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

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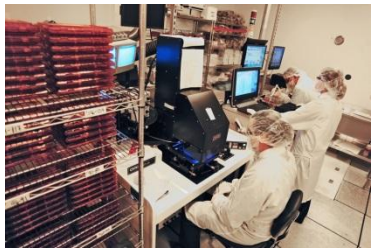
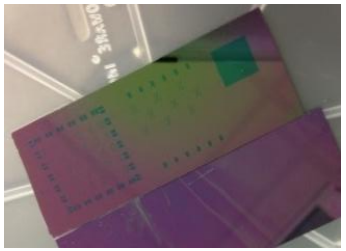
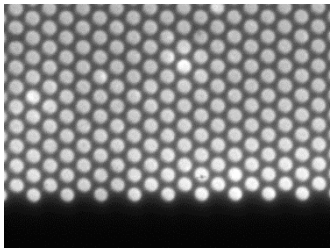
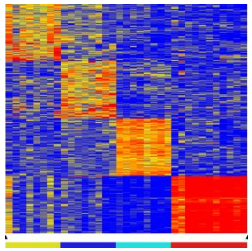

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Margot Kimura
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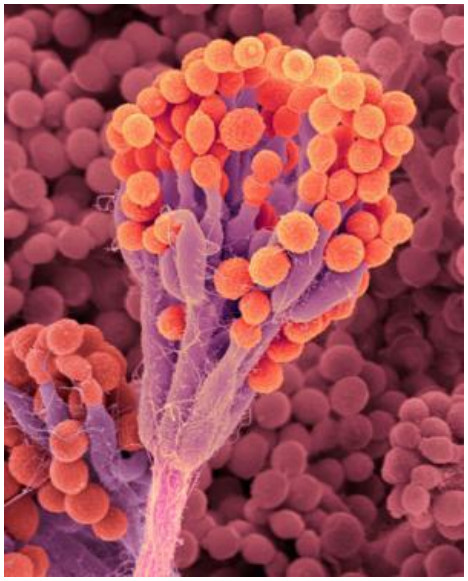
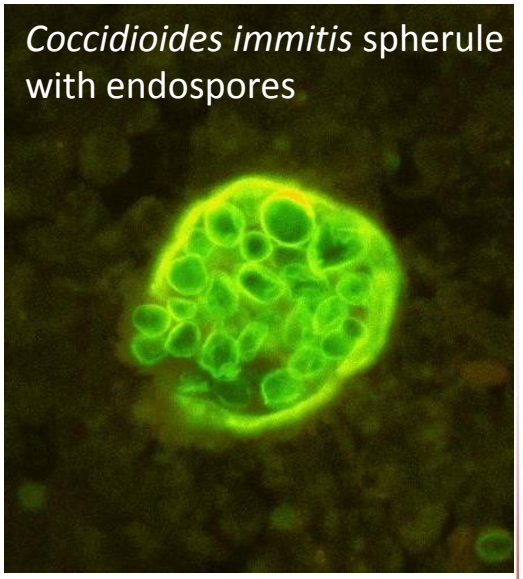
Infectious disease scenario

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

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





Coccidioidomycosis:

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

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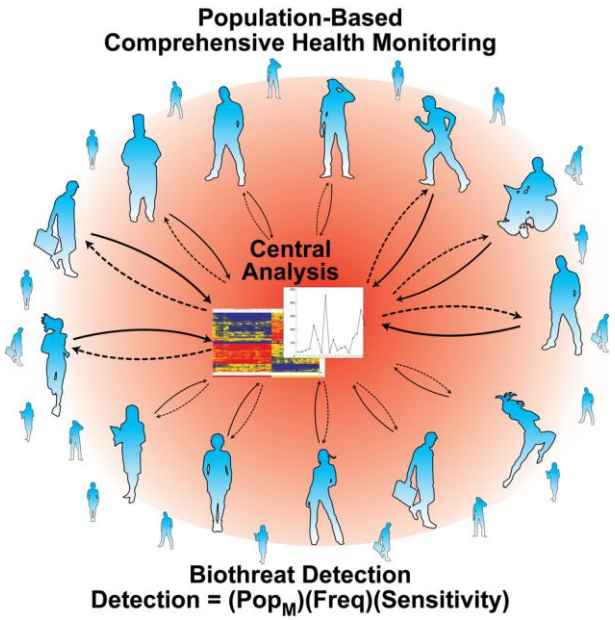
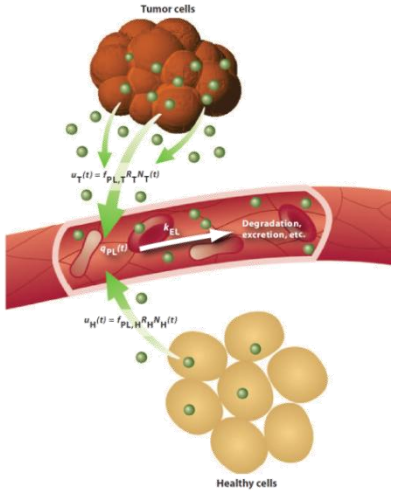
Challenges in biodetection and biosurveillance

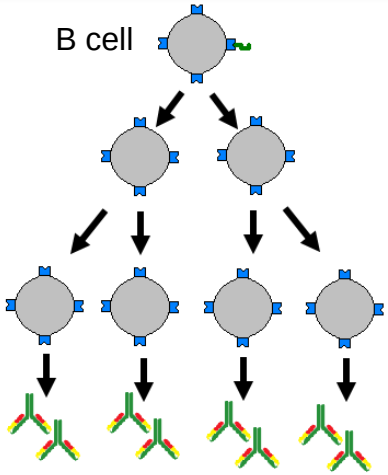


Blood dilution problem...



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

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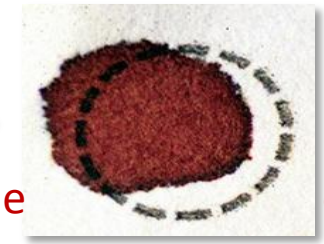
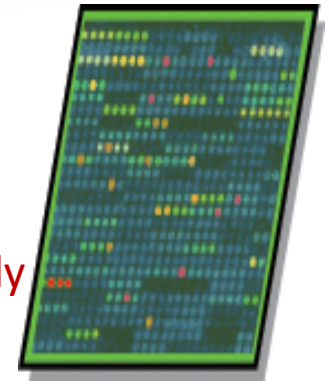
Need: a biodetector for rapid detection of ***ill*** and ***near-ill*** individuals

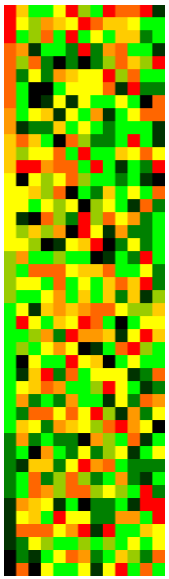
sample



dilute & apply



monitor



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

- *Produce a longitudinal profile of the suite of antibodies in an individual's blood to identify the ill and near-ill*
- Only microliters of blood needed; Easy - sample just diluted
- Can be self administered, minimally invasive
- Convenient - can be mailed on filter paper

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

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
Stafford, P., R. Halperin, J.B. et al., (2012). Molecular & Cellular Proteomics 11(4), 10.1074

