ASU Arizona State University

HealthTell™

Center for Innovations in Medicine

Immunosignatures: a Platform Technology for Diagnosis and Discovery

RUSNANO

Stephen Albert Johnston

Center for Innovations in Medicine

HealthTell

Russian American Anti Cancer Center





Current *Center for Innovations In Medicine* Projects

OBJECTIVE	INVENTION	COMPANY
Health Monitoring/ Early Diagnosis	Immunosignatures	HealthTell, Inc
Universal Preventative Cancer Vaccine	Frameshift Antigens	Calviri, LLC
NextGen Antibiotics, Anti-Virals	Synbodies	

Forbes Magazine, 1/19/2012

U.S. Healthcare Hits $3 Trillion

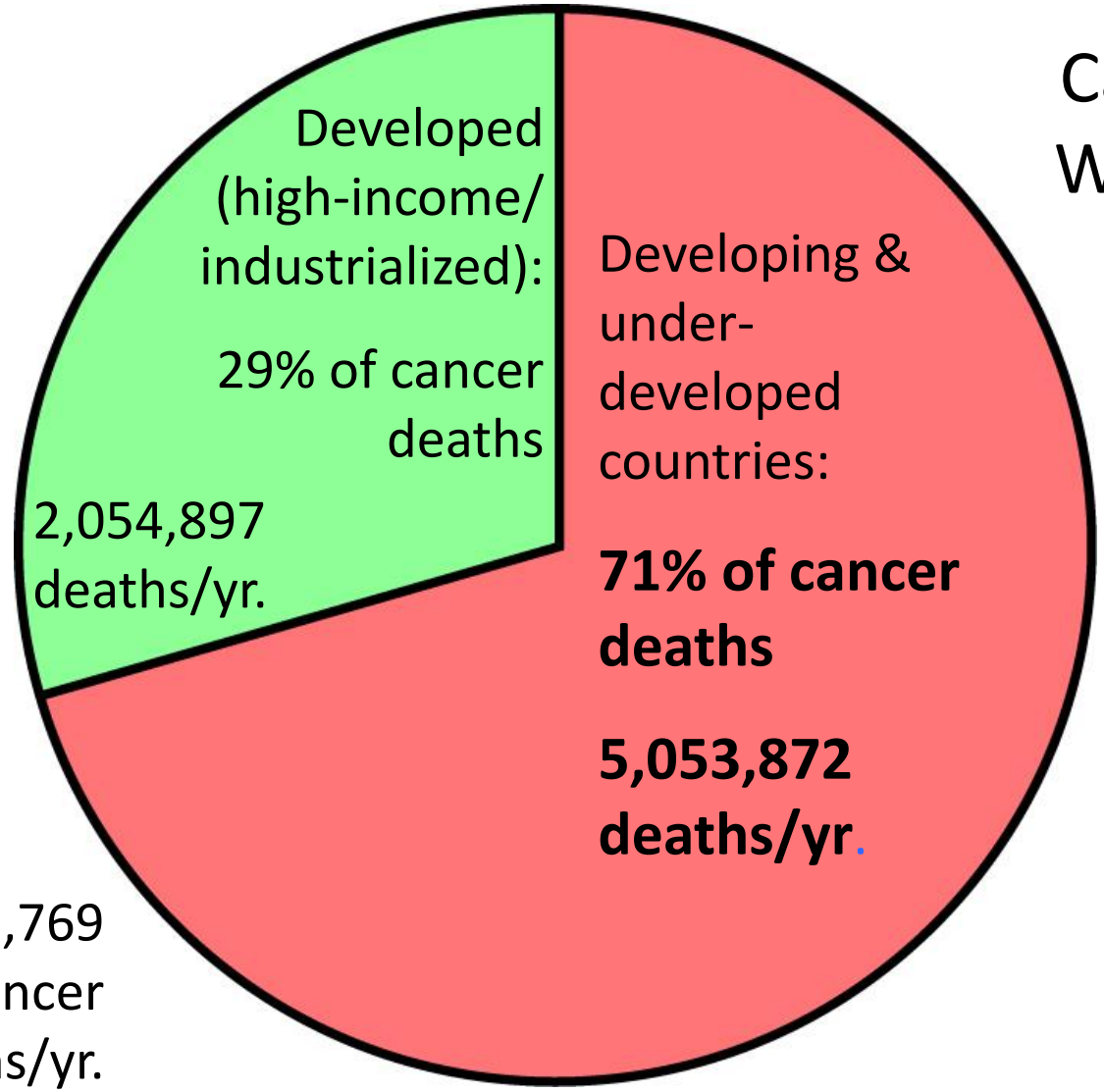
National Healthcare Expenditure – or NHE.

… NHE for 2012 is probably closer to $2.7 trillion but there's this nagging bookkeeping accrual of about $300 billion where we (narrowly) avoided those darn pesky SGR cuts to Medicare. … That puts the real NHE at about $3 trillion for 2012 (+ about 4% for each year forward – as far as the eye can see). **As one economist said – we don't have a debt problem in this country – we have a healthcare problem.**

http://www.forbes.com/sites/danmunro/2012/01/19/u-s-healthcare-hits-3-trillion/

$3 trillion is ~19% of the GDP for the US

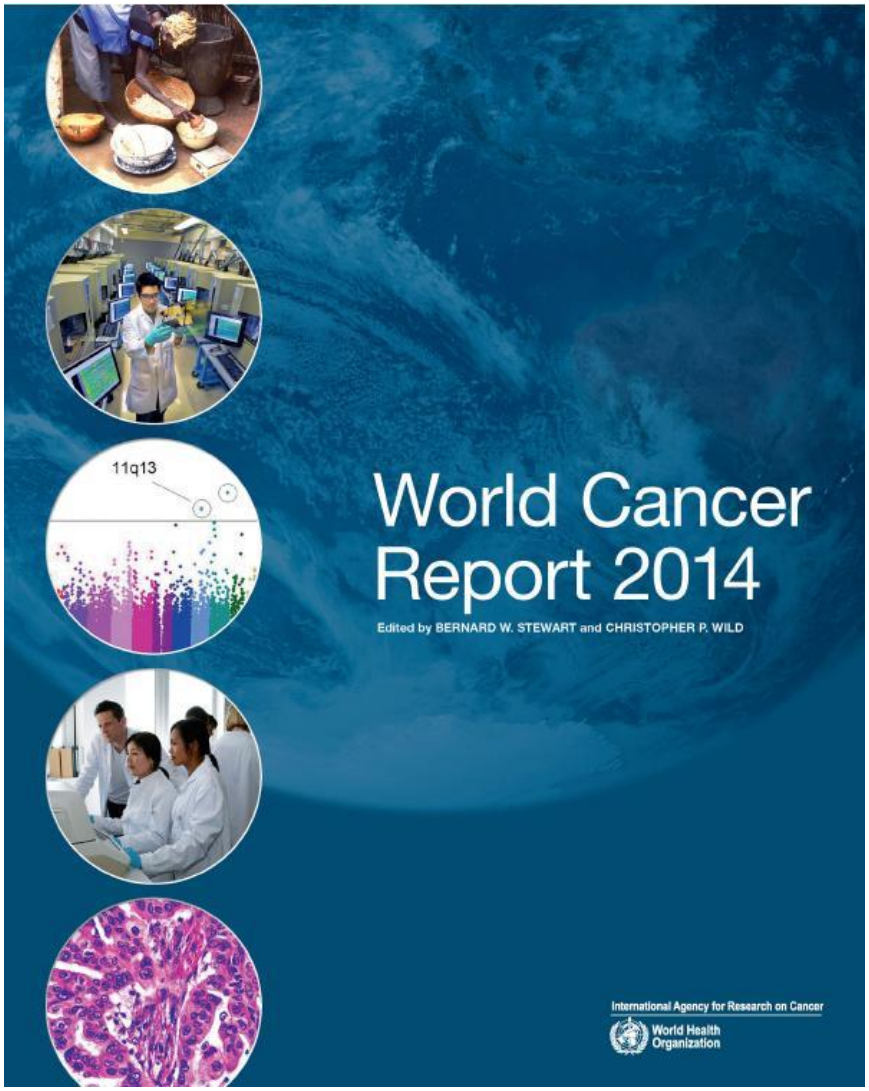
Cancer Deaths Worldwide



7,108,769 total cancer deaths/yr.

Source: *World Health Statistics 2006*, published by the World Health Organization (WHO). WHO.

