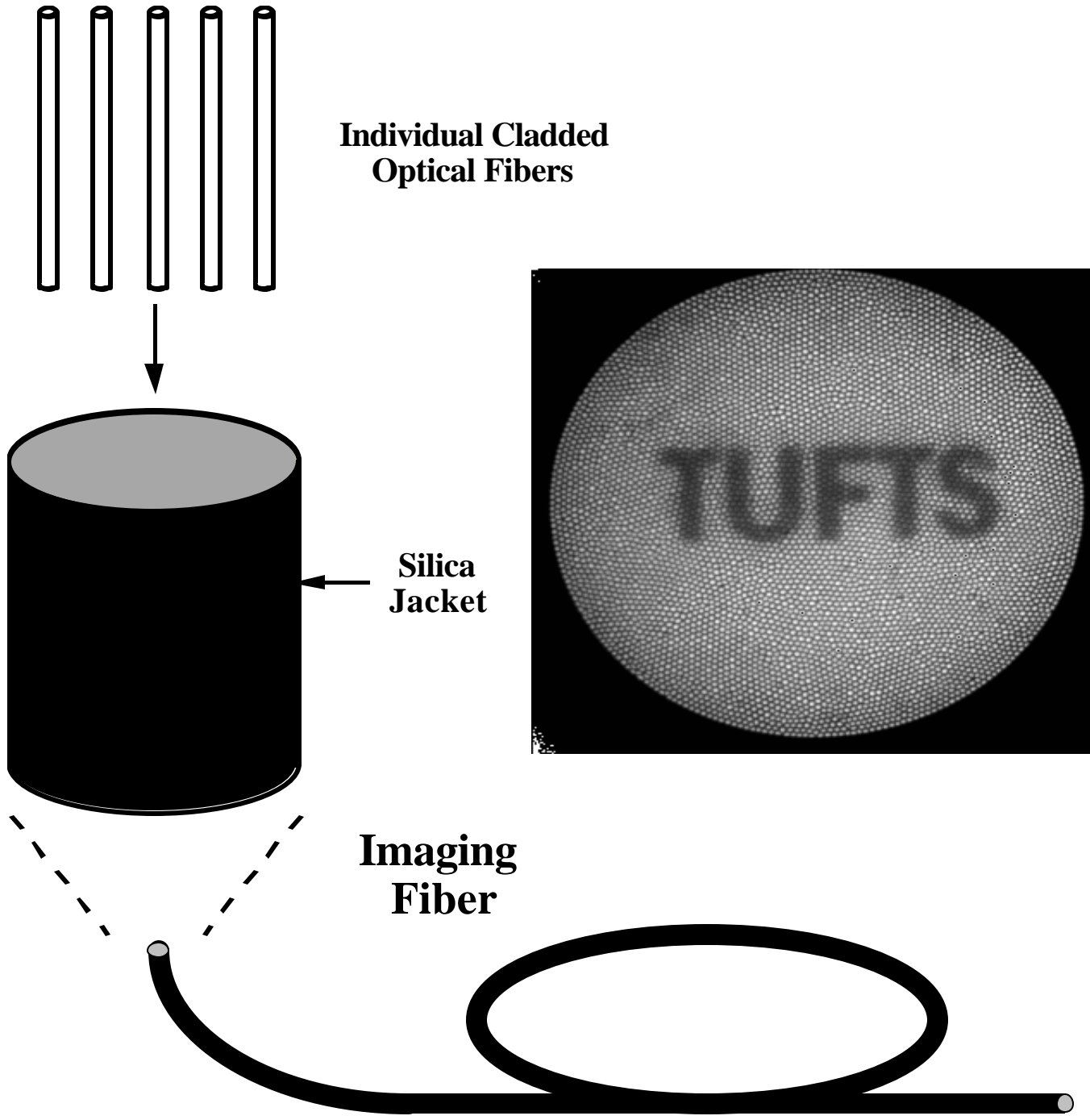
**Tufts University**

**4 April 2002**

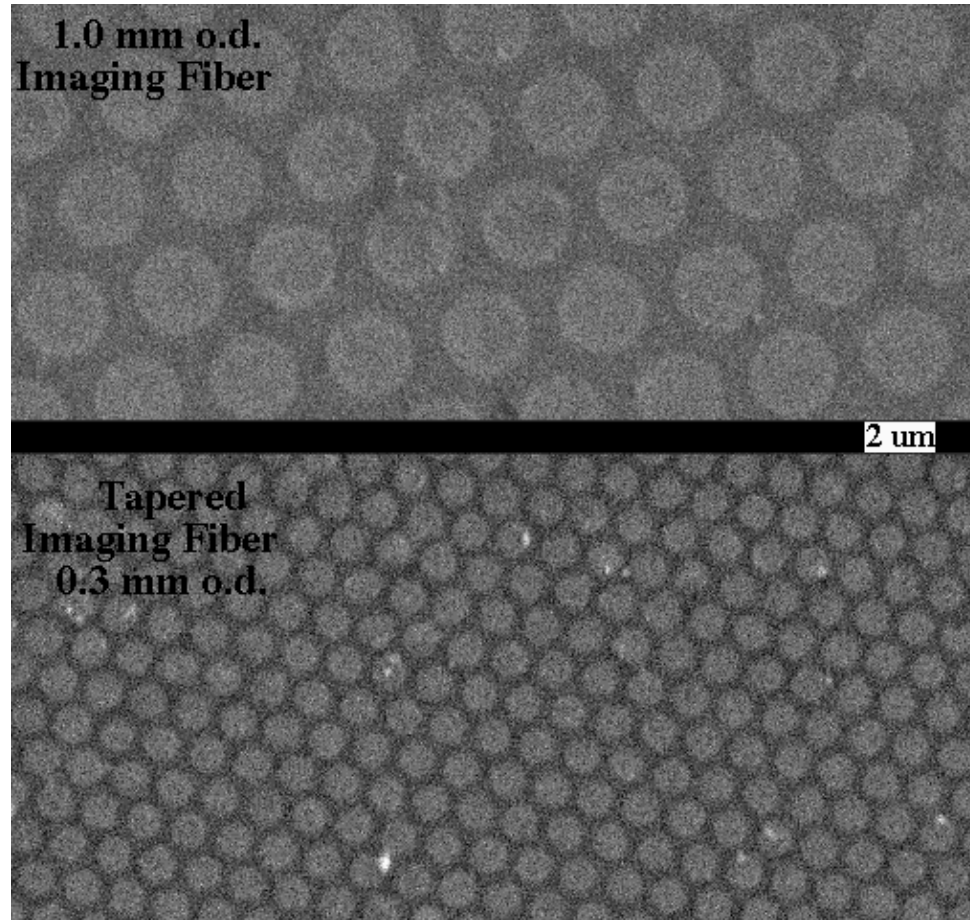
## Report Documentation Page

|   |  |  |
|---|--|--|
| <b>Report Date</b><br>04APR2002   | <b>Report Type</b><br>N/A                          | <b>Dates Covered (from... to)</b><br>03APR2002 - 04APR2002 |
| <b>Title and Subtitle</b><br>Microarrays  | <b>Contract Number</b><br>F19628-00-C-0002         |  |
|   | <b>Grant Number</b>                                |  |
|   | <b>Program Element Number</b>                      |  |
| <b>Author(s)</b><br>Walt, David   | <b>Project Number</b>                              |  |
|   | <b>Task Number</b>                                 |  |
|   | <b>Work Unit Number</b>                            |  |
| <b>Performing Organization Name(s) and Address(es)</b><br>Tufts University  | <b>Performing Organization Report Number</b>       |  |
| <b>Sponsoring/Monitoring Agency Name(s) and Address(es)</b><br>Air Force ESC/XPK (Richard Axtell) Hanscom AFB, MA<br>01731  | <b>Sponsor/Monitor's Acronym(s)</b>                |  |
|   | <b>Sponsor/Monitor's Report Number(s)</b>          |  |
| <b>Distribution/Availability Statement</b><br>Approved for public release, distribution unlimited   |  |  |
| <b>Supplementary Notes</b><br>Workshop paper from the New England Bioterrorism Preparedness Workshop held 3-4 april 2002 at MIT Lincoln Laboratory, Lexington, MA, The original document contains color images. |  |  |
| <b>Abstract</b>   |  |  |
| <b>Subject Terms</b>  |  |  |
| <b>Report Classification</b><br>unclassified  | <b>Classification of this page</b><br>unclassified |  |
| <b>Classification of Abstract</b><br>unclassified   | <b>Limitation of Abstract</b><br>SAR               |  |
| <b>Number of Pages</b><br>69  |  |  |





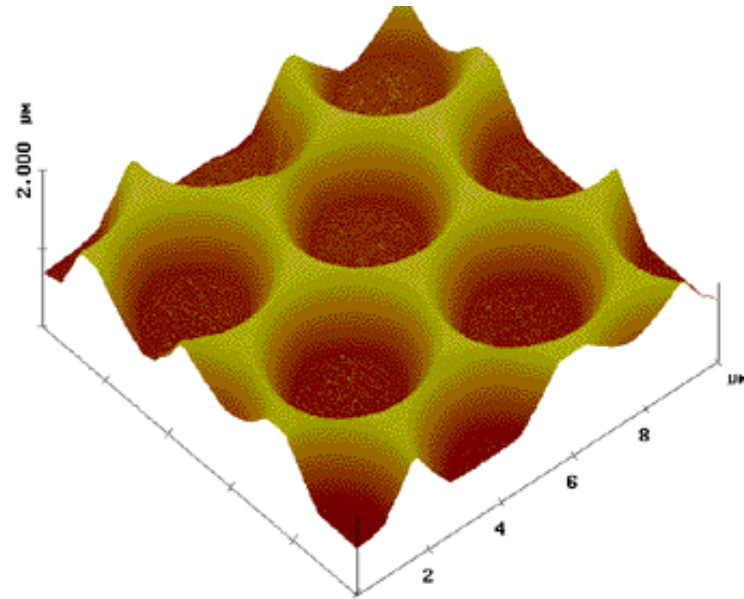
# Optical Imaging Fiber Before and After Tapering



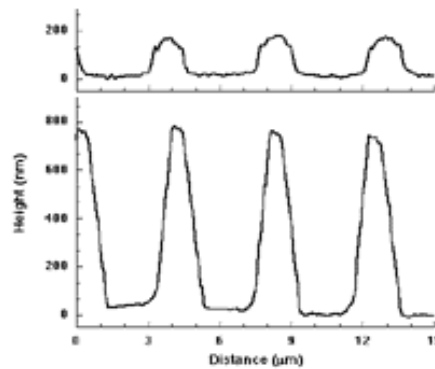
**Individual Core  
Diameter ~ 2.6  $\mu\text{m}$**

**Individual Core  
Diameter ~ 0.85  $\mu\text{m}$**

# AFM of a Chemically-Etched 1000- $\mu$ m Diameter Imaging Fiber



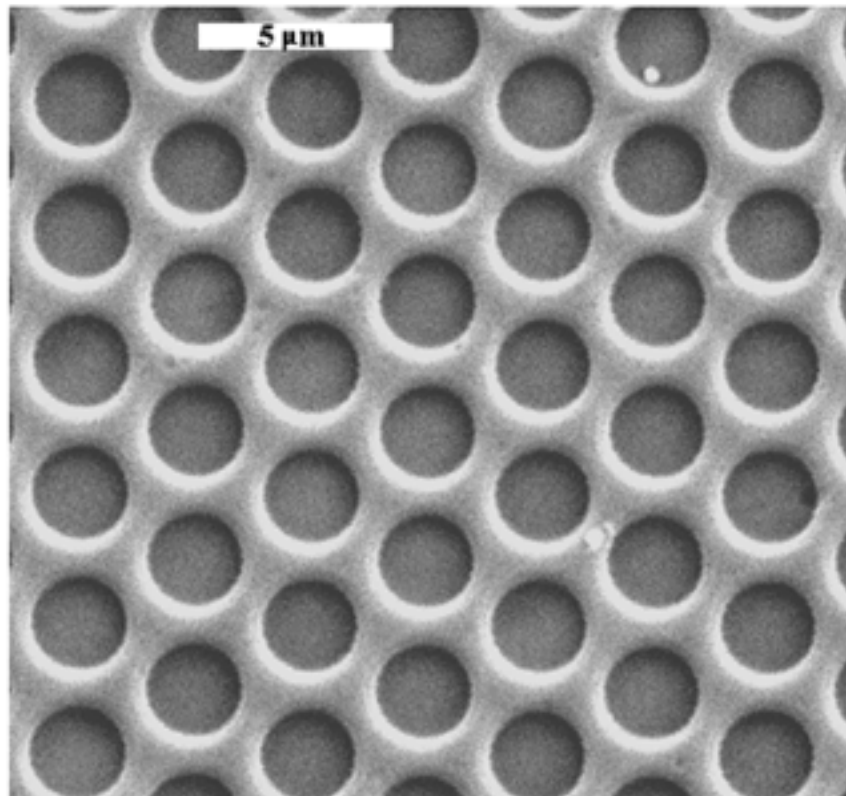
## Well Profiles



15 s etch

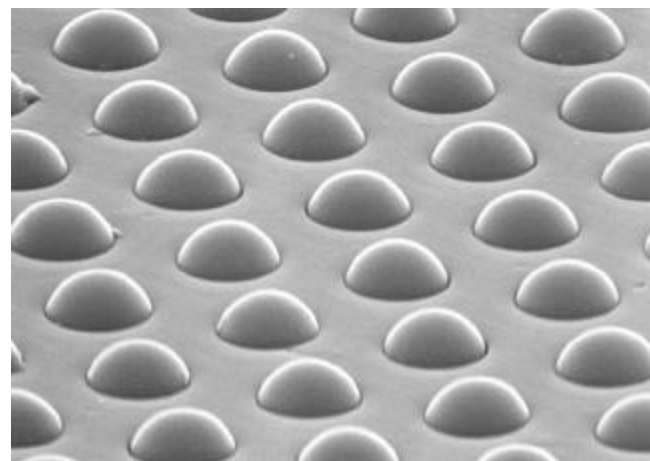
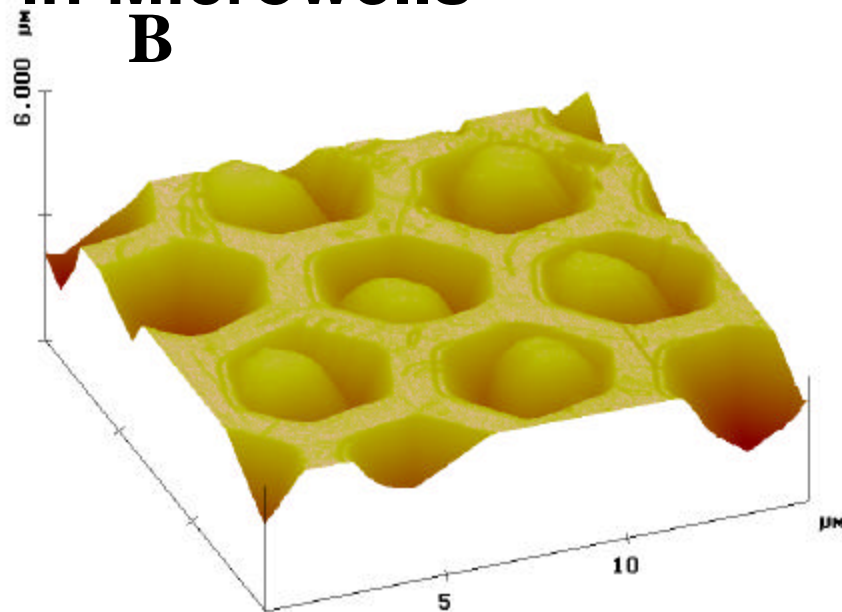
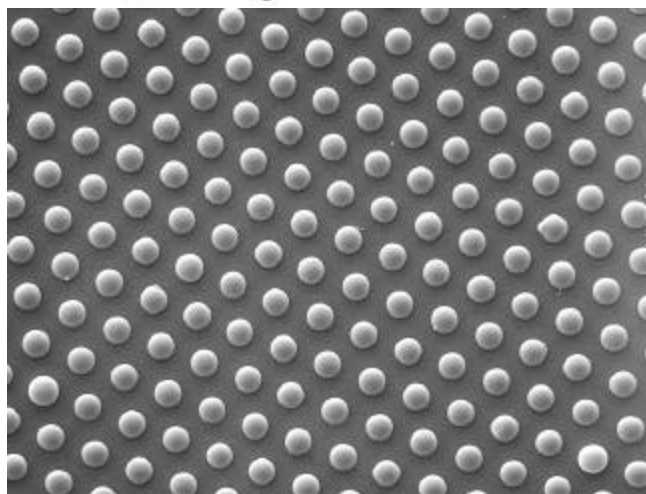
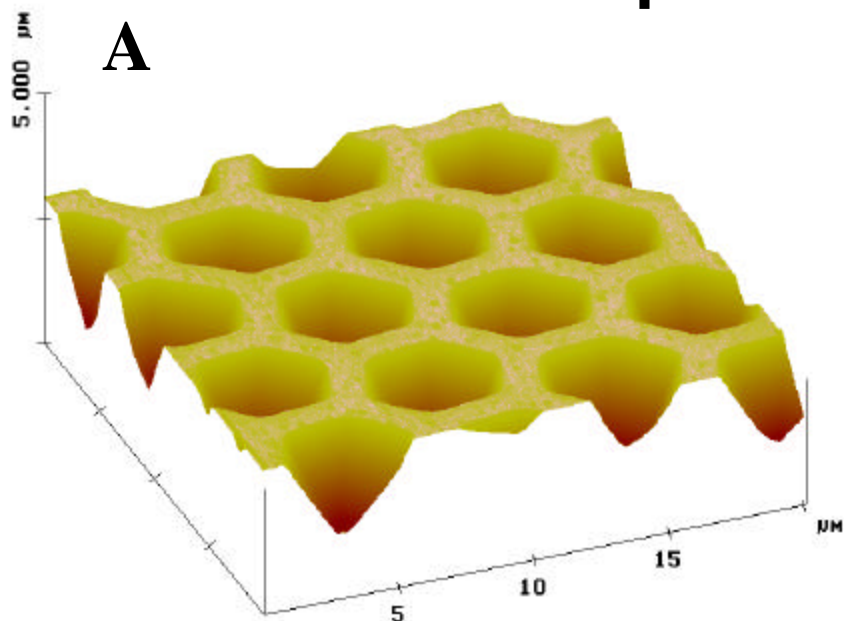
60 s etch

## SEM of a Chemically-Etched 1000- $\mu$ m Diameter Imaging Fiber

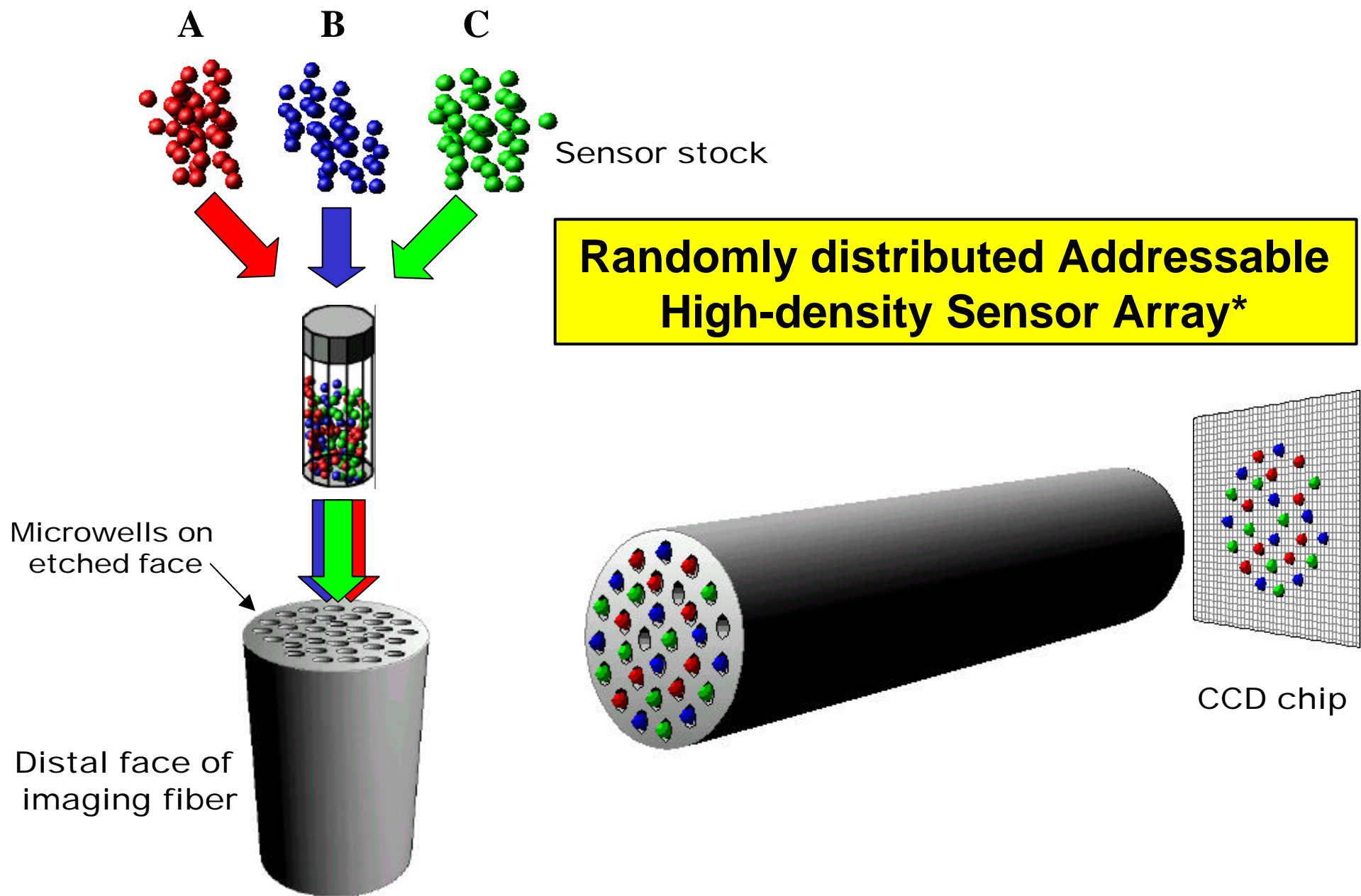


Pantano, P.; Walt, D.R. *Chem. Mater.* **1996**, 8, 2832-2835

# Microspheres in Microwells

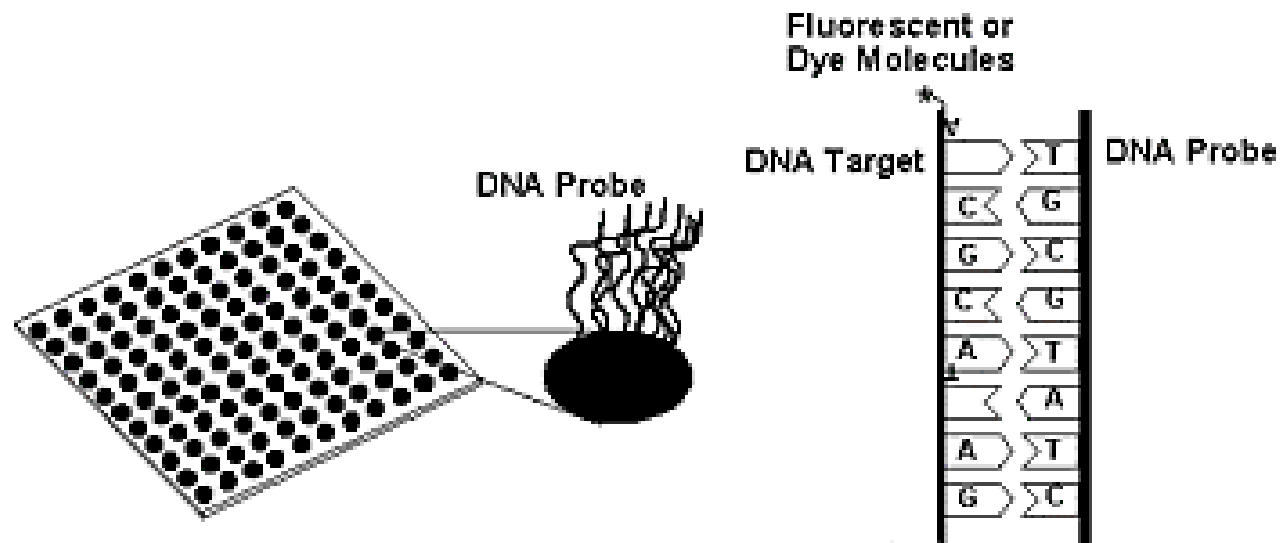


Michael, K.L. *et al. Anal. Chem.* 70 (7): 1242-1248 (1998).