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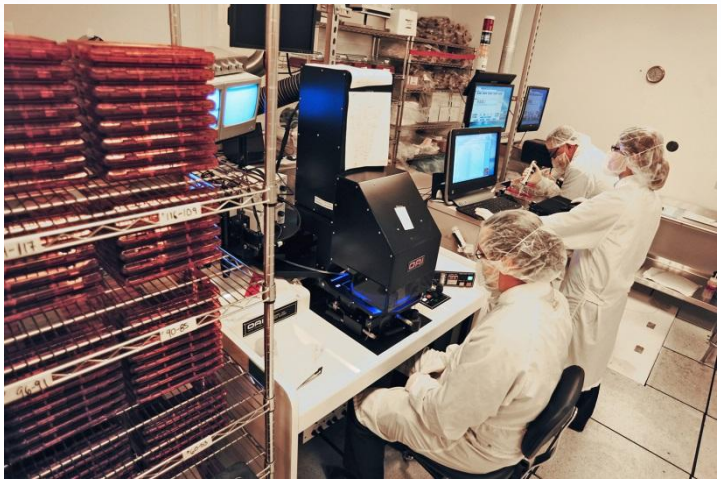
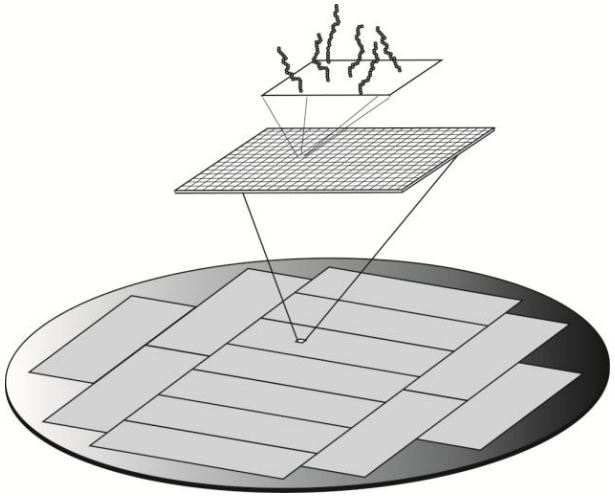
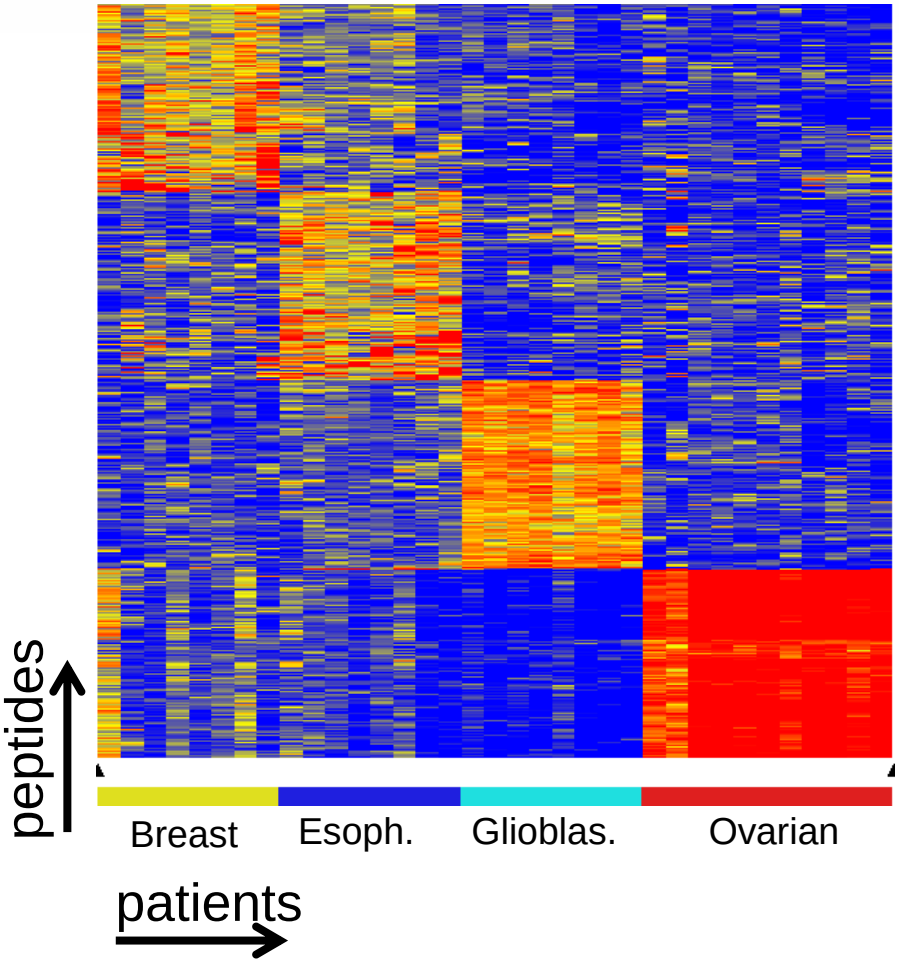
Immunosignaturing with peptide microarrays







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

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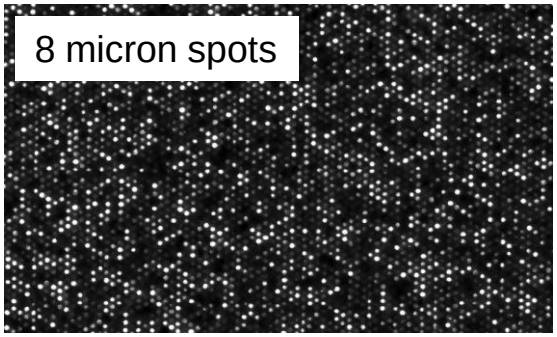
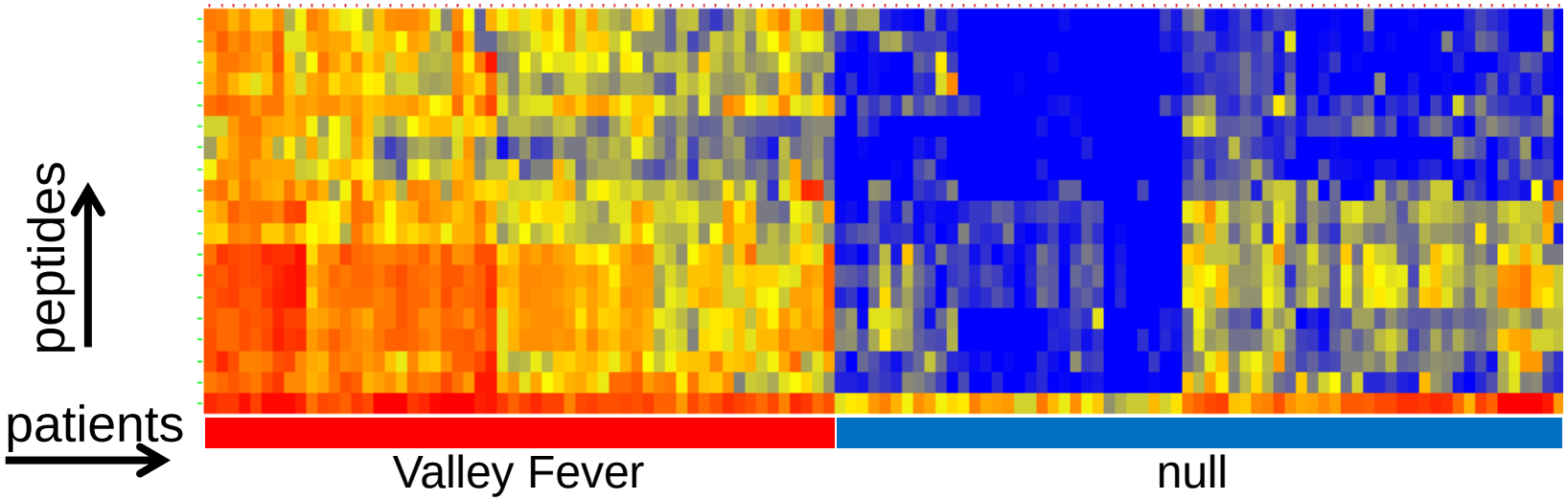
Detection of Valley fever

- 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

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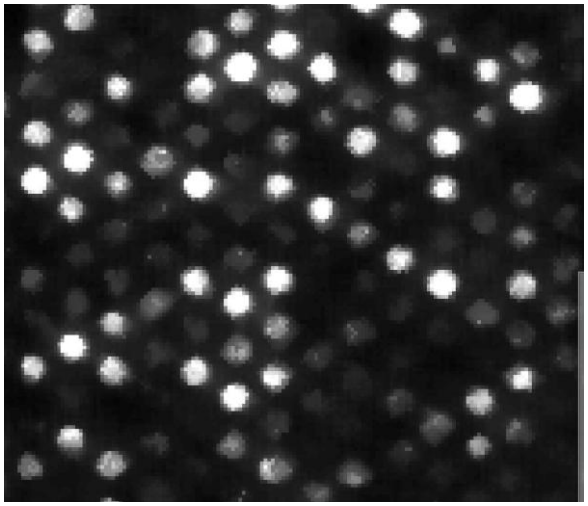
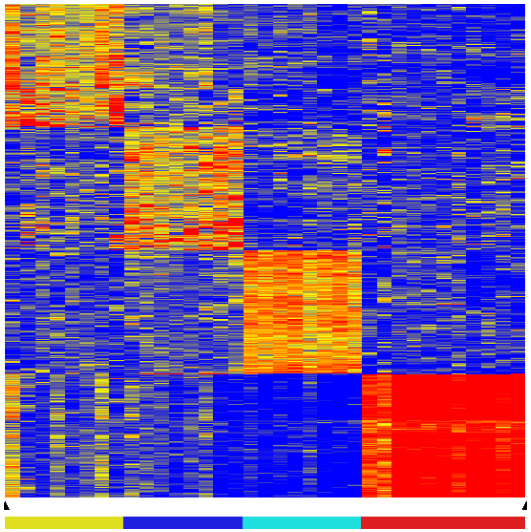
Technology maturation drivers