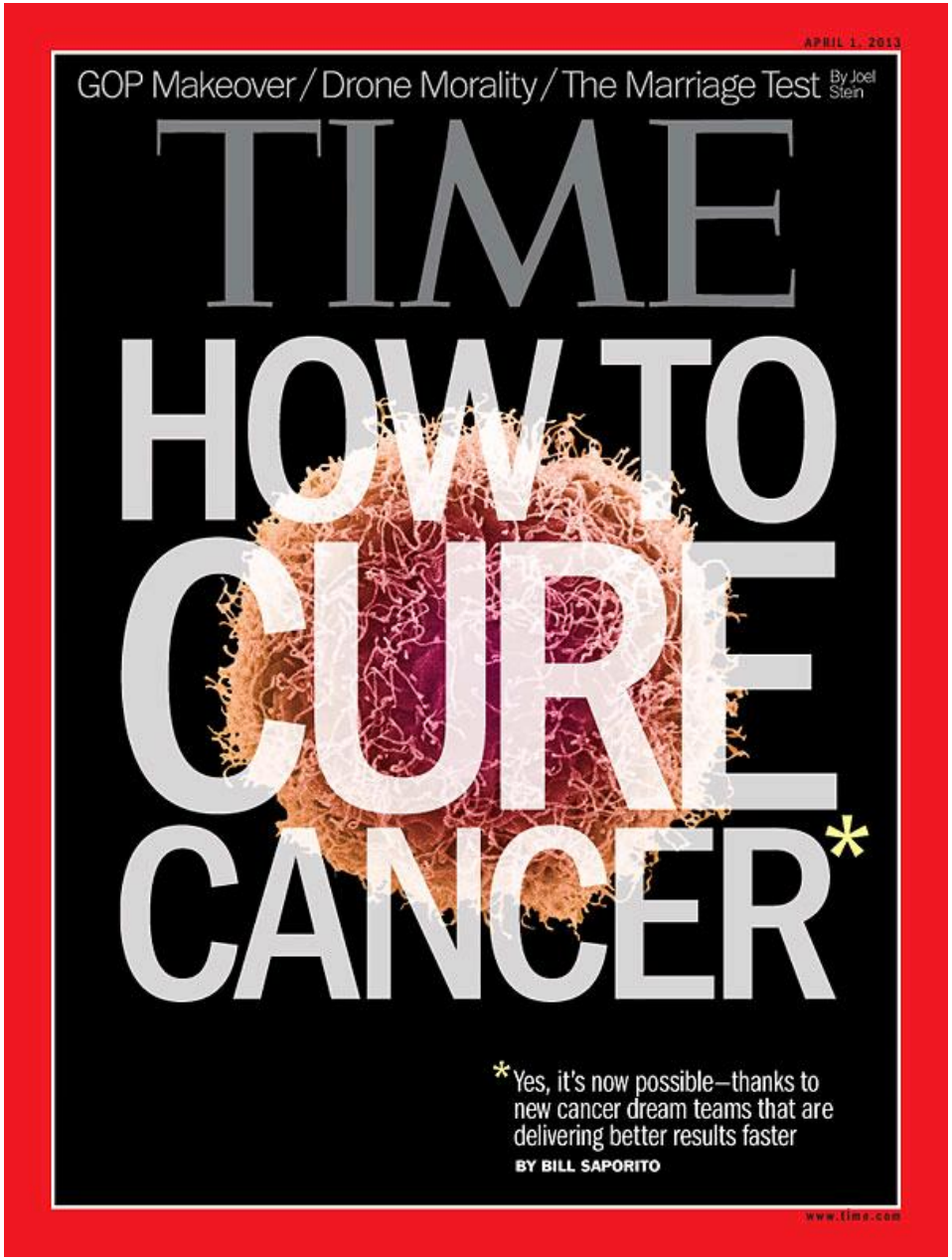
WHO: Imminent global cancer 'disaster'



THE GLOBAL ECONOMIC COST OF CANCER

“The total economic impact of premature death and disability from cancer worldwide was $895 billion in 2008.”

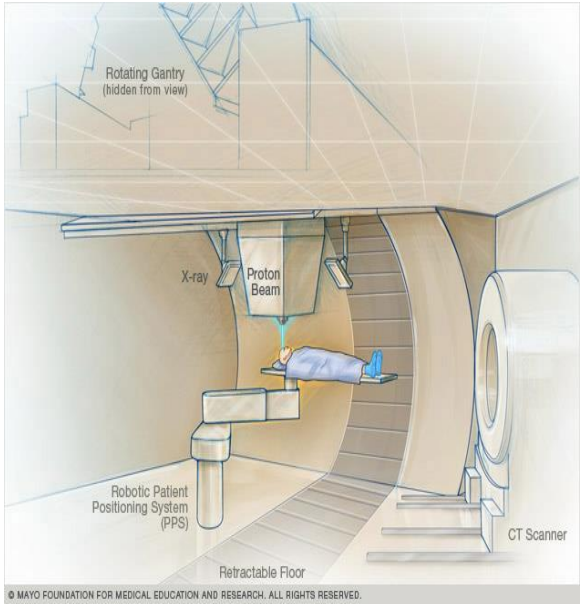
American Cancer Society®



Anne Weston, picture of “How to Cure Cancer”, Time magazine web, June,2013

“When the stars come together cancer doesn’t stand a chance”

Positron Emission Therapy



Development:	**$200B (1000 centers)**
Cost/Year:	**$50B**
Cost/Trtment	**~$30,000**
Physicists	**10,000**

Personalized Medicine

1. Tumor DNA and/or RNA from ONE Individual is Sequenced

2. Analysis of Sequence Indicates the Right Drug to Use

3. Treatments often >$100,000 US

Post-Symptomatic Medicine To Pre-Symptomatic Health

2009 GNP $14.7T
2009 Health Care Costs $2.5T

Per capita health expenditure ~$8000
Median adjusted gross income in 2007 ~$33,000*
Median federal taxes per capita in 2007 ~$1,000
Total Medicare expenditures in 2004 ~$3B
Medicare expenditure per capita ~$1000

Exhibit 1: National Health Expenditures per Capita, 1990-2018

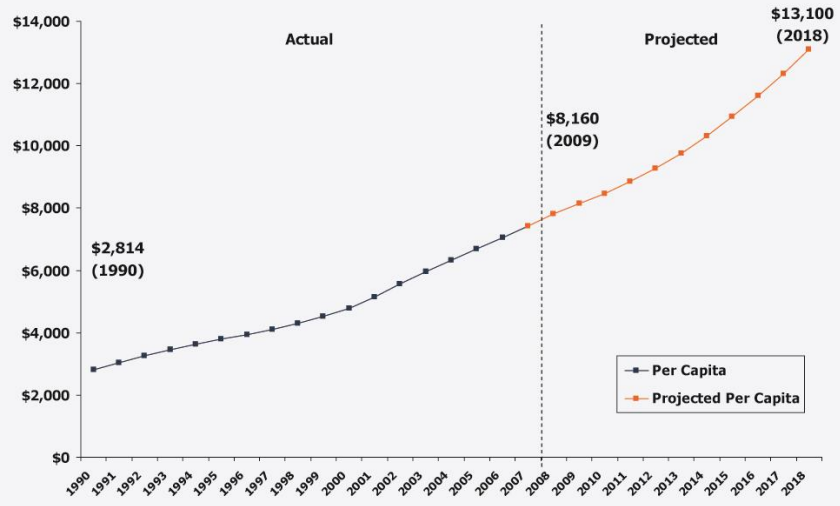
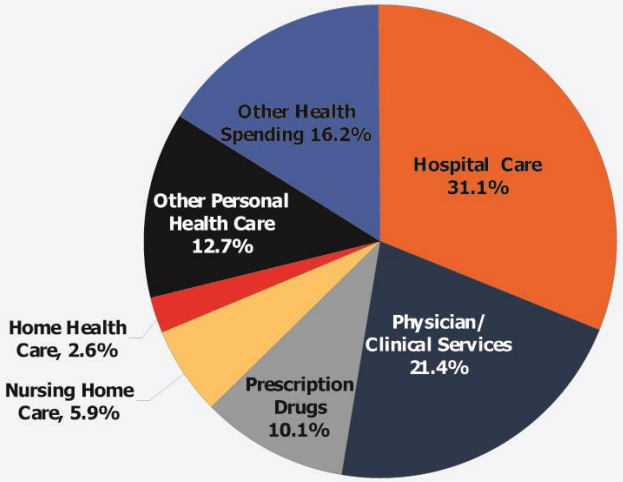


Exhibit 2: Distribution of National Health Expenditures, by Type of Service, 2007



http://www.kff.org/insurance/upload/7692_02.pdf
http://www.taxfoundation.org/news/show/250.html

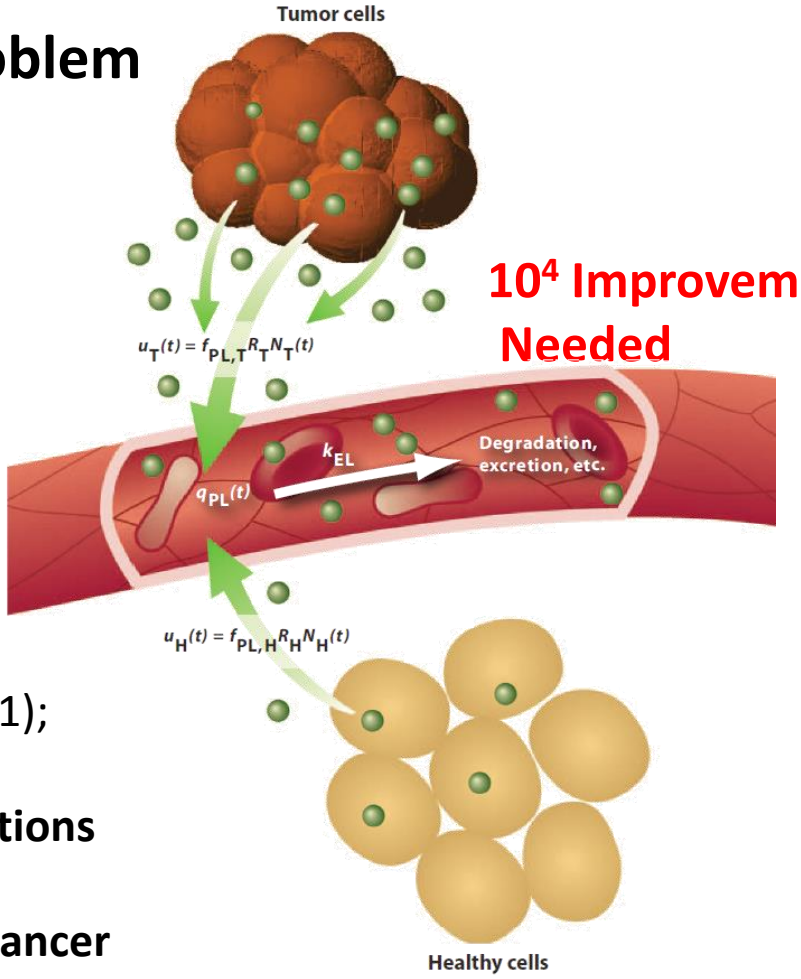
Transition from Post- to Pre-Symptomatic Medicine Requires System to Continuously Monitor Health of Well People

Specifications Required:

- Comprehensive
- Sensitive – Early Detection
- Simple
- Inexpensive
- Specificity – What is Wrong?

