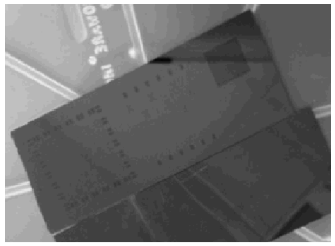
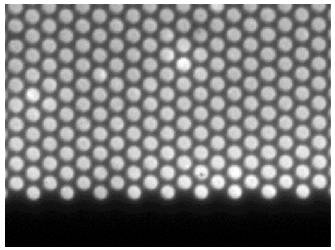
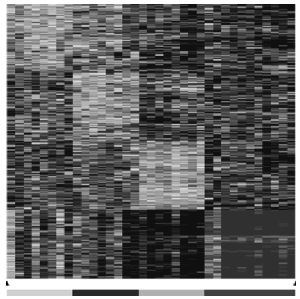
SAND2013-9446P

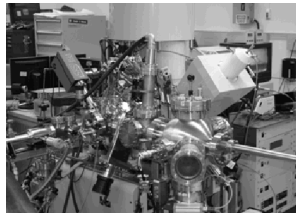
Population-Based Comprehensive Health Monitoring For Combating Infectious Disease and Bioterror Threats





Conrad D. James, Ph.D.
Biosensors and Nanomaterials Dept.
Sandia National Laboratories





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Ana Paula De Oliveira Sales
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HealthTell
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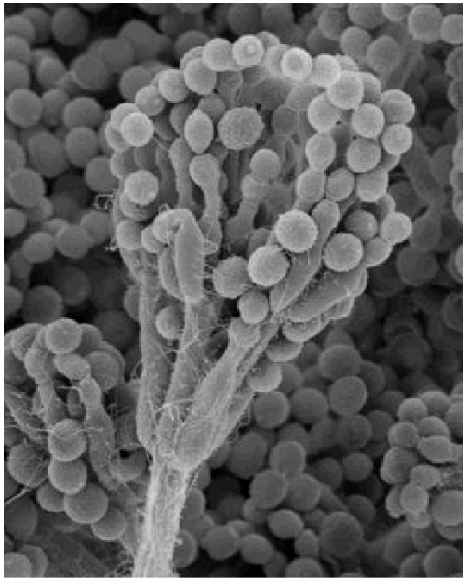
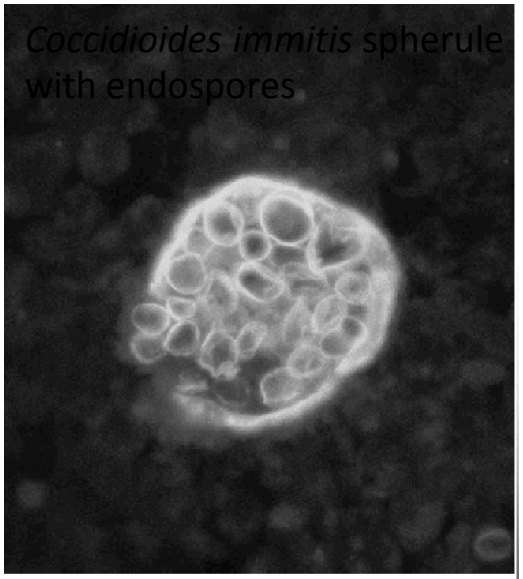
Army Research Office
Defense Threat Reduction Agency
NSF
DARPA

Infectious disease scenario

An outbreak of symptoms among a large population after an event (dust storm, etc.)

http://www.youtube.com/watch?v=8W4Cx44XKZ4





Coccidiomycosis:

- About 10,000 reported cases annually
- Mostly mild cases, can be life threatening
- Flu-like symptoms

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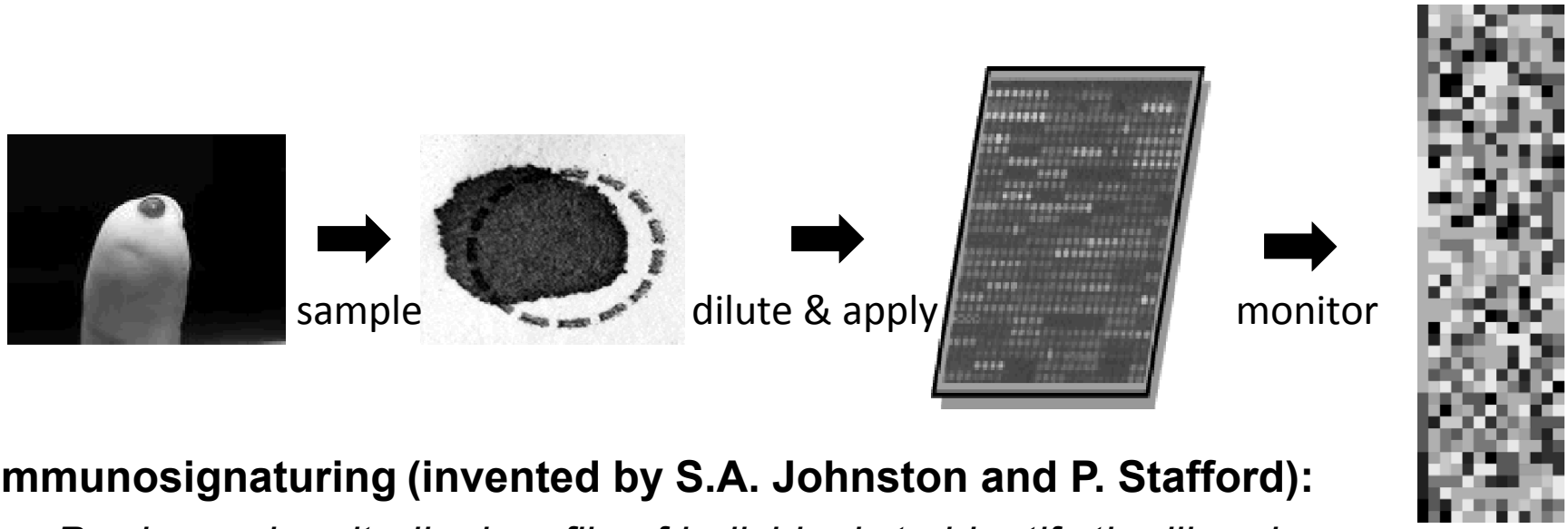
Need: a biodetector for rapid detection of ***ill*** and ***near-ill*** individuals



Immunosignaturing (invented by S.A. Johnston and P. Stafford):

- *Produce a longitudinal profile of individuals to identify the ill and predict individuals that will become ill*
- Only microliters of blood needed; Easy - sample just diluted
- Can be self administered, minimally invasive
- Convenient - can be mailed on filter paper

HealthTell™

Legutki, J. B., D. M. Magee, P. Stafford, S.A. Johnston (2010). Vaccine 28, 4529-4537
Kukreja, M., S.A. Johnston, P. Stafford (2012). J Proteomics Bioinform (6), 10.4172/jpb.S6-001
Stafford, P., R. Halperin, J.B. et al., (2012). Molecular & Cellular Proteomics 11(4), 10.1074/mcp.M111.011593

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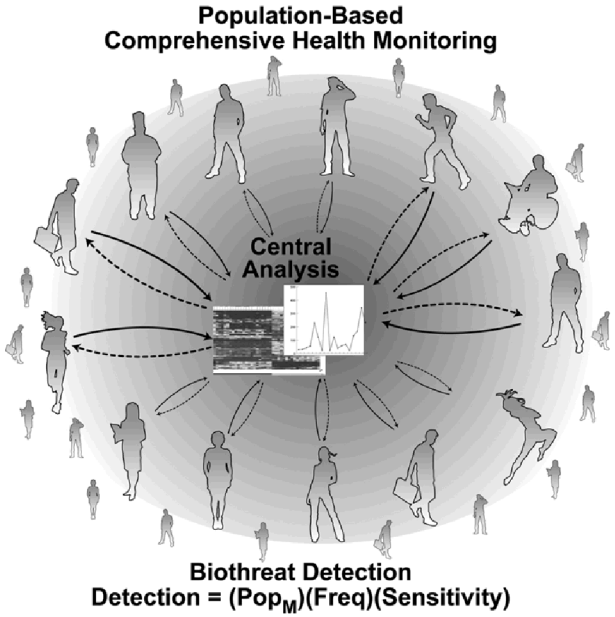
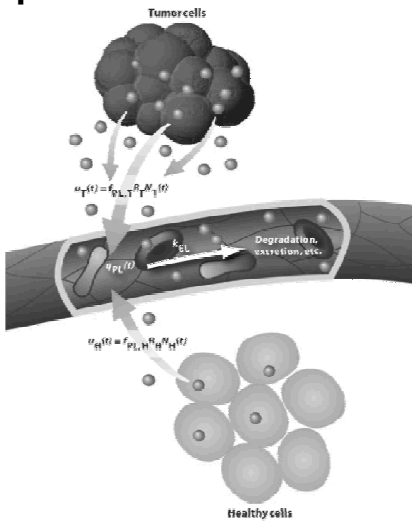
ARIZONA STATE UNIVERSITY

Challenges in biodetection and biosurveillance

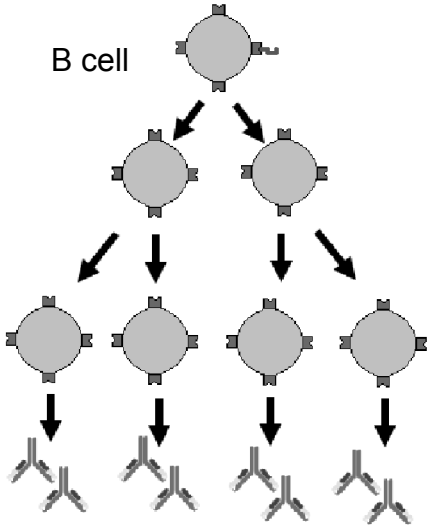


Blood dilution problem...