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





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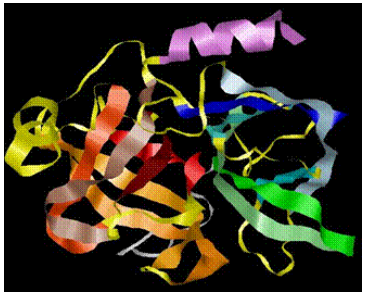
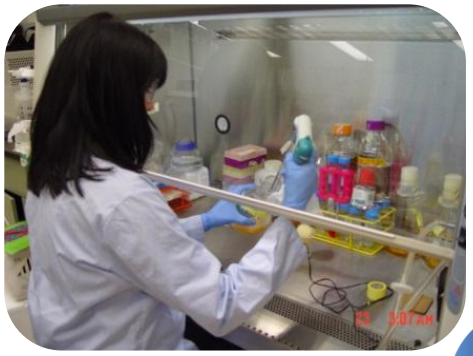
Technology maturation: U.S. National Laboratories

Peptide design & performance



Process development

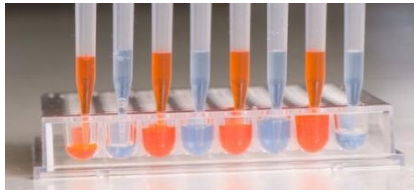


Characterization

Exploratory production

Low/mid-level production

Sample testing & assay optimization



Mature, optimize, and innovate

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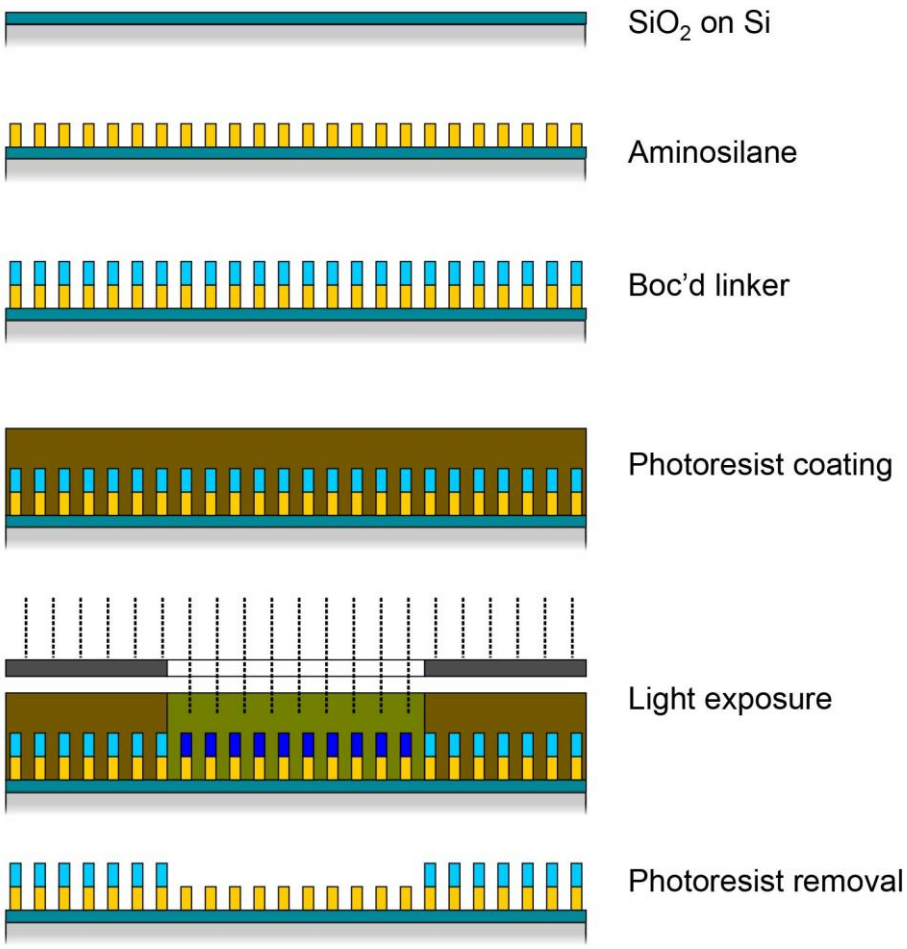
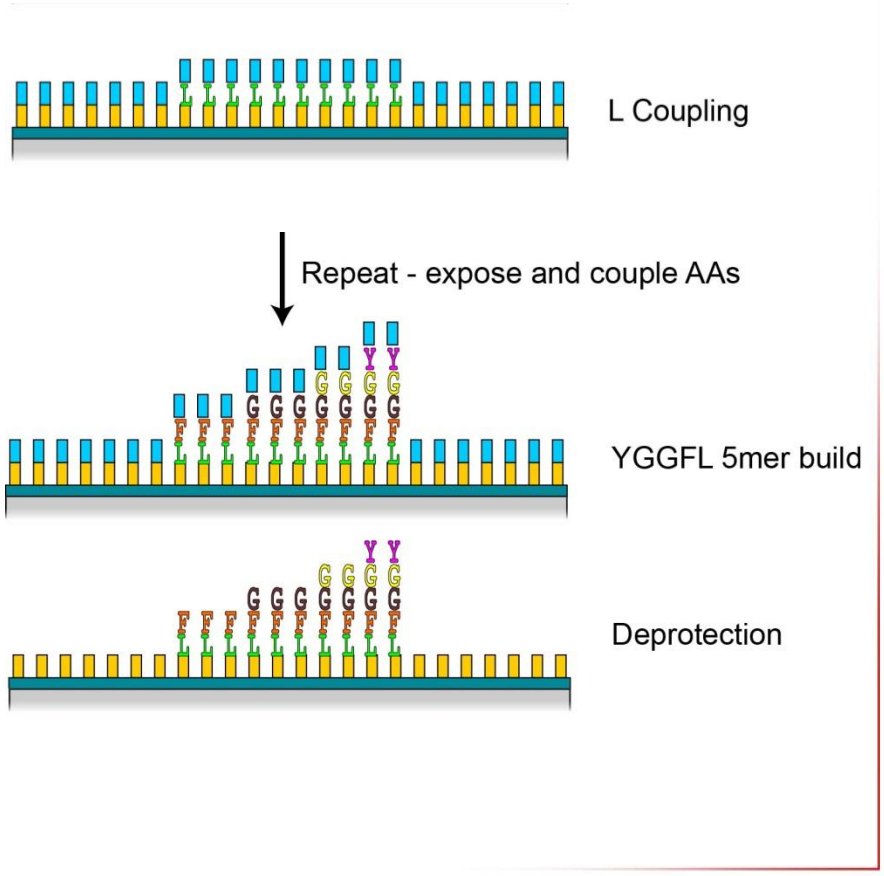
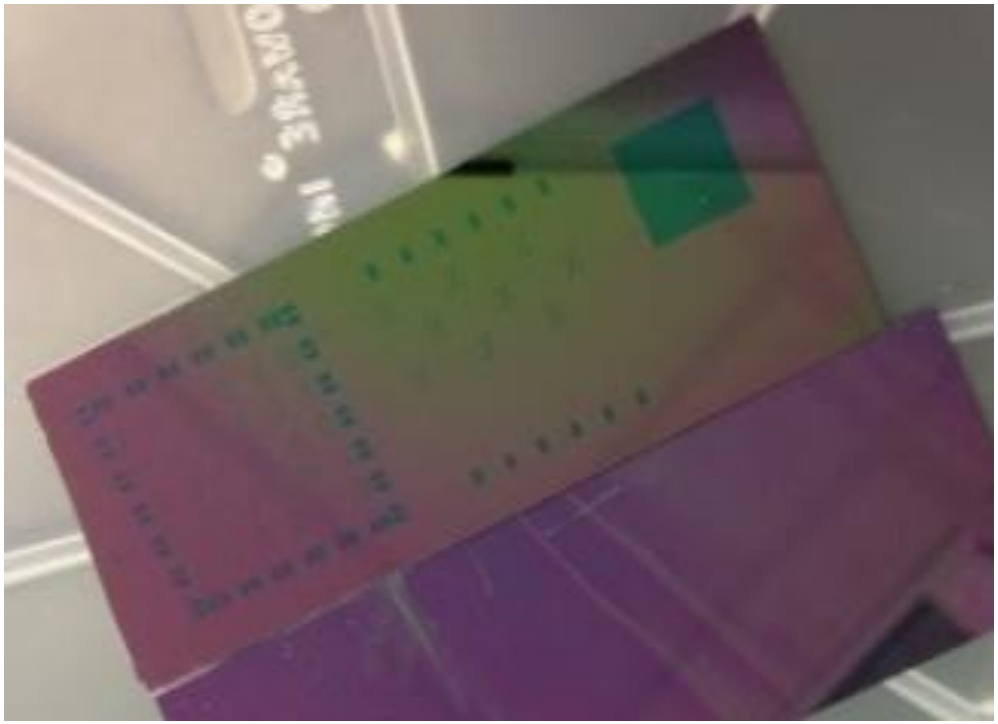
Microarray fabrication process- photolithography and reaction chemistry