Can Not Do Early Detection of Disease

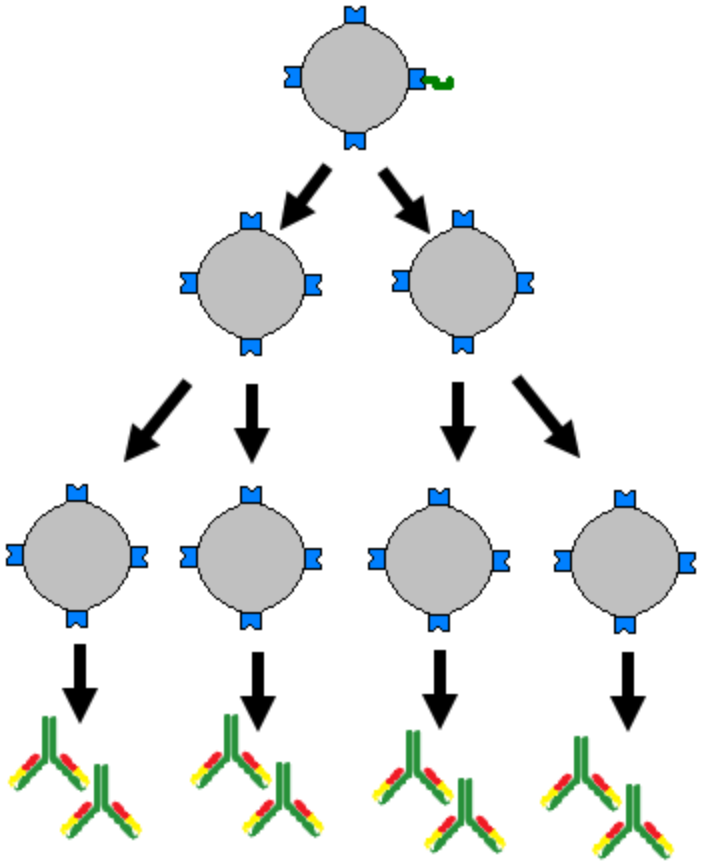
Blood Dilution Problem



10^4 Improvement in Detection Needed

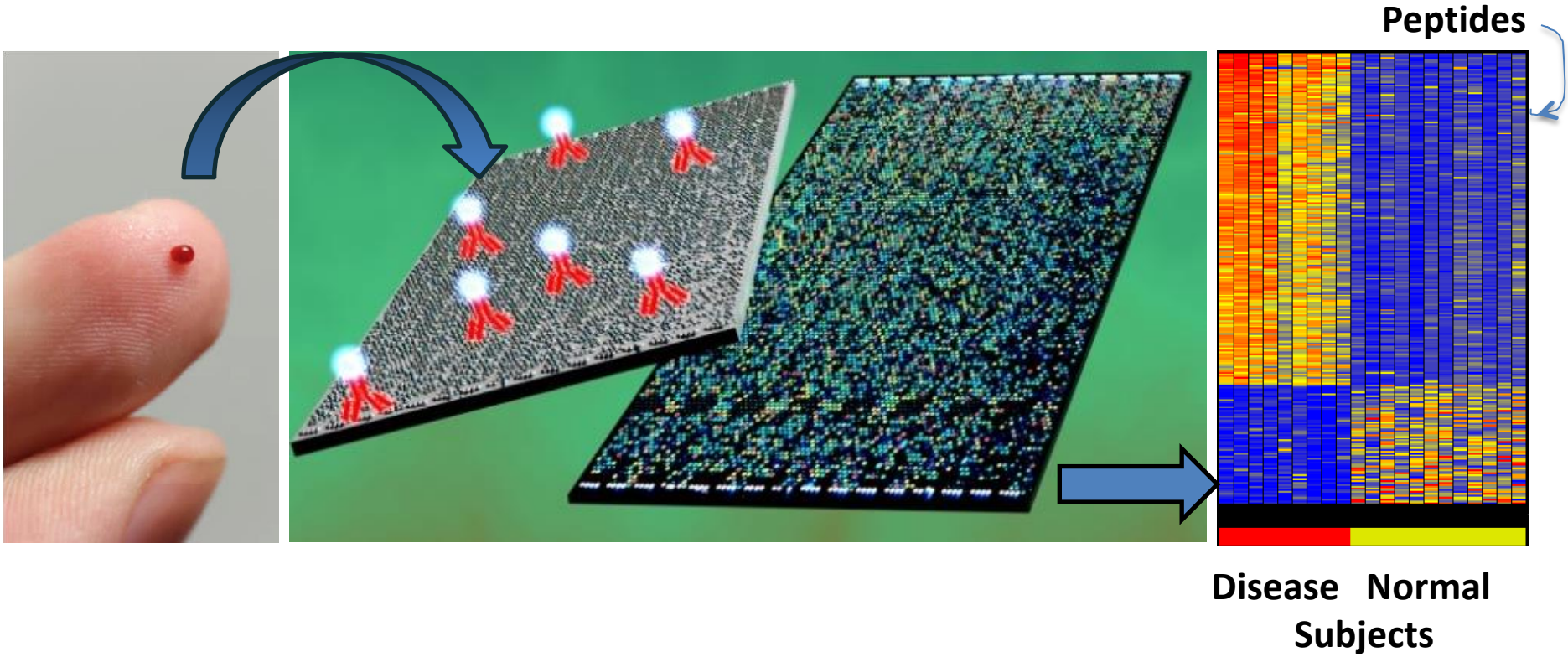
Sci Transl Med **3**, 109ra116 (2011);
Sharon S. Hori*, et al.*
Detection Strategies and Limitations Mathematical Model Identifies Blood Biomarker-Based Early Cancer

The Immune System Detects and Amplifies Signal



- **10^8 to 10^9 antibodies exist in serum**
- **A single reactive B cell encounters antigen and is activated**
- **Produces 5,000 to 20,000 antibodies per minute**
- **Divides every 70 hours**
- **Signal is amplified ~10^{11} times in one week**

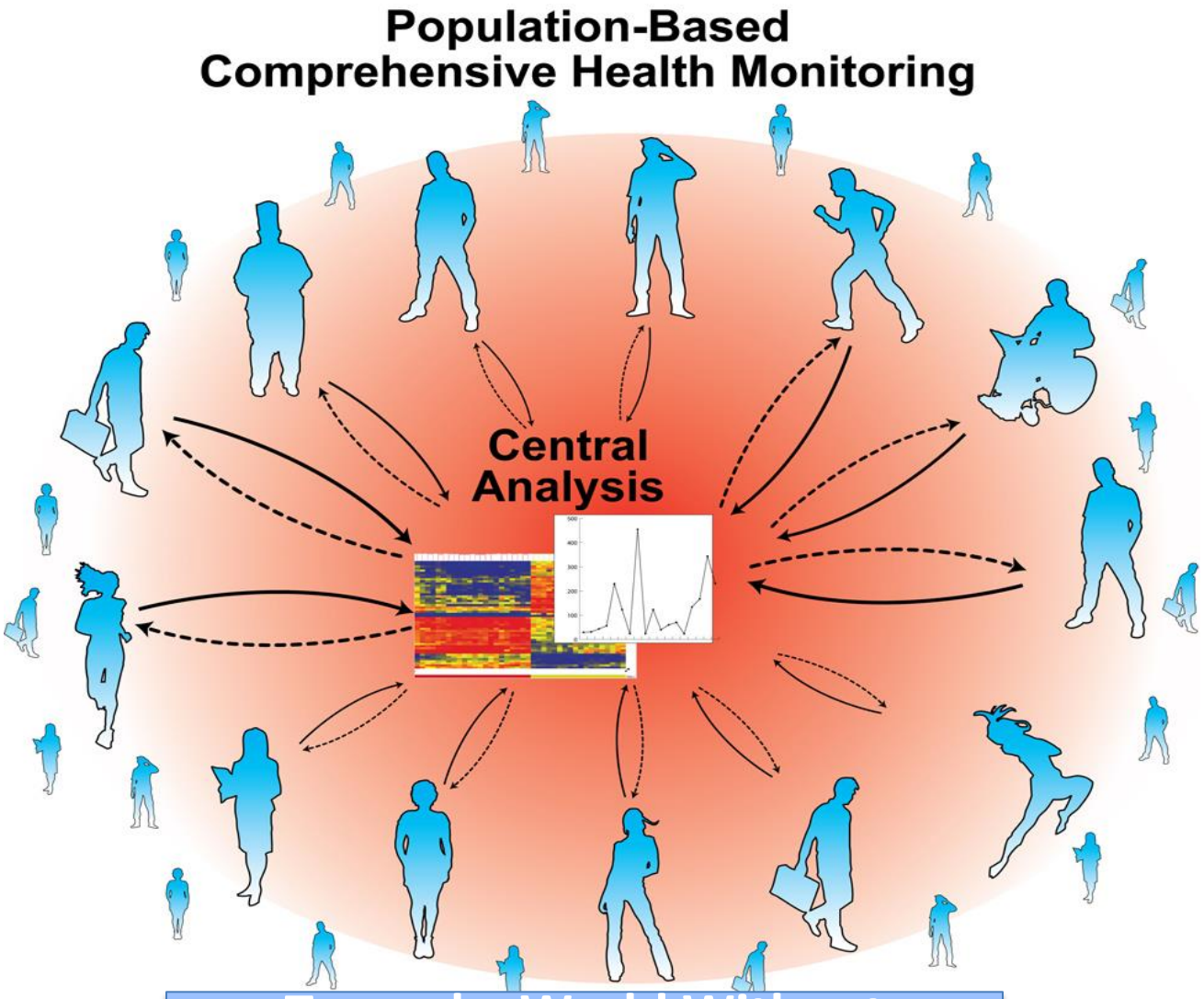
Immunosignatures: A universal, simple and cheap platform for disease diagnosis



CIM10K: 10,000 non-natural sequence peptides

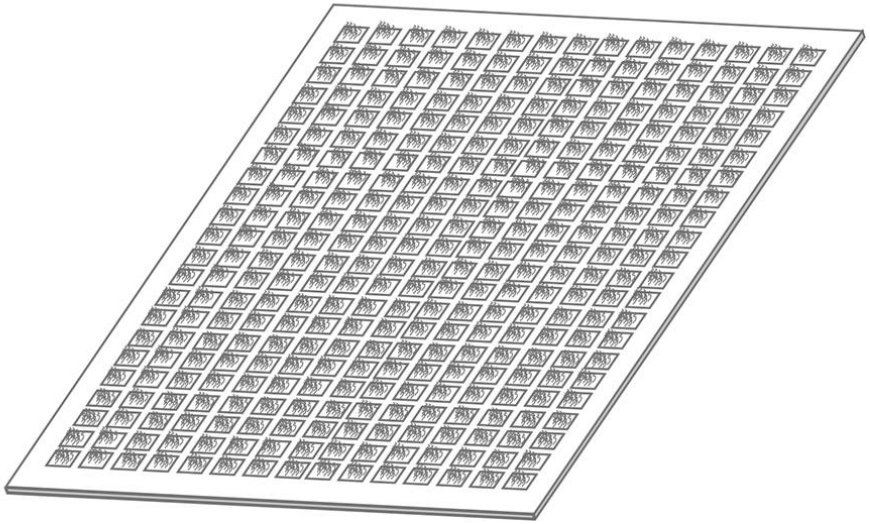
Sykes *et al.* 2013 Trends Biotechnol. 31(1):45-51