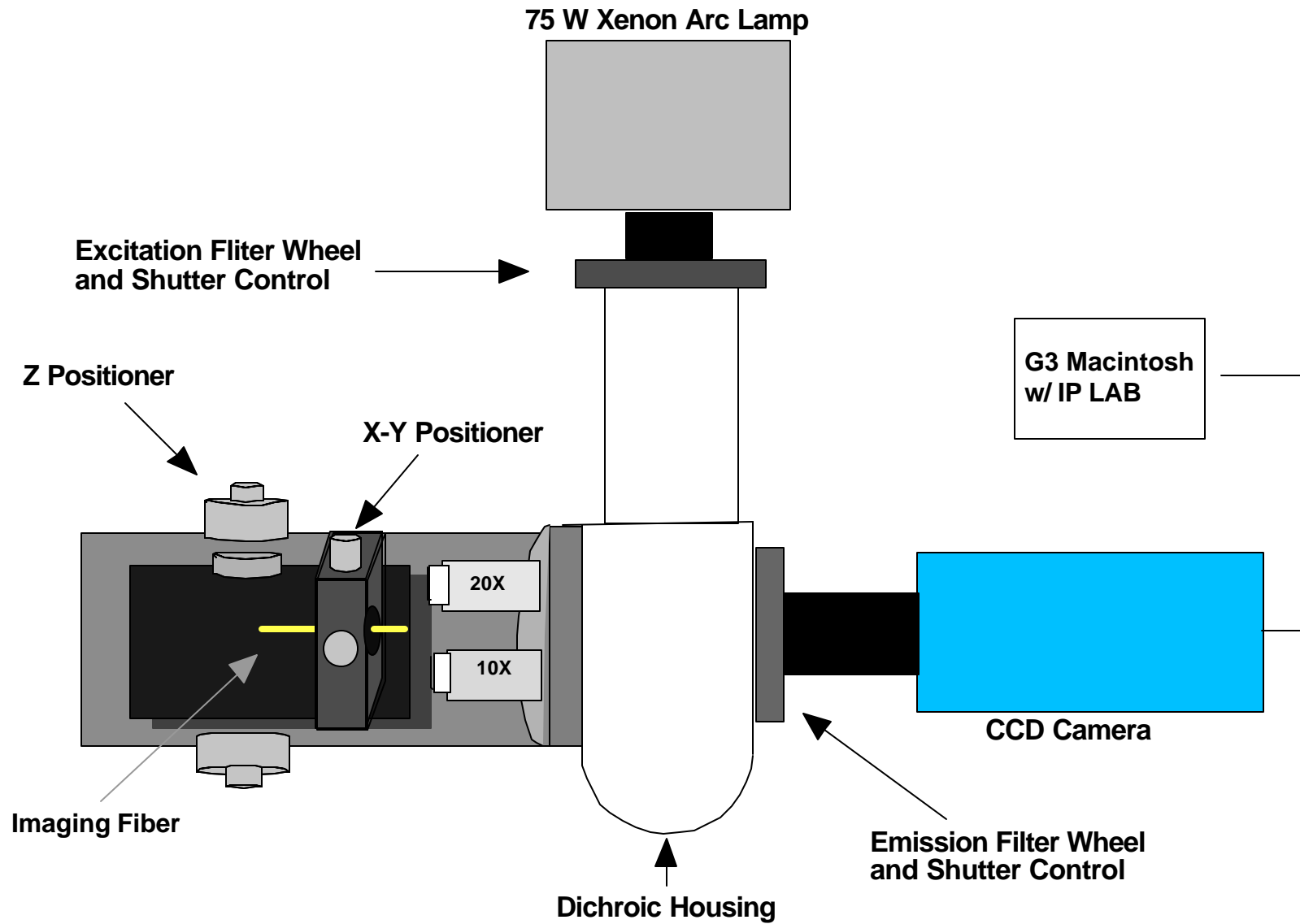


\*Michael et al. **1998** *Anal. Chem.* **70**: 1242-1248

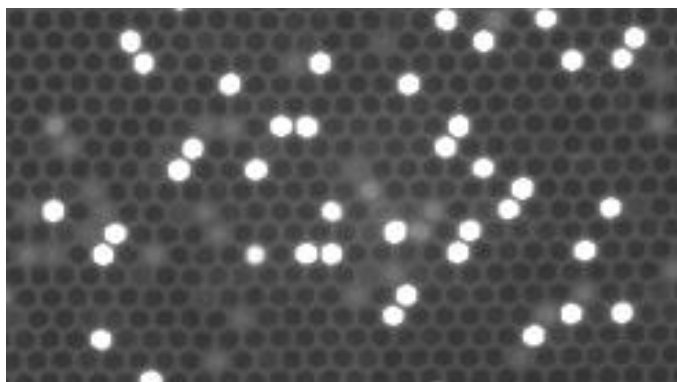
# DNA Array Principle



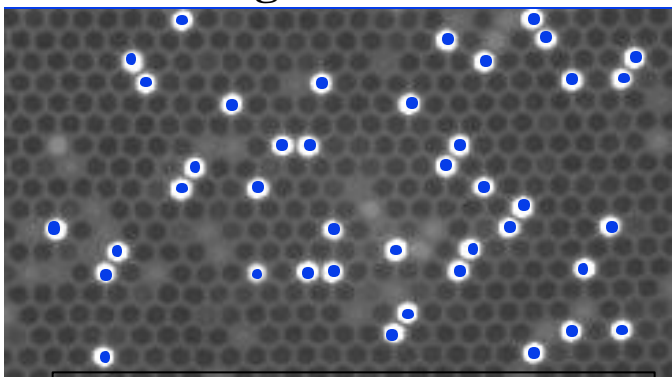
# Instrumentation: Modified Fluorescence Microscope



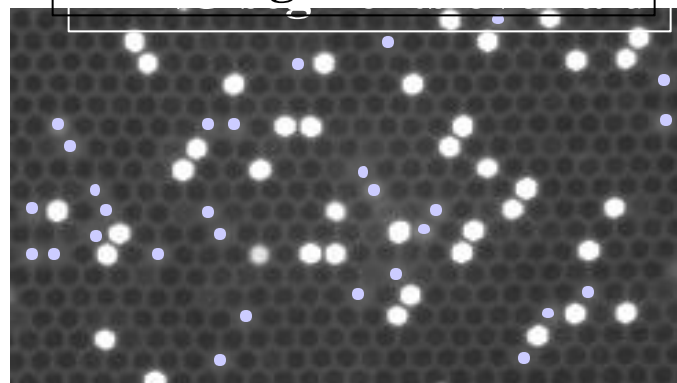
**Encoding Signal of Dye 1**



**Signal 530**

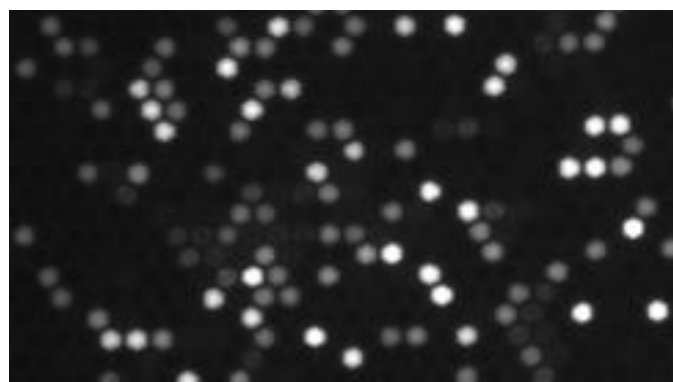


**IFNG segments overlaid**

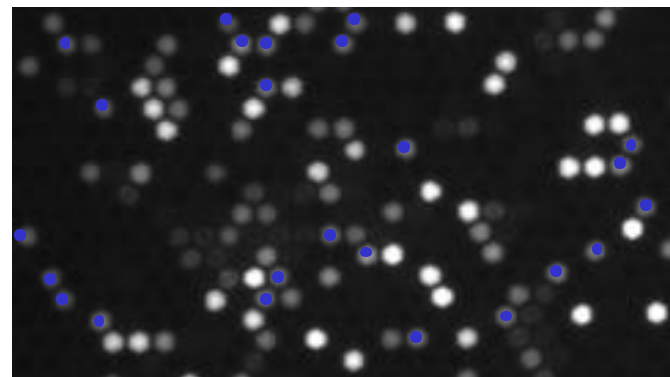


**IL2 segments overlaid**

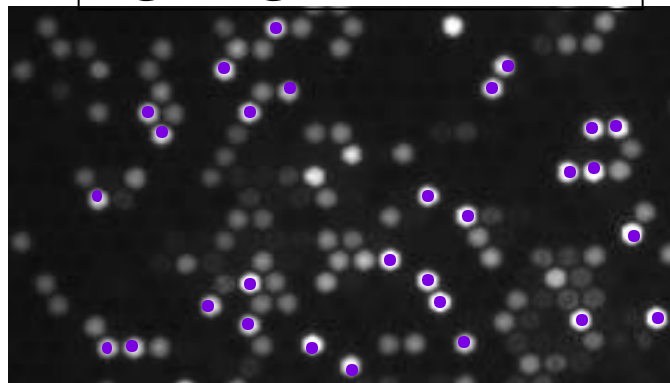
**Encoding Signal of Dye 2**



**Signal 620**



**Bglo segments overlaid**



**Hwt segments overlaid**

## Sequences of 25 Probes used together in a Microsphere Array

1) ?-glo (segment of human ?-globin)<sup>26</sup>  
TCA ACT TCA TCC ACG TTC ACC

2) IFNG (interferon gamma 1)<sup>26</sup>  
IFNG TGG GTT CTC TTG GCT GTT ACT

3) IL2 (interleukin-2)<sup>26</sup>  
TA CAA GAA TCC CAA ACT CAC CAG

4) IL4 (interleukin-4)<sup>26</sup>  
CC AAC TGC TTC CCC CTC TGT

5) IL6 (interleukin-6)<sup>26</sup>  
GT TGG GTC AGG GGT GGT TAT T

6) K-ras WT<sup>27</sup>  
GGA GCT GGT GGC GTA

7) H-ras WT<sup>27</sup>  
CCG GCG GTG T

8) CFTR (cystic fibrosis exon 11)<sup>13</sup>  
CAT TAT ACT TGT AGA G

9) R553X (cystic fibrosis exon 10)<sup>13</sup>  
TGT AGA ATT ATC TTC

10) PAN132<sup>16</sup> (human peripheral lymphocyte)  
CCT CTA TAC TTT AAC GTC AAG

11) Schena-2<sup>16</sup>  
AAG TTT AAC CTA TAC CCT GTC

12) Hakala-1<sup>20</sup>  
CCT ATG ATG AAT ATA G

13) Hakala-2<sup>20</sup>  
AAT ATG ATA ATG GCC T

14) complement to probe 1  
TG AAC GTG GAT GAA GTT G

15) complement to probe 2  
AG TAA CAG CCA AGA GAA CCC AAA

16) complement to probe 3  
CT GGT GAG TTT GGG ATT CTT GTA

17) complement to probe 4  
AC AGA GGG GGA AGC AGT TGG

18) complement to probe 5  
AA TAA CCA CCC CTG ACC CAA C

19) complement to probe 6  
TAC GCC ACC AGC TCC

20) complement to probe 7  
ACA CCG CCG G

21) complement to probe 8  
CTC TAC AAG TAT AAT G

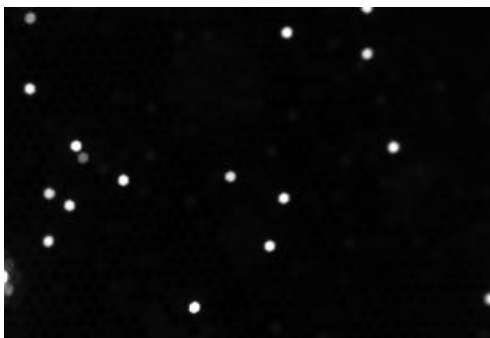
22) complement to probe 9  
GAA GAT GTT AAA GTA TAG AGG

23) complement to probe 10  
CTA GAC GTT AAA GTA TAG AGG

24) complement to probe 12  
CTA TAT TCA TCA TAG G

25) complement to probe 13  
AGG CCA TTA TCA TAT T

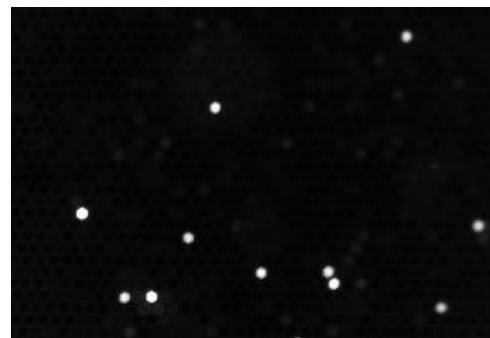
2



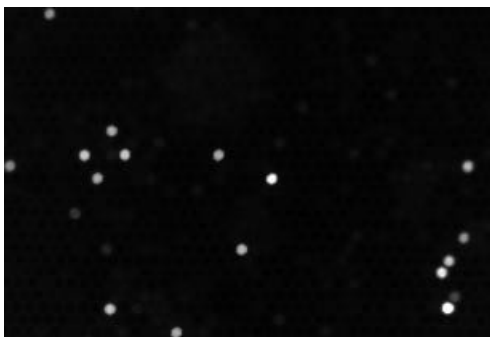
15



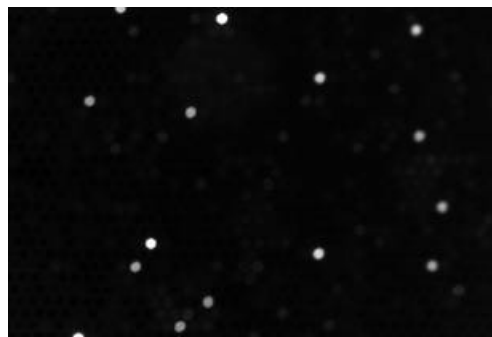
14



3



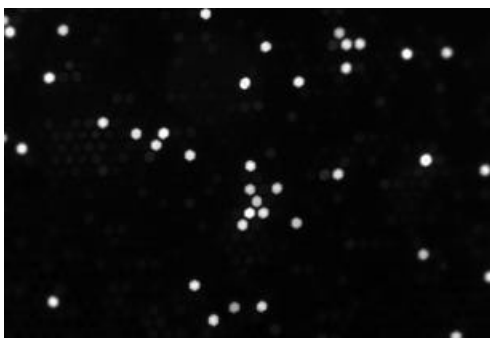
18



11



6



23



9



8



24



5

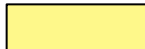


# *E. coli* Allelic Discrimination

## ycgW locus\*

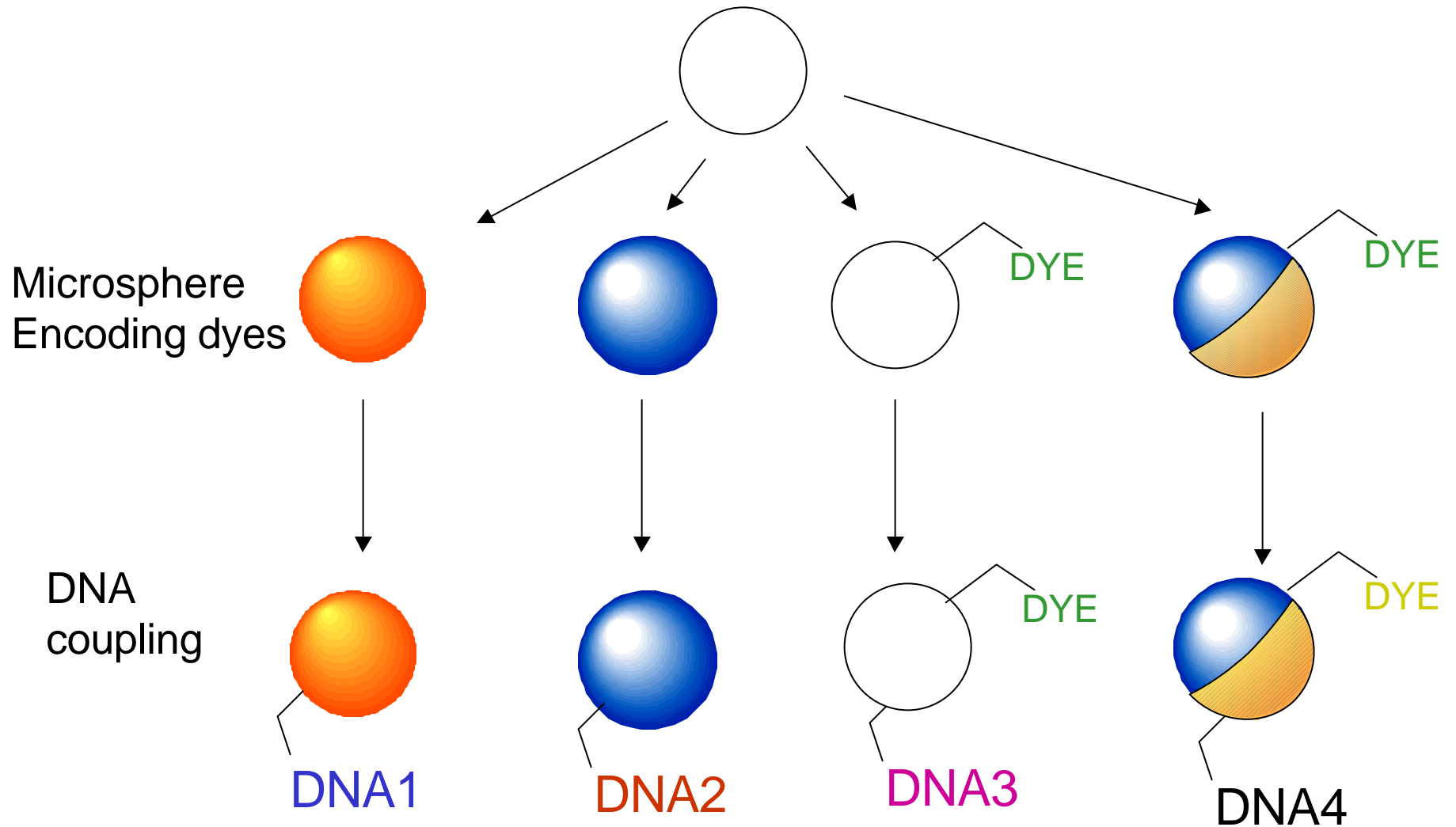
| No. | strain   | strain sequence             |  |  |  |  |  |  |  |  | No. | strain   | strain sequence             |  |  |  |  |  |  |  |  |
|-----|----------|-----------------------------|--|--|--|--|--|--|--|--|-----|----------|-----------------------------|--|--|--|--|--|--|--|--|
| 1   | BSR9b    | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  | 19  | HER1265  | no PCR product              |  |  |  |  |  |  |  |  |
| 2   | BSR9c    | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  | 20  | HER1266  | no PCR product              |  |  |  |  |  |  |  |  |
| 3   | "ETEC"   | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  | 21  | EC68     | no PCR product              |  |  |  |  |  |  |  |  |
| 4   | O111NM   | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  | 22  | EC69     | T T T T T T G A G G G G     |  |  |  |  |  |  |  |  |
| 5   | O113:H2  | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  | 23  | EC63     | no PCR product              |  |  |  |  |  |  |  |  |
| 6   | O157NM   | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  | 24  | EC54     | no PCR product              |  |  |  |  |  |  |  |  |
| 7   | HER1058  | no PCR product              |  |  |  |  |  |  |  |  | 27  | O86:H10  | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  |
| 8   | K12DH5a  | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  | 37  | O86:H18  | T T T G T T T T T T G       |  |  |  |  |  |  |  |  |
| 9   | K12W4100 | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  | 30  | O8:H9    | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  |
| 10  | O55:H7   | T T T T C G A A G G G G     |  |  |  |  |  |  |  |  | 34  | O9:H33   | T T T T T T G A G G A G     |  |  |  |  |  |  |  |  |
| 11  | "EPEC"   | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  | 38  | O153:H-  | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  |
| 12  | K12W3110 | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  | 43  | O26:H11  | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  |
| 13  | O22:H8   | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  | 48  | O127:H21 | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  |
| 14  | O26:H-   | T T T T T T G A A G G G G   |  |  |  |  |  |  |  |  | 52  | EC1      | T T T T T T G A G G G G     |  |  |  |  |  |  |  |  |
| 15  | O42:H2   | T T T T T G A A G G G G     |  |  |  |  |  |  |  |  | 53  | EC7      | T T T T T T G A G G G G     |  |  |  |  |  |  |  |  |
| 16  | O157:H7  | no PCR product              |  |  |  |  |  |  |  |  | 54  | EC18     | T T T T T T G A G G A G     |  |  |  |  |  |  |  |  |
| 17  | HER1057  | no PCR product              |  |  |  |  |  |  |  |  | 55  | EC47     | T T T G T T T T T T G G     |  |  |  |  |  |  |  |  |
| 18  | HER1261  | no PCR product              |  |  |  |  |  |  |  |  | 56  | EC52     | no PCR product              |  |  |  |  |  |  |  |  |
|     | Cons.    | T T T * * * * * * * * * * G |  |  |  |  |  |  |  |  |     | Cons.    | T T T * * * * * * * * * * G |  |  |  |  |  |  |  |  |