Test peptide microarrays



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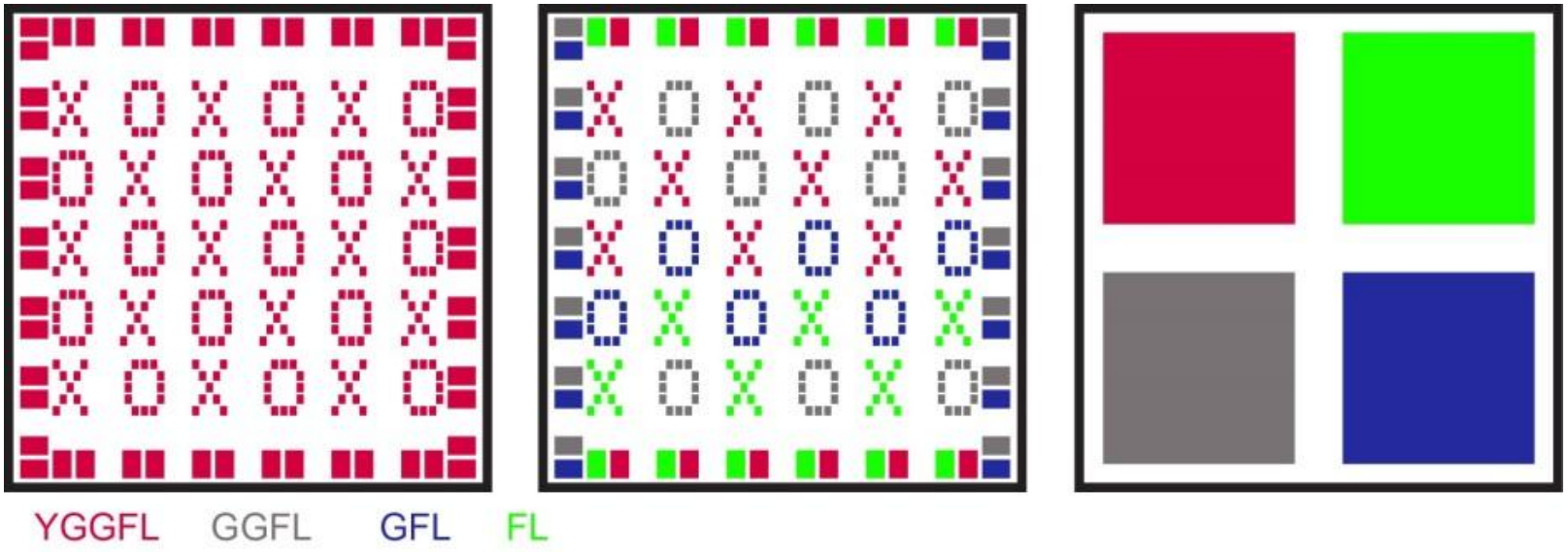
5mer peptide fabrication with control deletions



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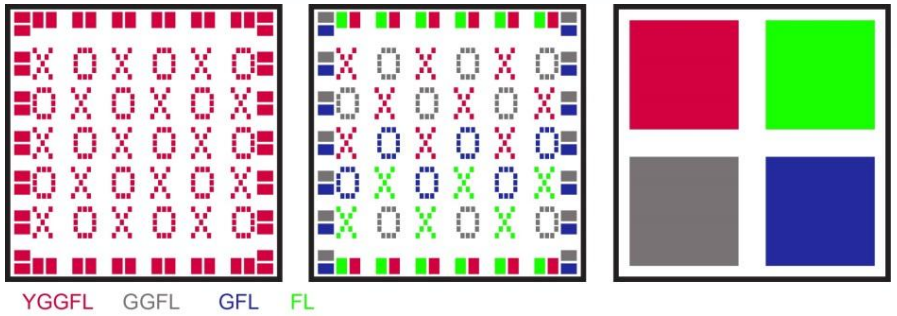
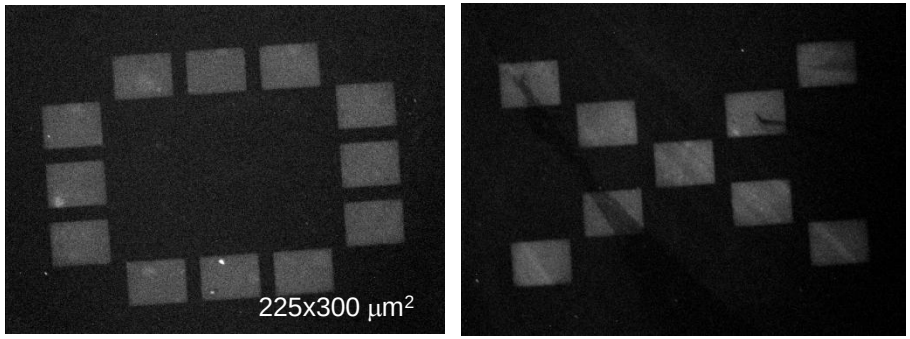
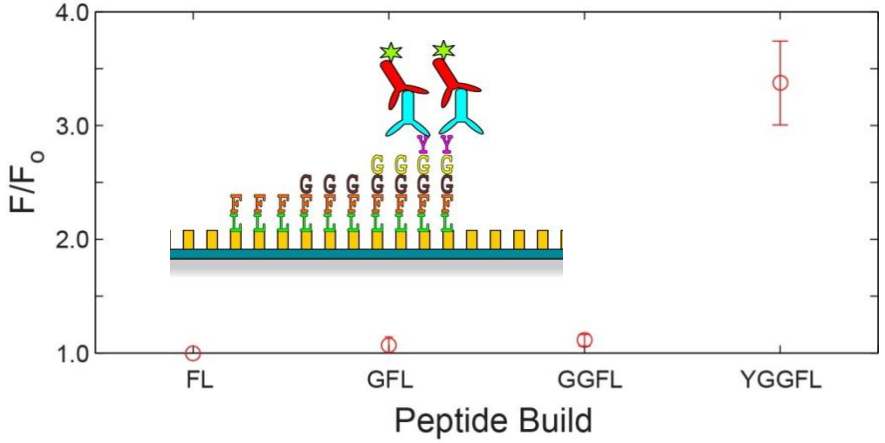
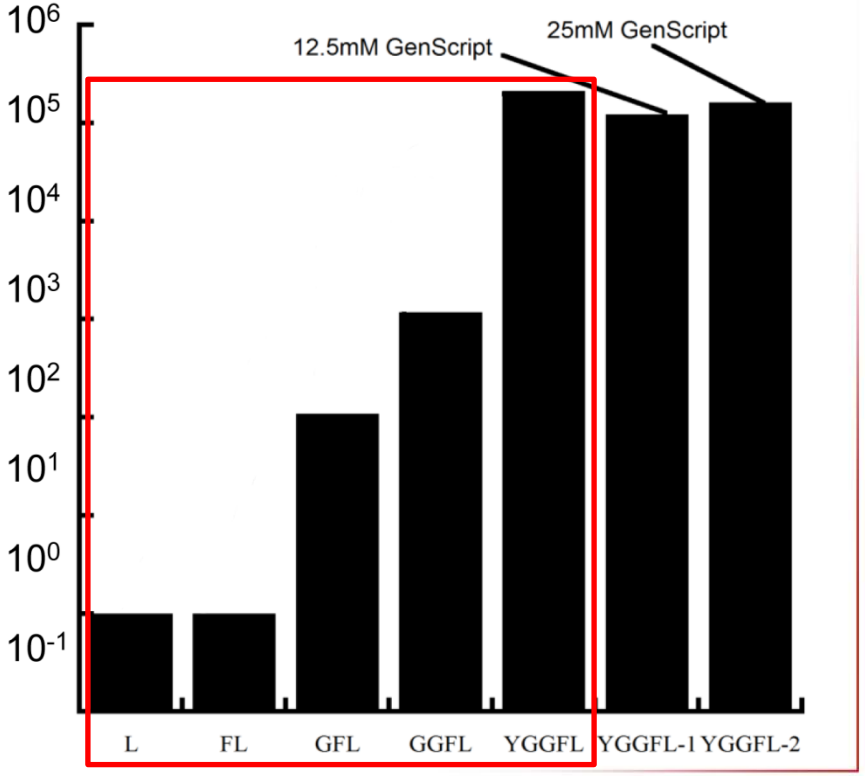
5mer peptide fabrication with control deletions