Hori and Gambhir, *Sci Transl Med*, 2011

The immune system detects & amplifies…



1000s of Abs produced/min;
~10^8 Abs in serum

B-cells respond quickly/specifically to changes in health by producing Abs

Sequence B- and/or T-cell variation in blood or…

Profile Abs over time

ASU

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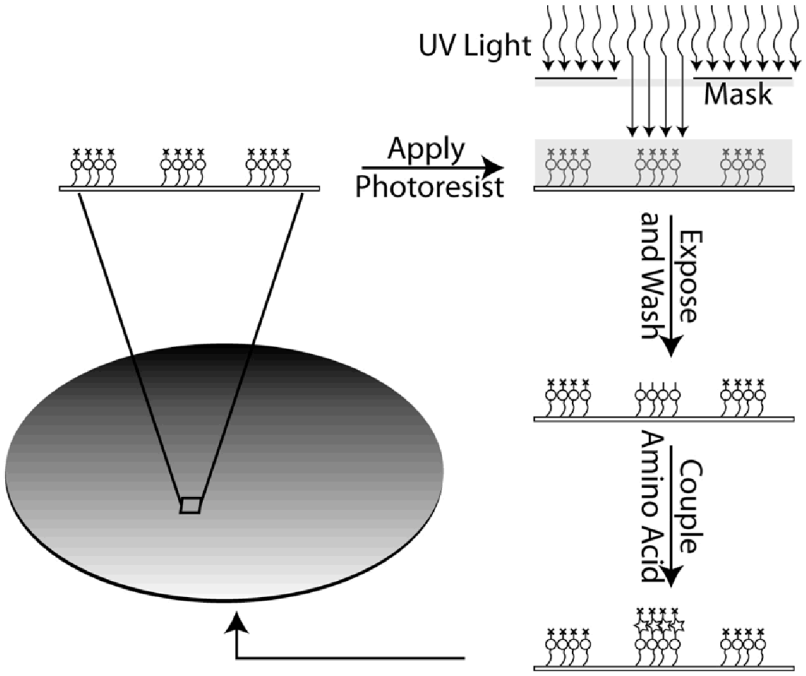
Immunosignaturing with peptide microarrays



- Standard fabrication instruments
- 330K peptide per array (assay)
- 312 assays per wafer

High density → High information content → High volume → Low cost

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ASU ARIZONA STATE UNIVERSITY

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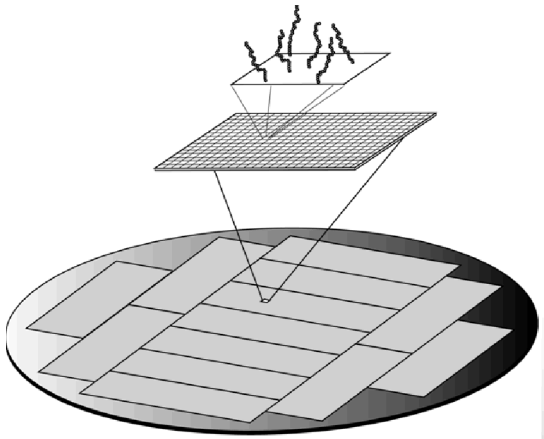
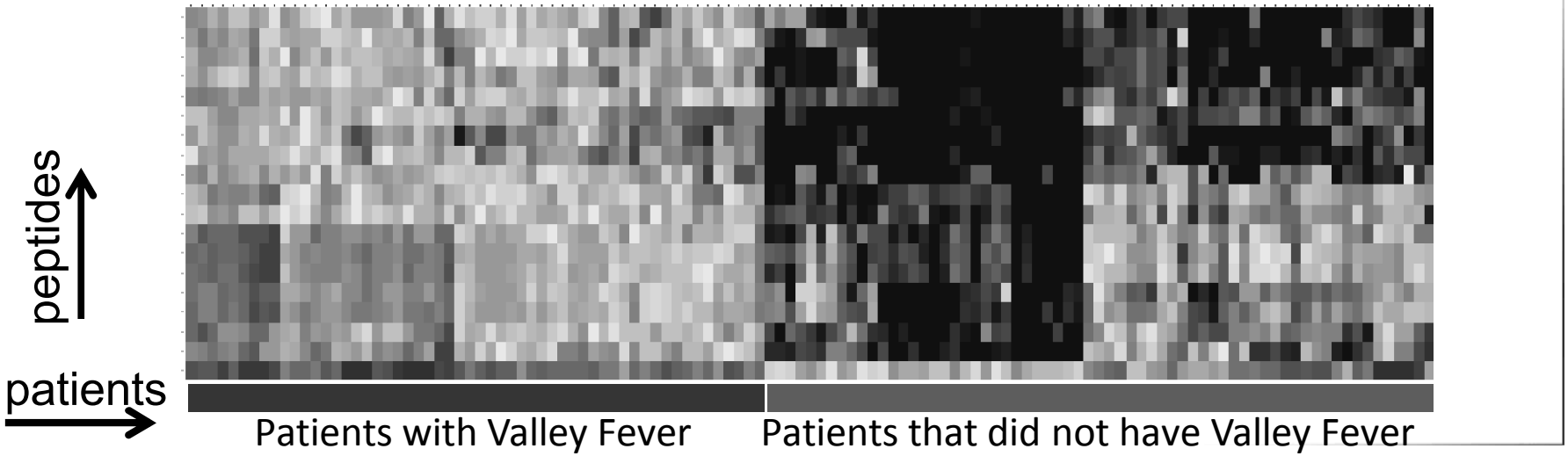
Detection of Valley fever

- 10K peptides on original array, 100 peptides selected
- 90 blinded samples from patients at the clinic
- Zero false positives (100% specificity)
- Zero false negatives (100% sensitivity)

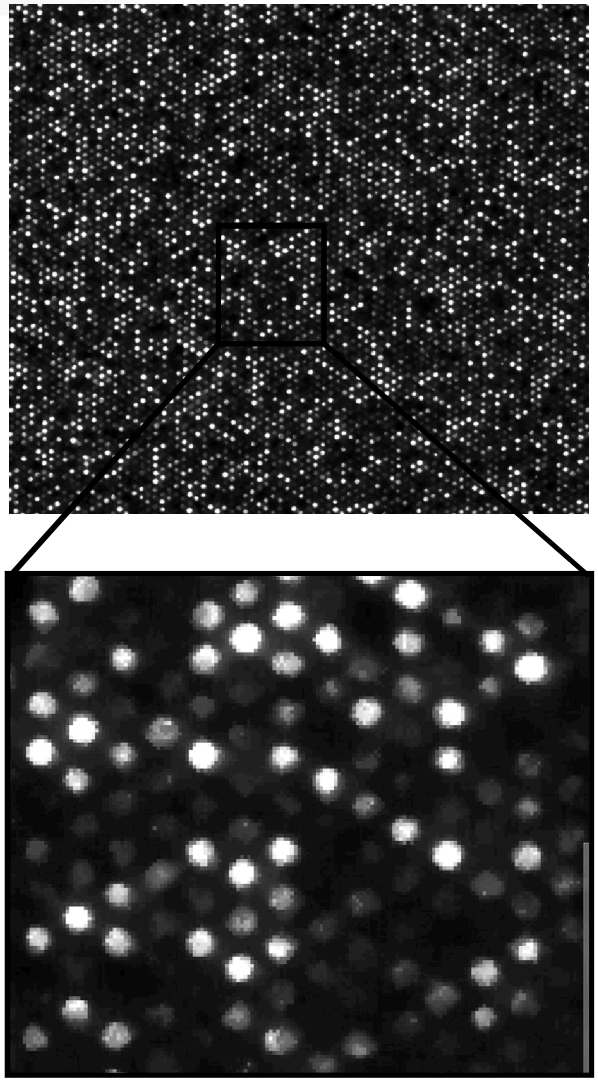
All Patients with Valley Fever Presented with Zero CF Titers, but were later shown to have the disease

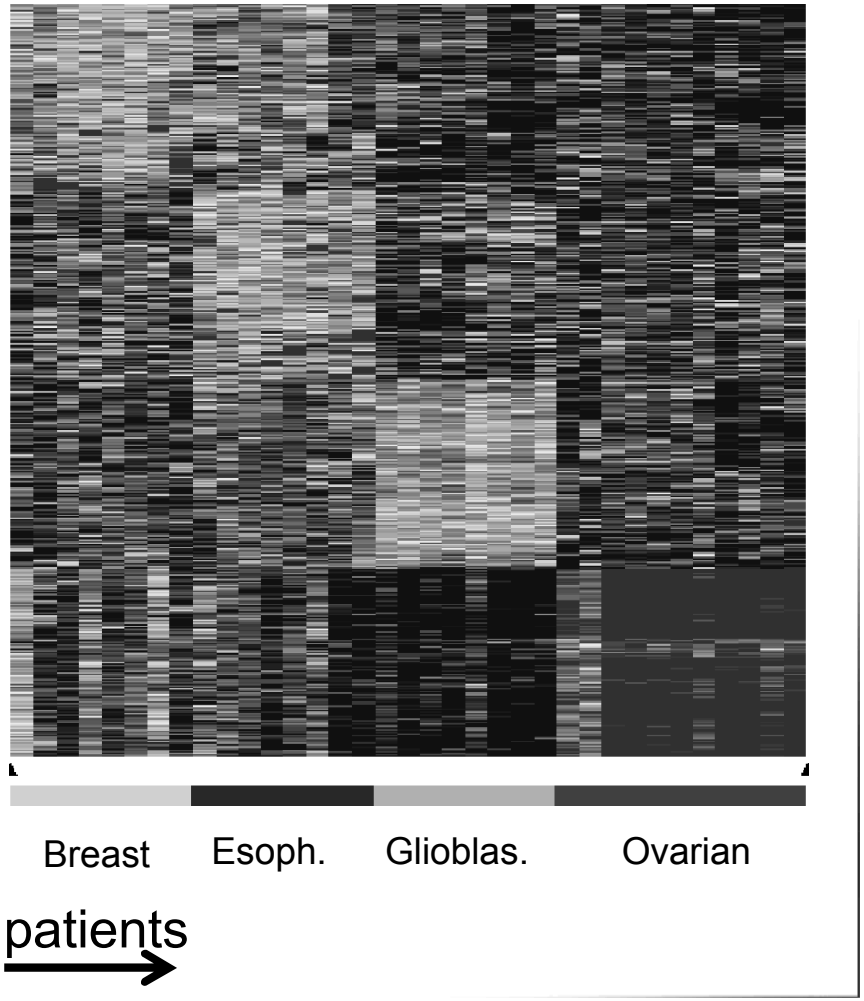




Discriminating cancers with the peptide array technology

Example array with 8 micron spots





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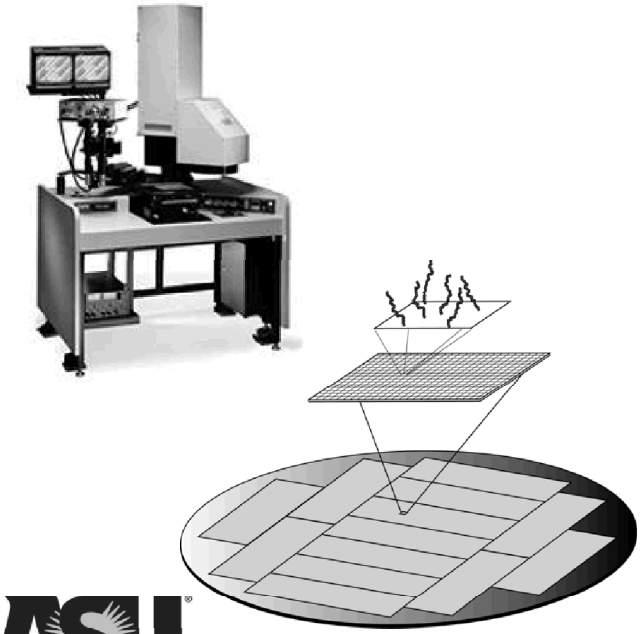
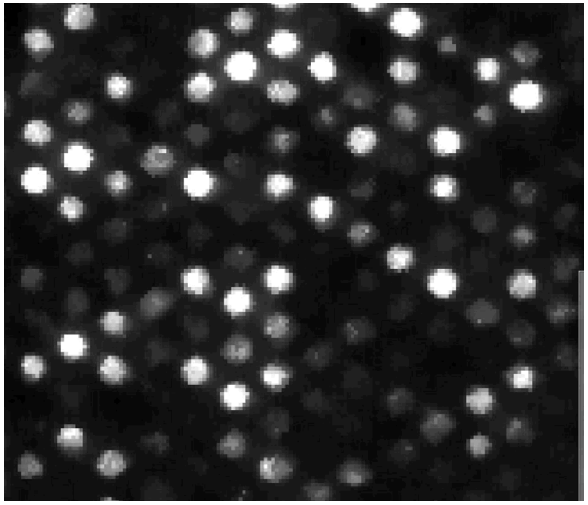
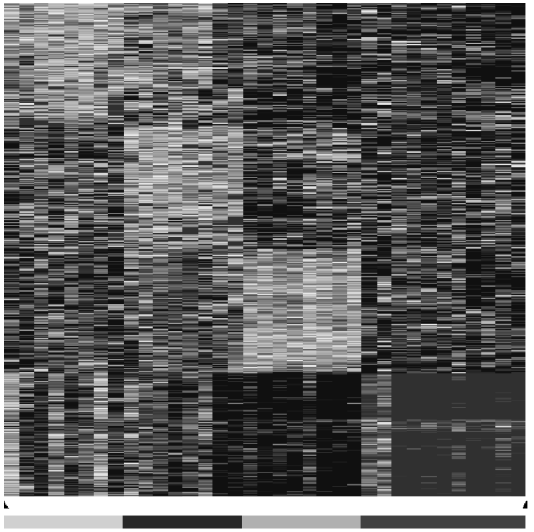
Technology maturation drivers