 = probe 1 signal

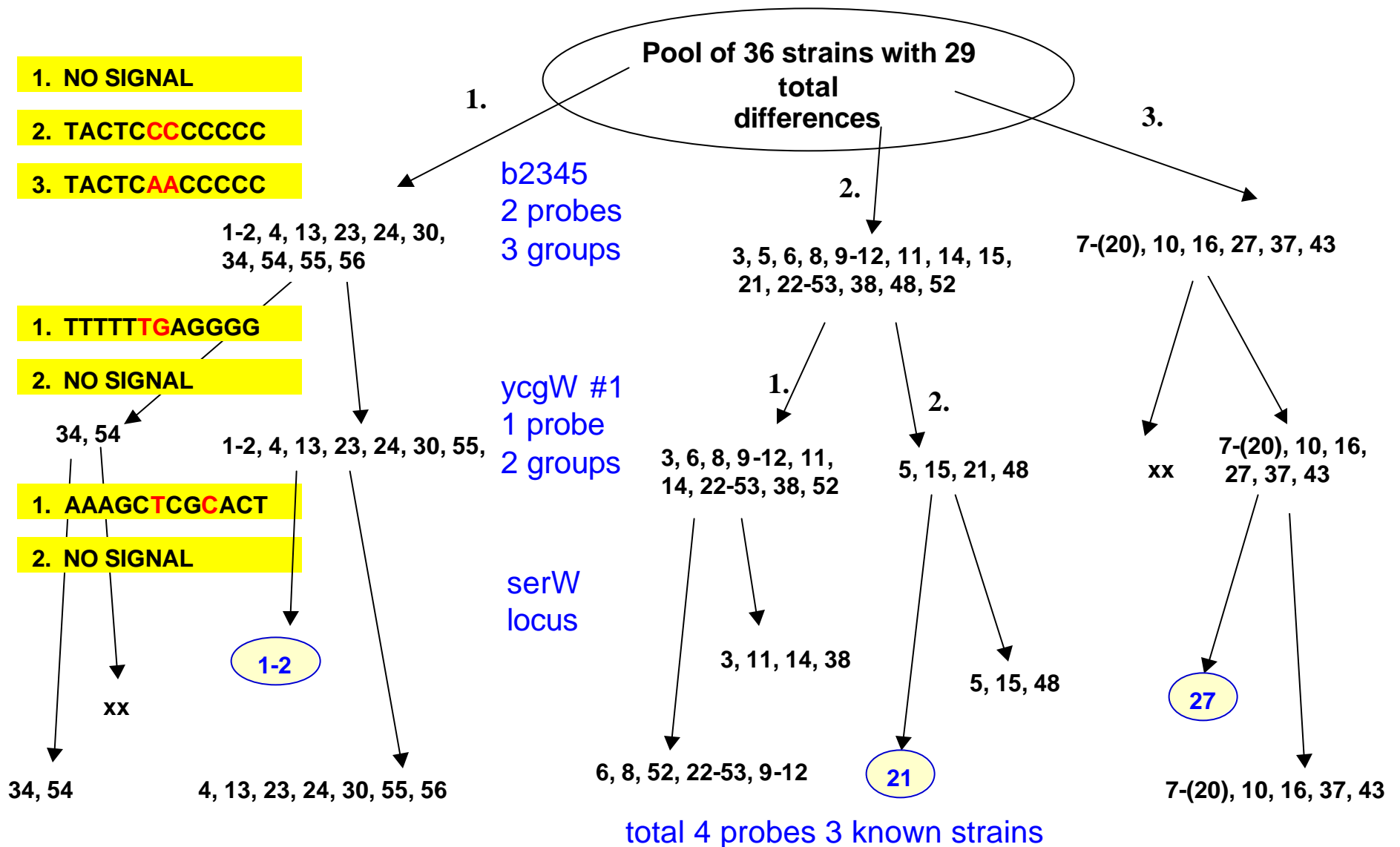
 = no signal

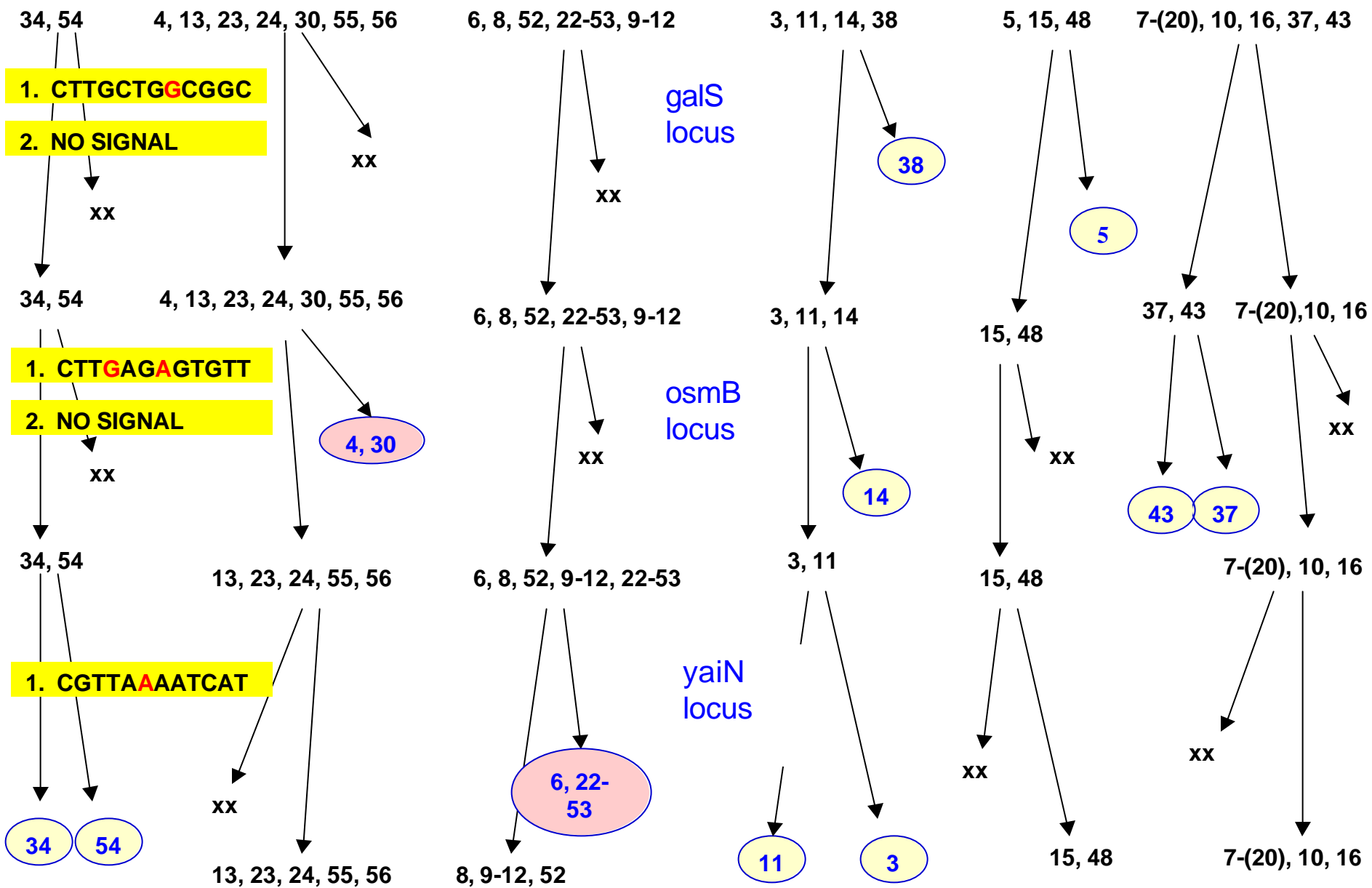
\*ycgW locus is 77 nucleotides long

# Microsphere Functionalization

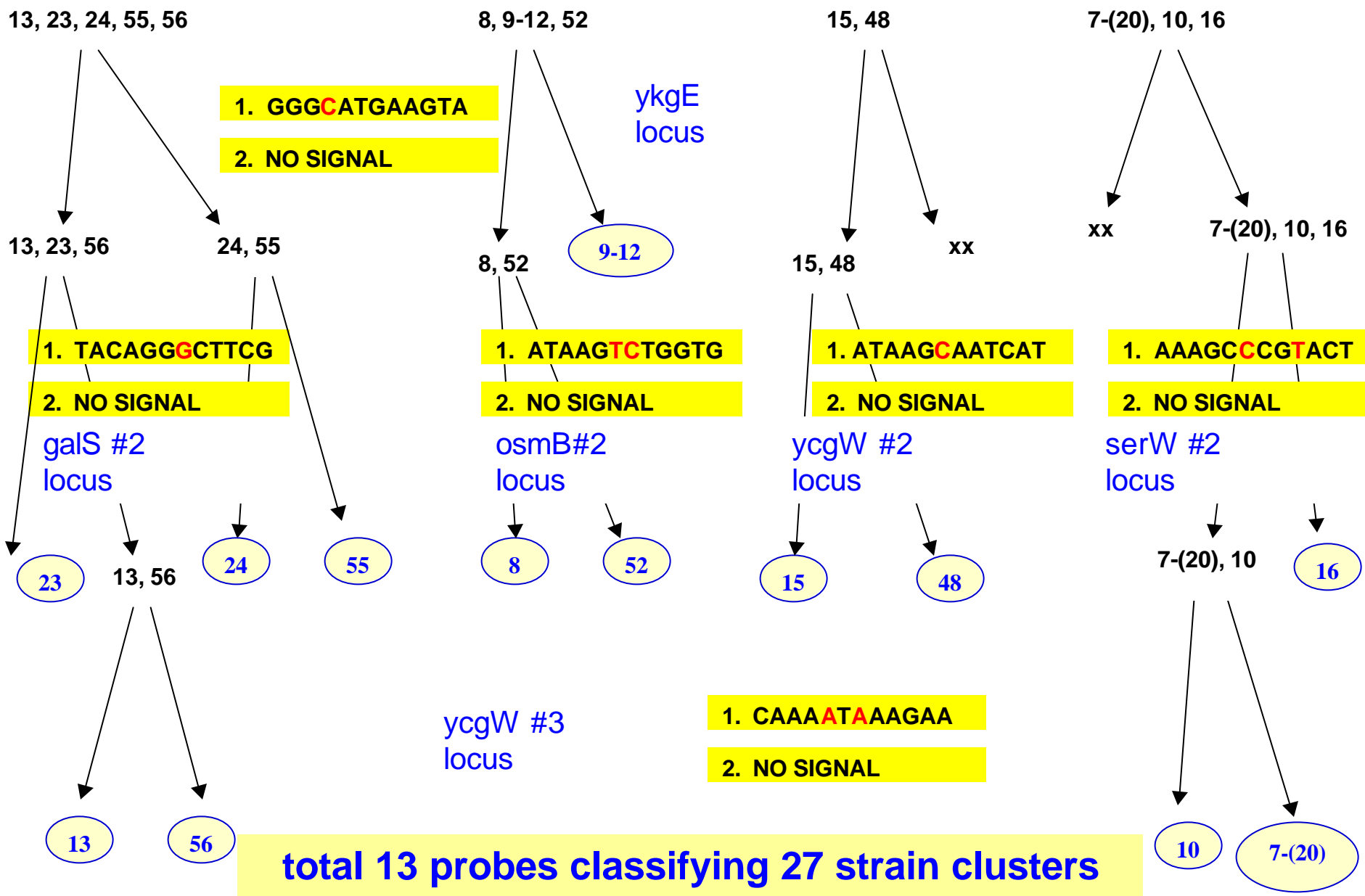


# *E. coli* Genomic Discrimination Flowchart






total 7 probes 12+ known strains



# *E. coli* Genomic Pattern Response

| No. | strain   | ycgW            | serW            | osmB            | yaiN            | ykgE            |
|-----|----------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 14  | O26:H-   | signal response | no signal       | no signal       | signal response | signal response |
| 11  | "EPEC"   | signal response | no signal       | signal response | signal response | signal response |
| 3   | "ETEC"   | signal response | no signal       | signal response | no signal       | signal response |
| 6   | O157NM   | signal response | signal response | signal response | no signal       | signal response |
| 22  | EC69     | signal response | signal response | signal response | no signal       | signal response |
| 9   | K12W4100 | signal response | signal response | signal response | signal response | no signal       |
| 12  | K12W3110 | signal response | signal response | signal response | signal response | no signal       |
| 8   | K12DH5a  | signal response | signal response | signal response | signal response | signal response |


 = signal response

 = no signal

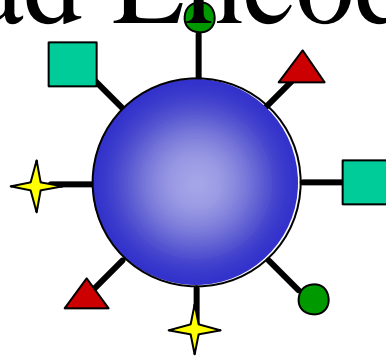
# *E. coli* Genomic Pattern Response

| No. | strain  | ycgW      | b2345           | serW            | galS            | Osmb            | yKgE            | ycgW#3          | serW#2          |
|-----|---------|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 21  | EC68    | no signal | signal response | signal response |                 |                 |                 |                 |                 |
| 1   | BSR9b   | no signal | no signal       | signal response |                 |                 |                 |                 |                 |
| 2   | BSR9c   | no signal | no signal       | signal response |                 |                 |                 |                 |                 |
| 15  | O42:H2  | no signal | signal response | no signal       | signal response |                 |                 |                 |                 |
| 5   | O113:H2 | no signal | signal response | no signal       | no signal       |                 |                 |                 |                 |
| 4   | O111NM  | no signal | no signal       | no signal       | signal response | no signal       |                 |                 |                 |
| 24  | EC54    | no signal | no signal       | no signal       | signal response | signal response | no signal       |                 |                 |
| 13  | O22:H8  | no signal | no signal       | no signal       | signal response | signal response | signal response | signal response |                 |
| 23  | EC63    | no signal | no signal       | no signal       | signal response | signal response | signal response | no signal       |                 |
| 10  | O55:H7  | no signal | no signal       | no signal       | no signal       | signal response | no signal       | signal response |                 |
| 16  | O157:H7 | no signal | no signal       | no signal       | no signal       | signal response | no signal       | no signal       | no signal       |
| 7   | HER1058 | no signal | no signal       | no signal       | no signal       | signal response | no signal       | no signal       | signal response |
| 17  | HER1057 | no signal | no signal       | no signal       | no signal       | signal response | no signal       | no signal       | signal response |
| 18  | HER1261 | no signal | no signal       | no signal       | no signal       | signal response | no signal       | no signal       | signal response |
| 19  | HER1265 | no signal | no signal       | no signal       | no signal       | signal response | no signal       | no signal       | signal response |
| 20  | HER1266 | no signal | no signal       | no signal       | no signal       | signal response | no signal       | no signal       | signal response |

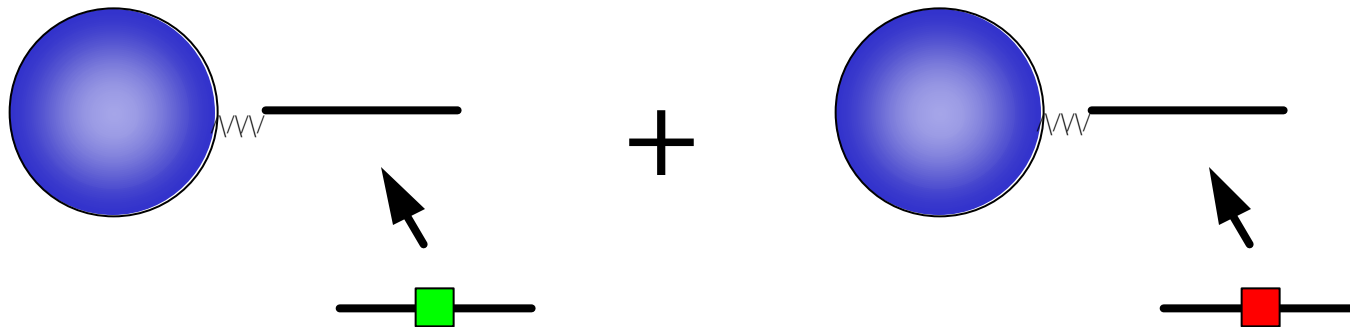
 = signal response

 = no signal

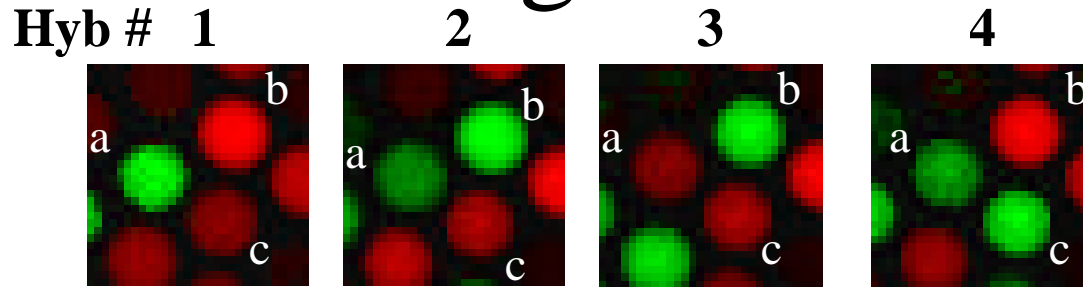
# Bead Encoding



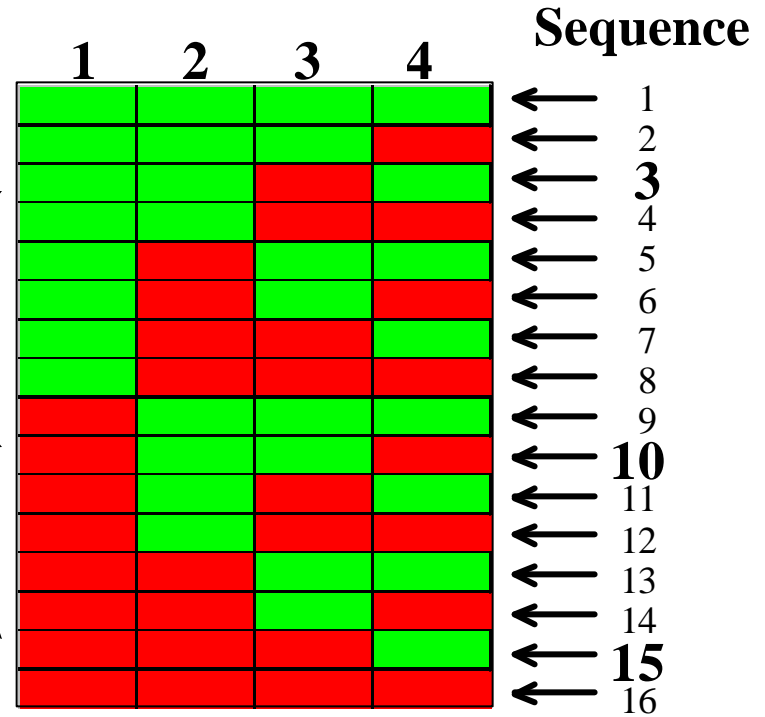
# Sequential Decoding



# Decoding 16 Probes



|          | Hyb.#1 | Hyb.#2 | Hyb.#3 | Hyb.#4 |
|----------|--------|--------|--------|--------|
| <b>a</b> | green  | green  | red    | green  |
| <b>b</b> | red    | green  | green  | red    |
| <b>c</b> | red    | red    | red    | green  |

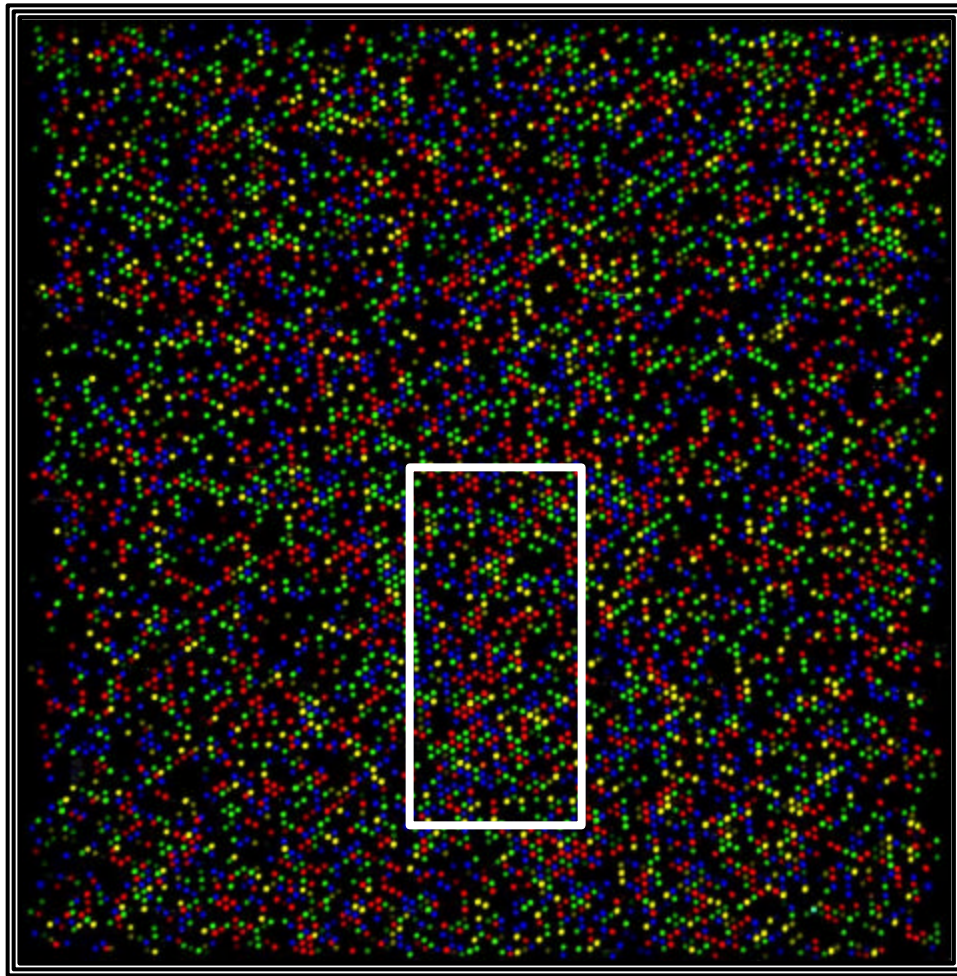


# Decoding is Exponential

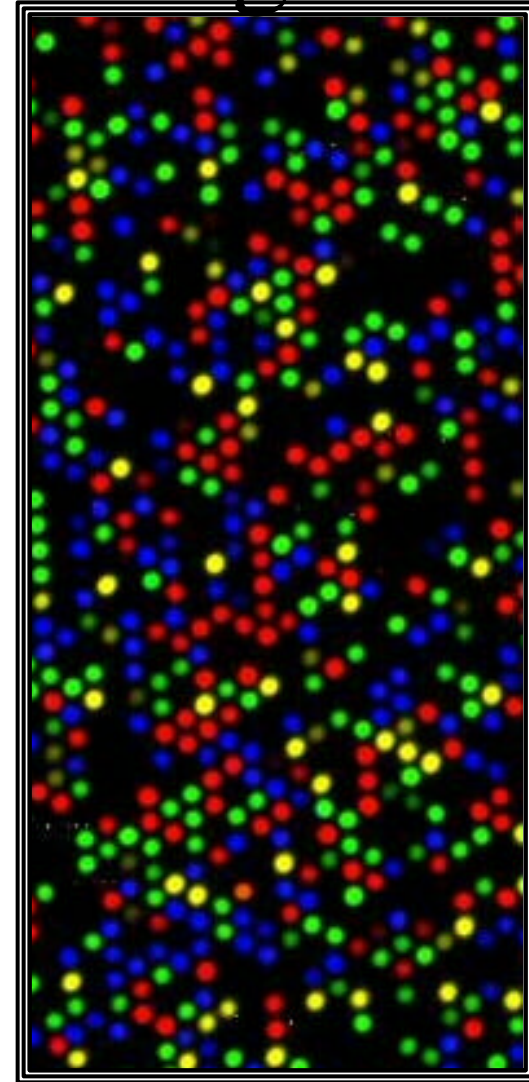
**$x$  labels ,  $z$  steps =  $x^z$  codes**

- **2** Dyes <sup>**4**</sup> Steps = **16** Codes
- **4** Dyes <sup>**6**</sup> Steps = **4,096** Codes