HealthTell™



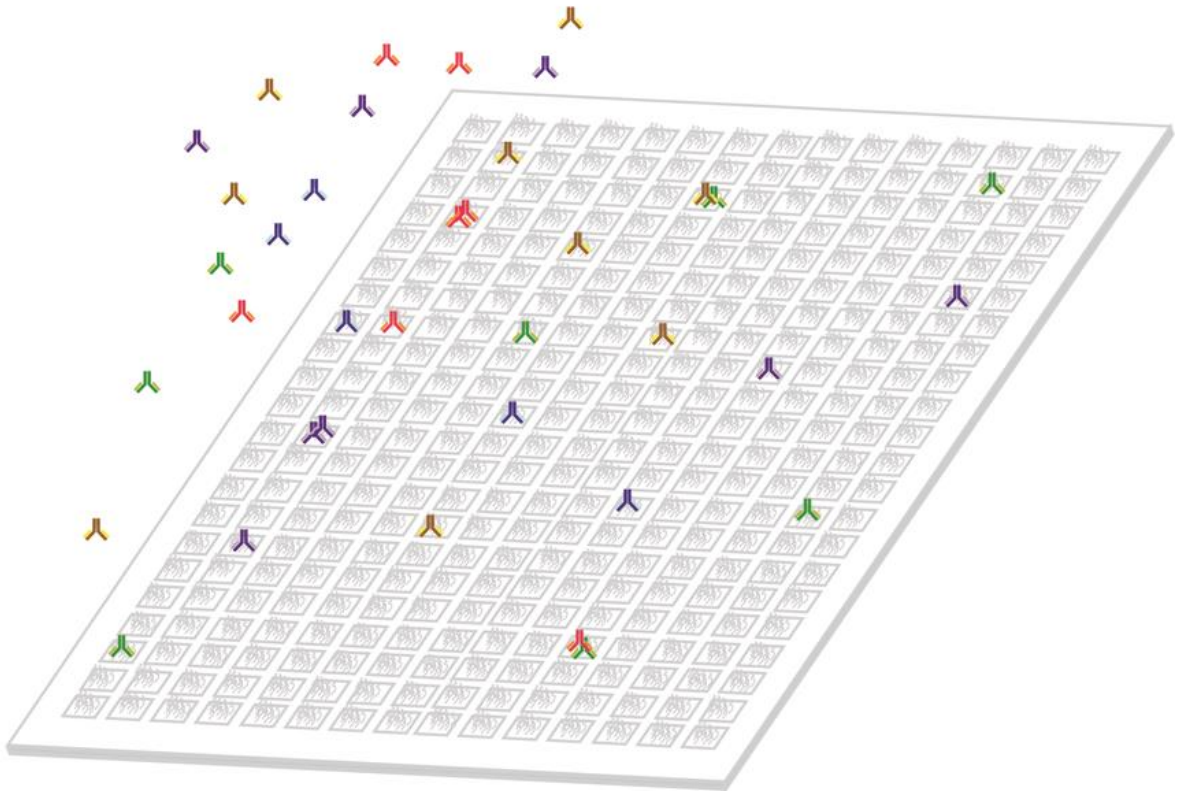
Toward a World Without Patients

Immunosignature Process



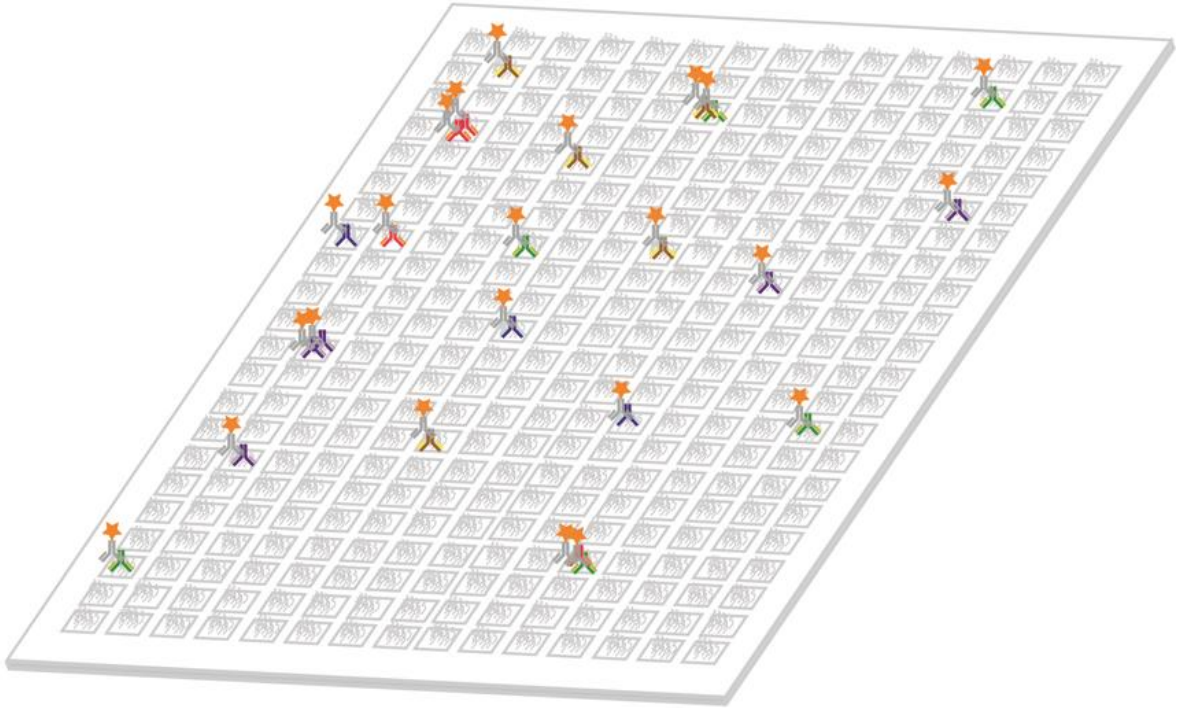
Array of 10K-350K,
Addressable,
Non-Natural Sequence Space
Peptides

Immunosignature Process



Add diluted blood

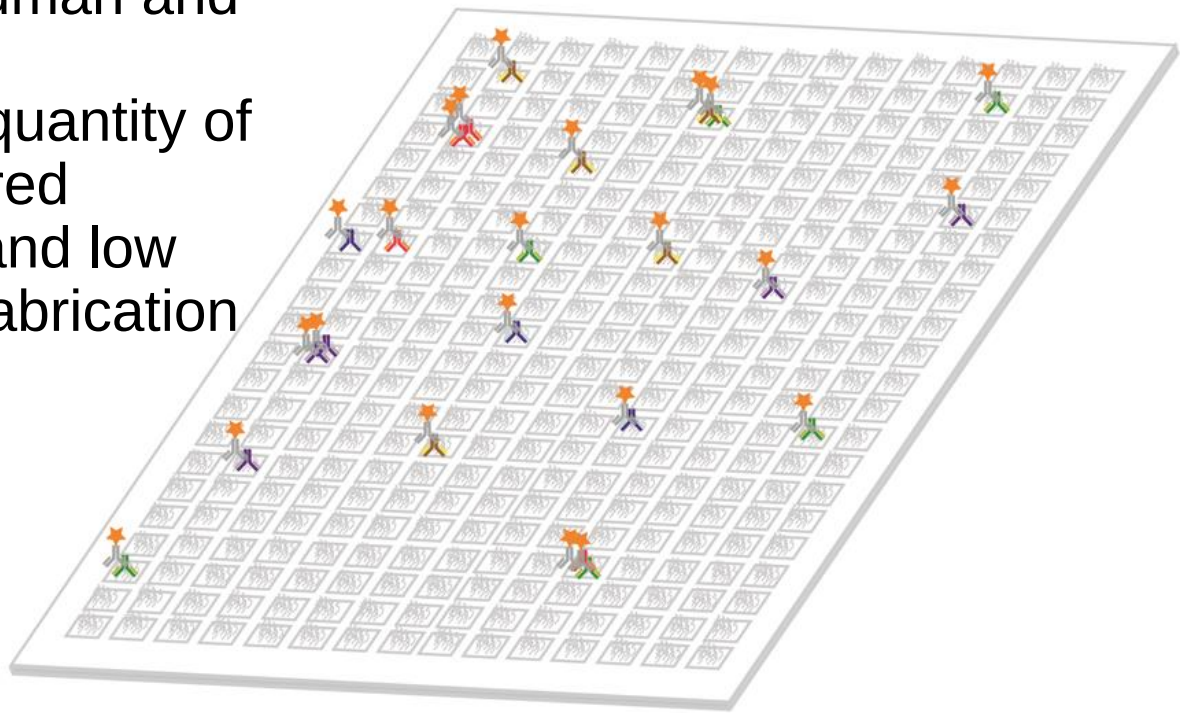
Immunosignature Process



Wash

Immunosignature Process

- One array for all samples, human and nonhuman
- Very small quantity of blood required
- Scalability and low cost array fabrication