- Manufacturability – product readiness for the market
- Robustness – consistent performance across platforms
- Quality assurance – accuracy of synthesized peptides

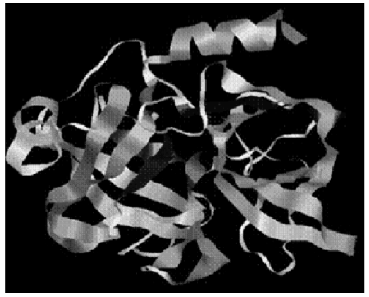




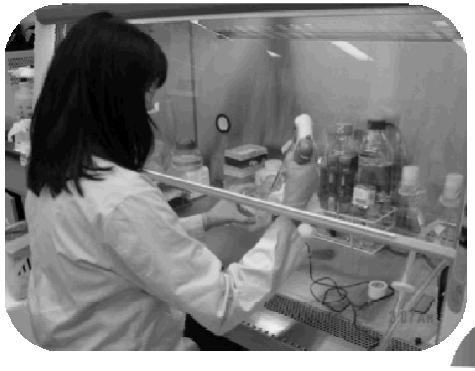


Technology maturation: U.S. National Laboratories

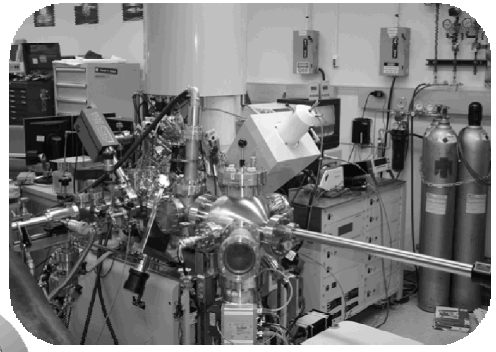
Peptide design & performance



Process development



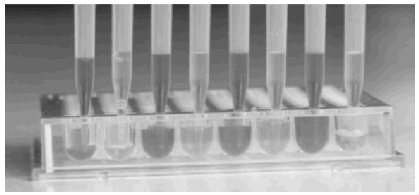
Characterization



Exploratory production

Low/mid-level production

Sample testing & assay optimization



Mature, optimize, and innovate

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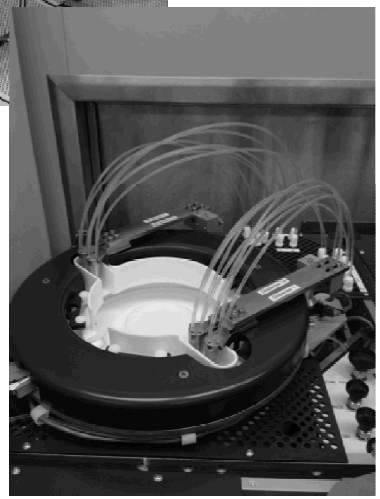
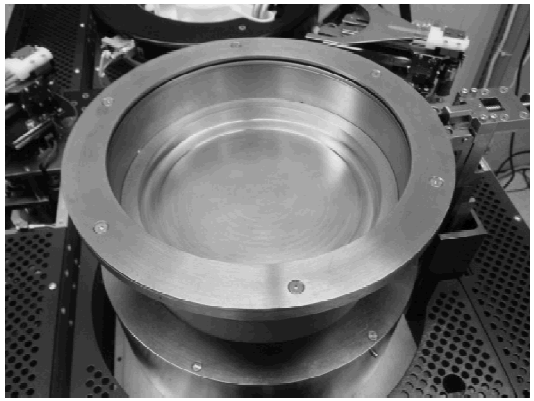
Pilot peptide array manufacturing





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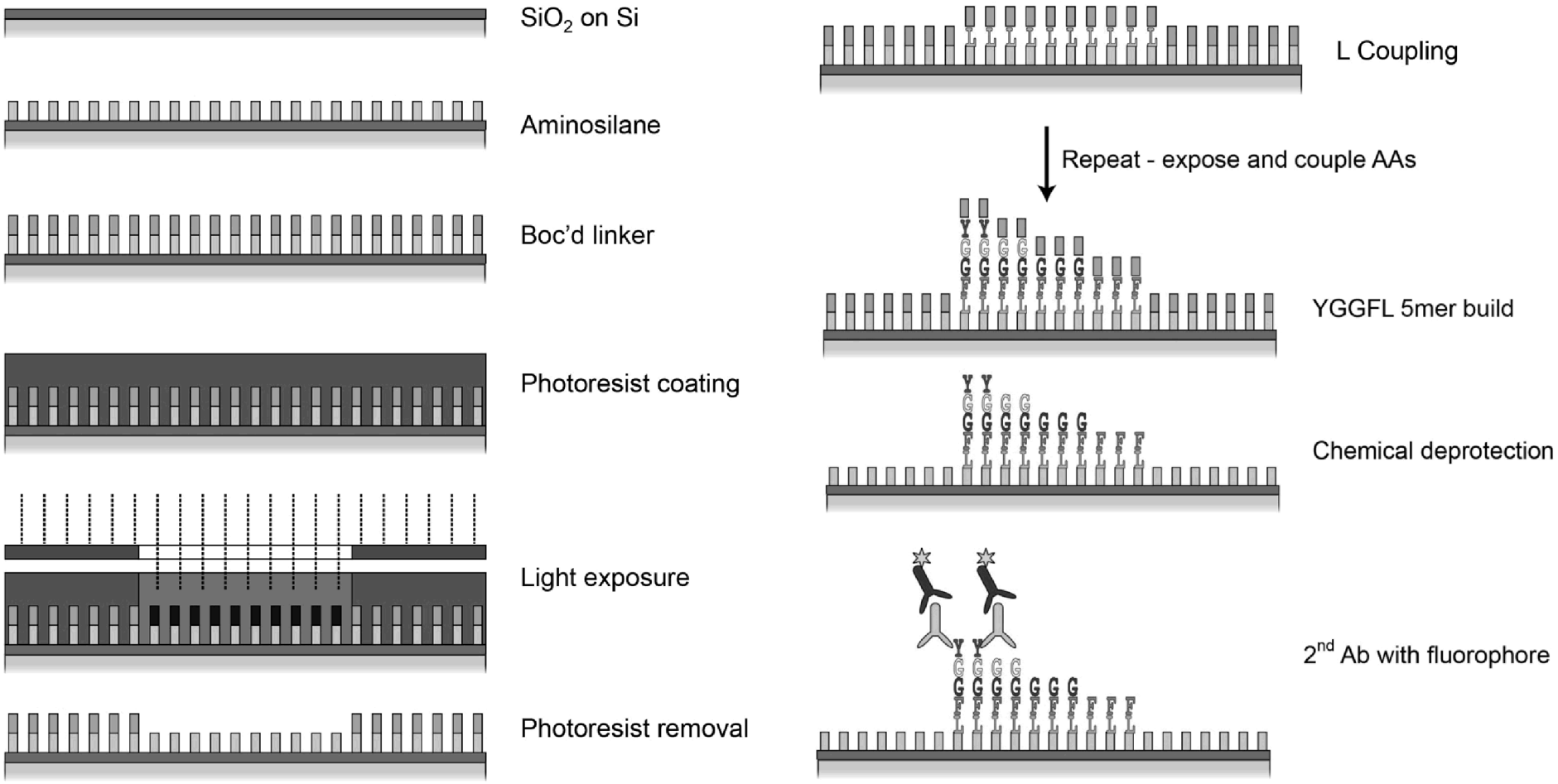
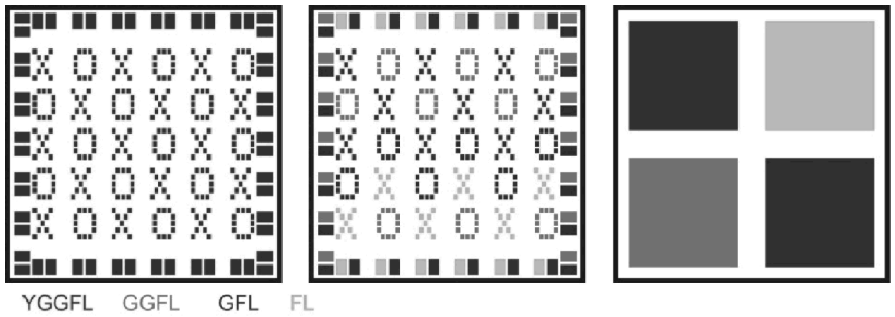
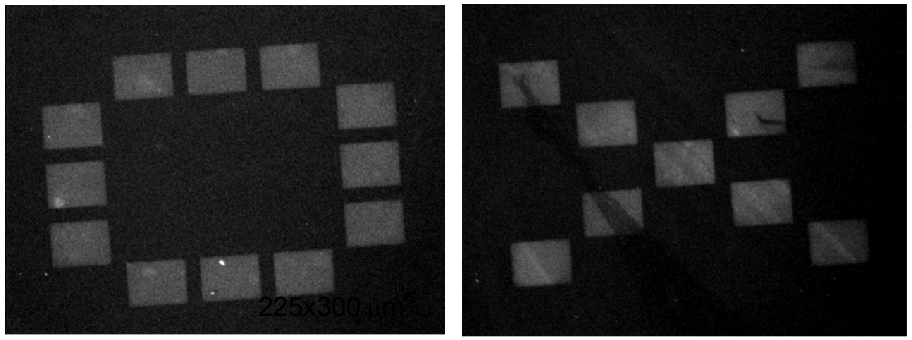
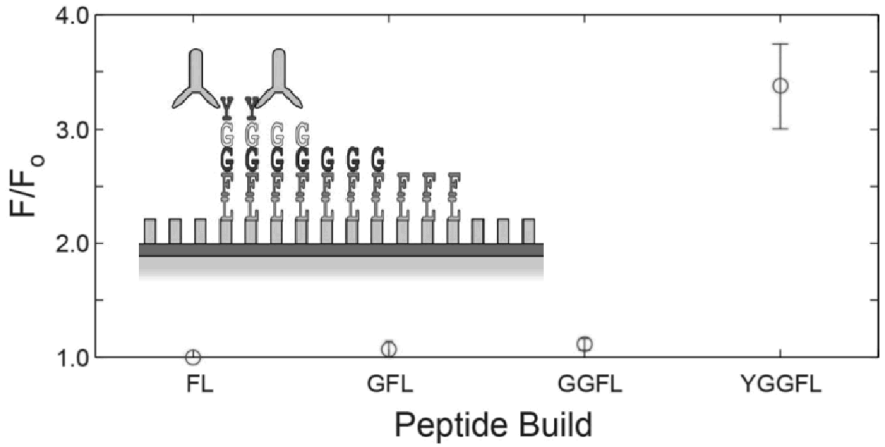
Microarray fabrication process- photolithography and reaction chemistry