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



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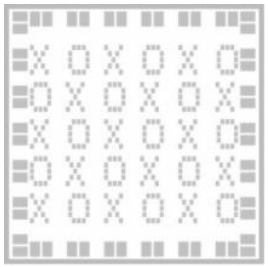
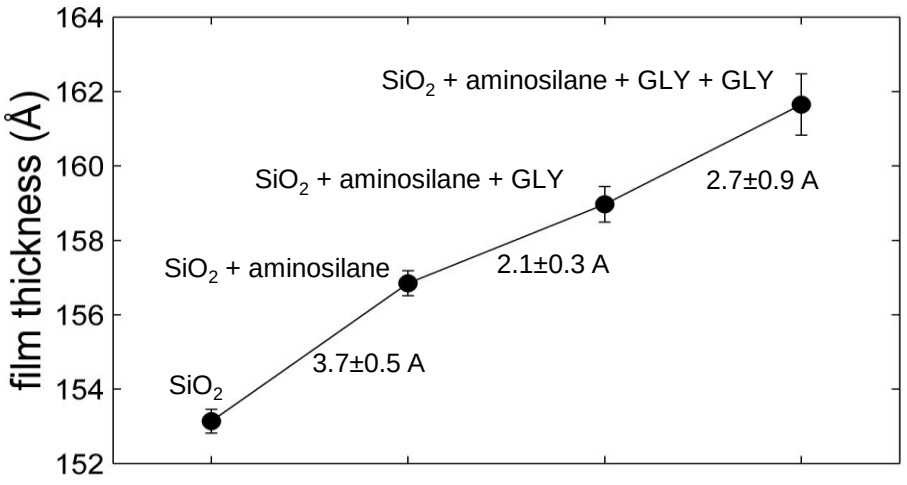
in situ ellipsometry for non-destructive sample assessment

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





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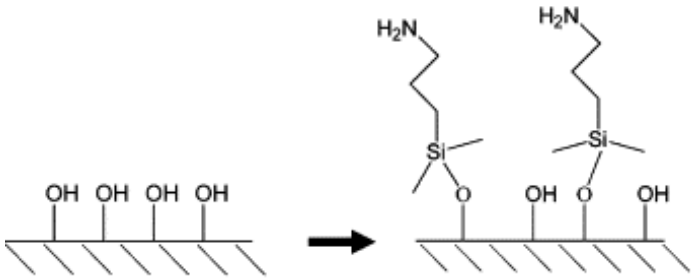
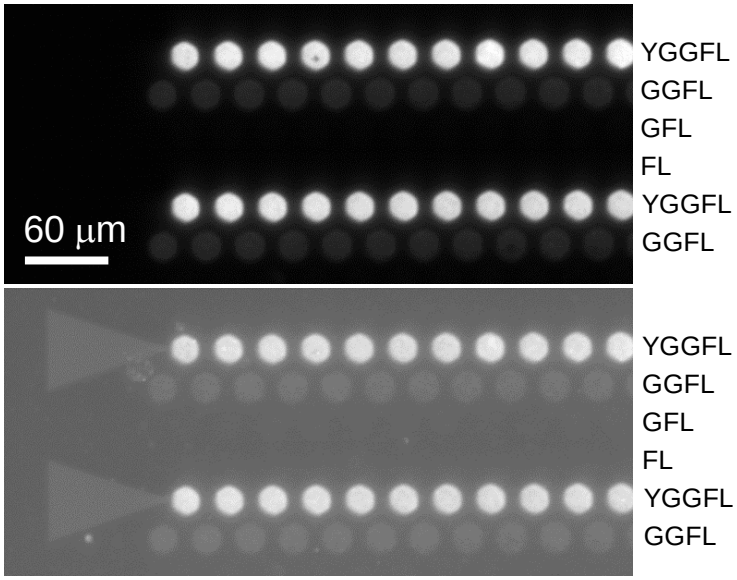
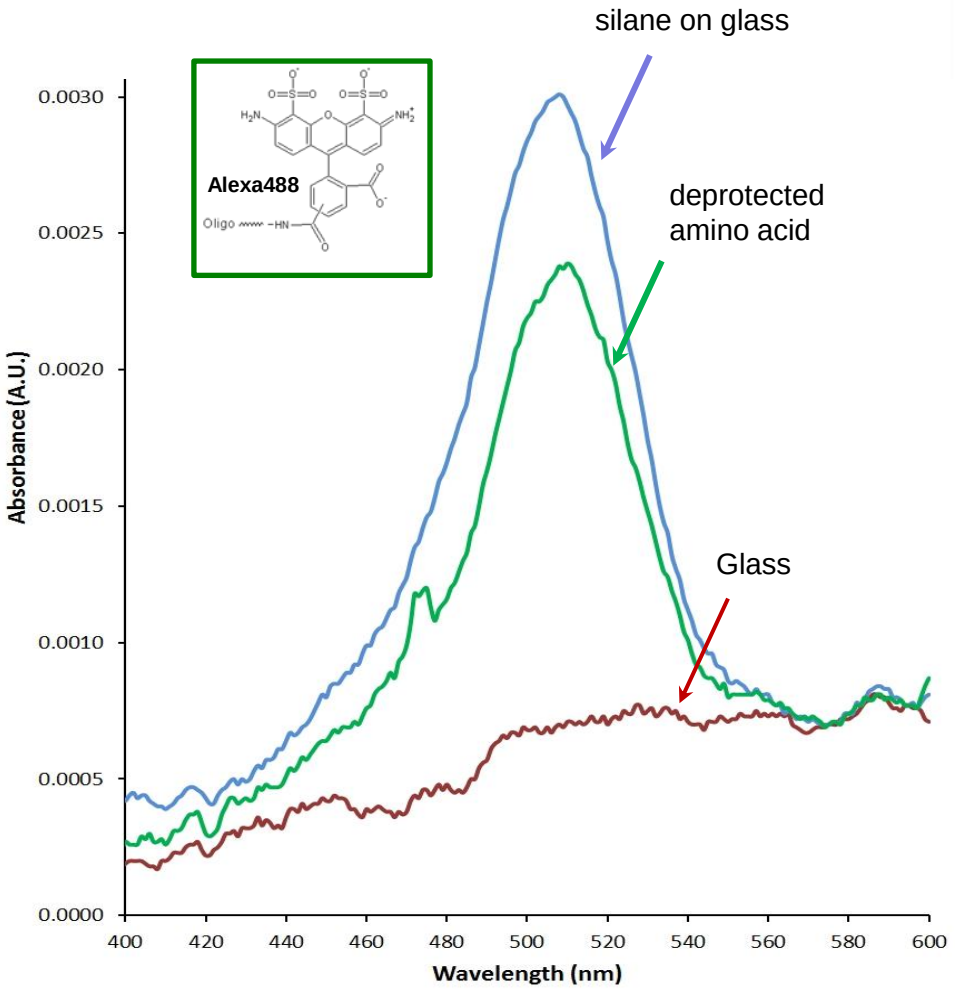
Quantitative measurement of amine-group surface density