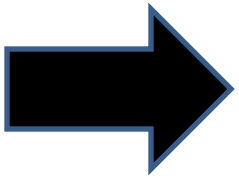
Wash

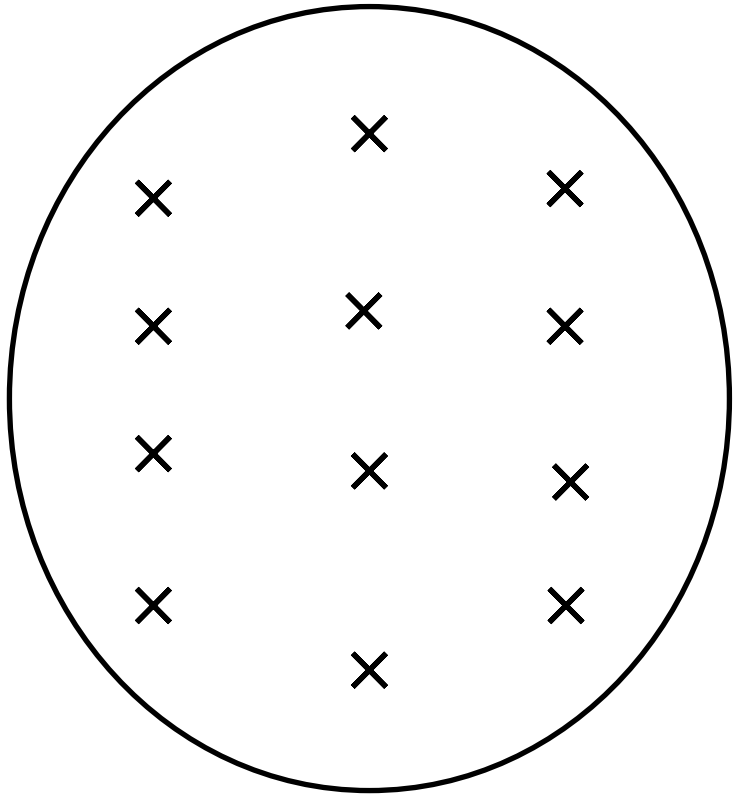
Problem: How to Display Ab Diversity

Antibody Diversity

10^9

Different Ab/person





10^{19} Peptide Sequence Space
In 3 x 10^5 peptides

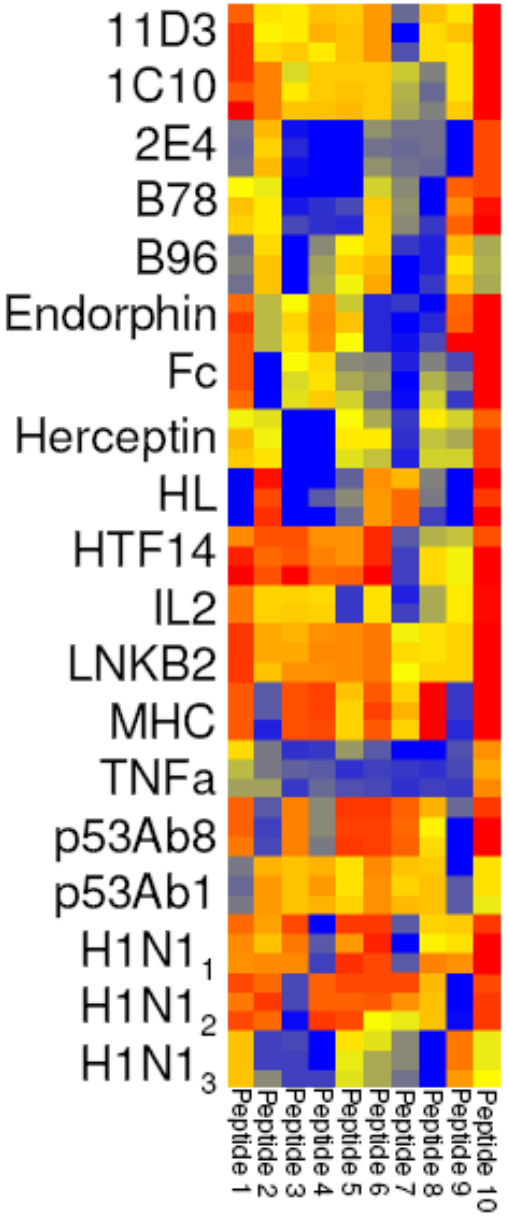
Nature Does Not Always Know Best

- Life occupies an infinitesimally small part of potential sequence space
- Therefore there are many other sequences that could be useful
- Peptides on array are chosen to evenly sample random sequence space(3.5×10^5/ 10^{21} possibilities)

Consequences: Same set of peptides can be used for any diagnosis

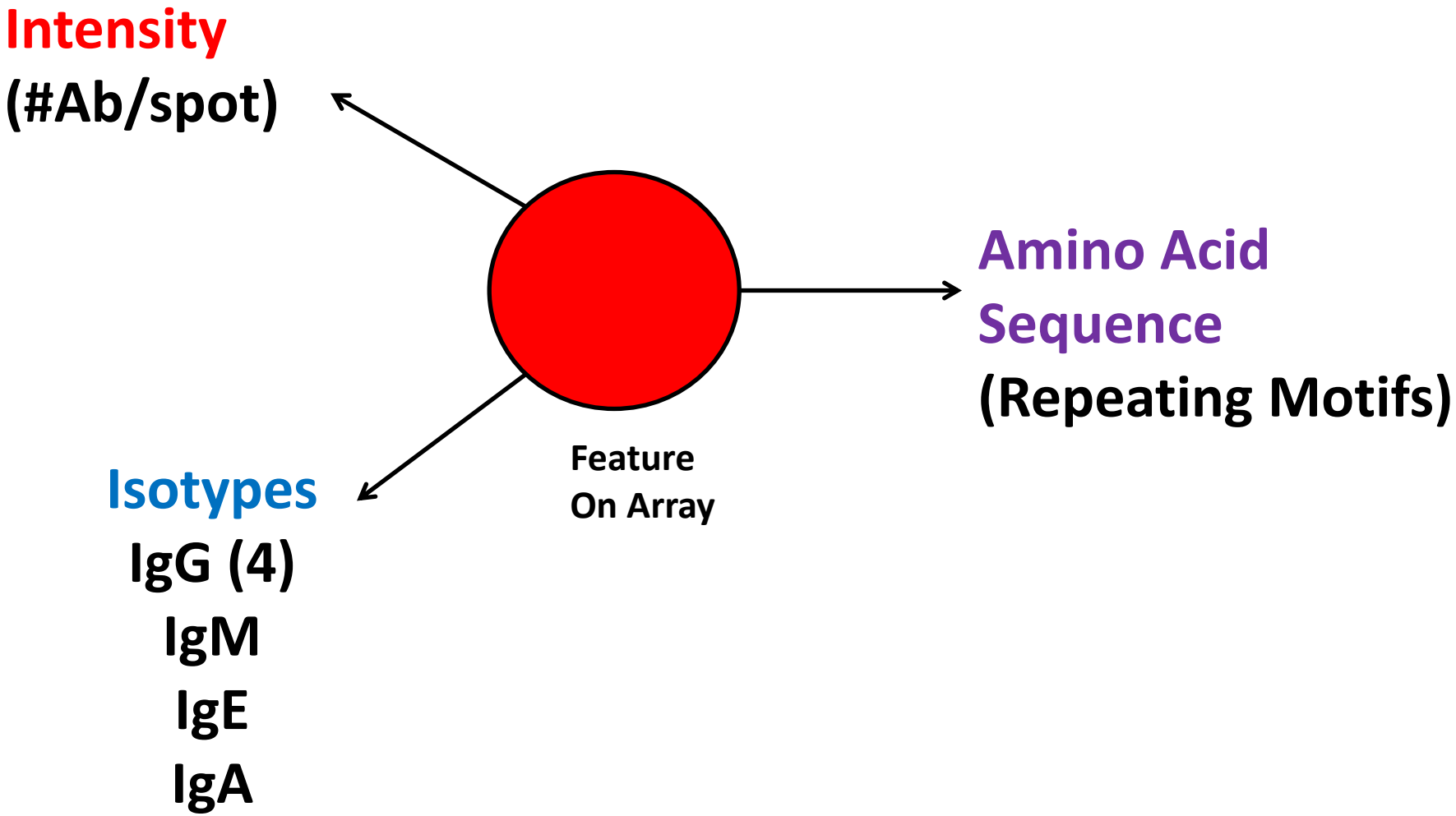
Super-fine resolution of antibody diversity

Monoclonal Antibodies Bind Distinct Patterns on the Array

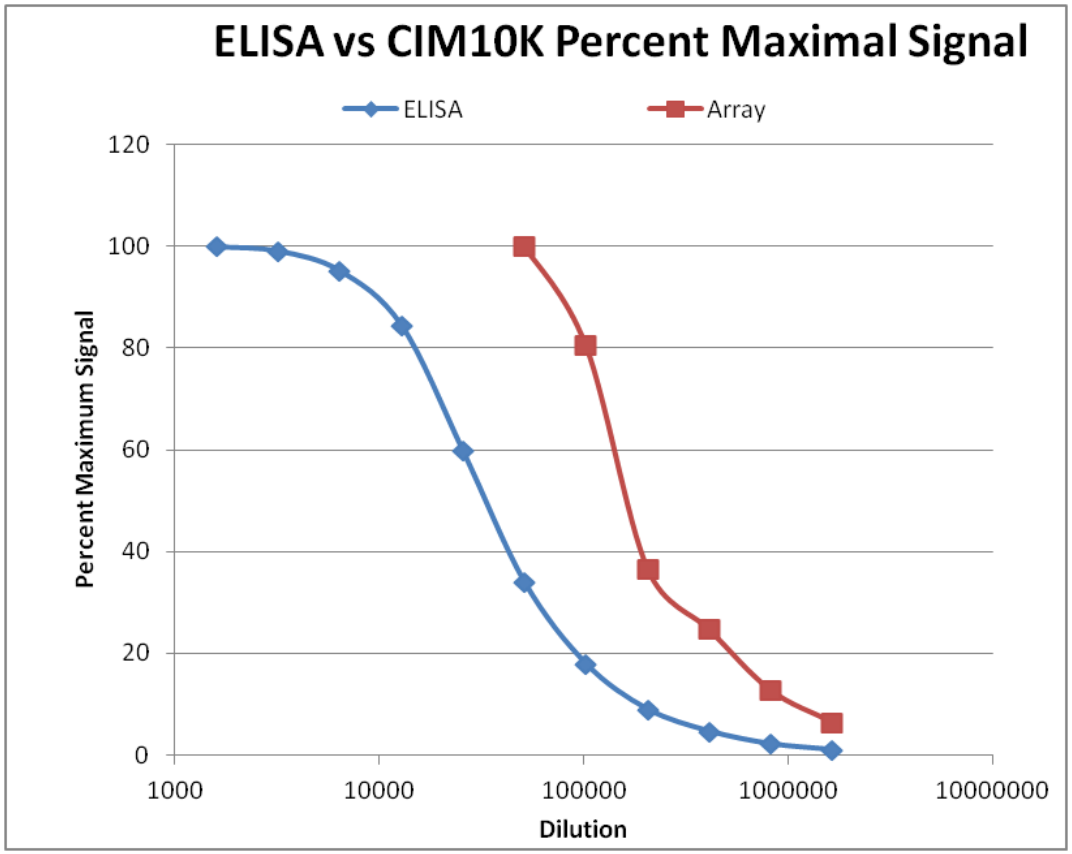


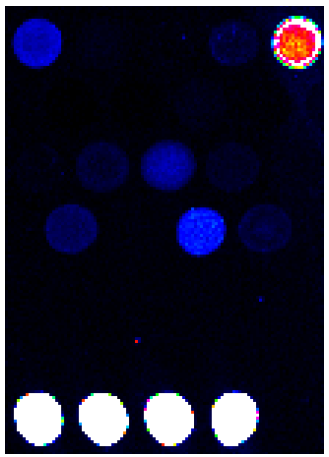
Stafford et al. Mol Cell Proteomics 2012. 11(4):M111.011593