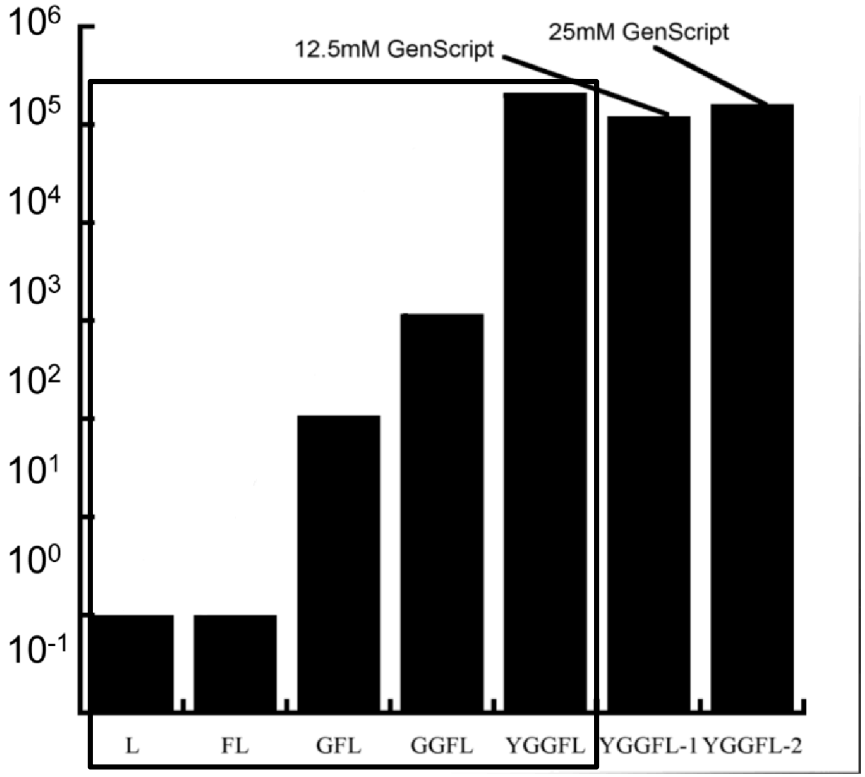
5mer peptide fabrication with control deletions







- Vehicle for testing/optimizing new peptide coupling chemistry
- Positive and negative controls



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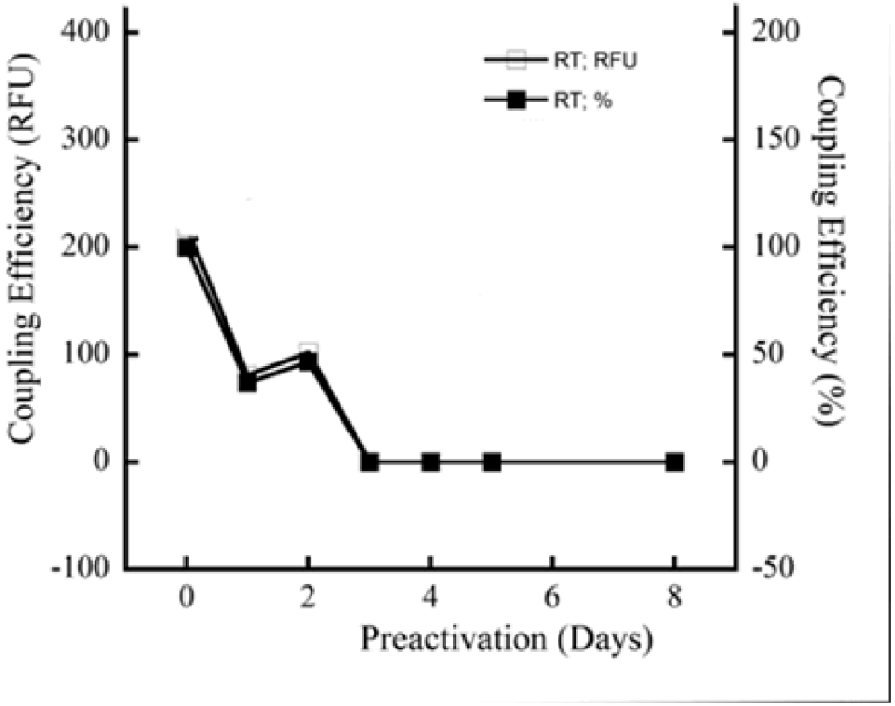
Stability of coupling reagents in a high-volume production environment

Examine reagent stability under storage conditions; potential need for *in situ* mixing



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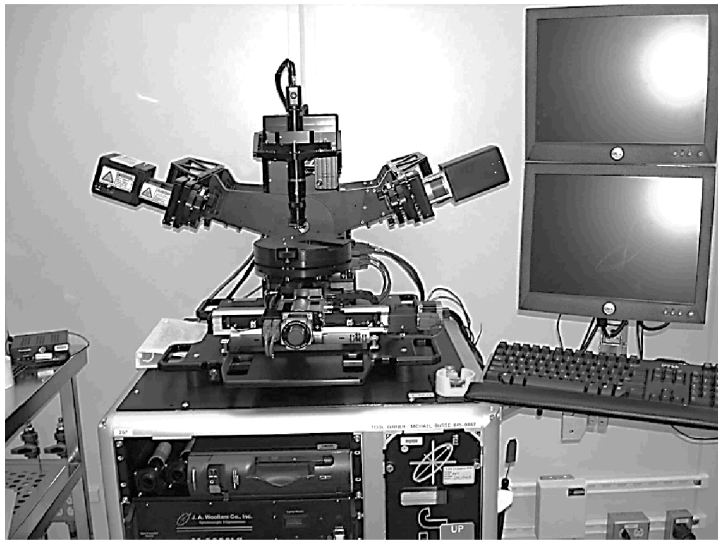
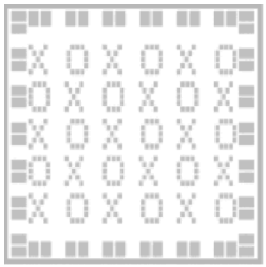
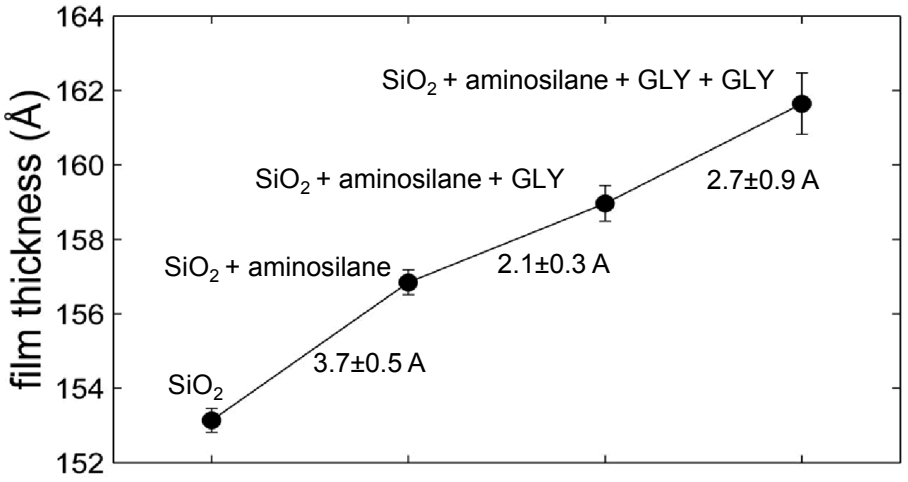
in situ ellipsometry for non-destructive sample assessment

Film analysis for sample-to-sample variability and longitudinal tracking of chemical processing





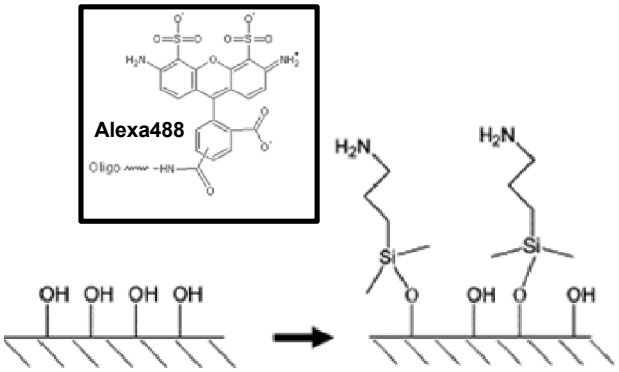
sample	film thickness (Å)	condition
1	155.25	oxide
2	157.21	oxide
3	157.24	oxide
4	154.78	oxide
5	160.83	silane
6	162.17	silane
7	163.93	silane
8	164.51	silane