# Four-Color Decoding

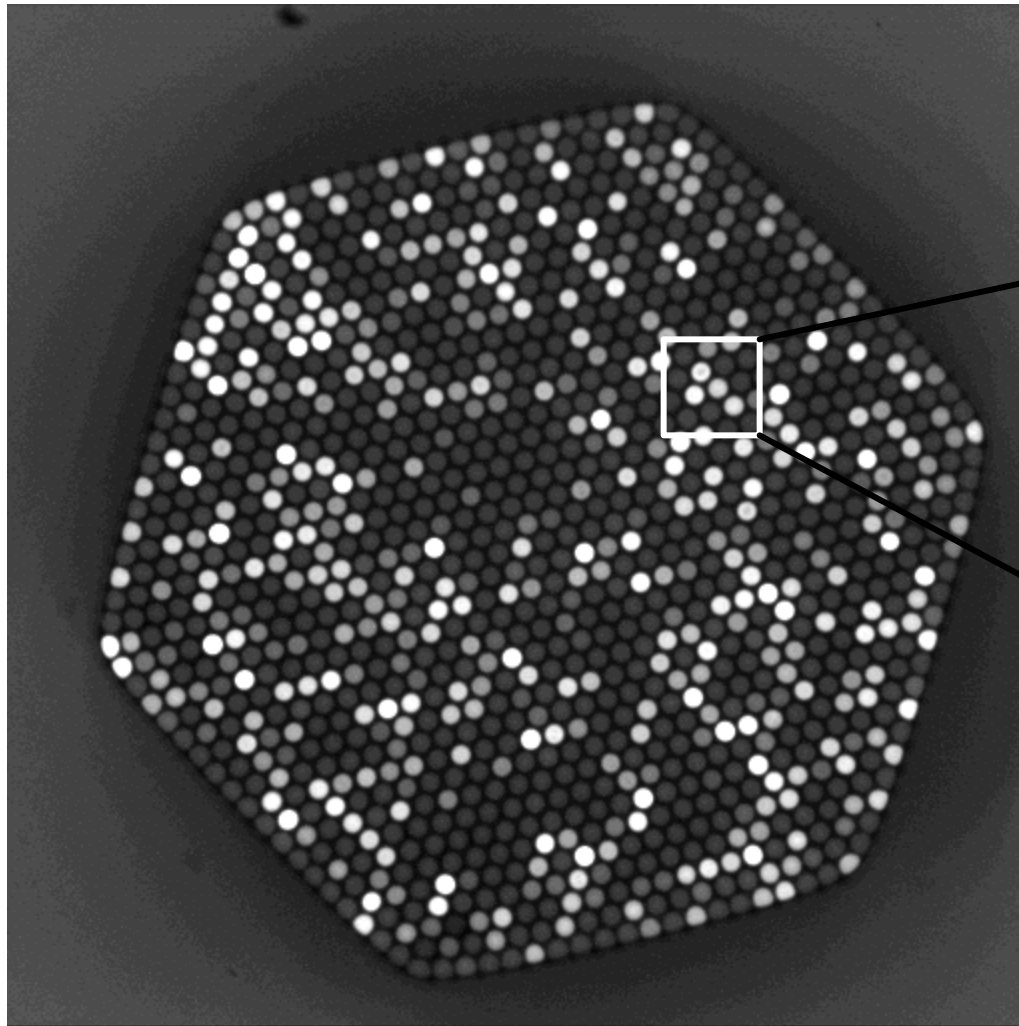


*~13,000 Wells, 16 Probe Sequences*

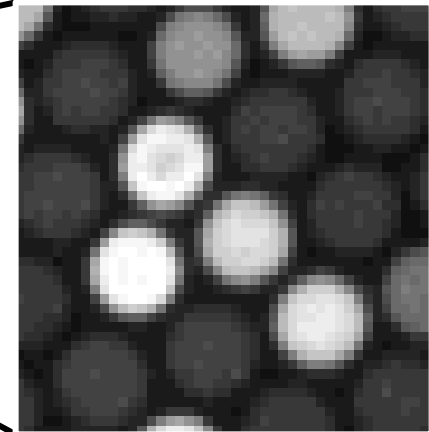


**D.R. Walt, *Science*, 2000**

# 1K Fiber Bundle



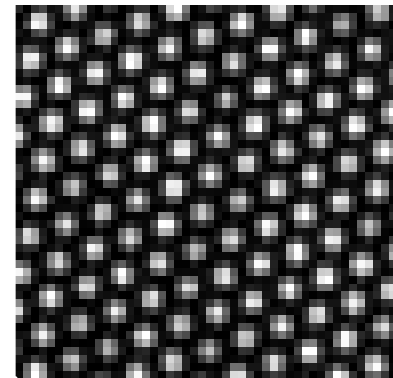
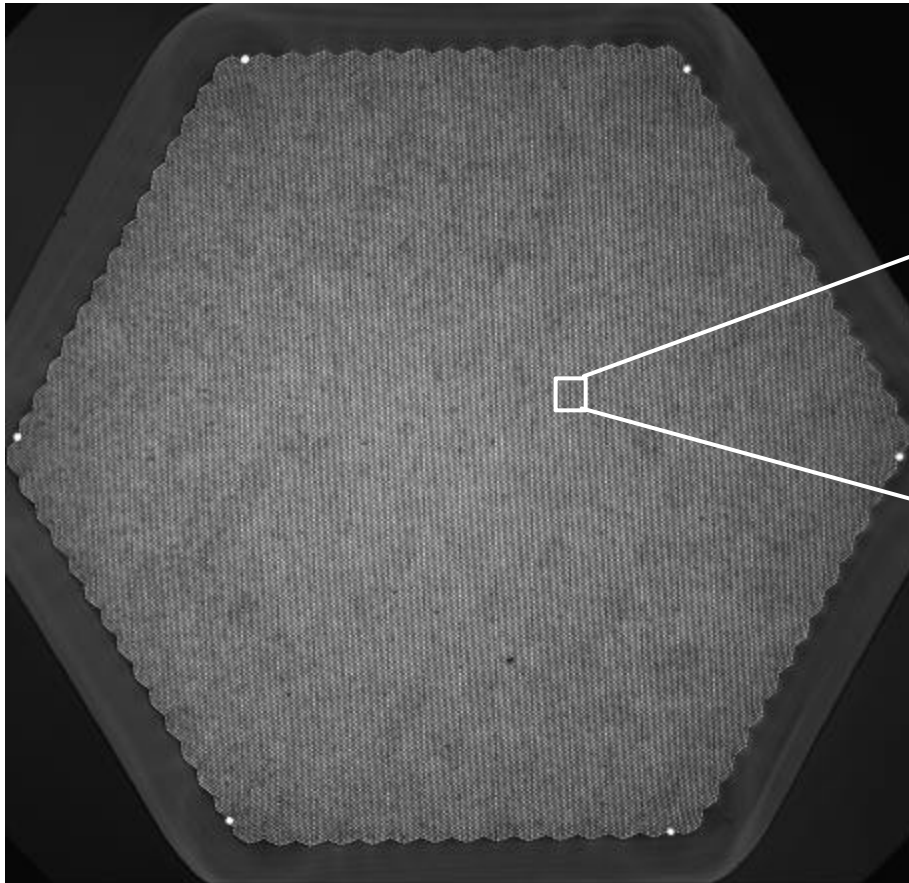
100? m



6? m

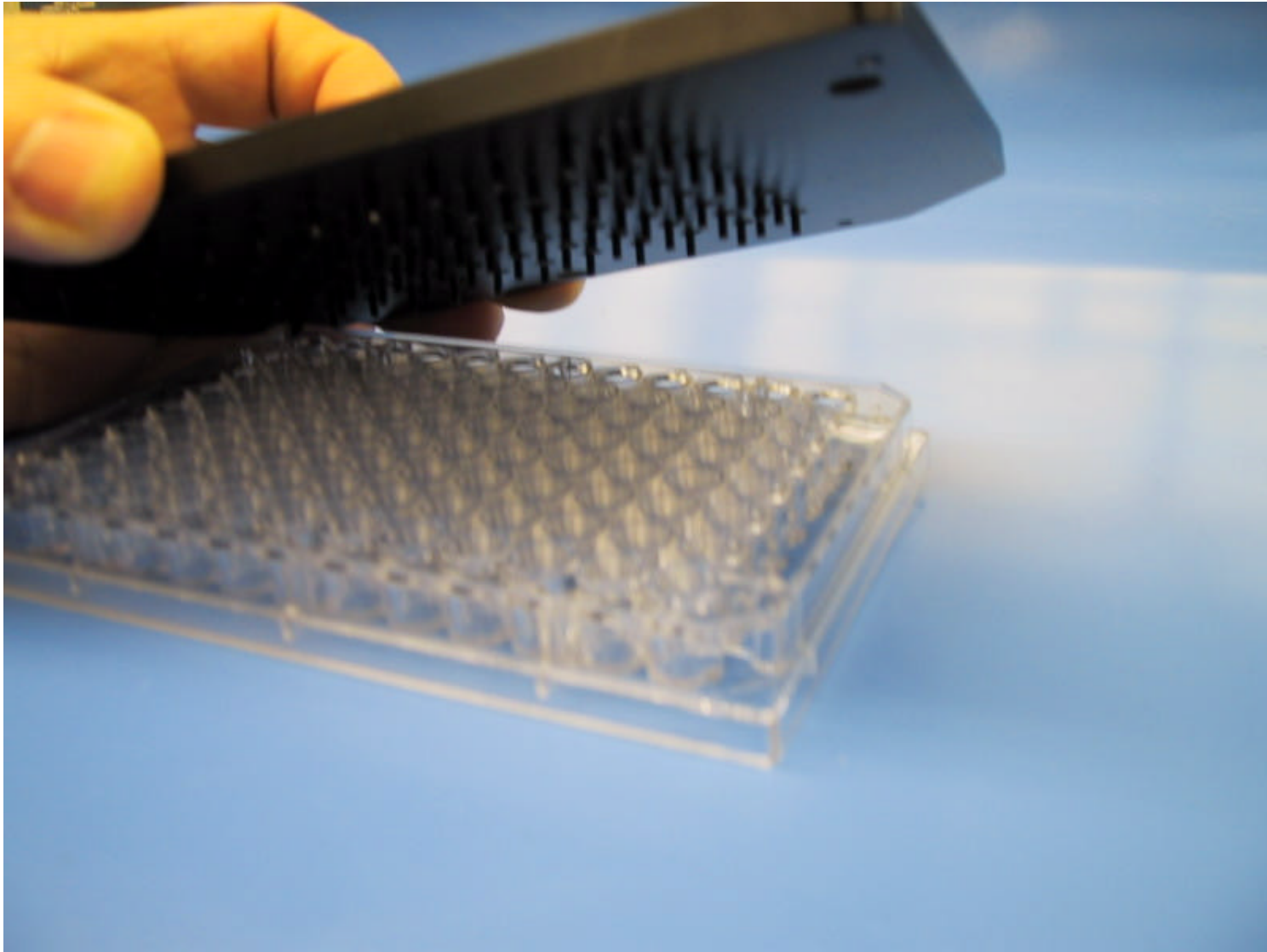


# 57K Fiber Bundle



??m

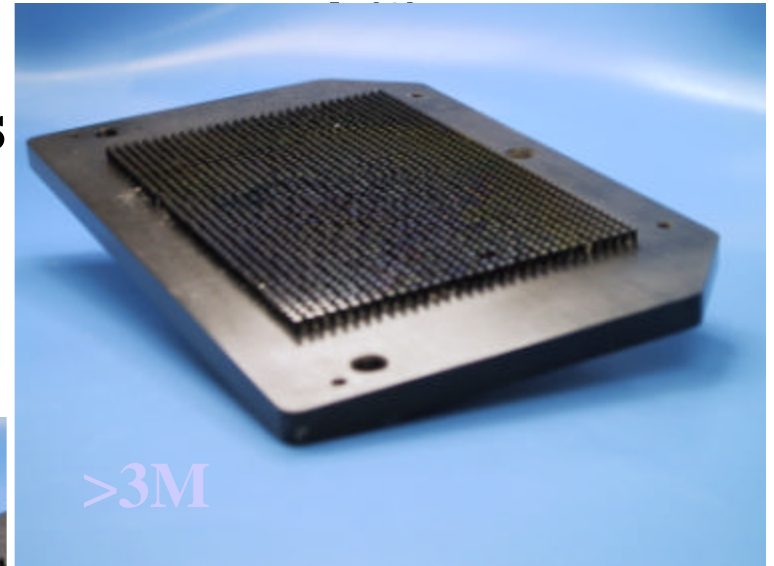
# Array of Arrays™



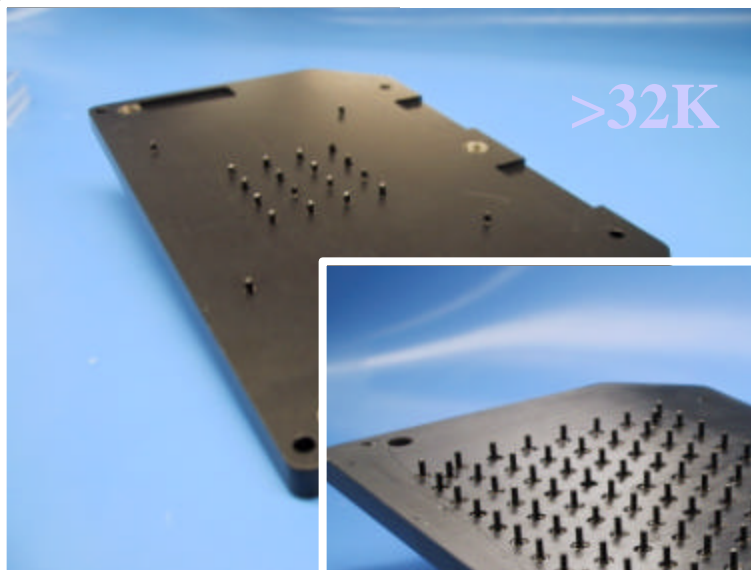
# Scalability of Technology

>2K

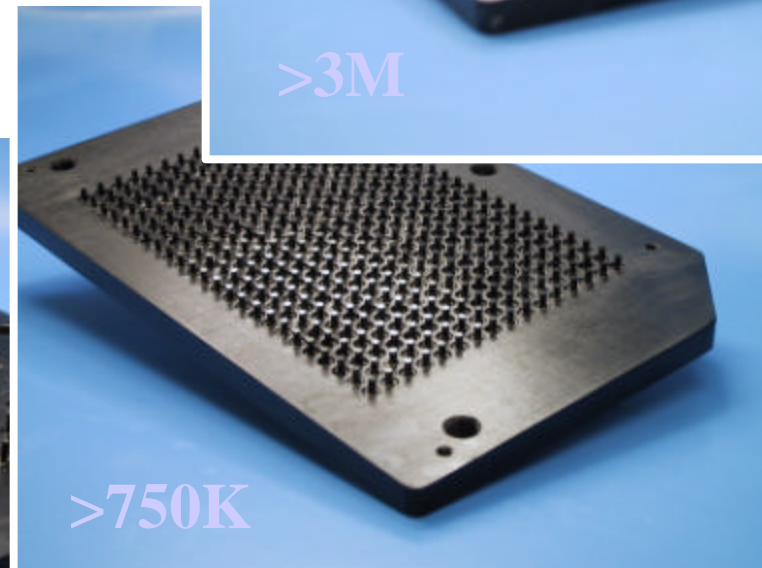
**Unique Experiments**  
(with ~20-fold redundancy)



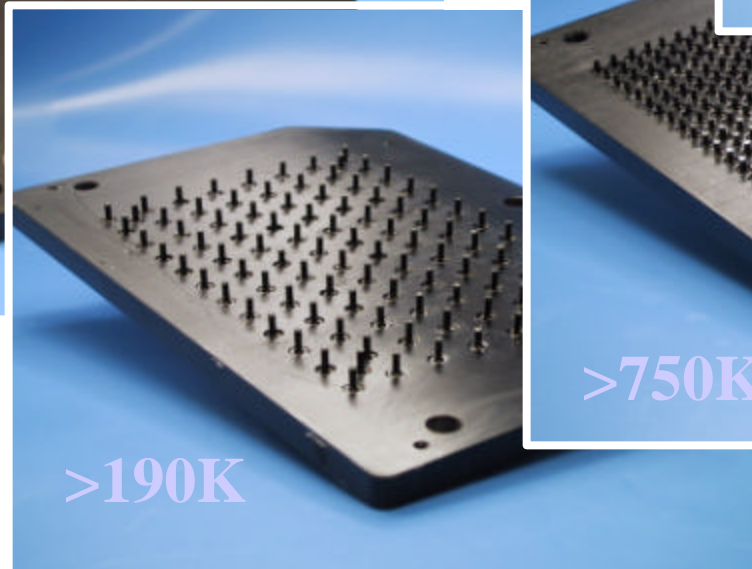
>3M



>32K



>750K



>190K

# Bead Summing

| Concentration | Hybridization Time: No Summing | Hybridization Time: 100 Bead Summed |
|---------------|--------------------------------|-------------------------------------|
| 100 pM        | 10 minutes                     | 4 minutes                           |
| 10 pM         | 30 minutes                     | 7 minutes                           |
| 100 fM        | 4 hours                        | 20 minutes                          |
| 10 fM         | 17 hours                       | 30 minutes                          |

# Size and Concentration

| Volume                         |      |                      | 1 M                | 1 nM               | 1 pM               |
|--------------------------------|------|----------------------|--------------------|--------------------|--------------------|
| $(1 \text{ m m})^3$            | 1 L  | $10^{-6} \text{ L}$  | $6 \times 10^{11}$ | $6 \times 10^8$    | $6 \times 10^5$    |
| $(100 \text{ } \mu\text{m})^3$ | 1 nL | $10^{-9} \text{ L}$  | $6 \times 10^8$    | $6 \times 10^5$    | $6 \times 10^2$    |
| $(10 \text{ } \mu\text{m})^3$  | 1 pL | $10^{-12} \text{ L}$ | $6 \times 10^5$    | $6 \times 10^2$    | $6 \times 10^{-1}$ |
| $(1 \text{ } \mu\text{m})^3$   | 1 fL | $10^{-15} \text{ L}$ | $6 \times 10^2$    | $6 \times 10^{-1}$ |                    |
| $(0.1 \text{ } \mu\text{m})^3$ | 1 aL | $10^{-18} \text{ L}$ | $6 \times 10^{-1}$ |                    |                    |

# Probe and Target Sequences for DNA Microarray Detection Limits

## Probe

IL2 (interleuken-2) 5'-TA-CAA-GAA-TCC-CAA-ACT-CAC-CAG-3'

IL6 (interleuken-6) 5'-GT-TGG-GTC-AGG-GGT-GGT-TAT-T-3'

F508C 5'-TAG-GAA-ACA-CCA-CAG-ATG-ATA-3'

## Target

IL2 (interleuken-2) 5'-CT-GGT-GAG-TTT-GGG-ATT-CTT-GTA-3'

IL6 (interleuken-6) 5'-AA-TAA-CCA-CCC-CTG-ACC-CAA-C-3'

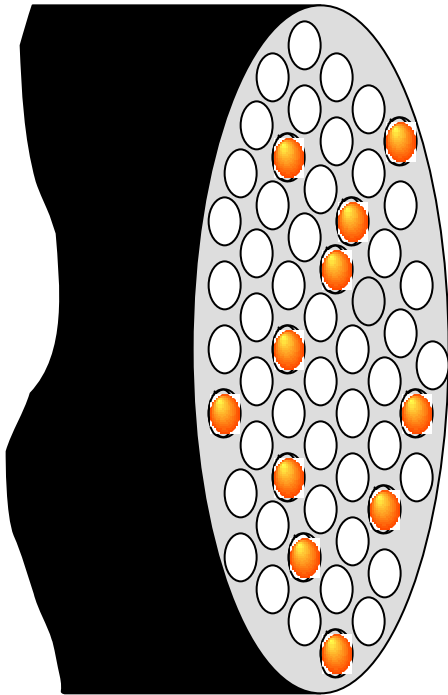
F508C 5'-TA-TCA-TCT-GTG-GTG-TTT-CCT-A-3'

## DNA Minimum Hybridization Time with ICCD Camera

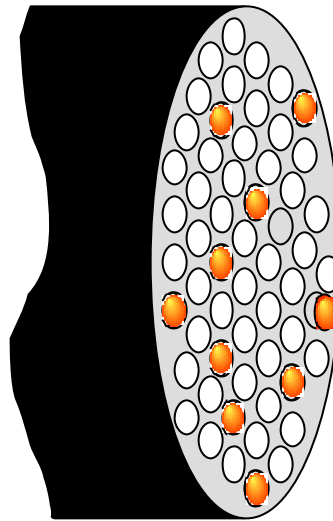
| DNA Concentration | Hybridization Time (min) |
|-------------------|--------------------------|
| 1 pM              | 10                       |
| 100 fM            | 20                       |
| 10 fM             | 30                       |
| 1 fM              | 60                       |

# Detection Limit Problem

Multiple beads provides a signal averaging benefit.



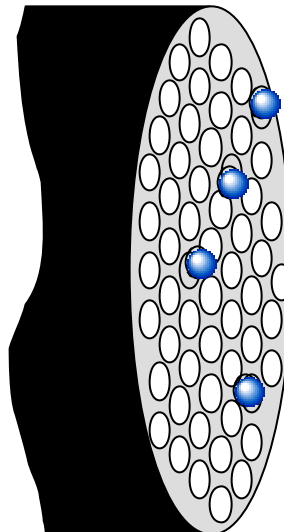
S/N increases by  $\sqrt{n}$



$\frac{1000 \text{ target molecules}}{10 \text{ beads}}$

=100 target molecules/bead

Fewer beads provide more target molecule numbers per bead.



$\frac{1000 \text{ target molecules}}{4 \text{ beads}}$

=250 target molecules/bead

# Multiplexed Array Sensitivity and Selectivity with 1 fM IL2 Target Solutions

IL2 Target - 1 fM concentration - 12 hour hybridization time

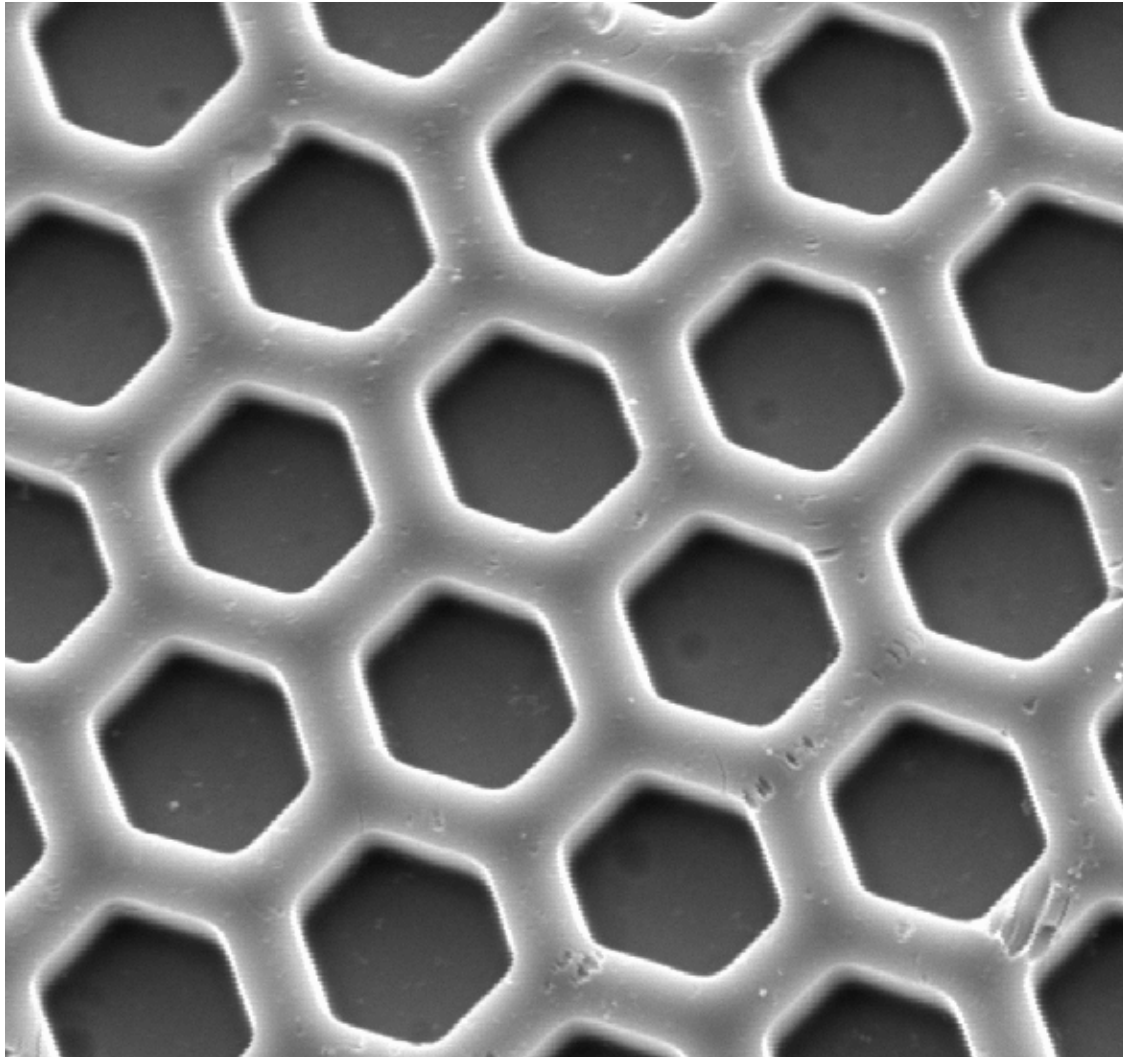
| Target/Probe |       | Mean background $\pm$ s.d | Hybridization $\pm$ s.d. | Signal $\pm$ s.d  |
|--------------|-------|---------------------------|--------------------------|-------------------|
| F508         | F508C | 530.43 $\pm$ 1.8          | 550.17 $\pm$ 7.5         | {19.74} $\pm$ 7.7 |
|              | IL2   | 563.99 $\pm$ 7.7          | 677.08 $\pm$ 8.1         | 113.09 $\pm$ 11   |
|              | IL6   | 445.99 $\pm$ 3.9          | 449.16 $\pm$ 1.4         | {3.17} $\pm$ 4.1  |
| IL2          | F508C | 439.64 $\pm$ 3.5          | 443.34 $\pm$ 5.6         | {3.70} $\pm$ 6.6  |
|              | IL2   | 432.52 $\pm$ 5.6          | 503.31 $\pm$ 6.6         | 70.79 $\pm$ 8.7   |
|              | IL6   | 431.11 $\pm$ 2.1          | 432.13 $\pm$ 2.8         | {1.02} $\pm$ 3.5  |
| IL6          | F508C | 454.84 $\pm$ 3.6          | 465.82 $\pm$ 1.4         | {10.98} $\pm$ 3.8 |
|              | IL2   | 429.42 $\pm$ 0.92         | 517.38 $\pm$ 2.6         | 87.96 $\pm$ 2.8   |
|              | IL6   | 459.81 $\pm$ 3.0          | 467.82 $\pm$ 5.3         | {8.01} $\pm$ 6.1  |