Information from Each Feature

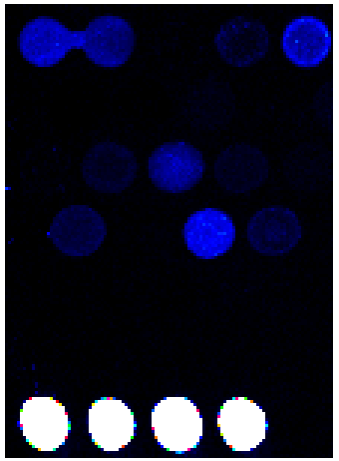


Peptide Microarray Vs ELISA





Sera
1:1,634,800



Secondary
Alone

Identifying The Immunosignature

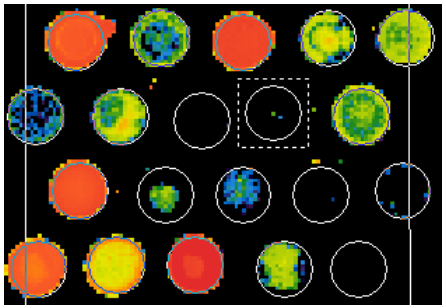
10,000 Peptides

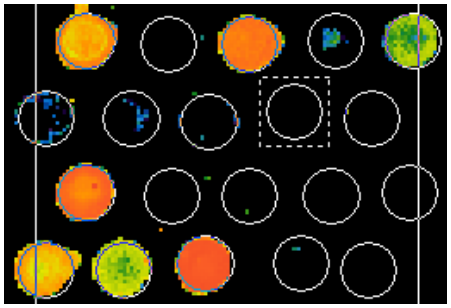
$p < 1x10^{-6}$
& Fold Change

~ 100 informative peptides

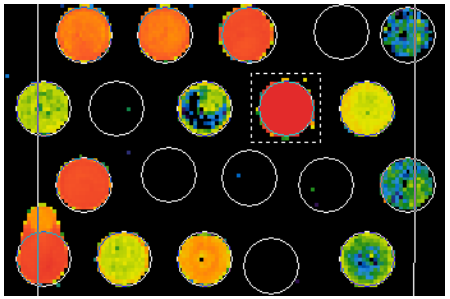
Train a Machine Learning Algorithm

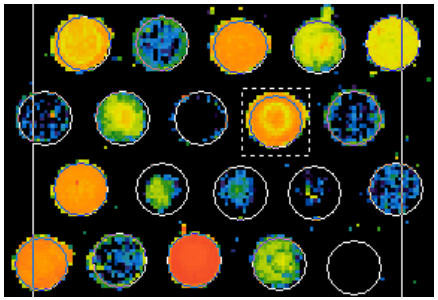
Control



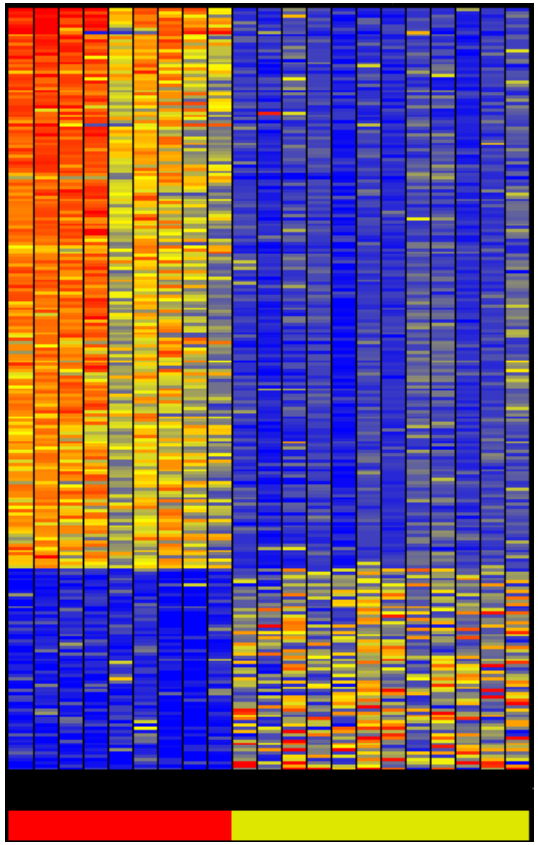


Disease

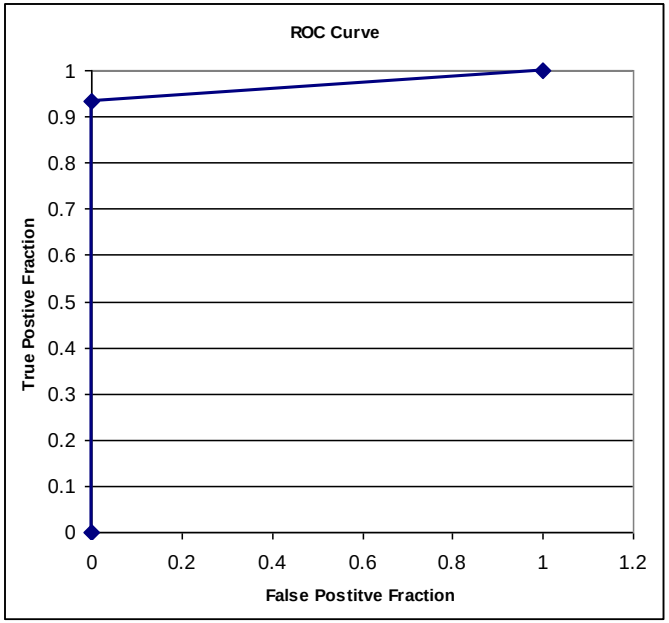




Performance is Tested on a Second Group



Disease Control



Features of Immunosignatures

Same chip used for all diseases, all species

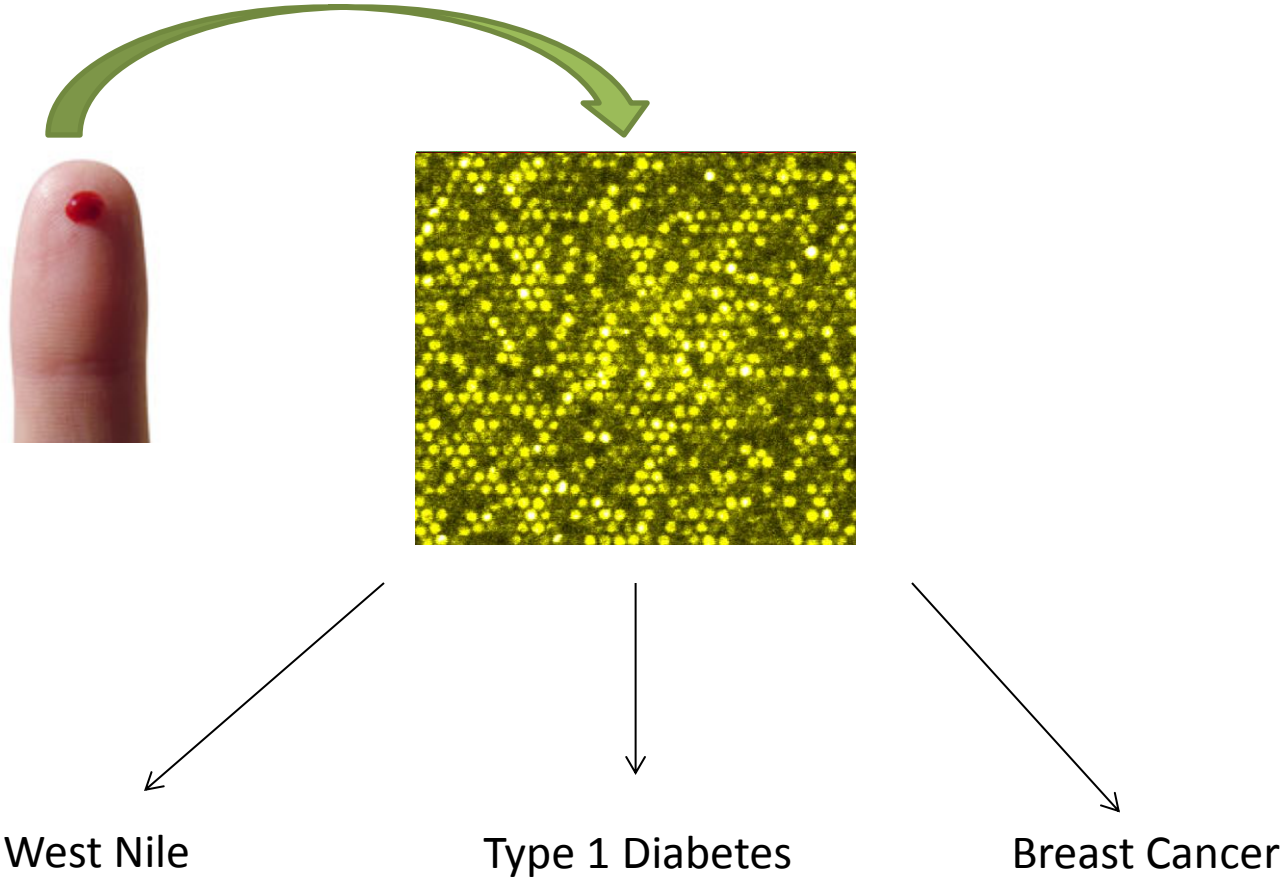
Detects all antibodies: sugars, non-linear, modifications