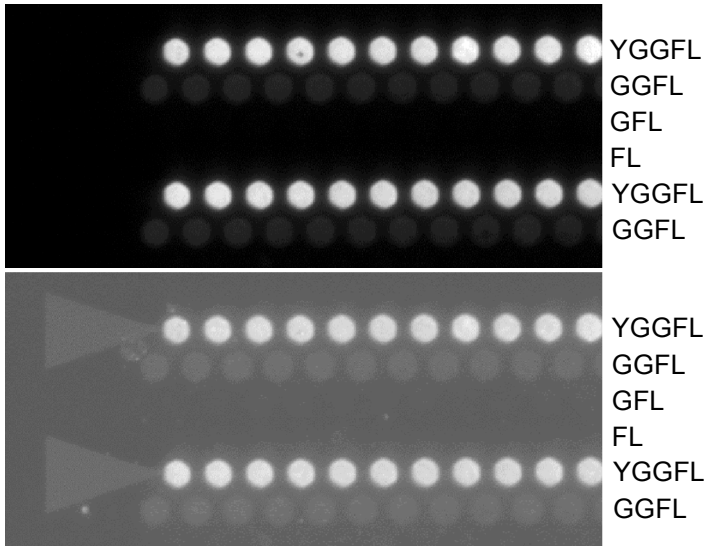


National Laboratories

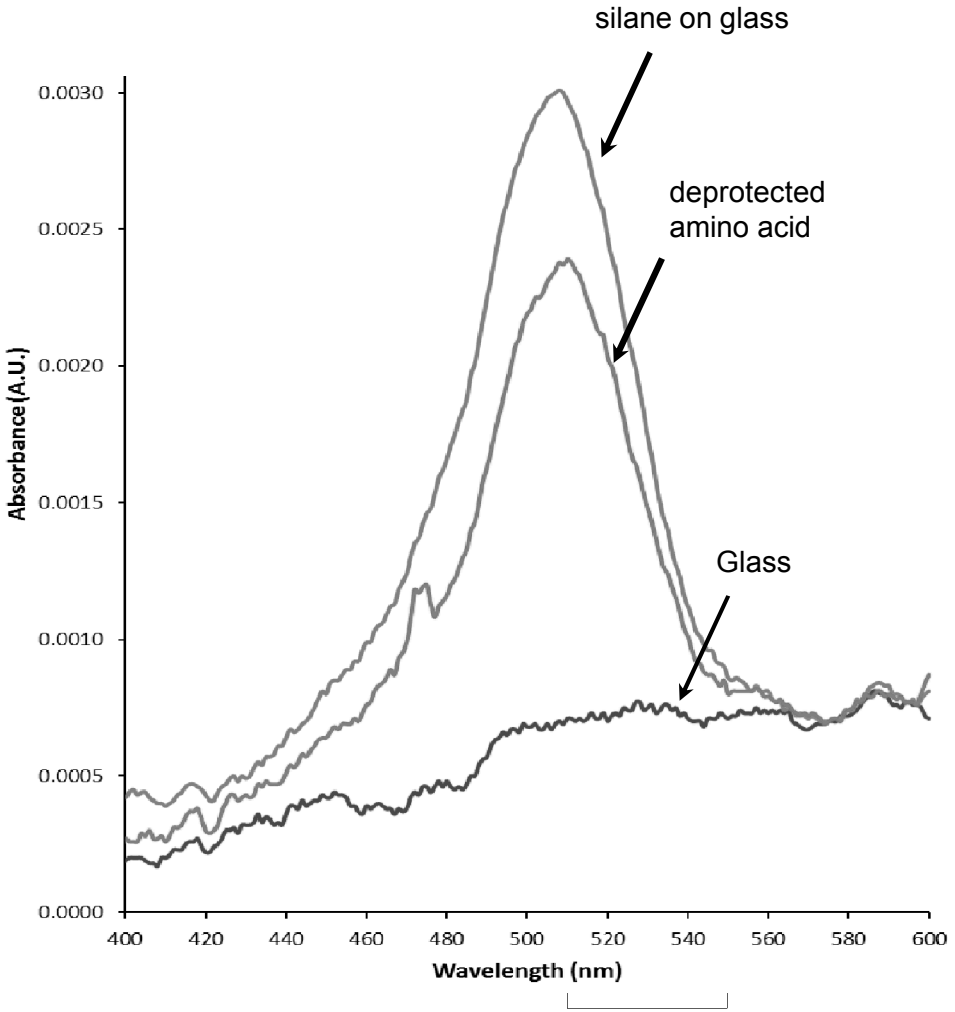
Quantitative measurement of amine-group surface density

Surface chemistry of peptide arrays:





UV/VIS/NIR Spectrometry:

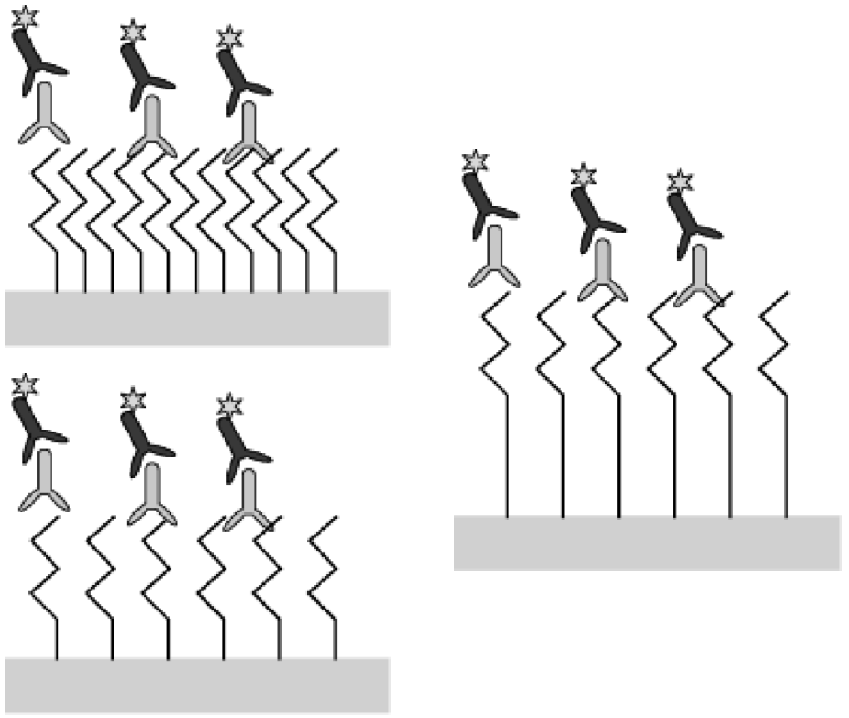


Affinity and avidity – impact on peptide array performance

Quantitative measurement of molecular surface density:

$$\Gamma = {}^{1}/_{2}\left[\frac{A_\lambda N_A}{\varepsilon_\lambda}\right] \text{ (molecules} \cdot \text{cm}^{-2}\text{)}$$

Surface	Molecules/cm^2 [10^{12}]	Surface amines [%]
Total free amines	9.12	100
Coupled Met-Boc	7.25	79.5
Neutralized	1.11	12.2
Unreacted free amines	0.76	8.4
Deprotected amines	4.54	49.8

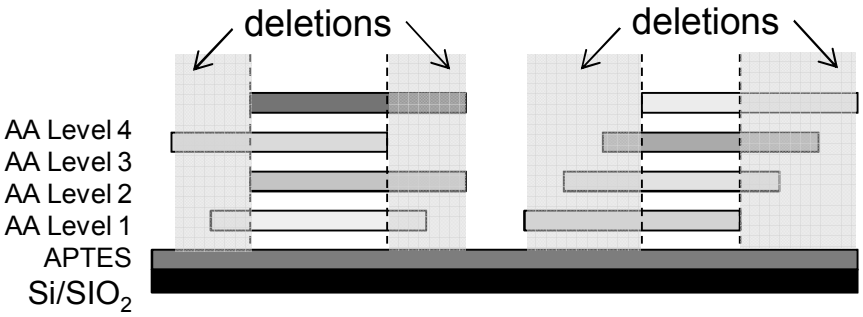


Many factors to explore to optimize Ab binding to peptide array: density, linker length, etc.

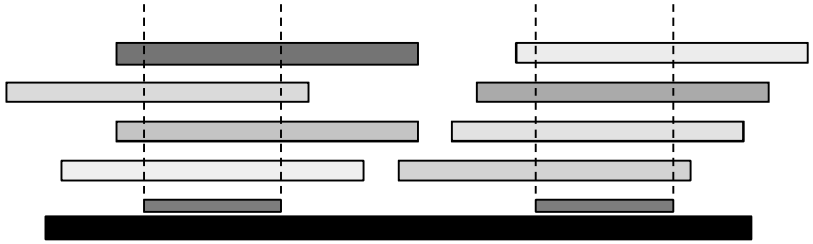
Alvarez et al. *Langmuir* **2011**, *27*, 2789; Bramblett et al. *Surf. Interface Anal.* **2002**, *373*, 91; Caruso et al. *Langmuir* **1998**, *14*, 3462.; Wang et al. *BioTechniques* **2005**, *38*, 127

Peptide deletion control with photolithographic confinement

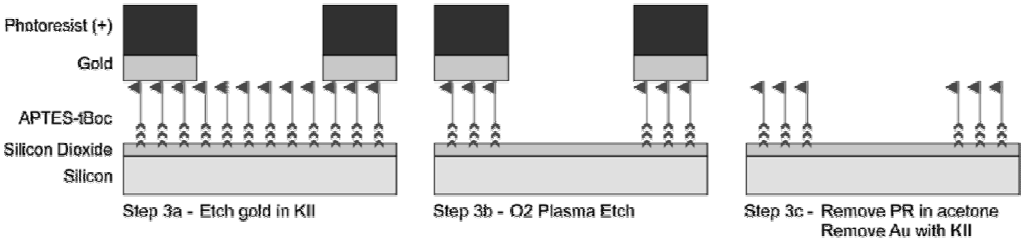
Photolithographic confinement of the aminosilane linker - reduces variation in array element size, improving array repeatablity, reduces occurrence of deletions

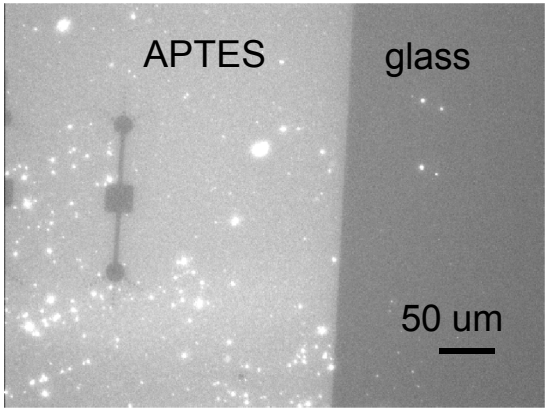


blanket aminosilane



isolated aminosilane





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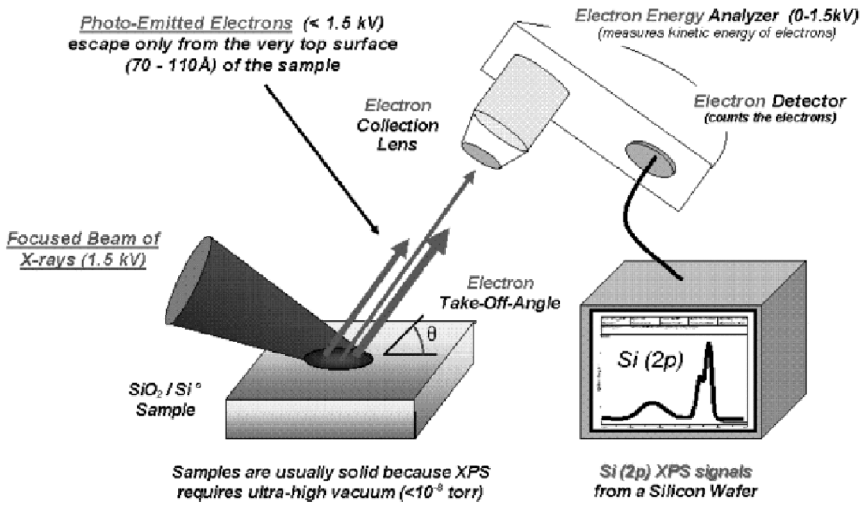
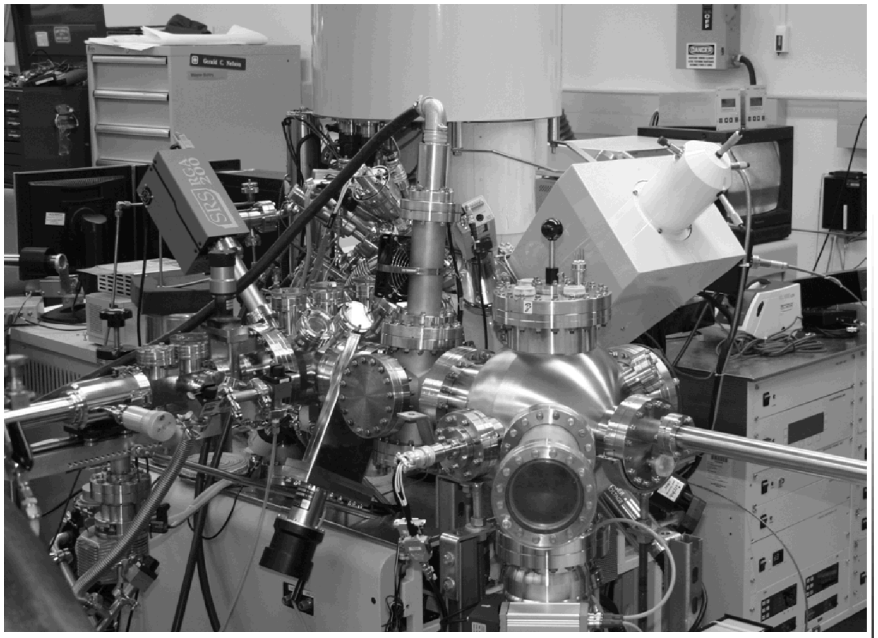
Spectroscopic analytical techniques for quality assurance and control