# Microsphere Array Sensitivity and Selectivity with 100 aM IL2 Target Solutions

IL2 Target - 100 aM concentration - 12 hour hybridization time

| Probe/Target | Mean background $\pm$ s.d | Hybridization $\pm$ s.d. | Signal $\pm$ s.d    |
|--------------|---------------------------|--------------------------|---------------------|
| IL2 F508C    | $386.97 \pm 3.2$          | $387.98 \pm 1.4$         | $\{1.01\} \pm 3.5$  |
| IL2 IL2      | $378.55 \pm 2.3$          | $394.00 \pm 3.7$         | $15.32 \pm 4.4$     |
| IL2 IL6      | $382.80 \pm 6.3$          | $393.81 \pm 6.1$         | $\{11.01\} \pm 7.1$ |
| IL2 F508C    | $268.66 \pm 2.3$          | $274.22 \pm 8.5$         | $\{5.56\} \pm 8.8$  |
| IL2 IL2      | $297.73 \pm 2.3$          | $310.02 \pm 2.3$         | $12.29 \pm 3.2$     |
| IL2 IL6      | $247.59 \pm 2.7$          | $248.70 \pm 6.9$         | $\{1.11\} \pm 7.4$  |
| IL2 F508C    | $410.73 \pm 2.6$          | $413.63 \pm 2.6$         | $\{2.90\} \pm 2.9$  |
| IL2 IL2      | $410.69 \pm 2.7$          | $455.26 \pm 6.5$         | $44.57 \pm 7.0$     |
| IL2 IL6      | $390.24 \pm 7.4$          | $392.88 \pm 2.8$         | $\{2.64\} \pm 7.9$  |

# SEM of a Microwell Array

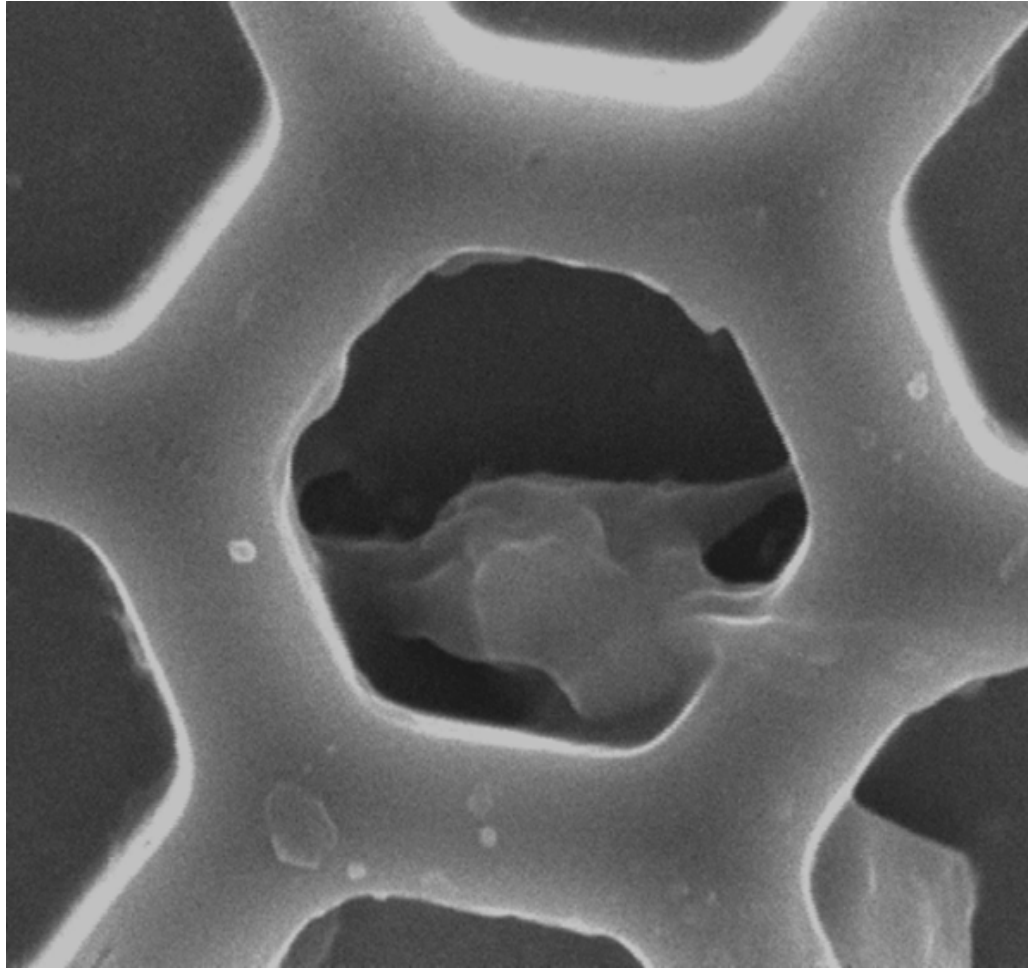


**7 ? m well diameter**

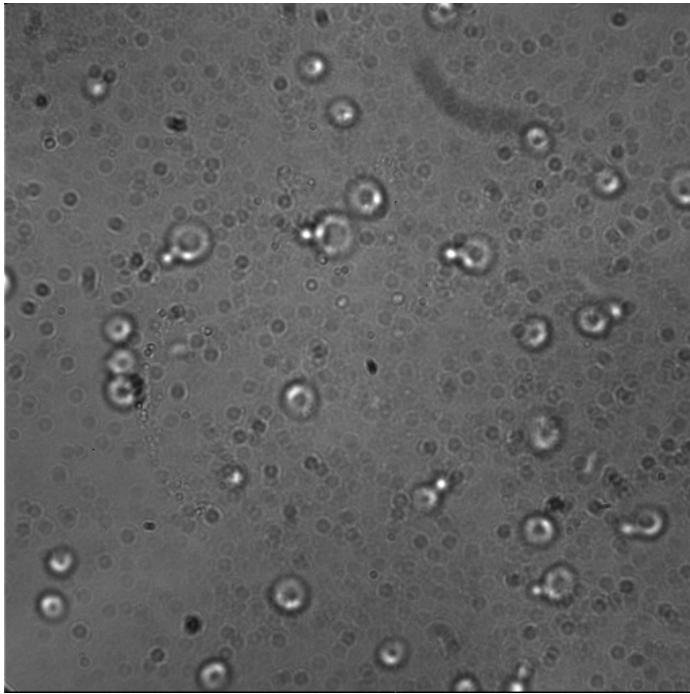
**~3 ? m well depth**

**~90 fL well volume**

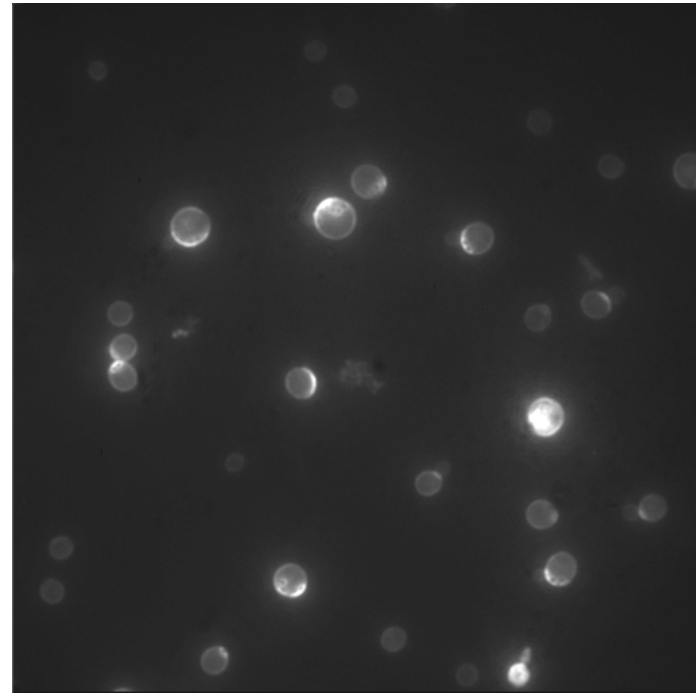
# Single NIH 3T3 Mouse Fibroblast Cell in a Fiber-optic Microwell



# Single Yeast (*Saccharomyces cerevisiae*) Cells Array

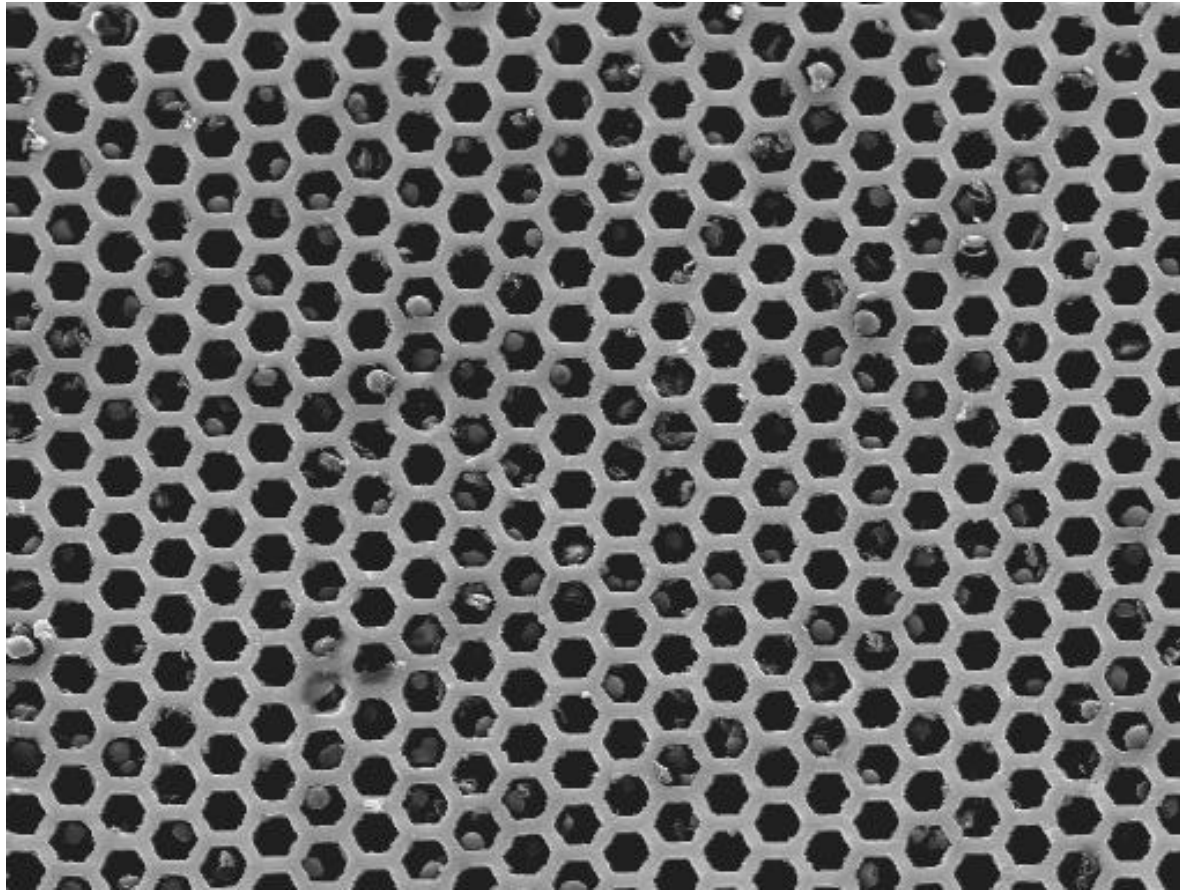


White light

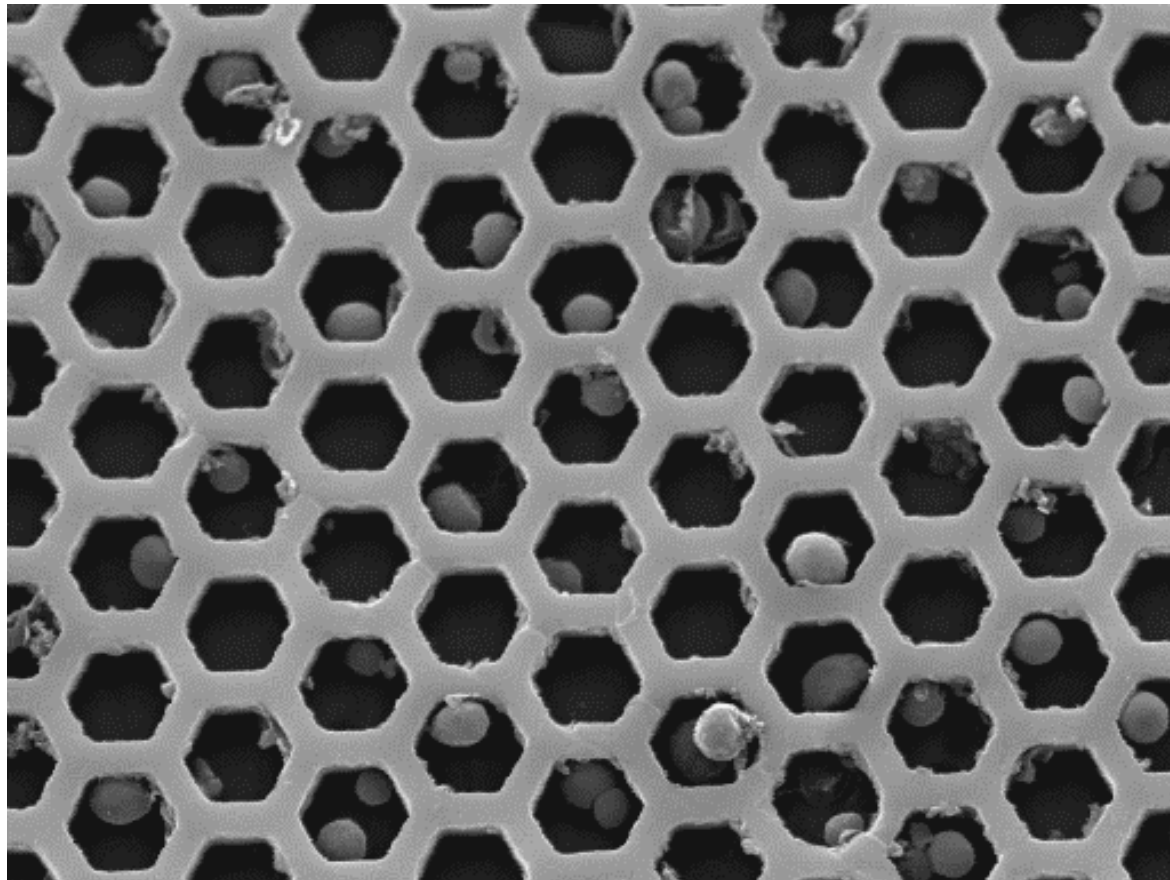


Calcofluor White 360/440

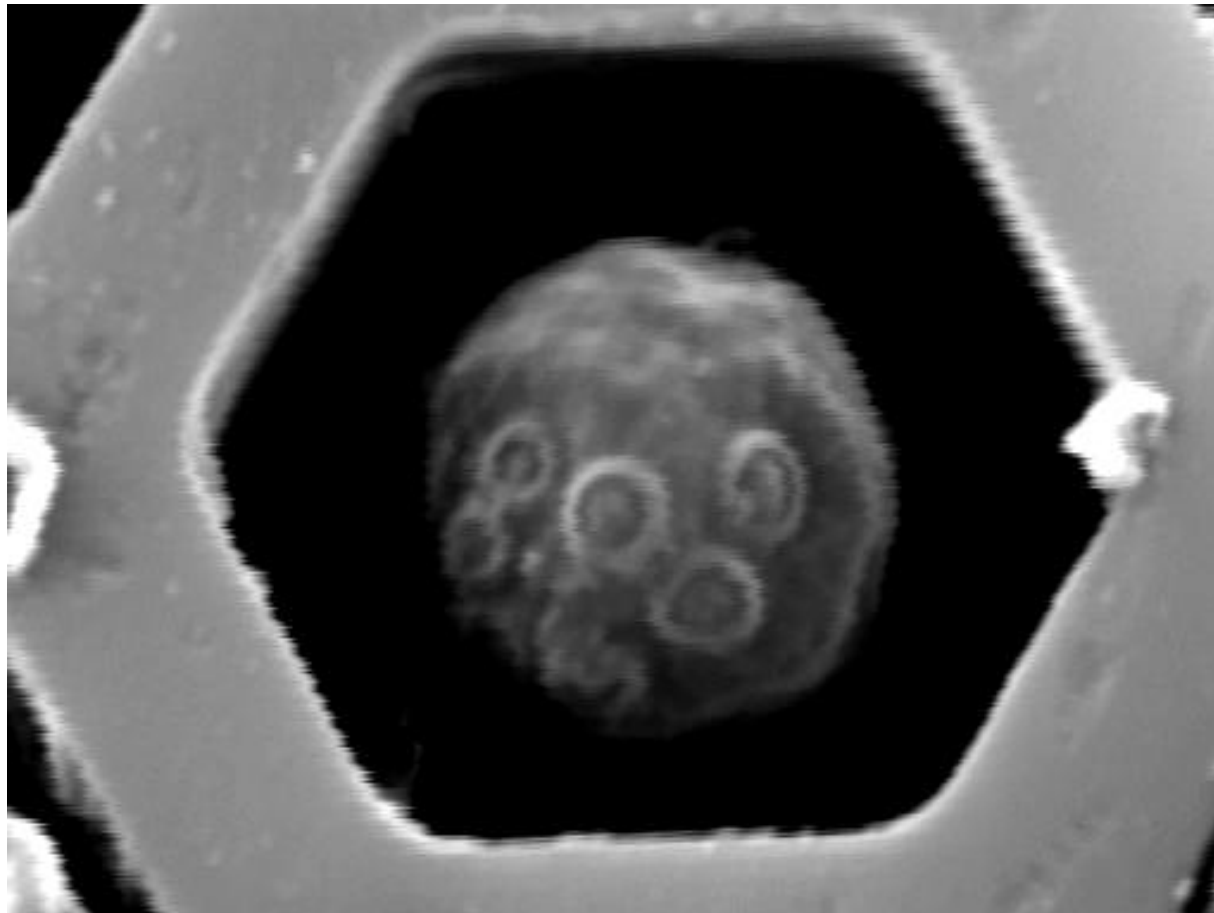
# SEM images of Single Yeast Cells on the Microwells array



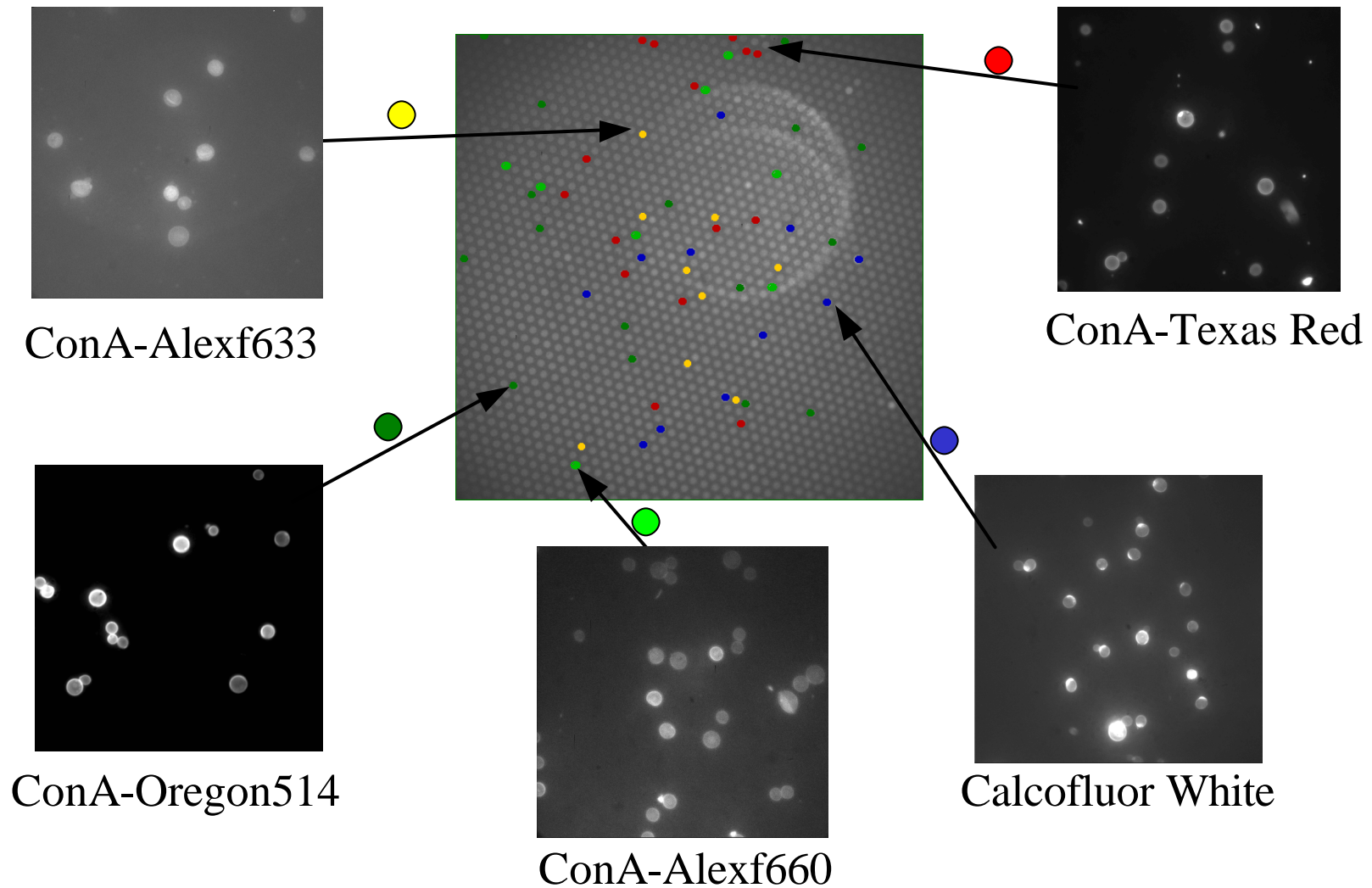
# SEM images of Single Yeast Cells on the Microwells array



## SEM images of Single Yeast Cells on the Microwells array

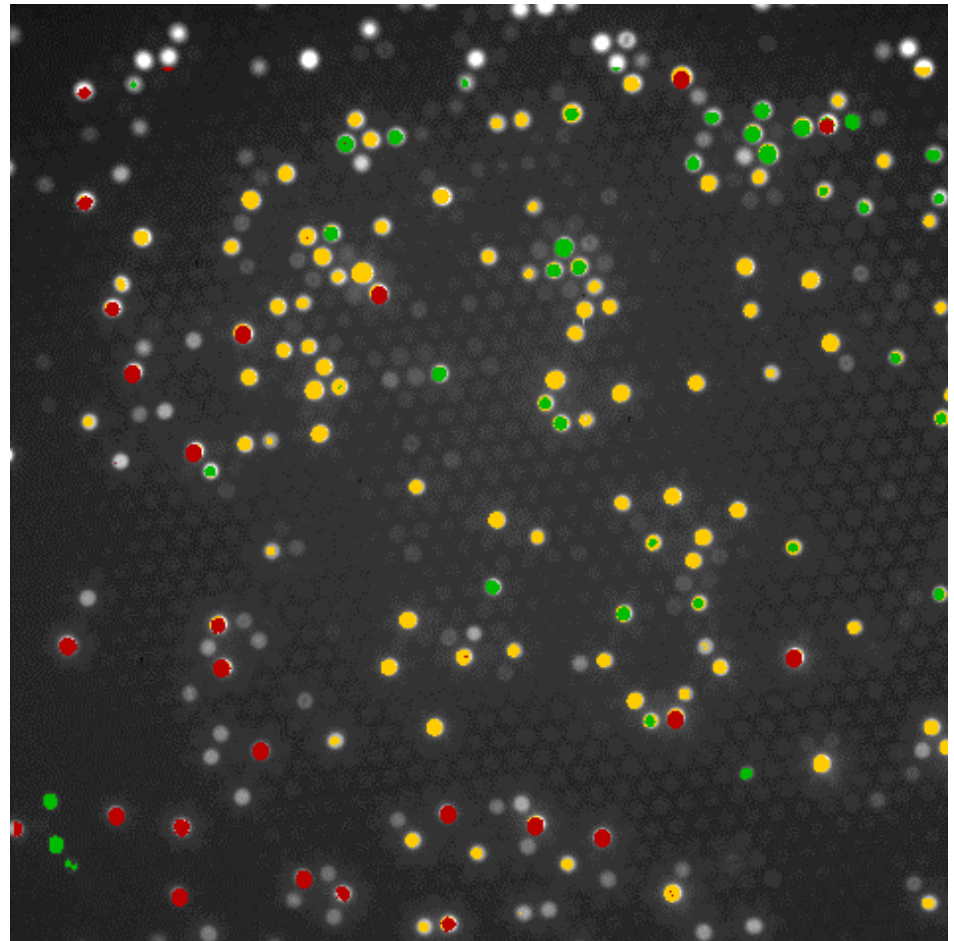


# Encoded Yeast Cells on the Fiber Array



# pH Measurement of Single Yeast Cells Microenvironment in the Array

- Concanavalin A-FITC
- Concanavalin A-FITC  
+  
Concanavalin A-Alexa fluor 660
- Concanavalin A-FITC  
+  
Concanavalin A-Texas Red



# Smarter Sensors- Anticipatory

Is it bad?