Historical sera samples work

No sample preparation

10-100x more sensitive than ELISA

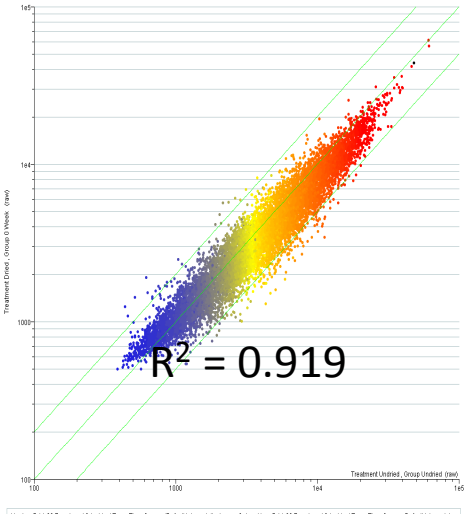
One Chip, Many Tests



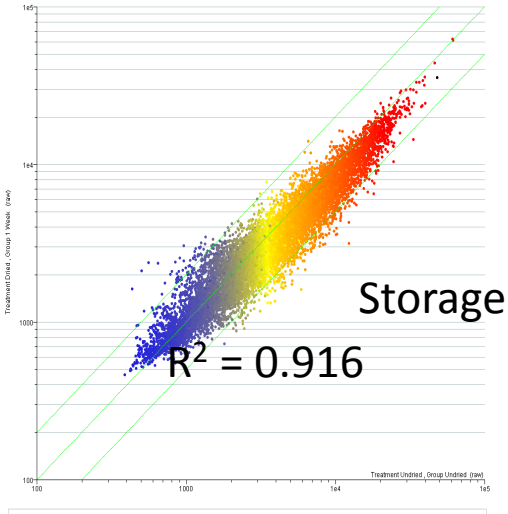
Dry Blood Works as Well as Fresh Blood

Dried vs. Undried Whole Blood Immunosignature Correlations

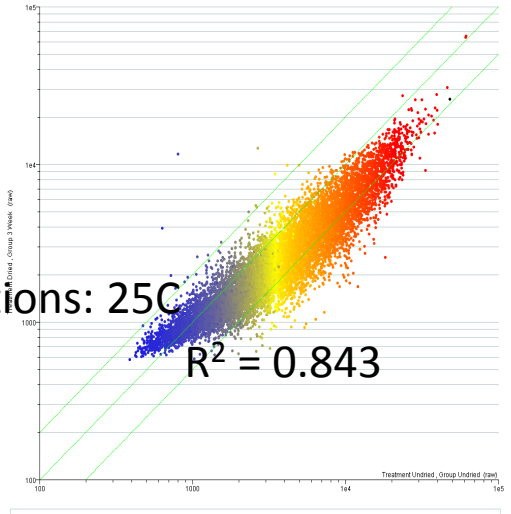
0 Week Dried vs.
Undried Whole Blood



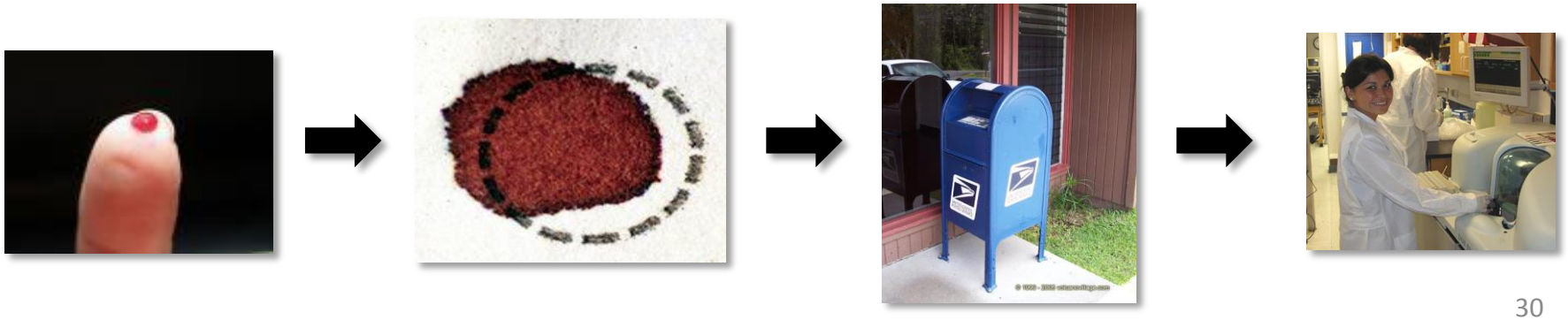
1 Week Dried vs.
Undried Whole Blood



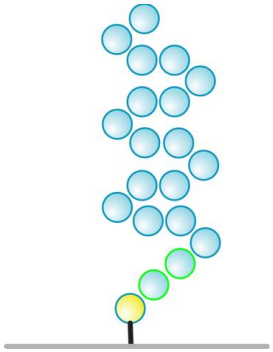
3 Week Dried vs.
Undried Whole Blood

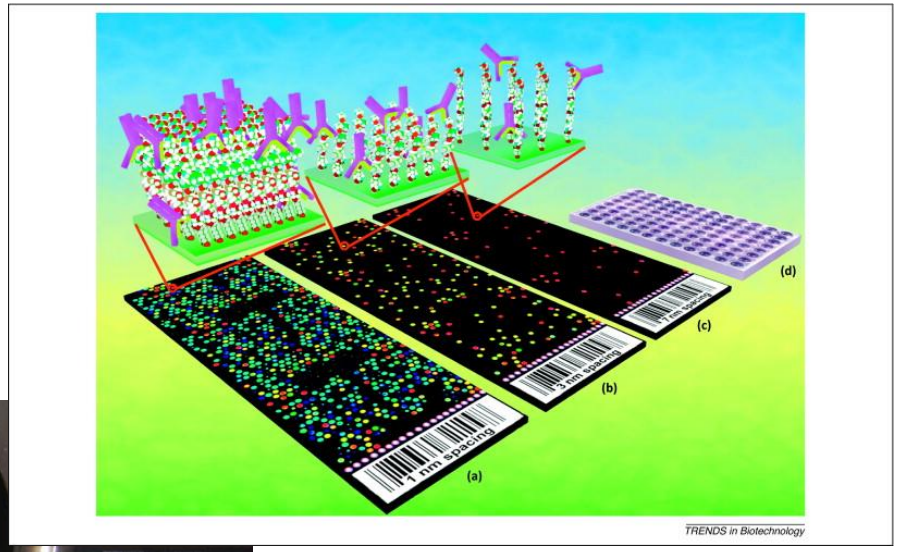


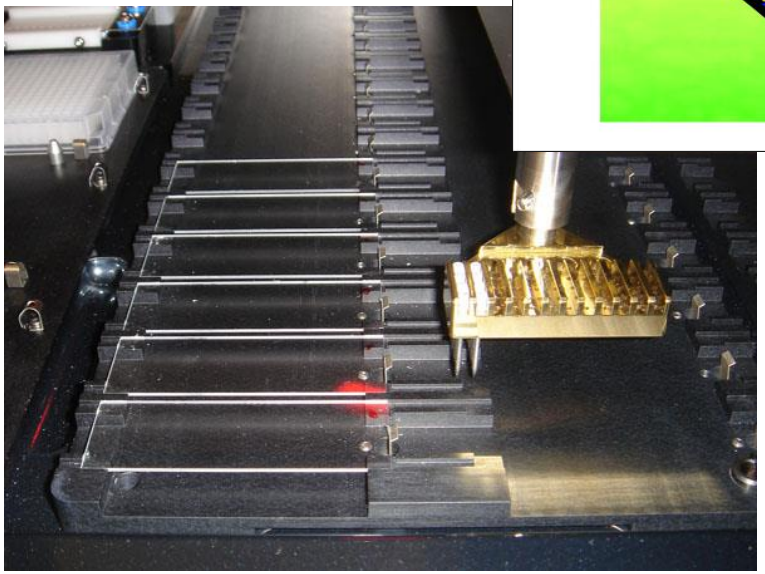
Storage conditions: 25C

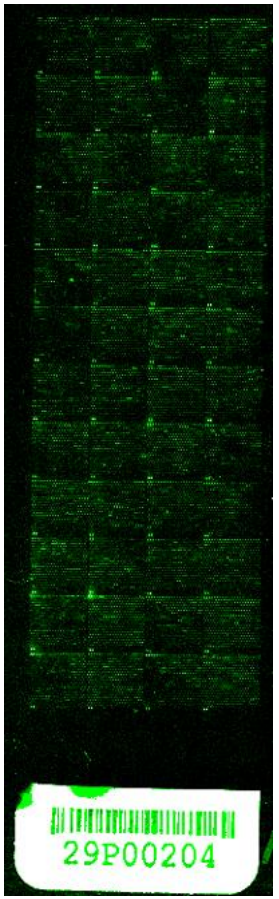


Platform 1: Printed Arrays of 10,000 Peptides





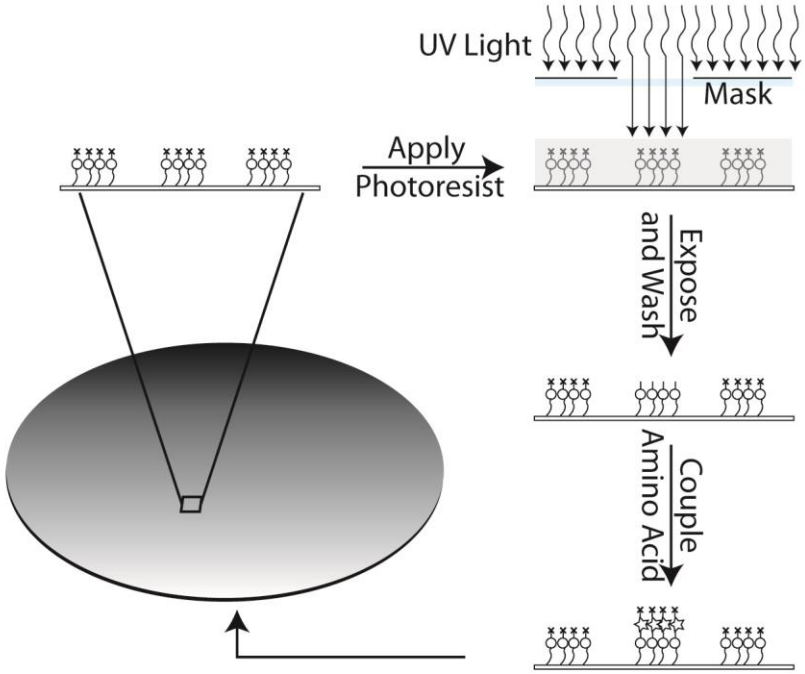


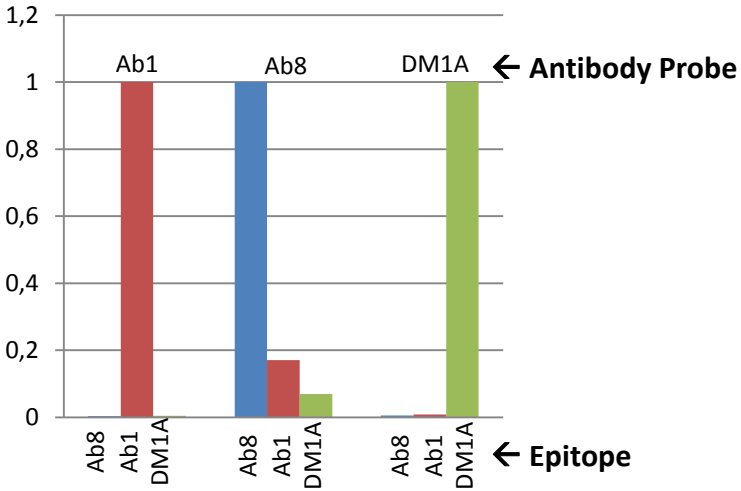


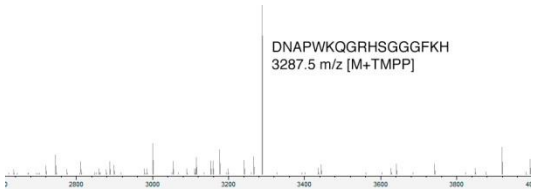