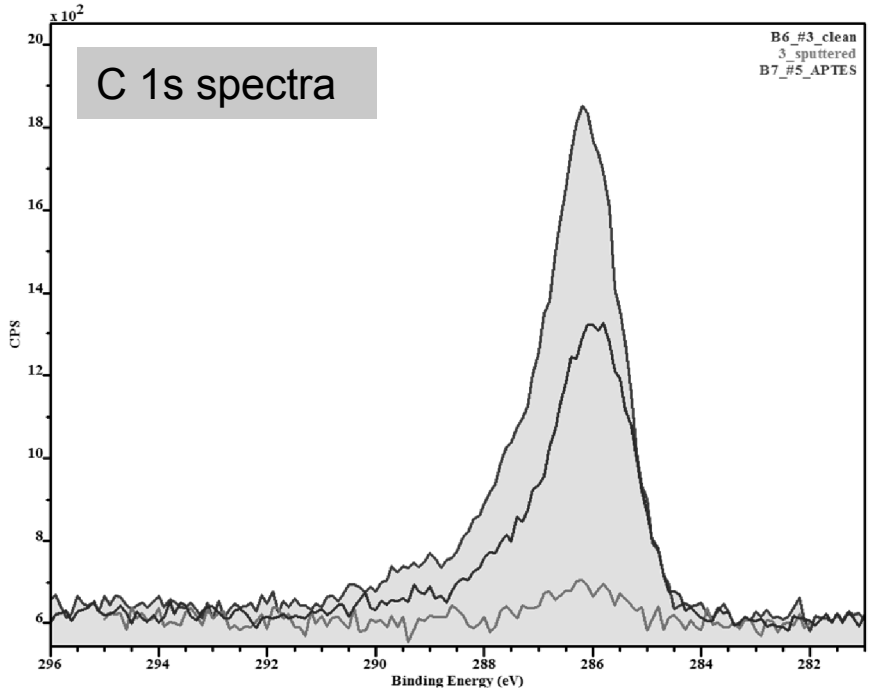
X-ray photoelectron spectroscopy (XPS):

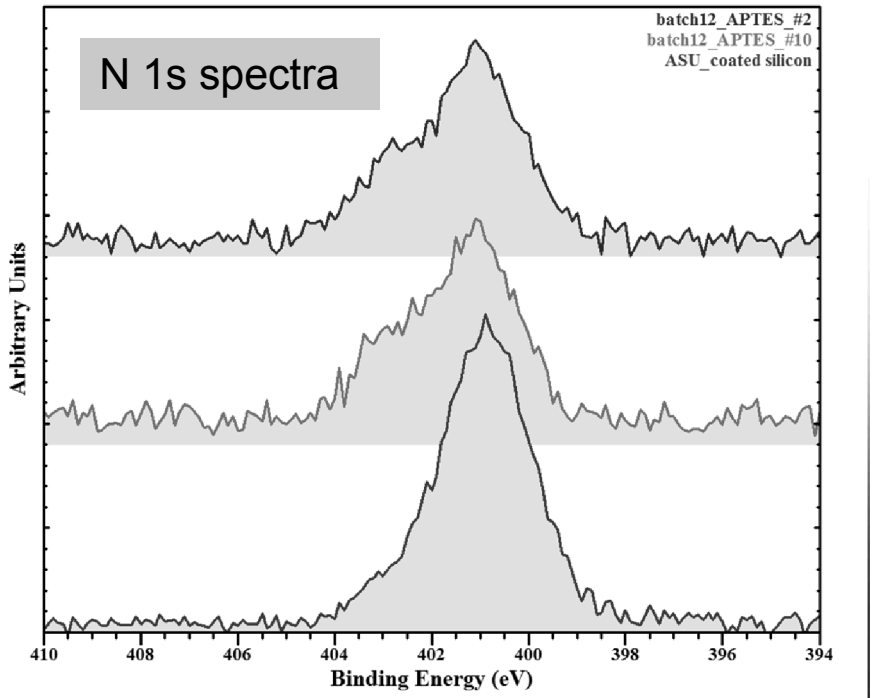




- XPS provides elemental and chemical surface analysis; ratiometric analysis to handle SNRs

Analysis of the substrate chemical state after peptide array processes





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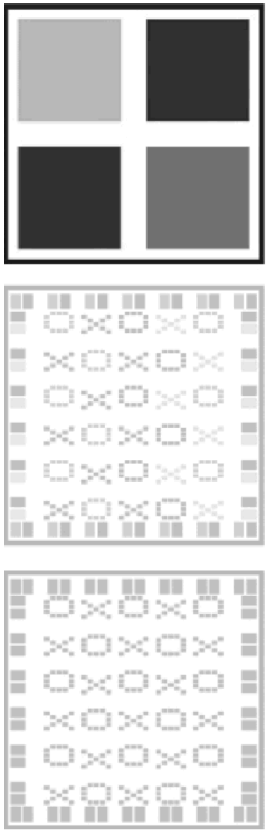
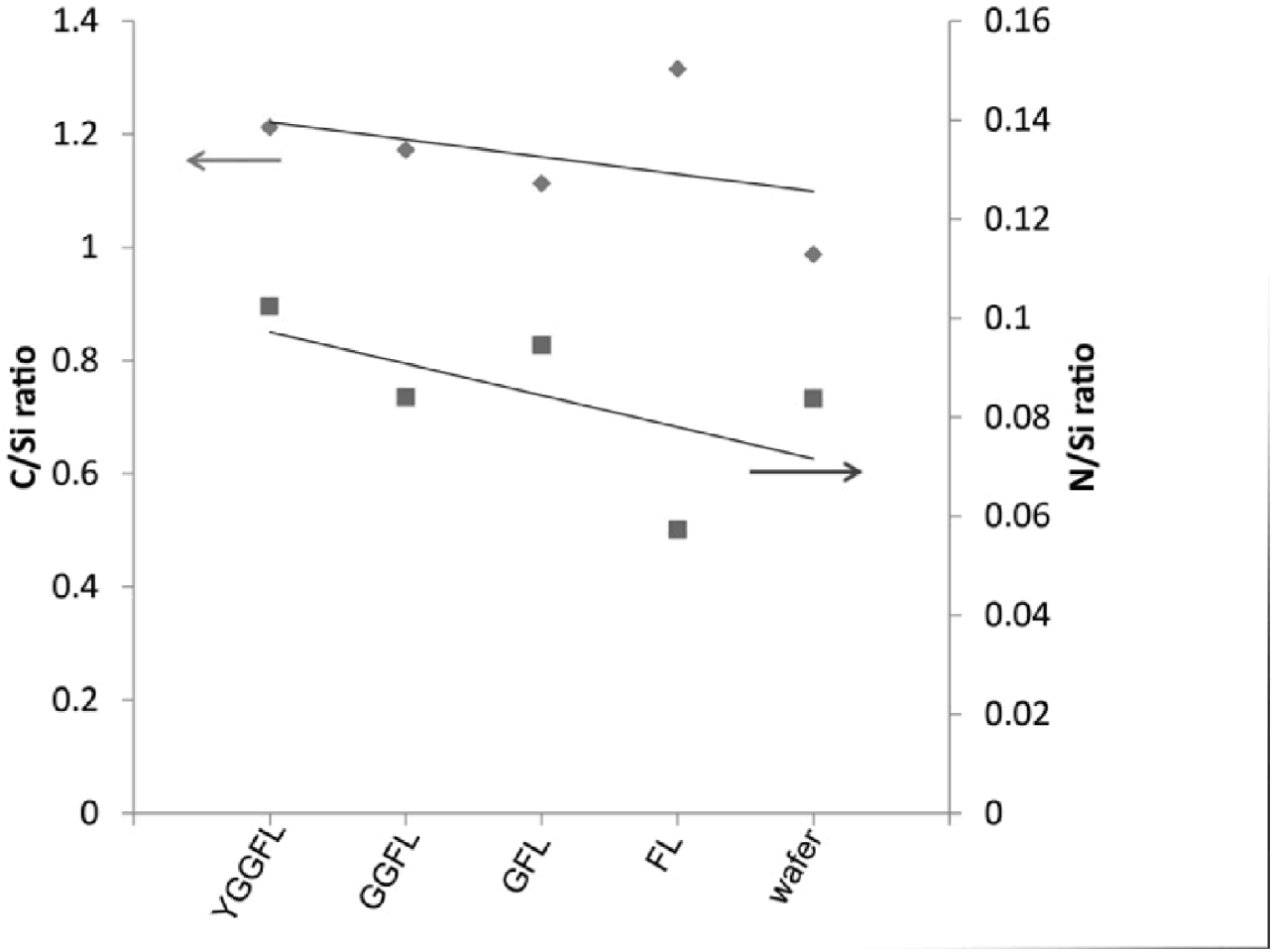
Quantitative quality control for peptide fabrication





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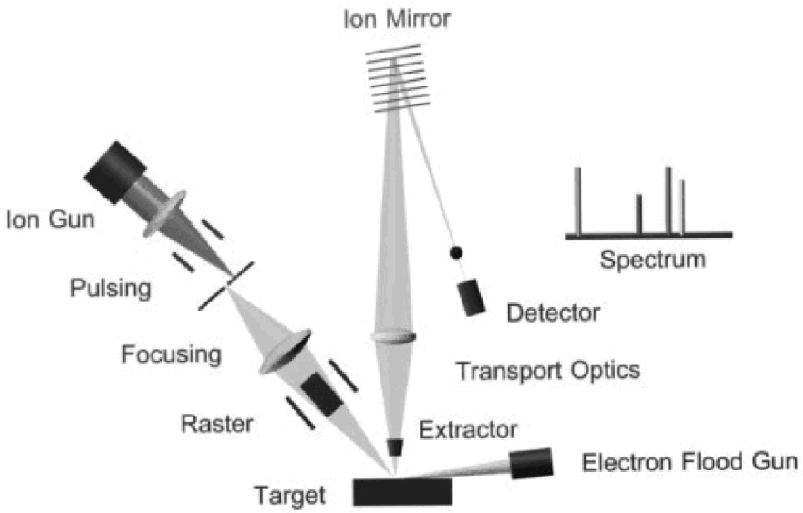
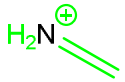
Identification of peptide signatures with mass spectrometry

- Time-of-flight secondary ion mass spectroscopy (TOF-SIMS) analyzes material *ion fragments* from ion bombardment; high mass resolution (ppt)

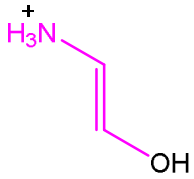




Gly

Chemical Formula: CH_4N^+

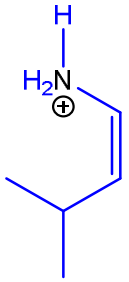
m/z: 30.03 (100.0%), 31.04 (1.1%)



Chemical Formula: $C_2H_6NO^+$

m/z: 60.04 (100.0%), 61.05 (2.3%)

G-Immonium Ion



Chemical Formula: $C_5H_{12}N$

L-Immonium Ion:

Henry and Bertrand, *Surface & Interface Analysis* **2009**, 41, 105
Sole-Domenech, et al., *Analytical Chemistry* **2010**, 82, 1964