Surface chemistry of peptide arrays:





UV/VIS/NIR Spectrometry:

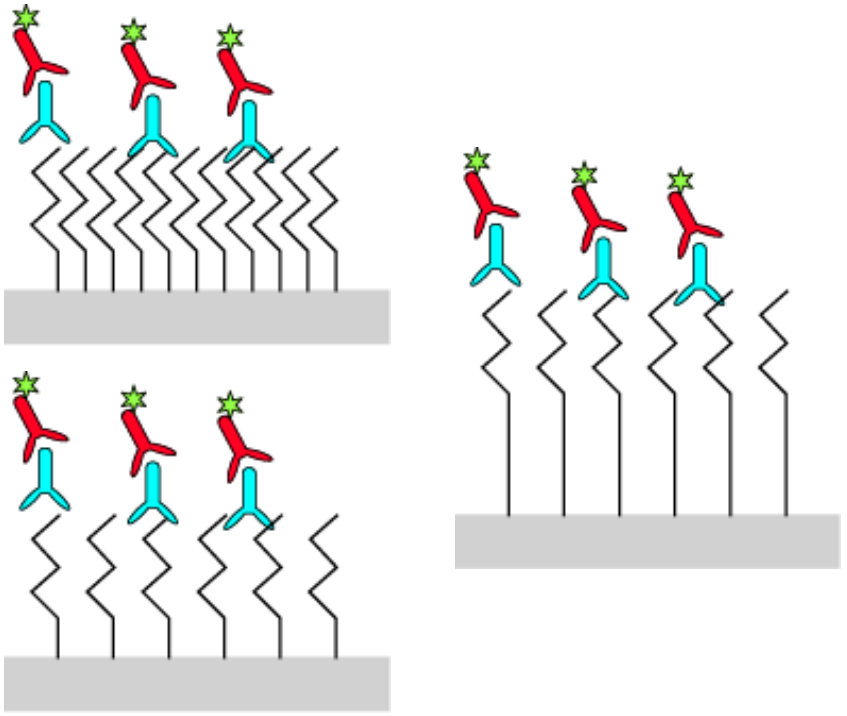


Affinity and avidity – impact on peptide array performance

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



Optimize Ab binding to peptide array: density, linker length, etc.

Alvarez et al. *Langmuir* **2011**, *27*, 2789;
Wang et al. *BioTechniques* **2005**, *38*, 127

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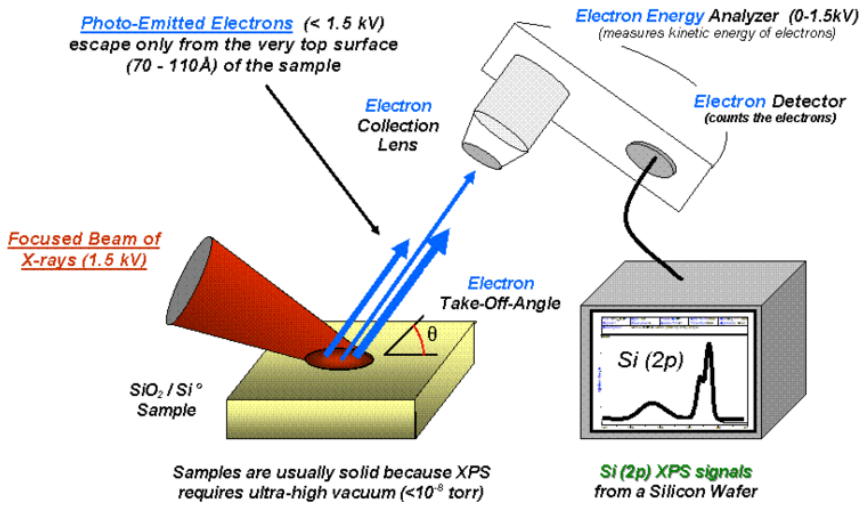
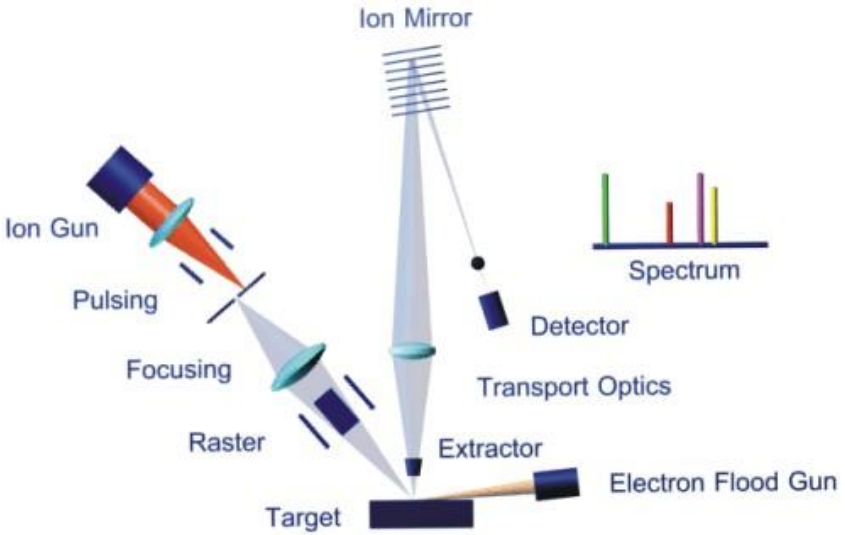
Spectroscopic analytical techniques for quality assurance and control

X-ray photoelectron spectroscopy (XPS):



http://en.wikipedia.org/wiki/X-ray_photoelectron_spectroscopy

Time of flight secondary ion mass spectroscopy (TOF-SIMS):

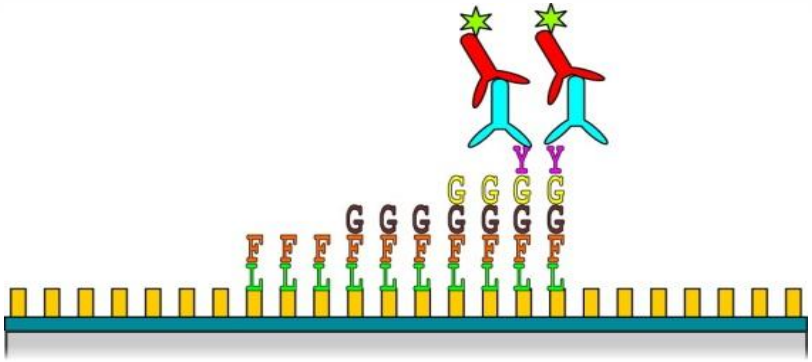


http://serc.carleton.edu/research_education/geochemsheets/techniques/ToFSIMS.html

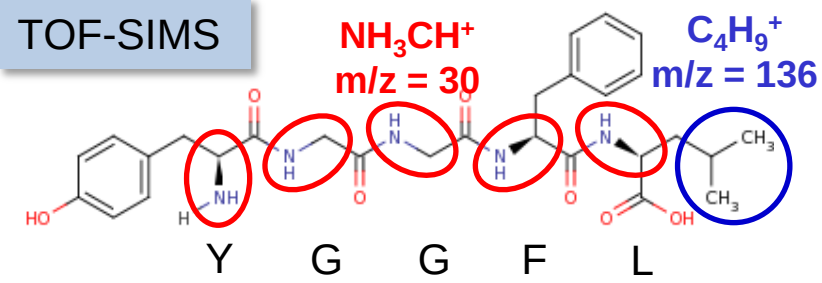
- XPS: elemental and chemical surface analysis
- TOF-SIMS: molecular information