What does it resemble?

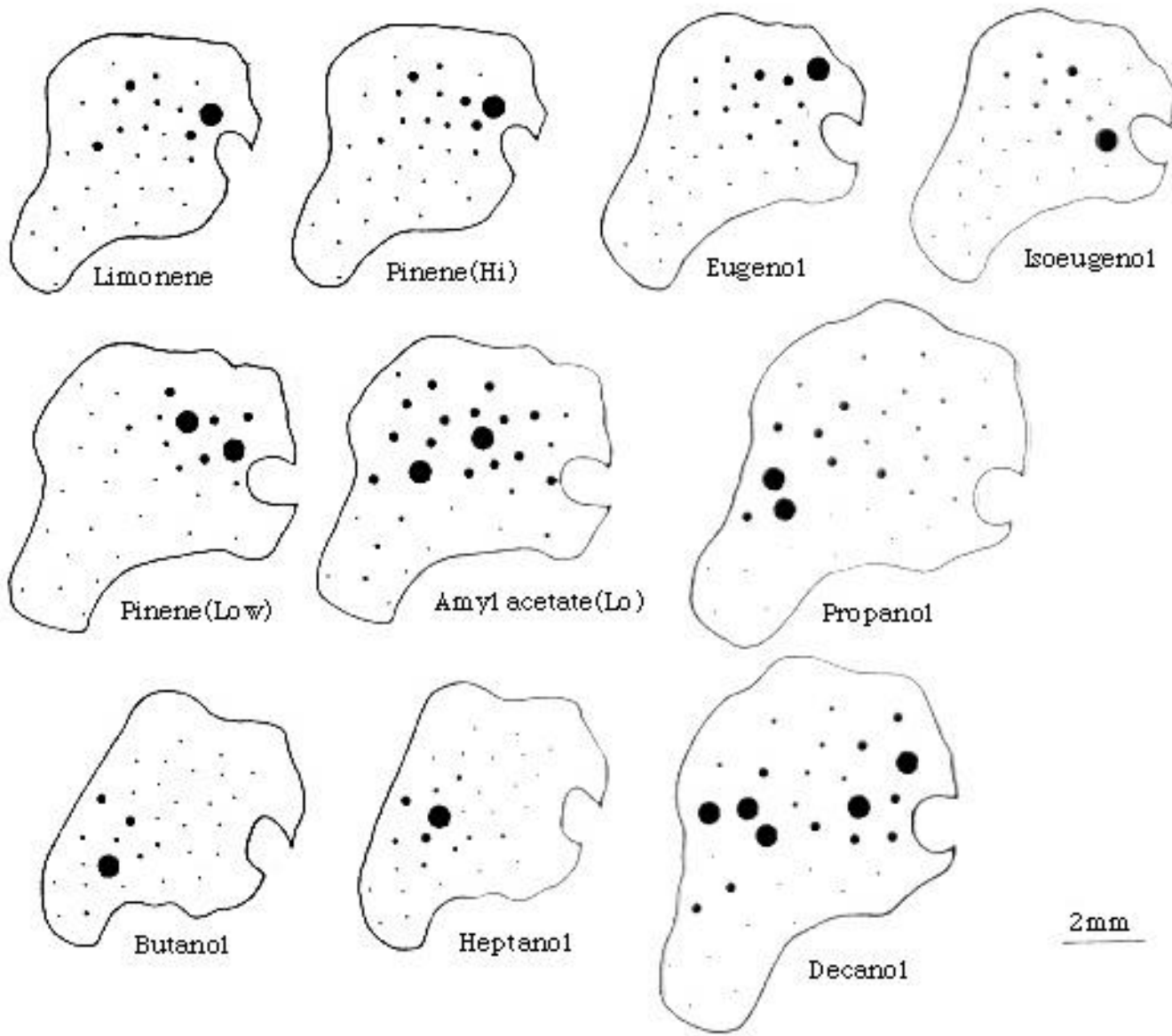
What will it do?

e.g. GI, neurotoxic, etc.

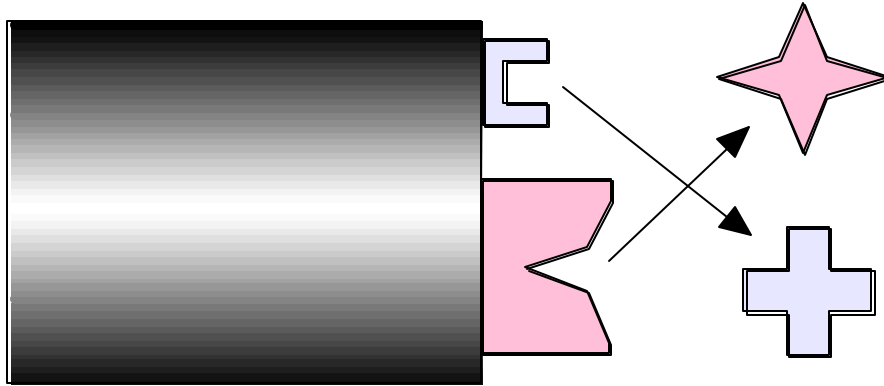
“common virulence mechanisms”



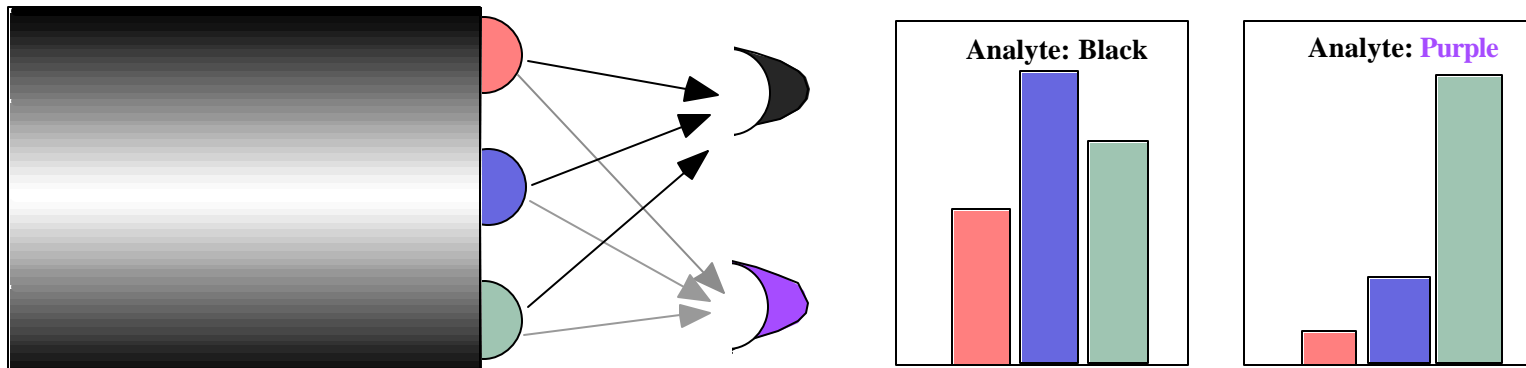
surrogates



# Sensor Design

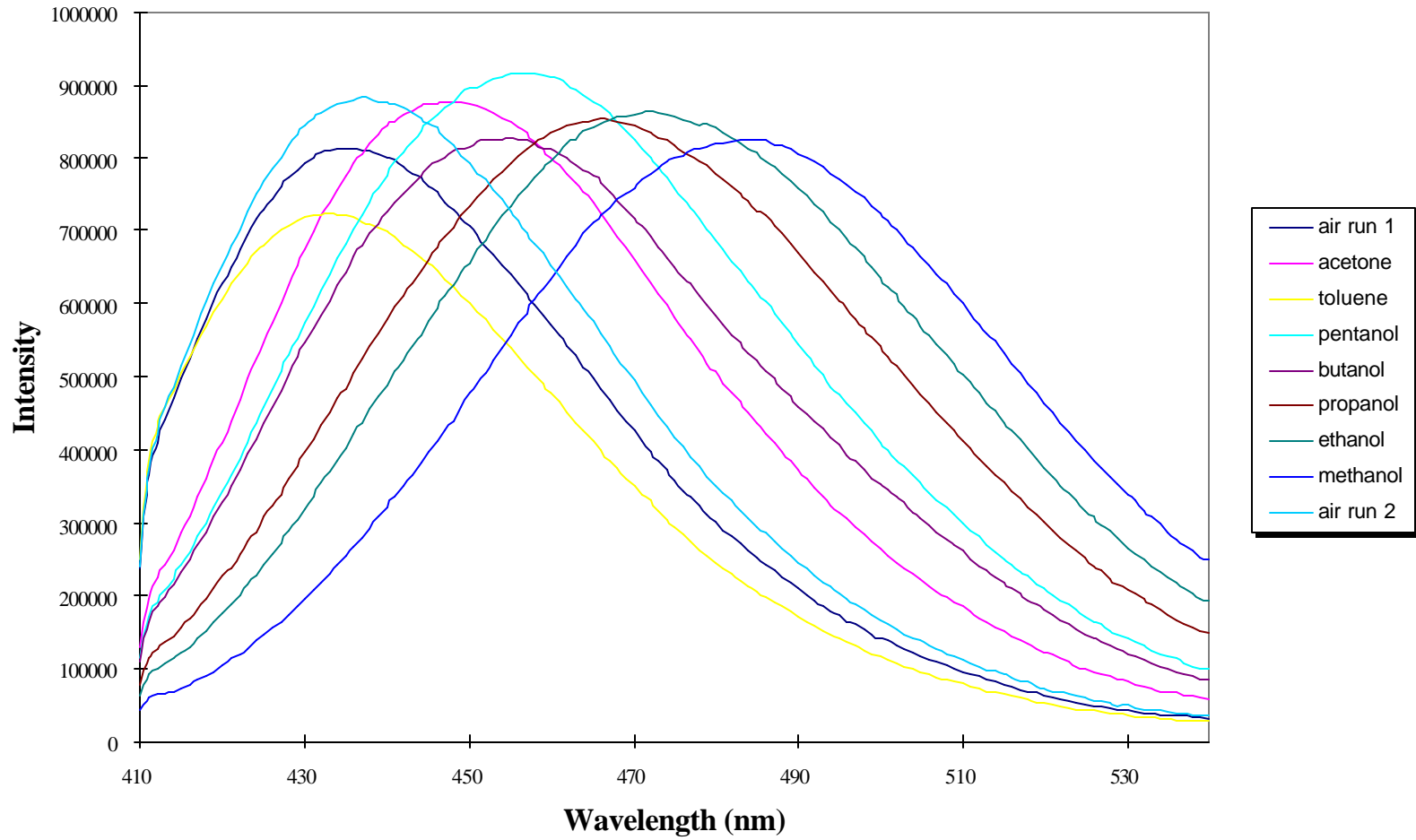


A) Lock-and-key Sensor

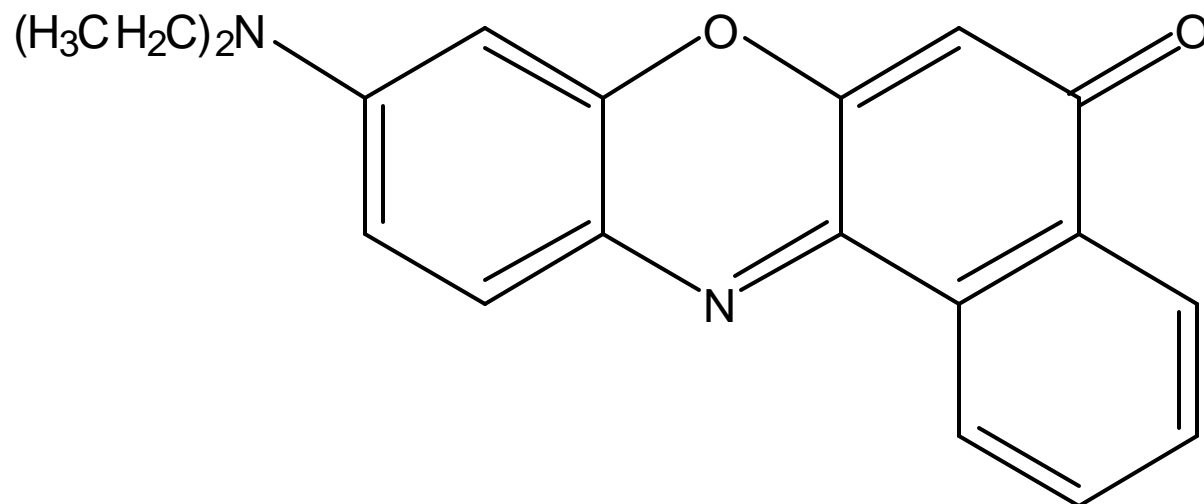


B) Cross-reactive Sensor

# Solvatochromic Effect

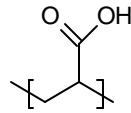


# Nile Red

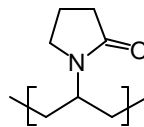


# Role of Polymer Polarity

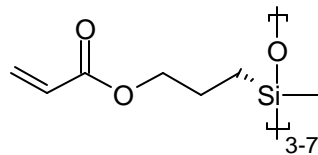
poly(acrylic acid), **PAA**



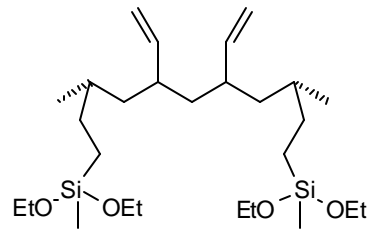
poly(N-vinyl pyrrolidone), **PVP**



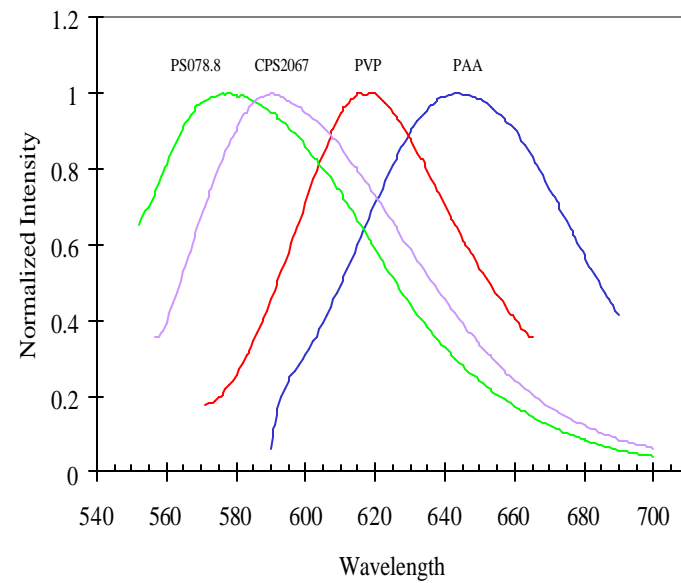
acryloxypropylmethyl-  
cyclosiloxane, **CPS2067**



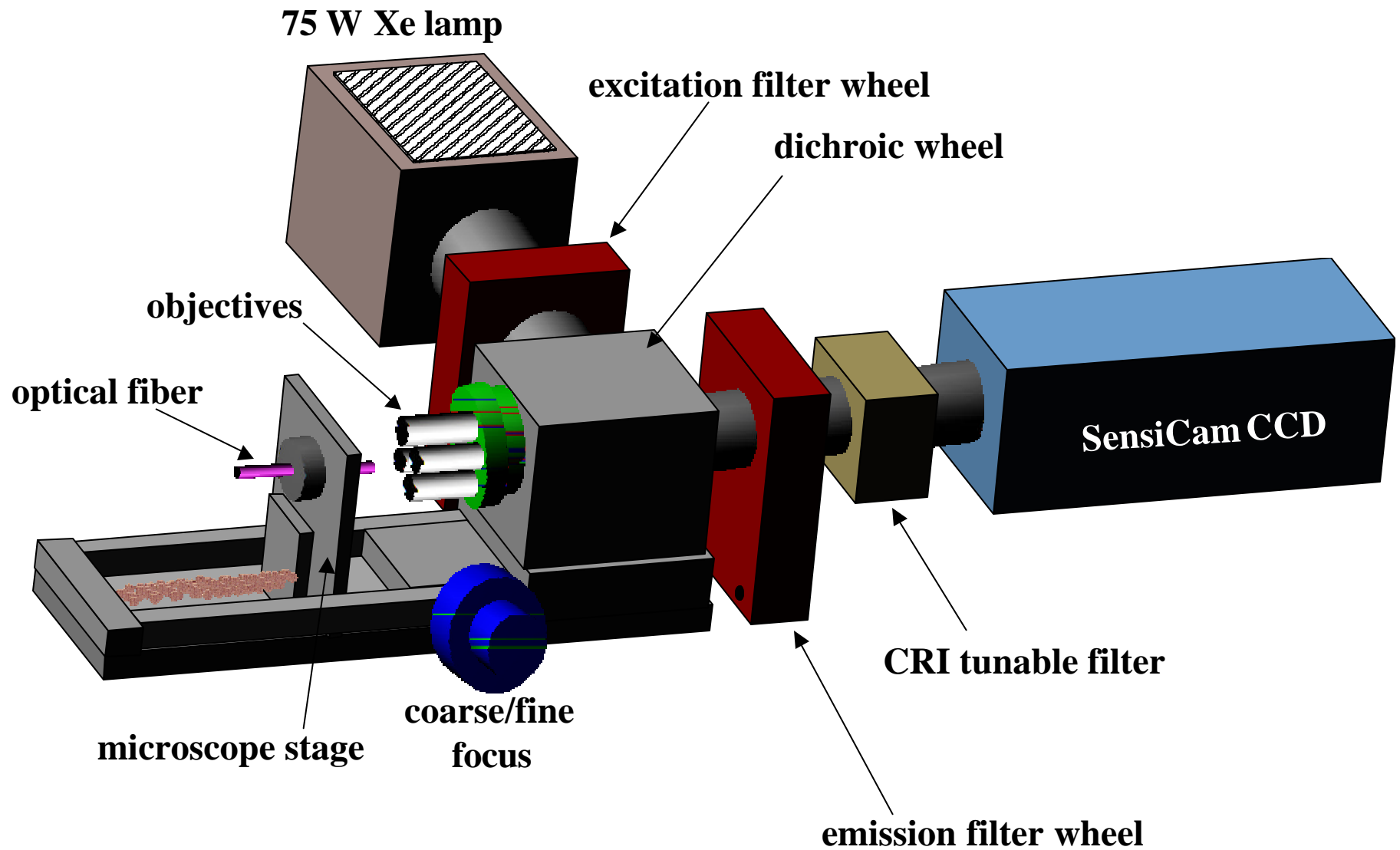
diethoxymethylsilyl-modified  
polybutadiene, **PS078.8**



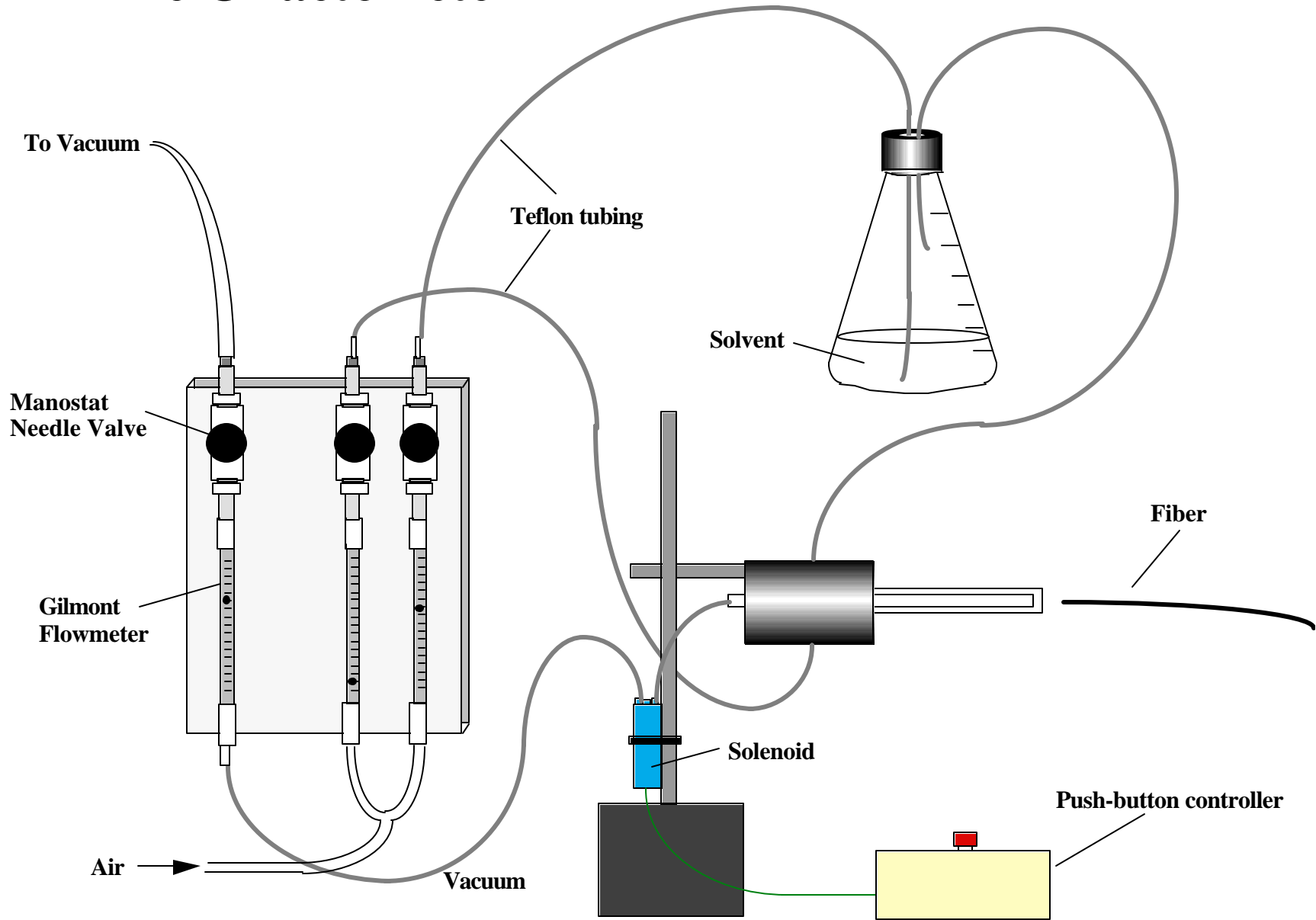
Decreasing  
polarity



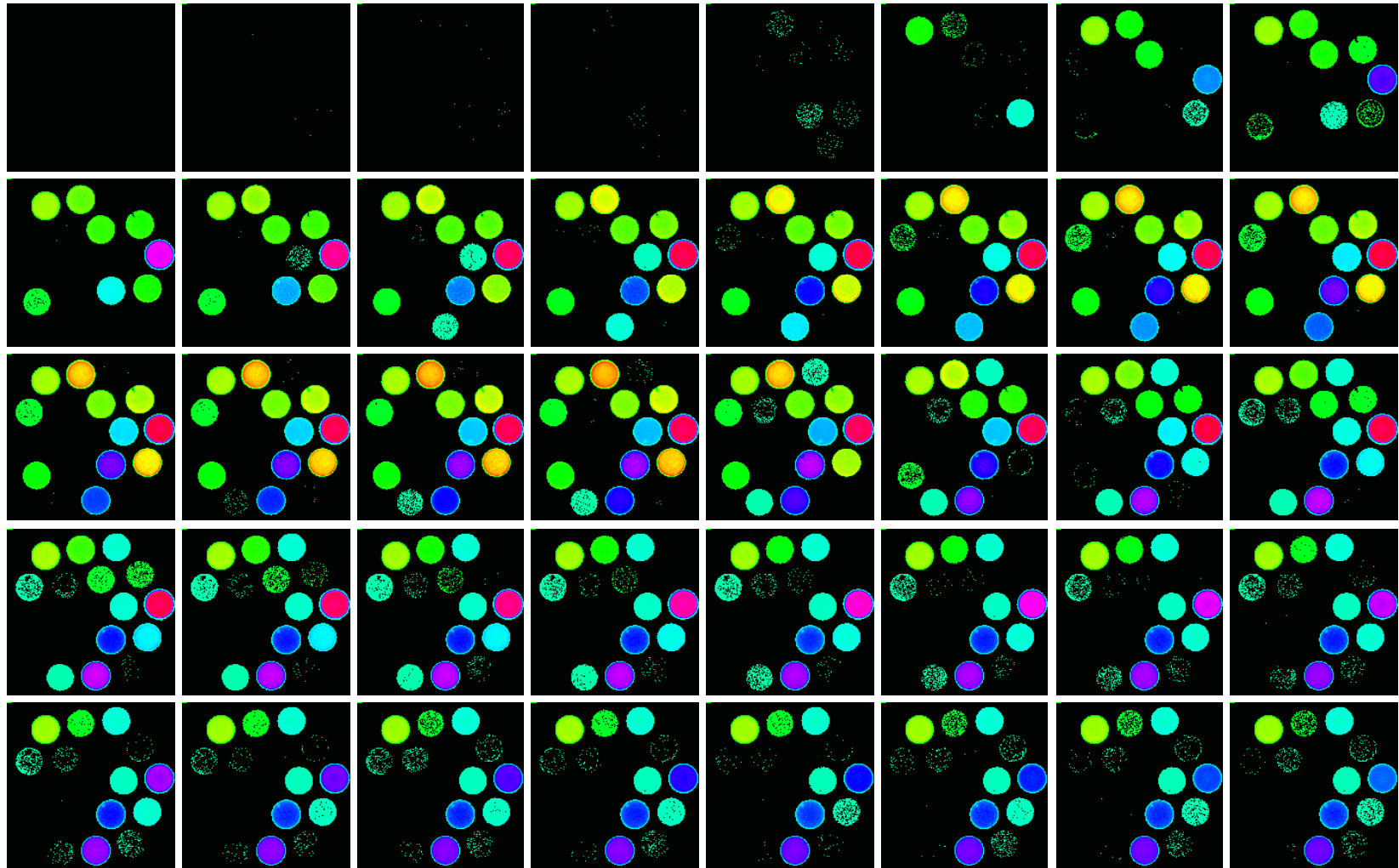
# CCD-based imaging system



# The Olfactometer — J. Kauer



# Sensor Array Response to Benzene Vapor Pulse



Dickinson, T. A., *et al.* (1996) *Nature*, 382: 697-700.