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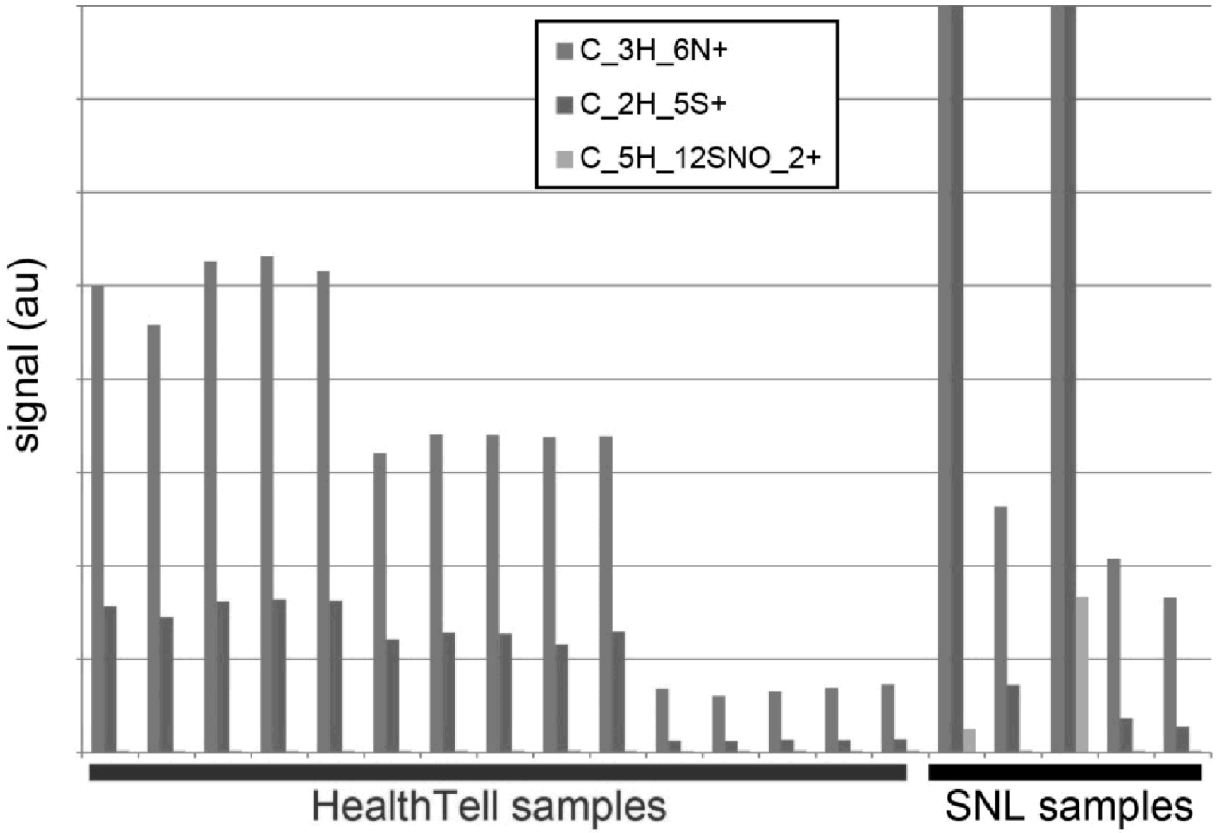
Identification of elemental & molecular signatures of amino acids



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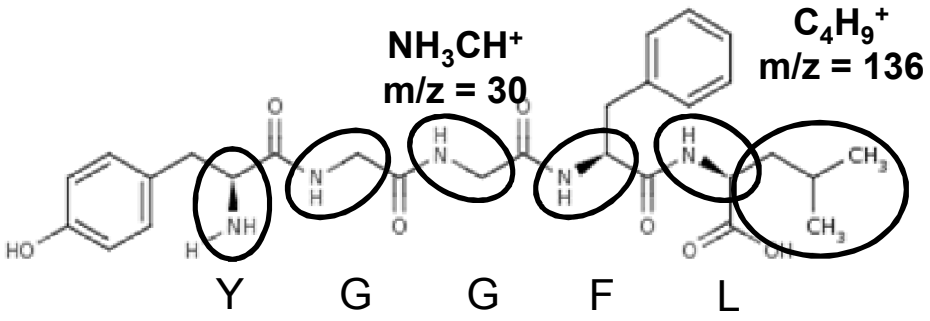
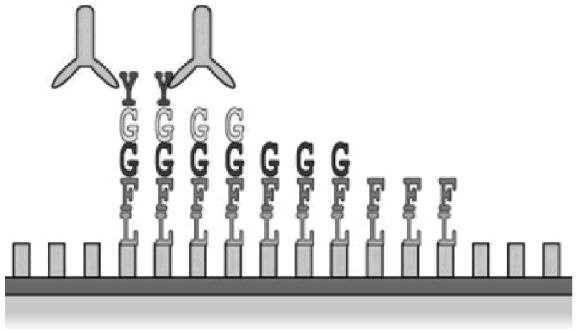
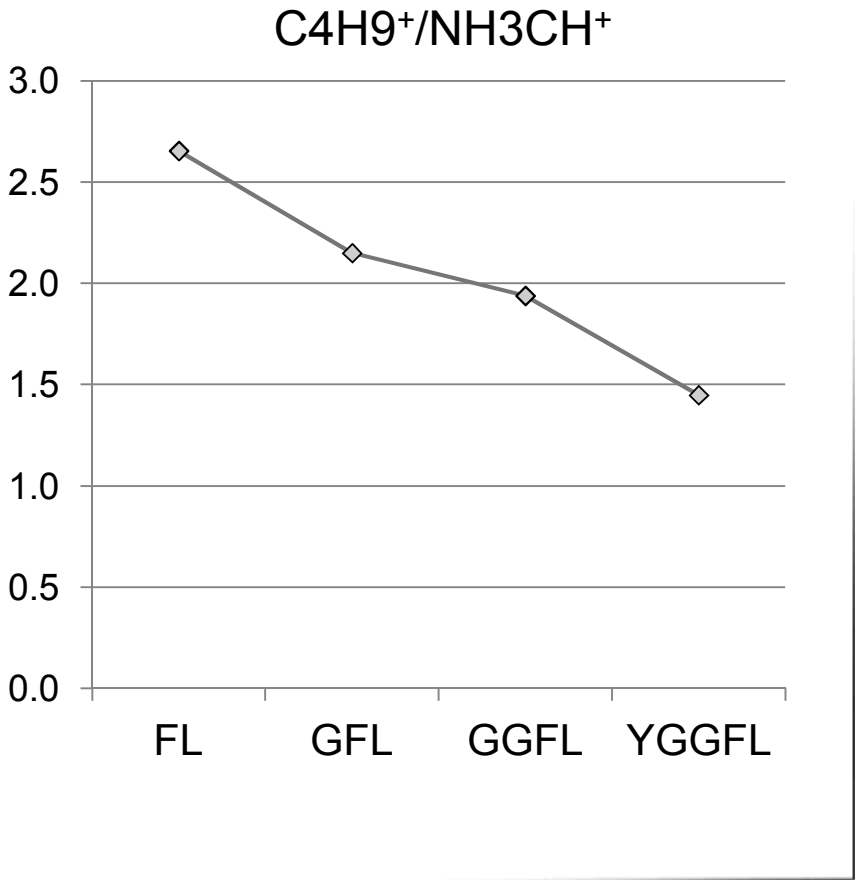
Tracking of peptide synthesis with TOF-SIMS



http://pepbank.mgh.harvard.edu/interactions/details/57761





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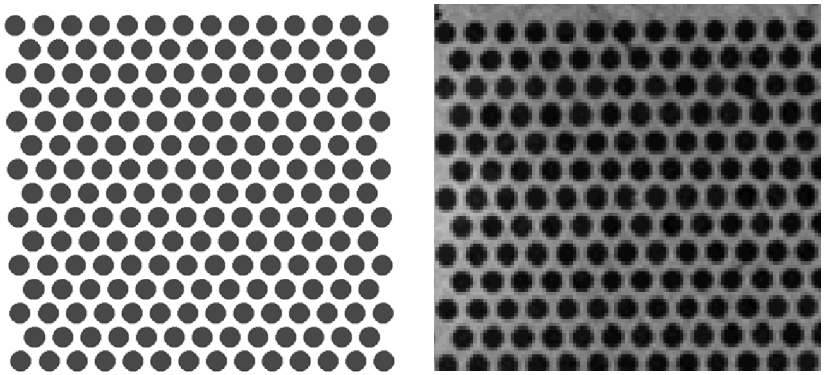
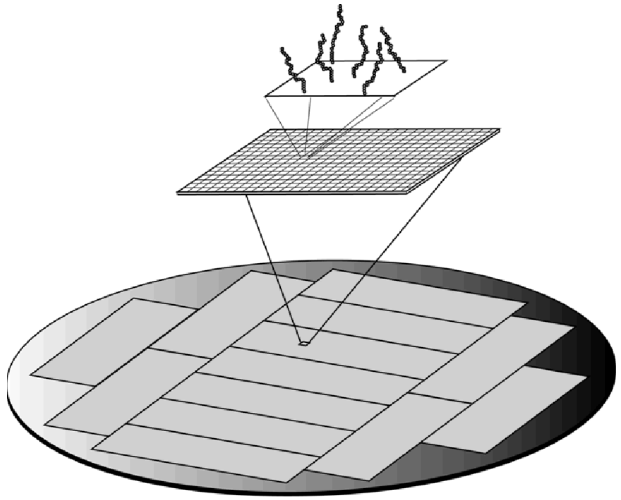
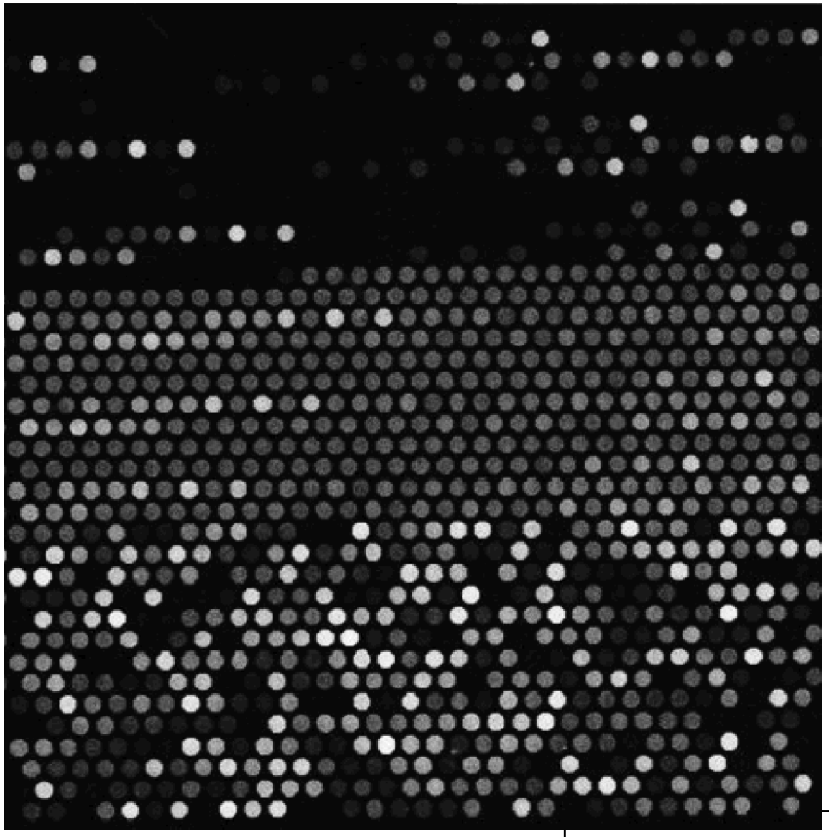
TOF-SIMS imaging for peptide content analysis