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

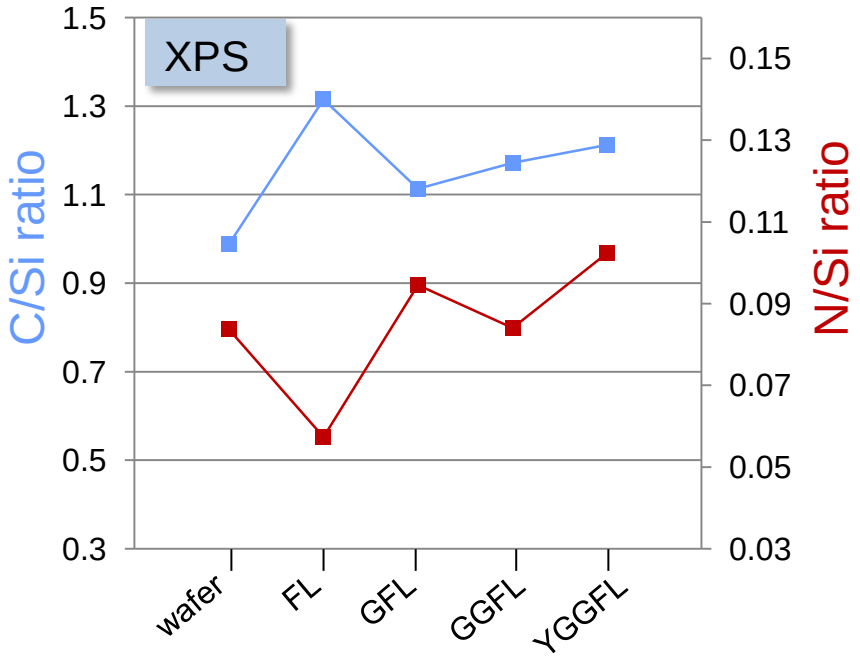
Quantitative quality control of peptide builds using elemental and molecular analysis

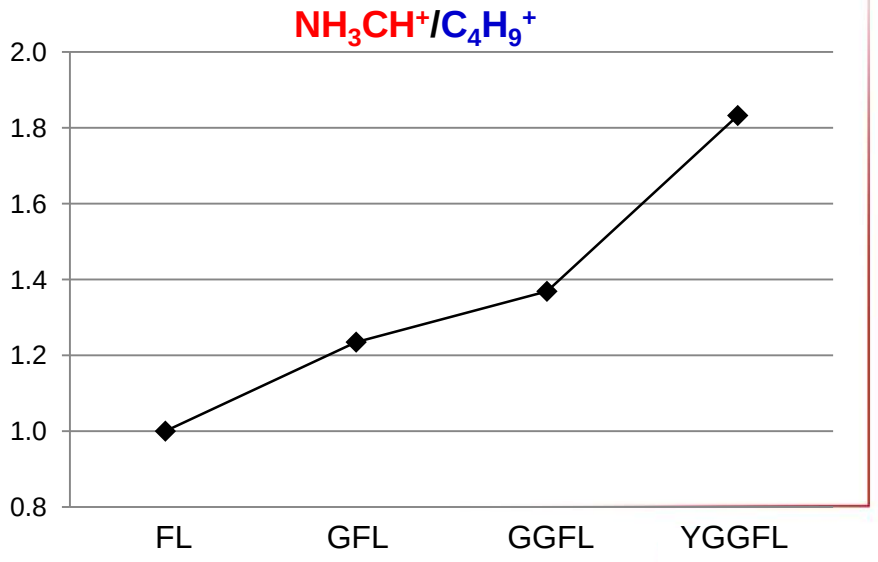


TOF-SIMS



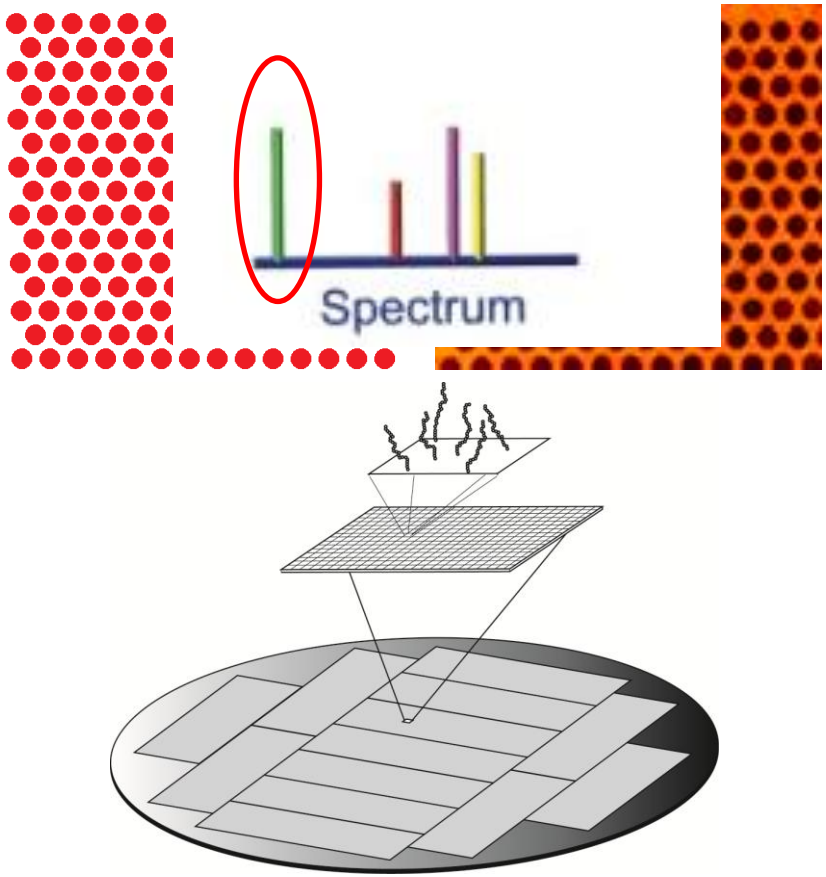
http://pepbank.mgh.harvard.edu/interactions/details/57761