# Classification Results

## Learning Vector Quantization Approach

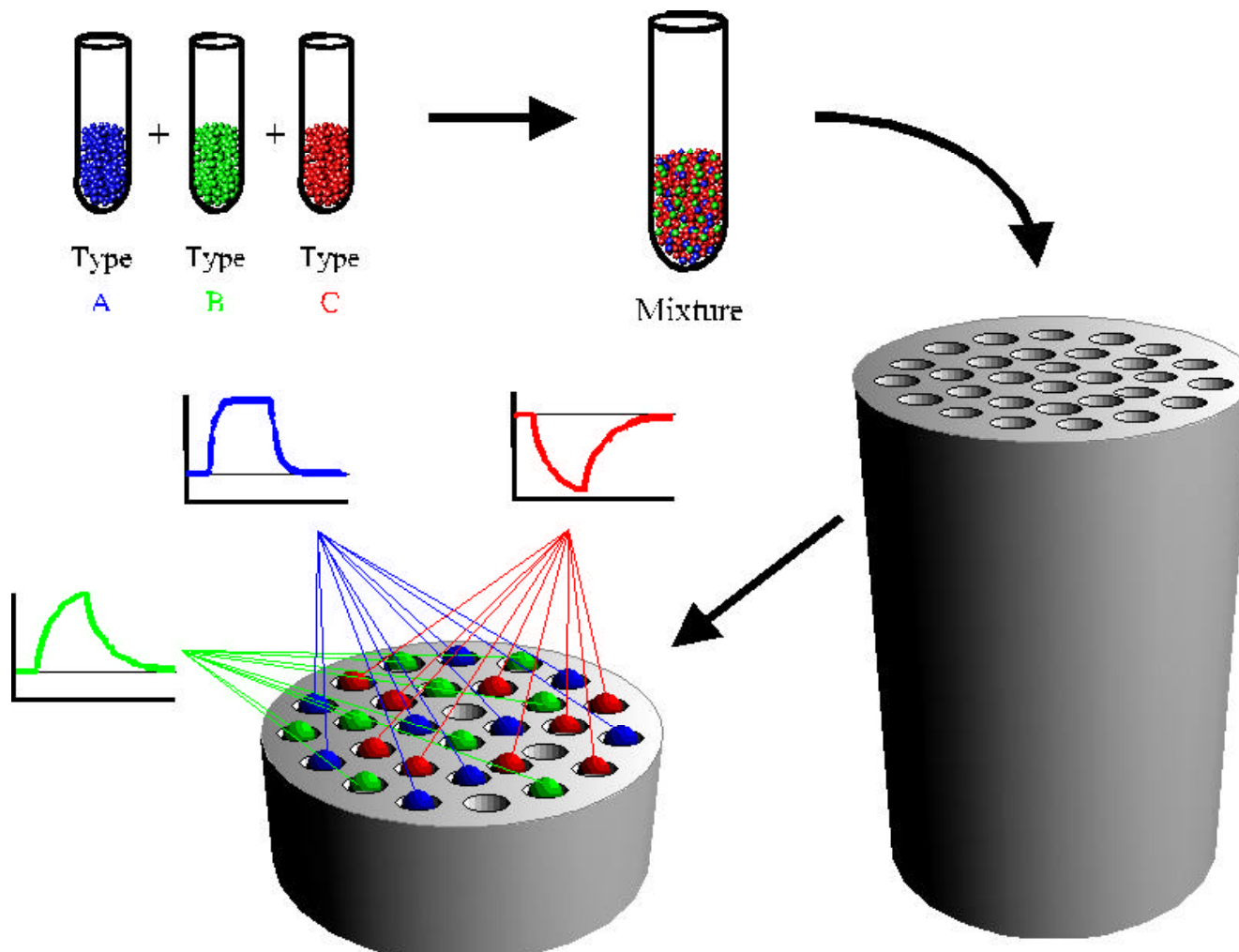
### Network Output

True  
Identity

|    | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1  | 24 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| 2  |    | 23 |    |    |    |    |    |    |    | 1  |    |    |    |    |    |    |    |    |    |    |
| 3  |    |    | 20 |    |    |    |    |    | 3  |    | 1  |    |    |    |    |    |    |    |    |    |
| 4  |    |    | 1  | 19 |    |    |    |    | 1  |    |    |    | 1  |    |    |    |    | 2  |    |    |
| 5  |    |    | 1  |    | 17 | 4  |    |    | 2  |    |    |    |    |    |    |    |    |    |    |    |
| 6  |    |    |    | 1  | 2  | 19 |    |    |    |    | 1  |    |    |    | 1  |    |    |    |    |    |
| 7  |    |    |    |    |    |    | 18 | 4  |    | 1  |    |    | 1  |    |    |    |    |    |    |    |
| 8  |    |    |    |    |    |    | 2  | 21 |    |    |    |    | 1  |    |    |    |    |    |    |    |
| 9  |    |    |    | 1  |    |    |    |    | 23 |    |    |    |    |    |    |    |    |    |    |    |
| 10 |    |    | 3  |    |    |    |    |    |    | 19 |    |    | 1  |    |    | 1  |    |    |    |    |
| 11 |    |    |    |    |    |    |    |    |    |    | 21 |    |    |    |    | 1  |    |    | 2  |    |
| 12 |    |    |    |    |    |    |    |    |    |    |    | 23 |    |    |    |    |    |    | 1  |    |
| 13 |    |    |    |    |    |    |    |    | 1  |    |    |    | 23 |    |    |    |    |    |    |    |
| 14 |    |    |    |    |    |    |    |    | 1  |    |    |    |    | 20 |    |    | 2  |    | 1  |    |
| 15 |    | 1  |    |    |    |    |    |    |    |    |    |    |    |    |    | 23 |    |    |    |    |
| 16 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 24 |    |    |    |
| 17 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 24 |    |    |
| 18 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 24 |    |
| 19 |    |    |    |    |    |    |    |    |    |    | 4  |    |    |    |    |    |    |    |    | 20 |
| 20 |    | 1  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    | 23 |

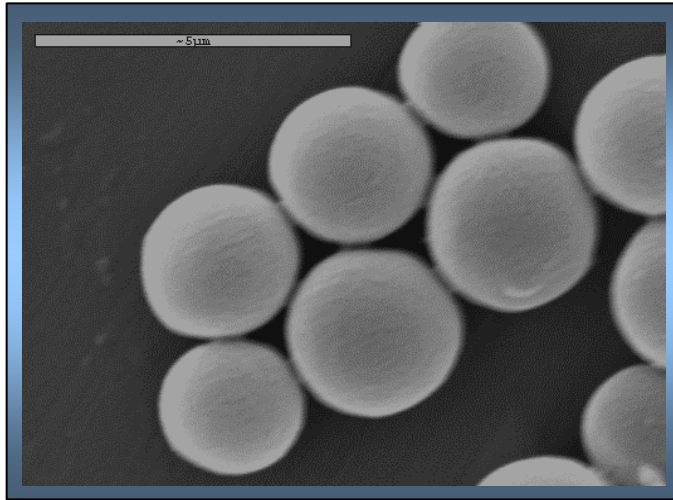
- 1) Acetone
- 2) Butyl Acetate
- 3) Beauty
- 4) Camphor
- 5) Carvone -
- 6) Carvone +
- 7) Chloroform
- 8) Dichloroethane
- 9) DMSO
- 10) Drakkar Noir
- 11) Water
- 12) Heptane
- 13) Isopropanol
- 14) Indole
- 15) Mercaptoethanol
- 16) Methanol
- 17) Propanol
- 18) Propionic Acid
- 19) Pseudoexplosive
- 20) Toluene

# SENSOR ARRAYS are Assembled 'Randomly' in ONE Fabrication Step

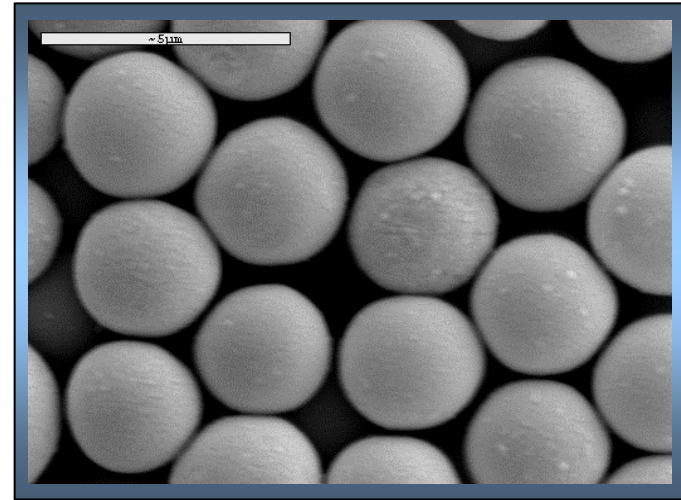


**NOTE: the Sensor Array is a 'Self-Encoding' Bead Array (SEBA). Billions of Sensors are Fabricated at Once.**

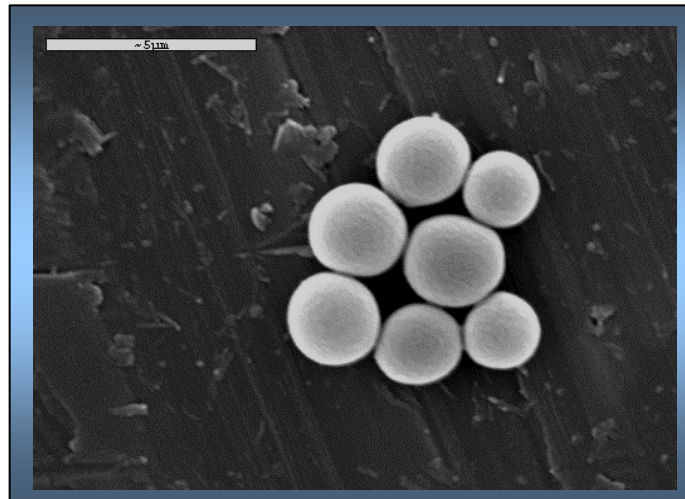
# Hollow Poly(benzyl methacrylate) Spheres



3.5 h polymerization



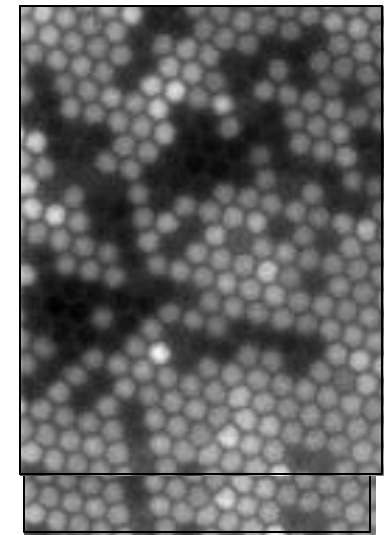
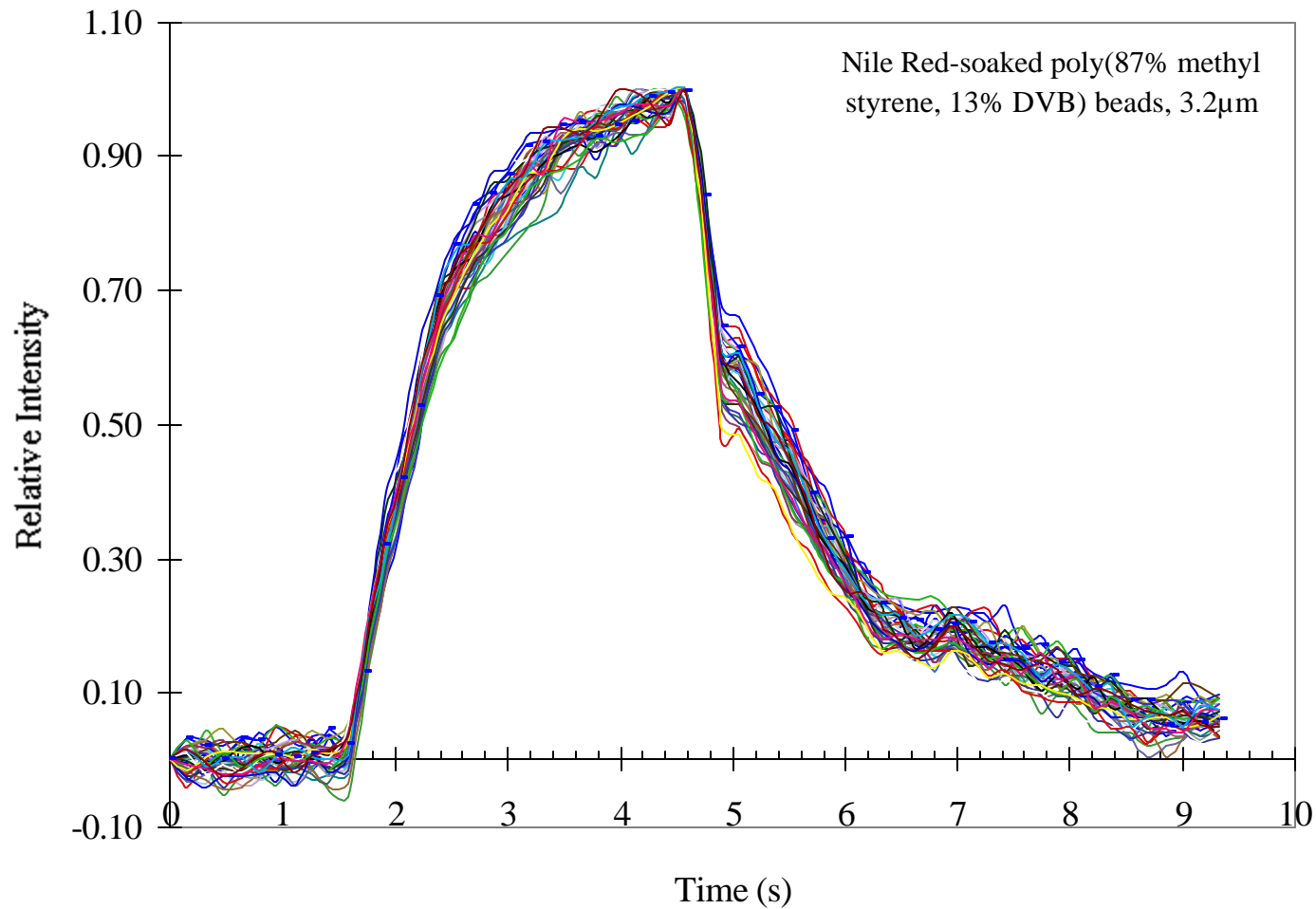
6.5 h polymerization



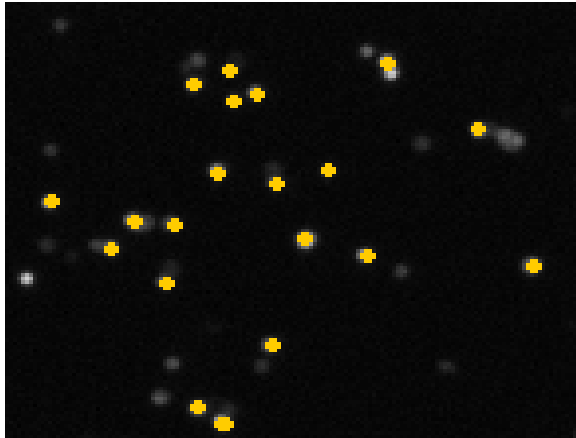
14h polymerization

Chem. Mater. 2000

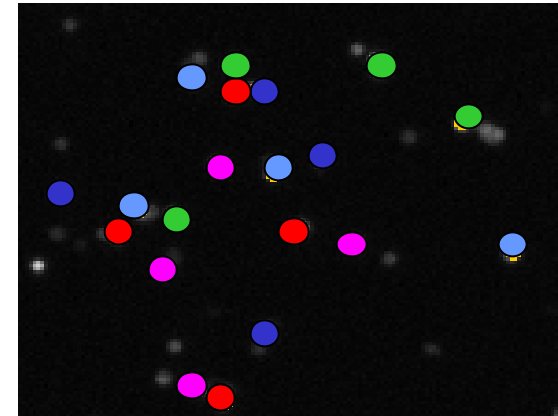
# Nile Red/PolyMethylStyrene Beads in Wells: Response of 40 beads to methanol pulse



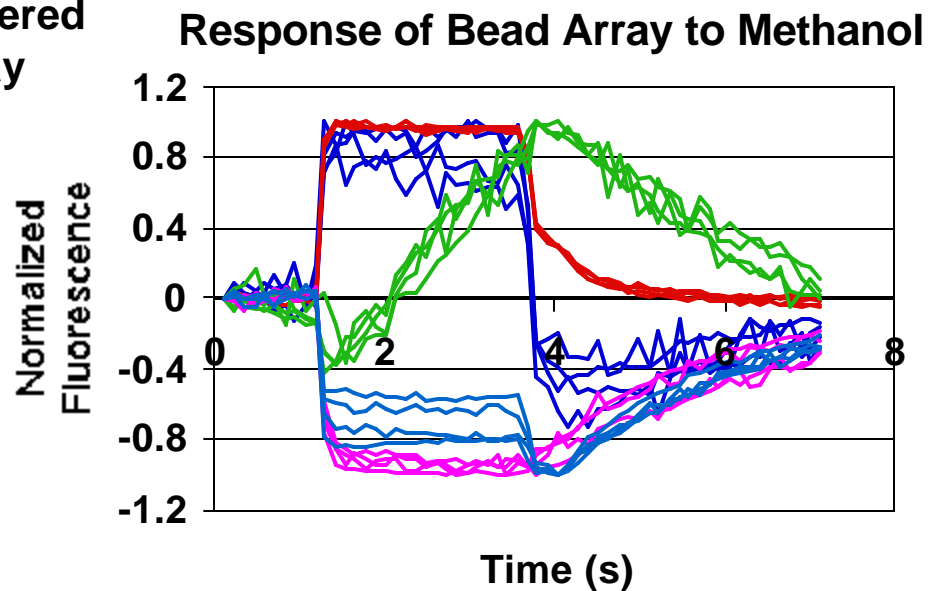
# Sensor Registration Problem



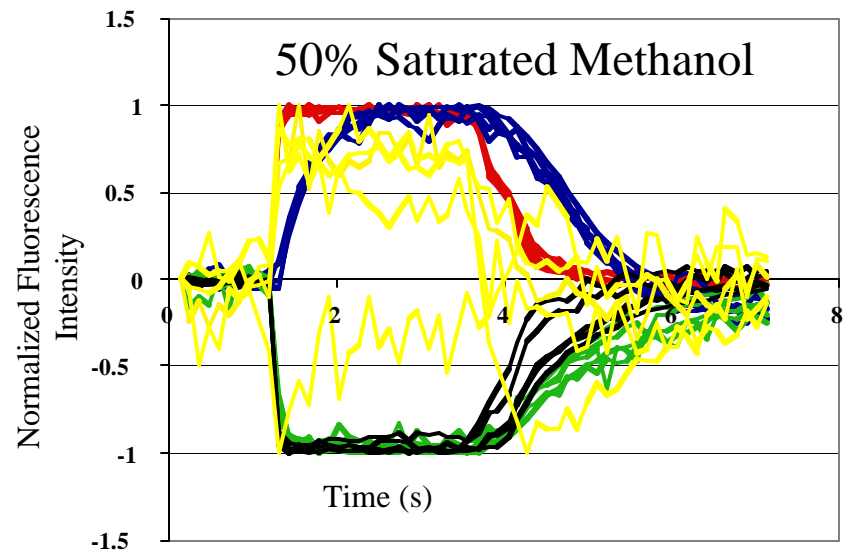
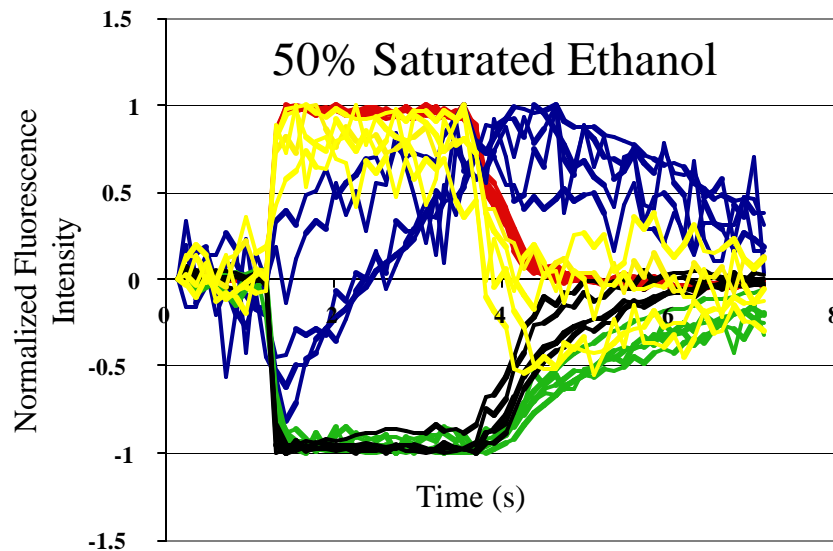
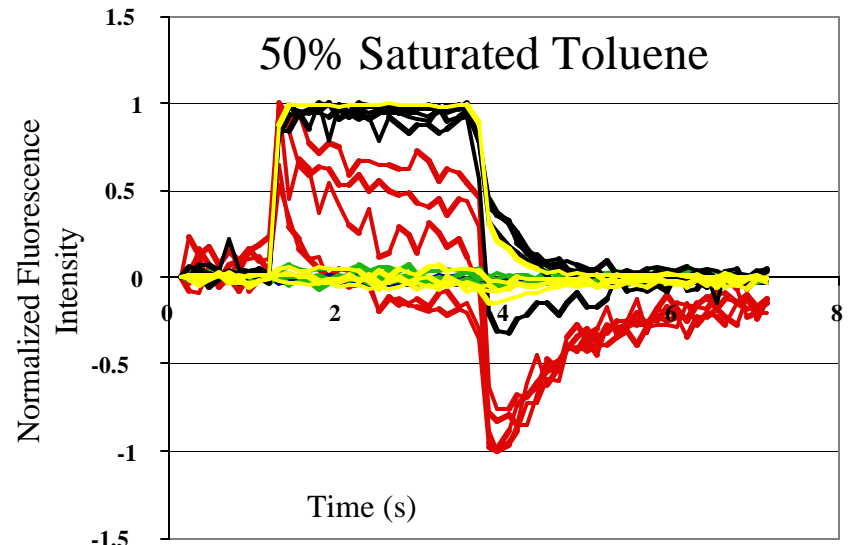
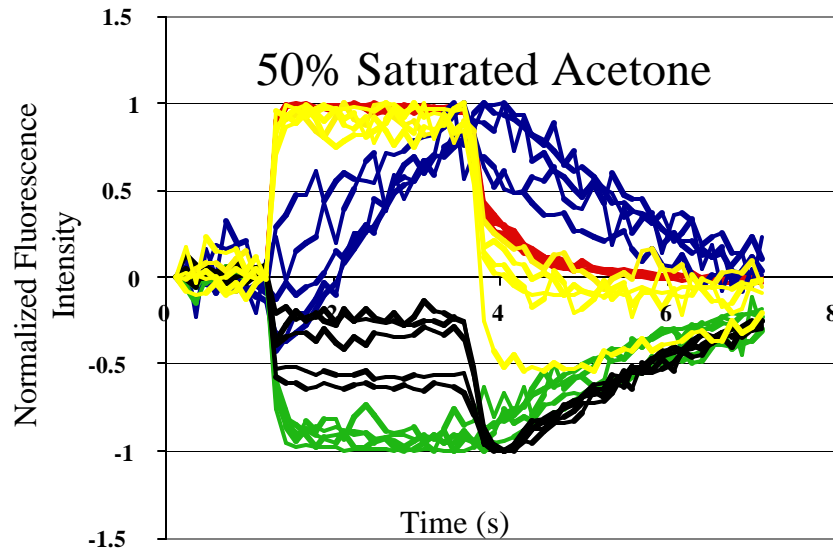
Randomly Ordered  
5 Bead Array