- Scanning mode, 5 μm/pixel
- Identify amino acid secondary ion signatures



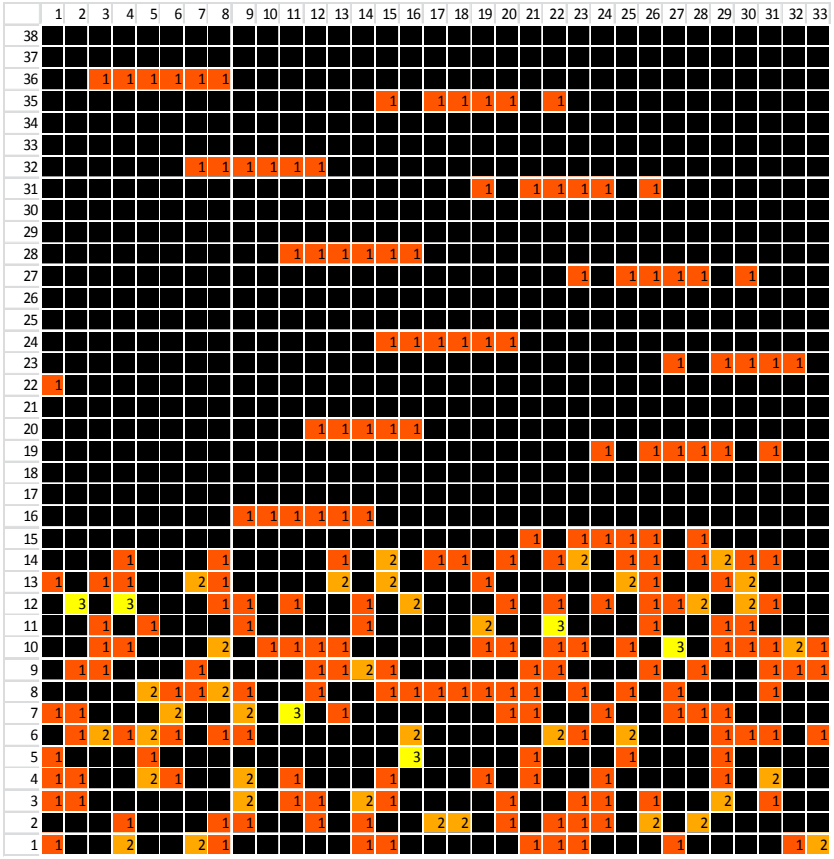




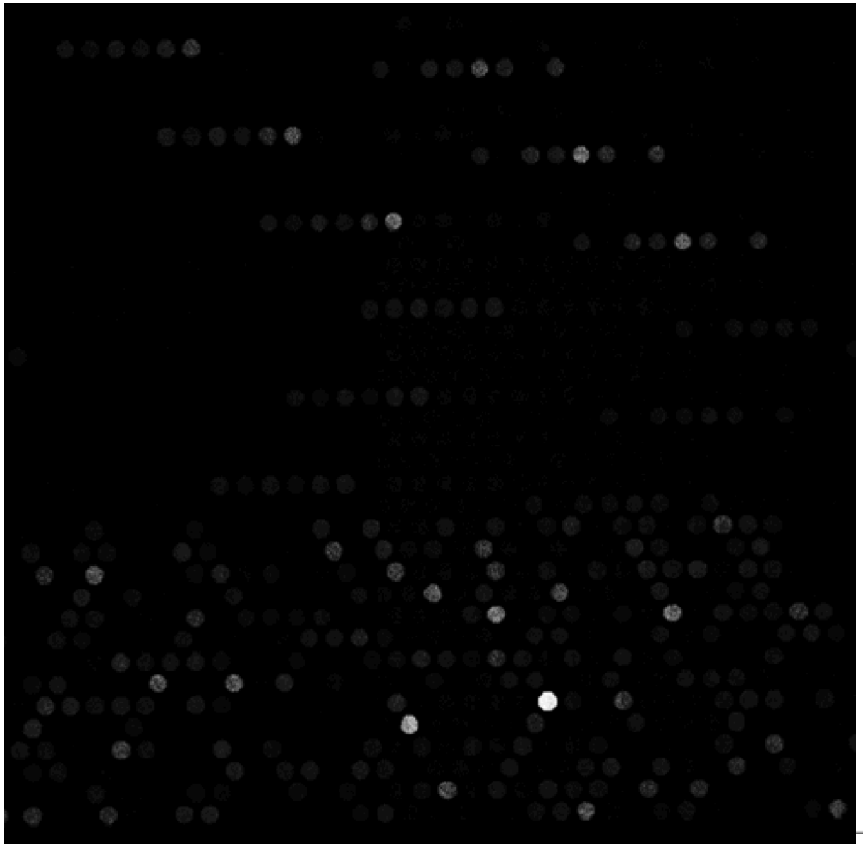
$C_4H_8N^+$ 70D (P Immonium Ion)

Correlation of ion signatures to peptide array map

Phenylalanine map of peptide array:



TOF-SIMS scan for $C_8H_{10}N^+$ 120D (F Immonium Ion)



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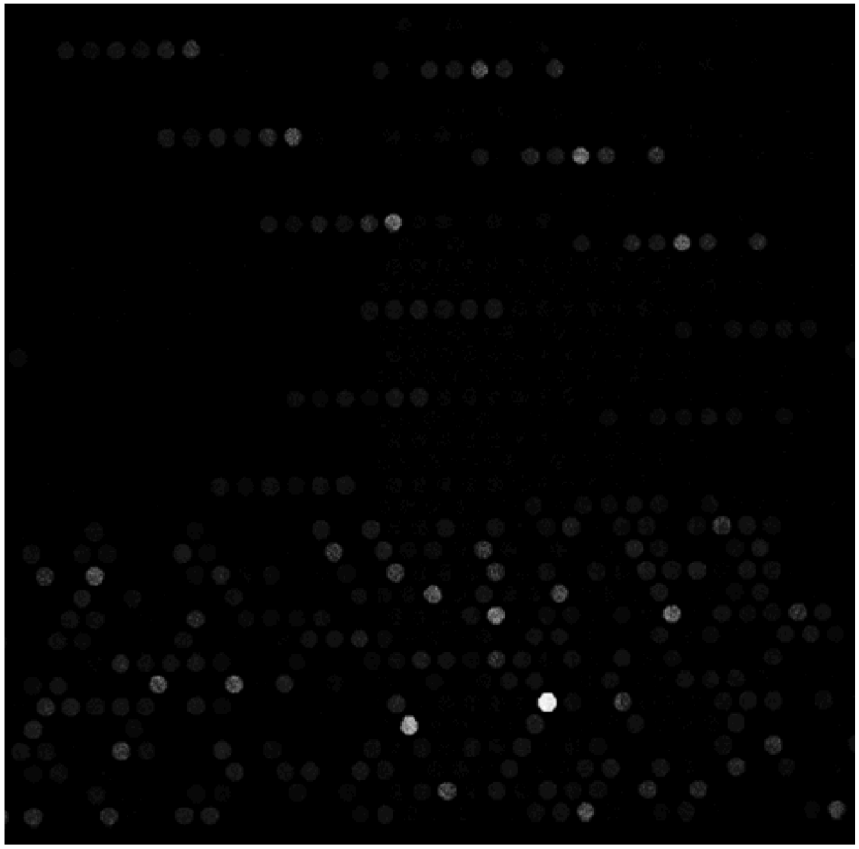
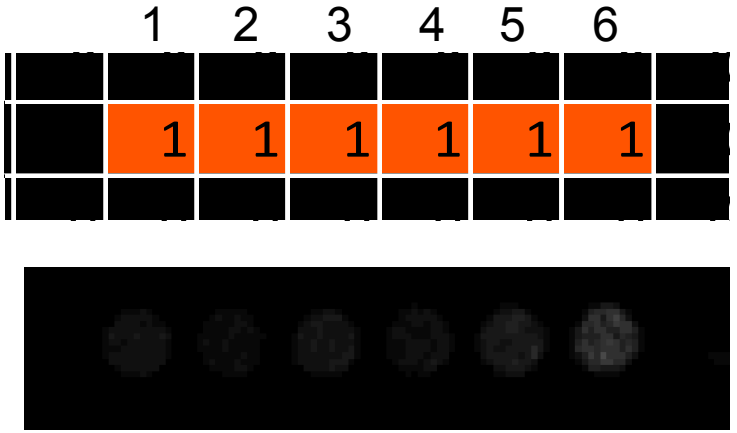
Ion signature intensity correlates to amino acid position

TOF-SIMS scan for $C_8H_{10}N^+$ 120D
(F Immonium Ion)



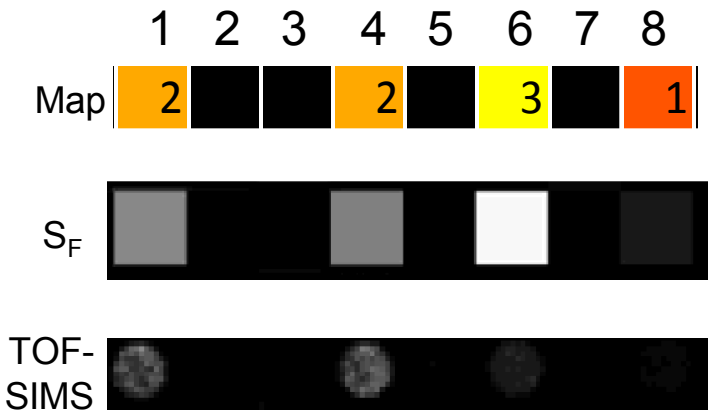


Spot	peptide	TOF-SIMS	
1	LNW**F**GSGG	84.3	17
2	LPH**F**GSGG	65.0	13
3	LW**F**NGSGG	96.7	18
4	LH**F**KGSGG	74.6	15
5	L**F**NQGSGG	105	25
6	L**F**KEGSGG	134	36

Ion intensity varies with position, copy number, and environment

- Develop an *expectation score* for the ion intensity of an amino acid as a function of position within a peptide, peptide length, and copy number

$$S_{AA} = \frac{n_{AA}}{l_{pep}} \sum_{AA_i}^{i} (l_{pep} - p_{AA_i} + 1)$$



Spot	peptide	S_F	TOF-SIMS	
1	LDYAW**FF**YLRHRGGSGG	2.71	73.4	13
2	LLVYVGNPRREQEGSGG	0	4.9	1.2
3	LSARWRPRRYEYNGSGG	0	7.5	1.5
4	LWW**F**KSNG**F**YDGSGG	2.53	69.8	20
5	LVGGHSLHLWRGSGG	0	7.3	1.5
6	LRHPNP**FF**KEHPN**F**YGSGG	4.89	43.8	10
7	LWDYSVWPGAKGWGGSGG	0	4.0	1.6
8	LHAHGNPH**F**NLHPQVWGSGG	0.60	11.4	2.1

Conclusions

- Peptide microarray imunosignaturing technology has immense promise for providing unique biodetection capabilities
- Additional characterization and optimization are necessary to further develop this technology and maximize impact
- Combined with complementary analytical techniques (e.g. genetic sequencing), new operational applications can be developed