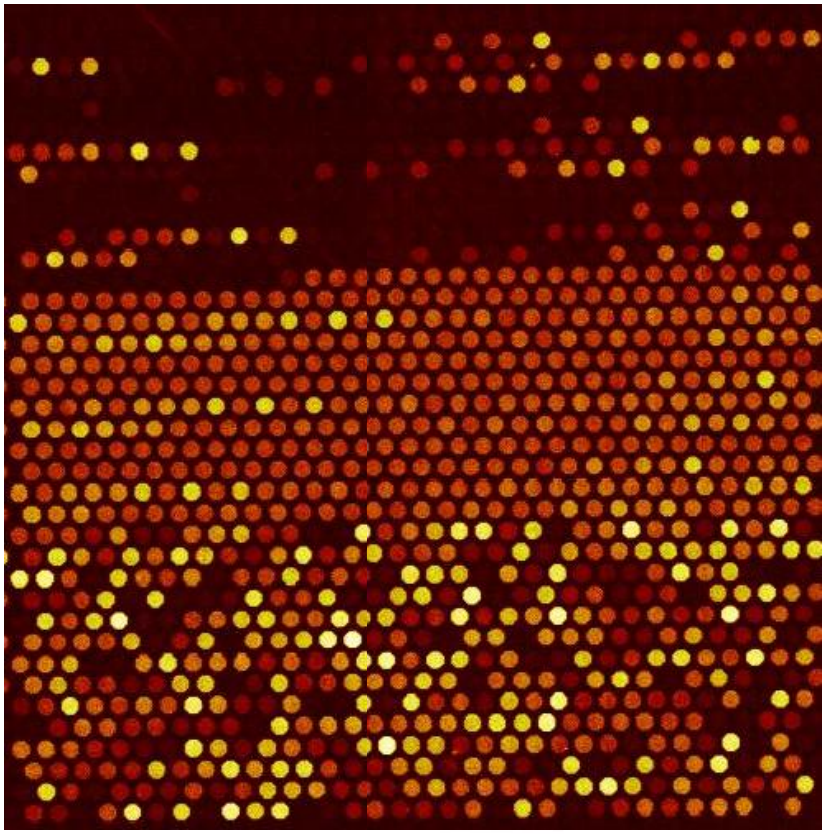




TOF-SIMS imaging for peptide content analysis

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





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

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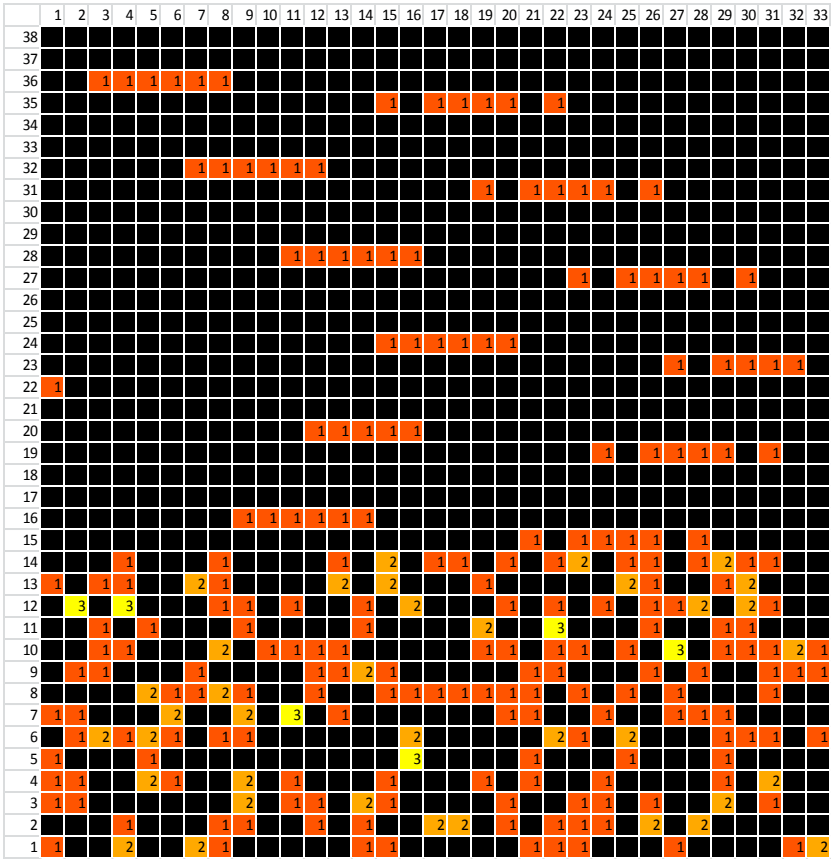
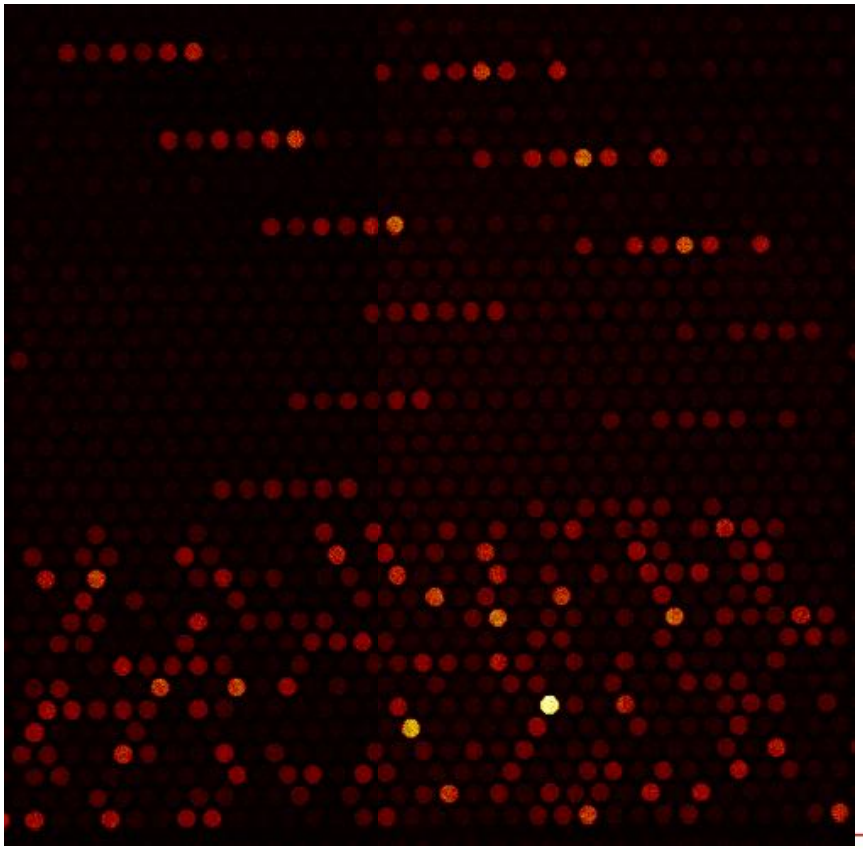
Correlation of ion signatures to peptide array map

Phenylalanine map of peptide array:



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



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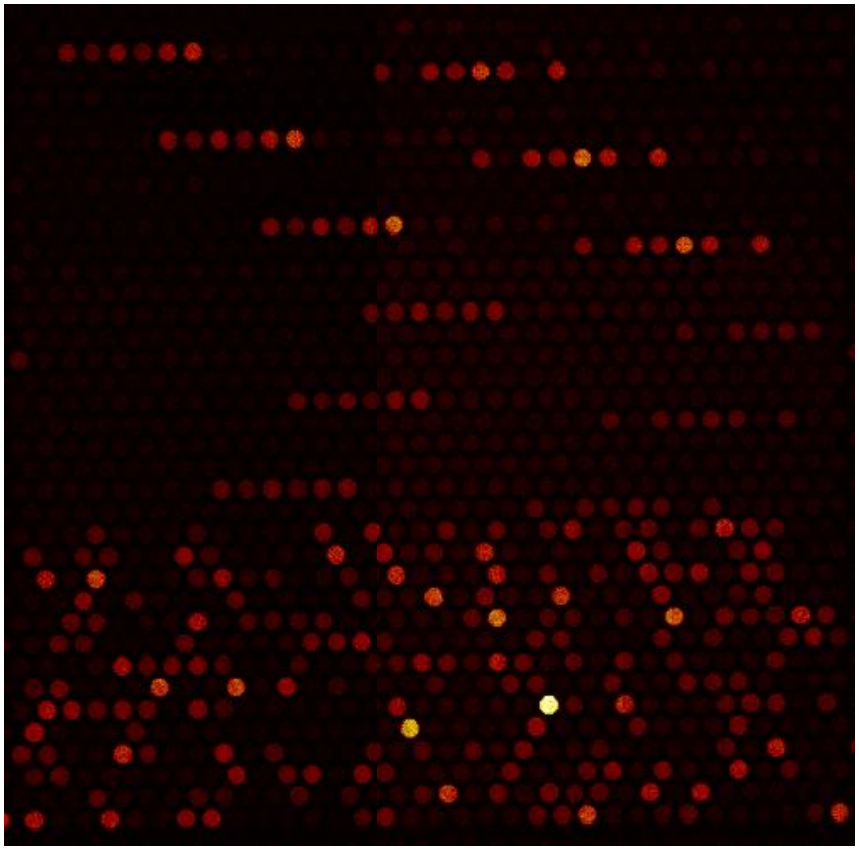
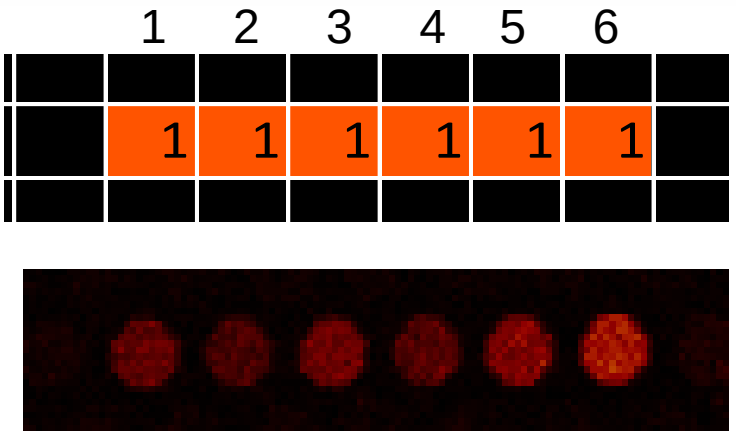
Ion signature intensity correlates to amino acid position

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

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Conclusions

- Peptide microarray immunosignaturing 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

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