Decoded 5  
Bead Array



# 5 Sensor Types with 4 Analytes

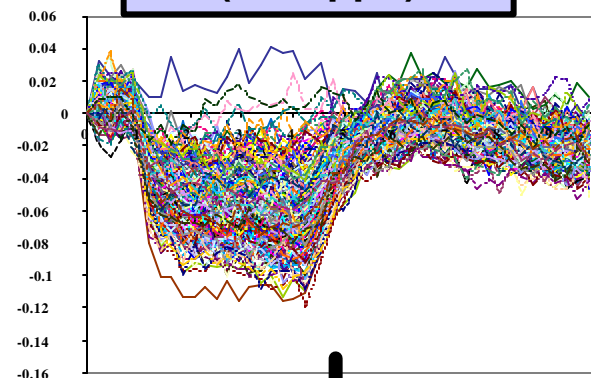
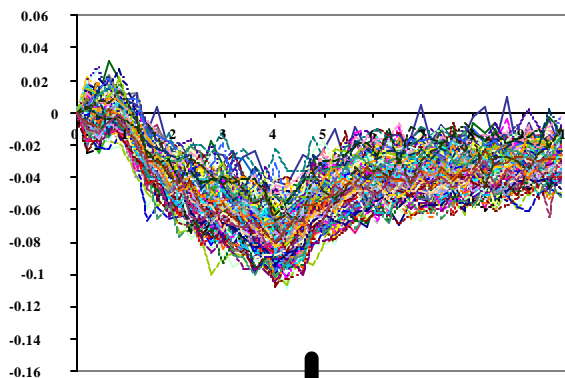
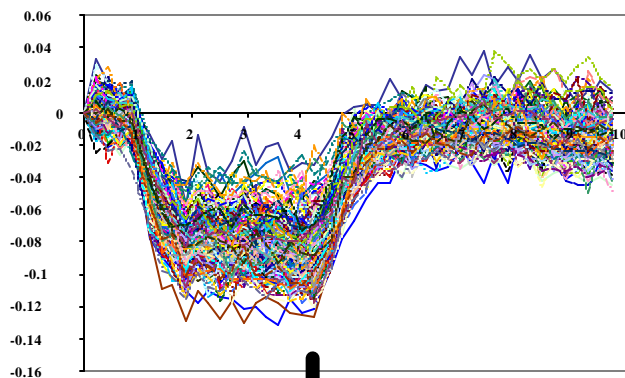


# TIME (s) vs. FLUORESCENCE RESPONSE: 250 INDIVIDUAL Bead Sensors

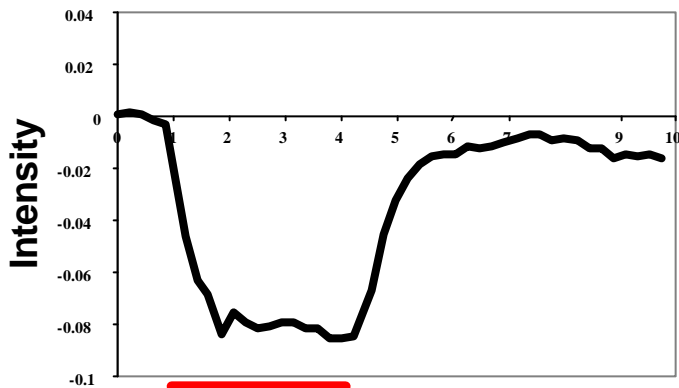
~23 ppb 2,4-DNT

~80 ppb 1,3-DNB

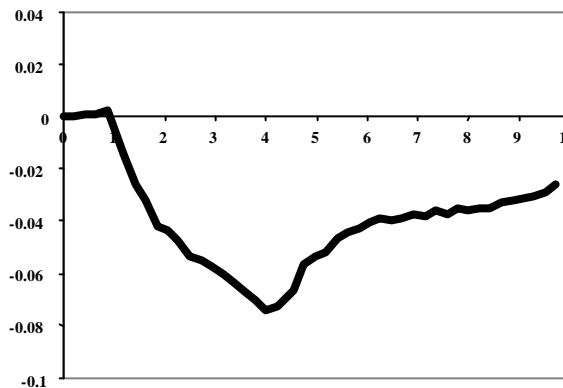
8% saturated  
TNT vapor Strips  
(~0.4 ppb)



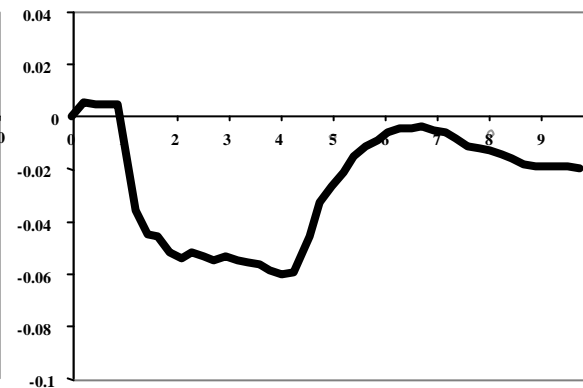
Signal/Noise Improvement:  
Average of 1000 Sensors



3.25 s vapor exposure



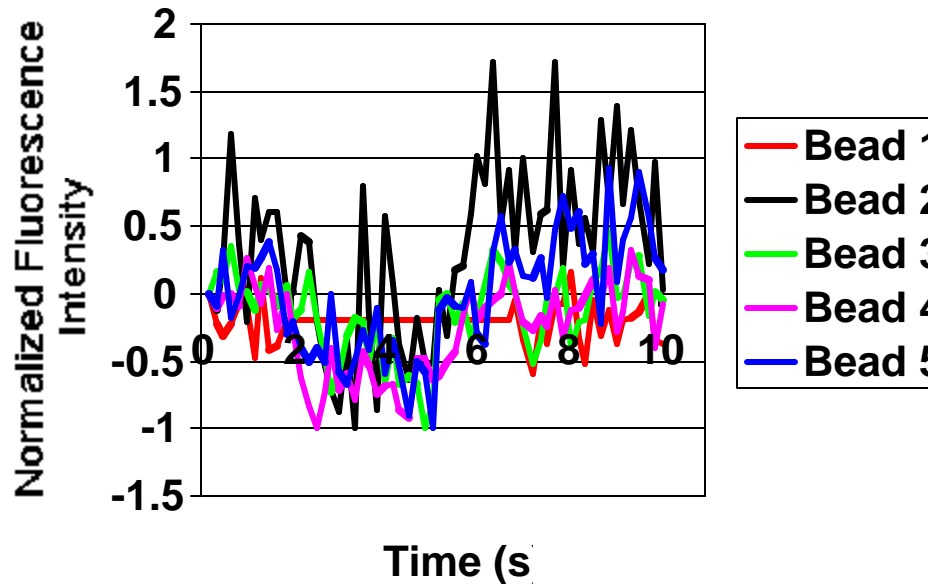
Time (s)



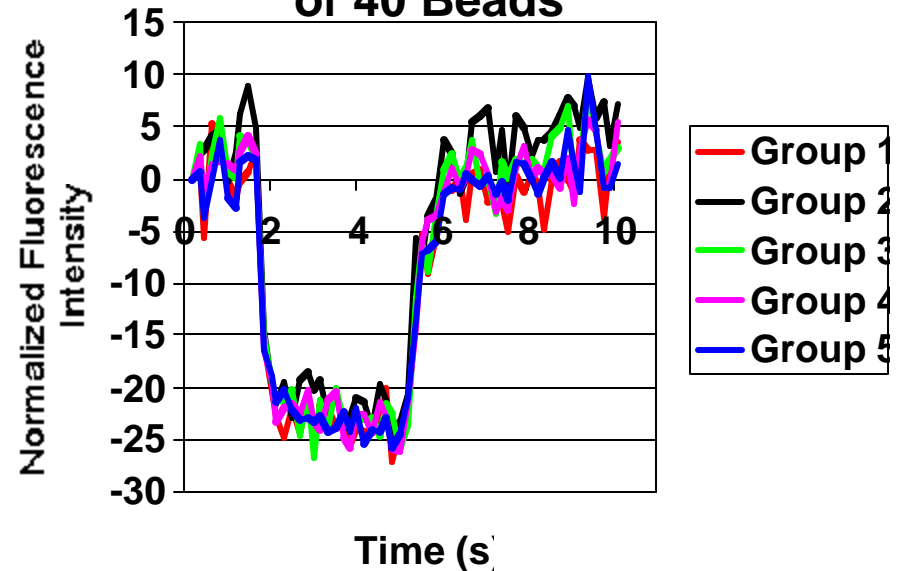
Albert, K. J. and D. R. Walt (2000) *Anal. Chem.* in press.

# Signal Summing

**Individual Response of  
5 Bead Sensors**



**Summed Responses of  
5 Random Groups  
of 40 Beads**



**Summing improves signal-to-noise ratio.**

# Analytes for Two Class Problem

- Pure Analytes
  - Acetone
  - Benzene
  - Chloroform
  - Ethanol
  - Ethyl Acetate
  - Heptane
  - Methanol
  - Toluene
  - 1,3-Dinitrobenzene
  - 4-Nitrotoluene
- Binary Mixtures
  - Ethyl Acetate/Heptane
  - Methanol/Benzene
  - 4-NT/Benzene
  - 4-NT/Heptane
  - 4-NT/Methanol
  - 1,3-DNB/Ethyl Acetate
  - 1,3-DNB/Heptane

# Concentrations of Analytes

Table 1: The concentration of the pure analytes  $\pm 15\%$ .

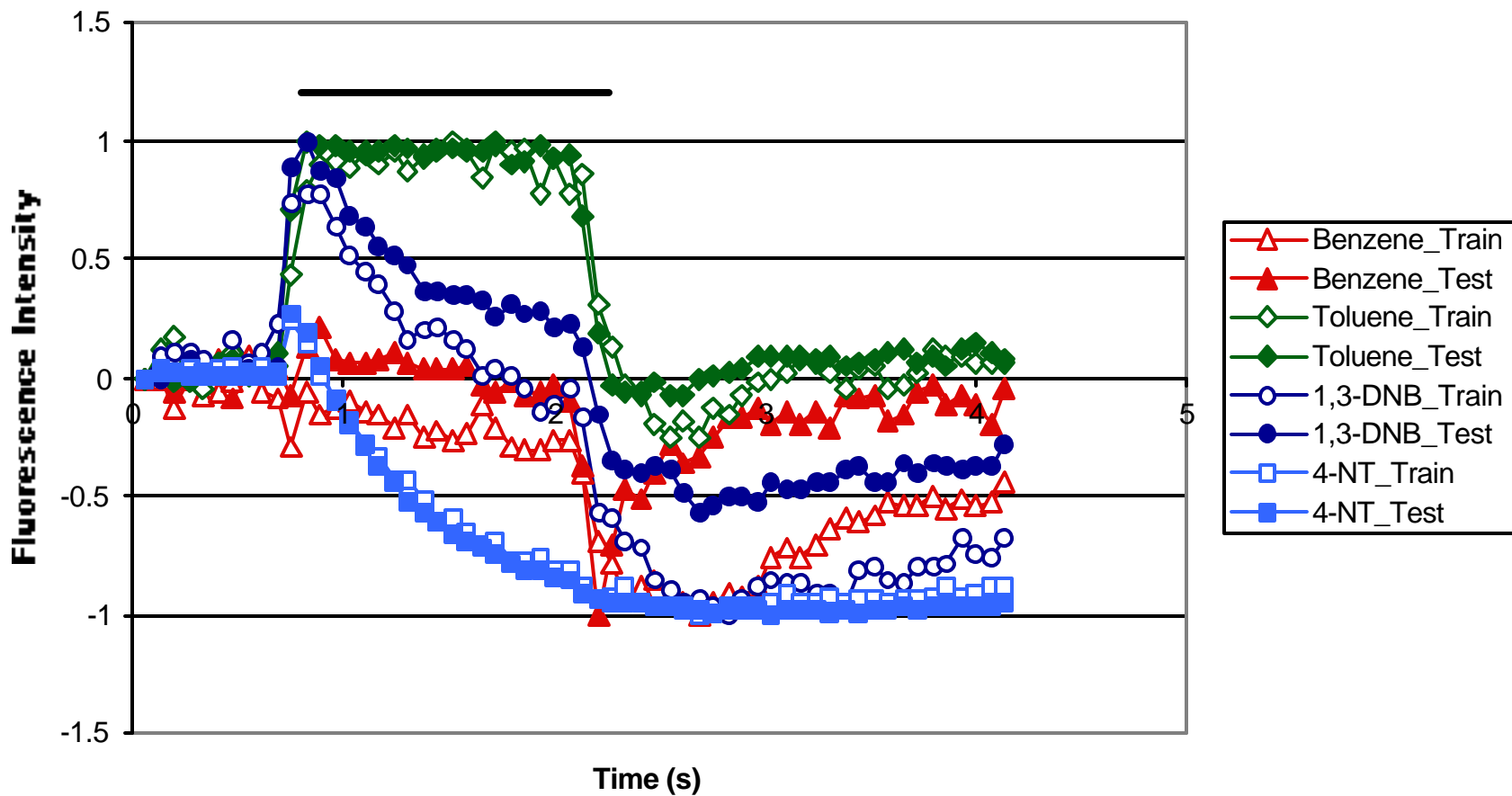
The concentrations were calculated based on literature values for analyte vapor pressures.

| Analyte            | Vapor Pressure @25 °C (mmHg) | Concentration (ppm) |
|--------------------|------------------------------|---------------------|
| Acetone            | 2.31E+02                     | 7.6E+04             |
| Benzene            | 9.53E+01                     | 3.1E+04             |
| Chloroform         | 1.97E+02                     | 6.5E+04             |
| Ethanol            | 5.90E+01                     | 1.9E+04             |
| Ethyl Acetate      | 9.45E+01                     | 3.1E+04             |
| Heptane            | 4.57E+01                     | 1.5E+04             |
| Methanol           | 1.27E+02                     | 4.2E+04             |
| Toluene            | 2.84E+01                     | 9.4E+03             |
| 1,3-Dinitrobenzene | 9.00E-04                     | 6.0E-01             |
| 4-Nitrotoluene     | 1.64E-01                     | 1.1E+02             |

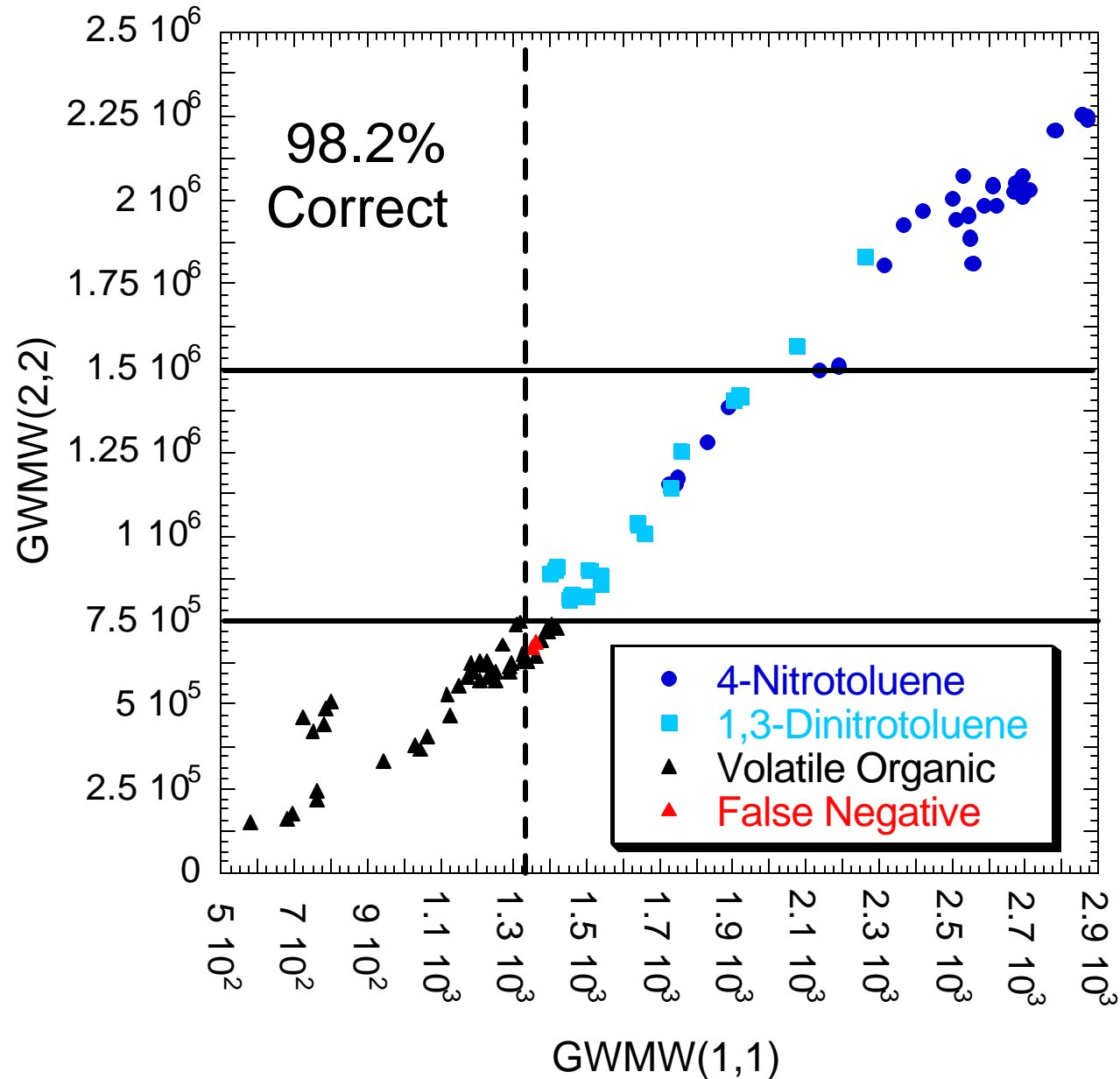
Table 2: The concentration of the binary mixtures  $\pm 15\%$ .

| Analyte 1     | Analyte 2          | Concentration analyte1 (ppm) | Concentration analyte2 (ppm) |
|---------------|--------------------|------------------------------|------------------------------|
| Benzene       | Methanol           | 3.1E+04                      | 4.2E+04                      |
| Benzene       | 4-Nitrotoluene     | 3.1E+04                      | 5.5E+01                      |
| Benzene       | 4-Nitrotoluene     | 3.1E+04                      | 1.1E+02                      |
| Ethyl Acetate | Heptane            | 3.1E+04                      | 1.5E+04                      |
| Ethyl Acetate | 1,3-Dinitrotoluene | 3.1E+04                      | 3.0E-01                      |
| Ethyl Acetate | 1,3-Dinitrotoluene | 3.1E+04                      | 6.0E-01                      |
| Heptane       | 1,3-Dinitrotoluene | 1.5E+04                      | 6.0E-01                      |
| Heptane       | 4-Nitrotoluene     | 1.5E+04                      | 1.1E+02                      |
| Methanol      | 4-Nitrotoluene     | 4.2E+04                      | 5.5E+01                      |
| Methanol      | 4-Nitrotoluene     | 4.2E+04                      | 1.1E+02                      |

# Reproducible Responses from Training to Testing array



# First Testing Array (1 Month)





# Live/Dead Bacteria Discrimination

## Calculated Identity

| Actual Identity | Calculated Identity |         |         |         |         |          |         |         |         |         |        |
|-----------------|---------------------|---------|---------|---------|---------|----------|---------|---------|---------|---------|--------|
|                 | Live B10            | Live B4 | Live B5 | Live B8 | Live B9 | Dead B10 | Dead B4 | Dead B5 | Dead B8 | Dead B9 | Medium |
| Live B10        | 4                   | 1       | 0       | 0       | 0       | 0        | 0       | 0       | 0       | 0       | 0      |
| Live B4         | 2                   | 3       | 0       | 0       | 0       | 0        | 0       | 0       | 0       | 0       | 0      |
| Live B5         | 0                   | 0       | 5       | 0       | 0       | 0        | 0       | 0       | 0       | 0       | 0      |
| Live B8         | 0                   | 0       | 0       | 4       | 0       | 0        | 1       | 0       | 0       | 0       | 0      |
| Live B9         | 0                   | 0       | 0       | 0       | 5       | 0        | 0       | 0       | 0       | 0       | 0      |
| Dead B10        | 0                   | 0       | 0       | 0       | 0       | 5        | 0       | 0       | 0       | 0       | 0      |
| Dead B4         | 0                   | 0       | 0       | 0       | 0       | 0        | 5       | 0       | 0       | 0       | 0      |
| Dead B5         | 0                   | 0       | 0       | 0       | 0       | 0        | 0       | 5       | 0       | 0       | 0      |
| Dead B8         | 0                   | 0       | 0       | 0       | 0       | 0        | 0       | 0       | 5       | 0       | 0      |
| Dead B9         | 0                   | 0       | 1       | 0       | 2       | 0        | 0       | 2       | 0       | 0       | 0      |
| Medium          | 0                   | 0       | 0       | 0       | 0       | 0        | 0       | 0       | 0       | 0       | 10     |

85% Correct, 87% Variance (7PCs)

B10: *Acintobacterium*

B4: *M. luteus*

B5: *E. coli*

B8: *Salmonella*

B9: *Klebsiella peumoniae*

# Acknowledgements

ONR



DARPA



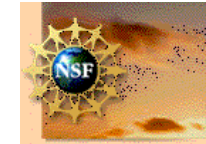
DOE



NIH



NSF



## Walt Group

Myoyong Lee  
Karri Michael  
Paul Pantano  
Caroline Schauer  
Jenny Tam

Keith Albert  
Todd Dickinson  
Jane Ferguson  
Michael Fleming  
Jason Epstein

Ferenc Szurdoki  
Frank Steemers  
Shannon Stitzel  
Laura Taylor  
Tarun Mandal

## Lawrence Livermore

Fred Milanovich

## Pennsylvania State University

Peter C. Jurs

## Tufts University School of Medicine

John S. Kauer     Joel White

## Johns Hopkins University

Lenore Cowen

## Illumina

Mark Chee

Kevin Gunderson