17 amino acids long, random sequence, and all amino acids except C are used.
Two copies of the library are printed on a glass slide (~1200 peptides/cm^2).
Mass spectra available for all peptides spotted on the arrays.

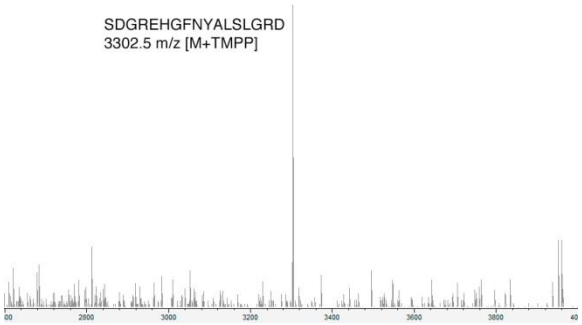
Fabrication Approach

- UV photolithography
- All chemistry performed using coater/developer
- Large number of cycles makes the process challenging



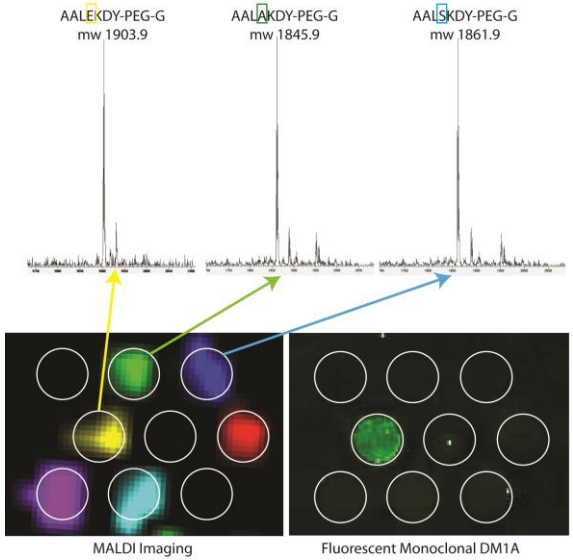


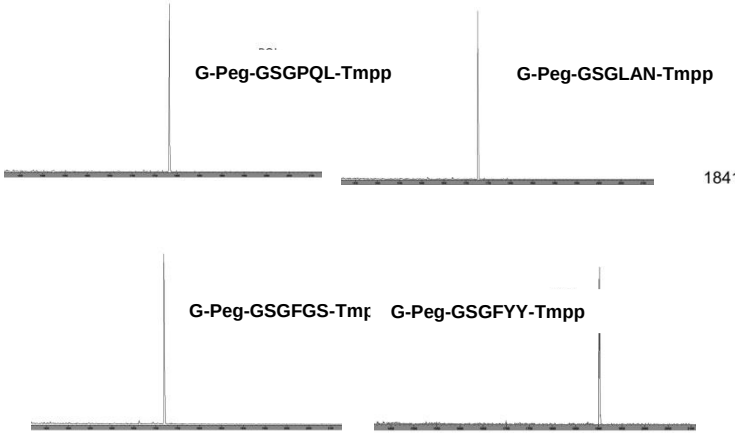


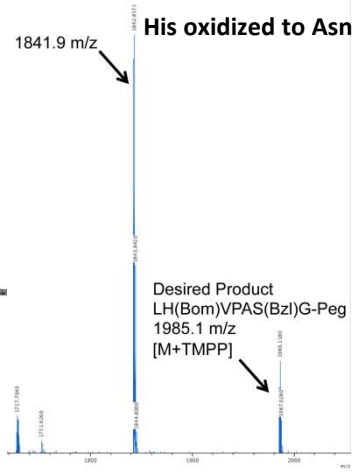


Stepwise Yield Analysis

A	0.998
V	0.999
P	0.999
L	0.992
G	0.991
Y	0.992
F	0.998
S	0.998
N	0.998
Q	0.986
K	0.972
D	0.997
E	0.998
H	0.711

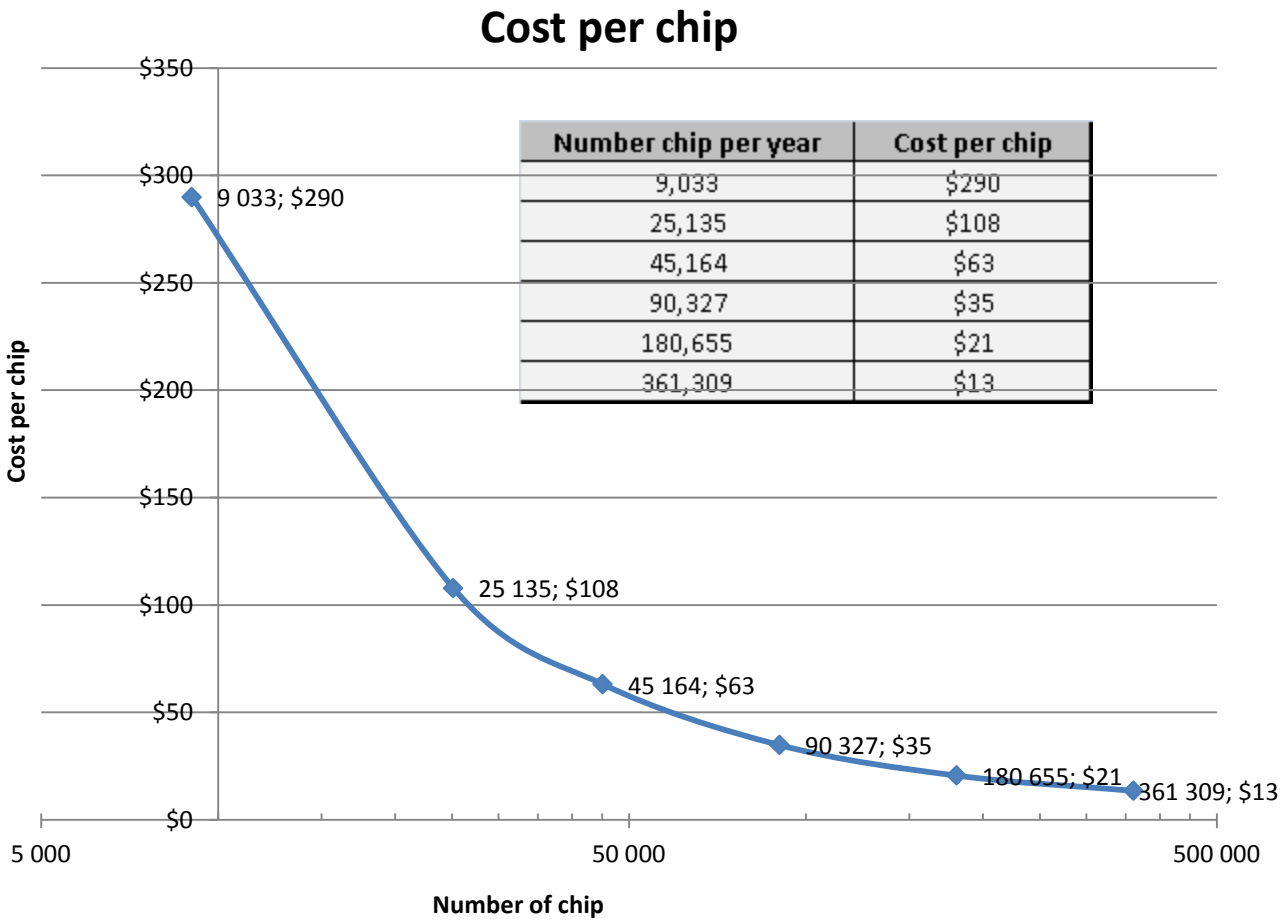






Material in this presentation contains ASU and HealthTell Intellectual Property and is Confidential

Cost per chip Vs volume

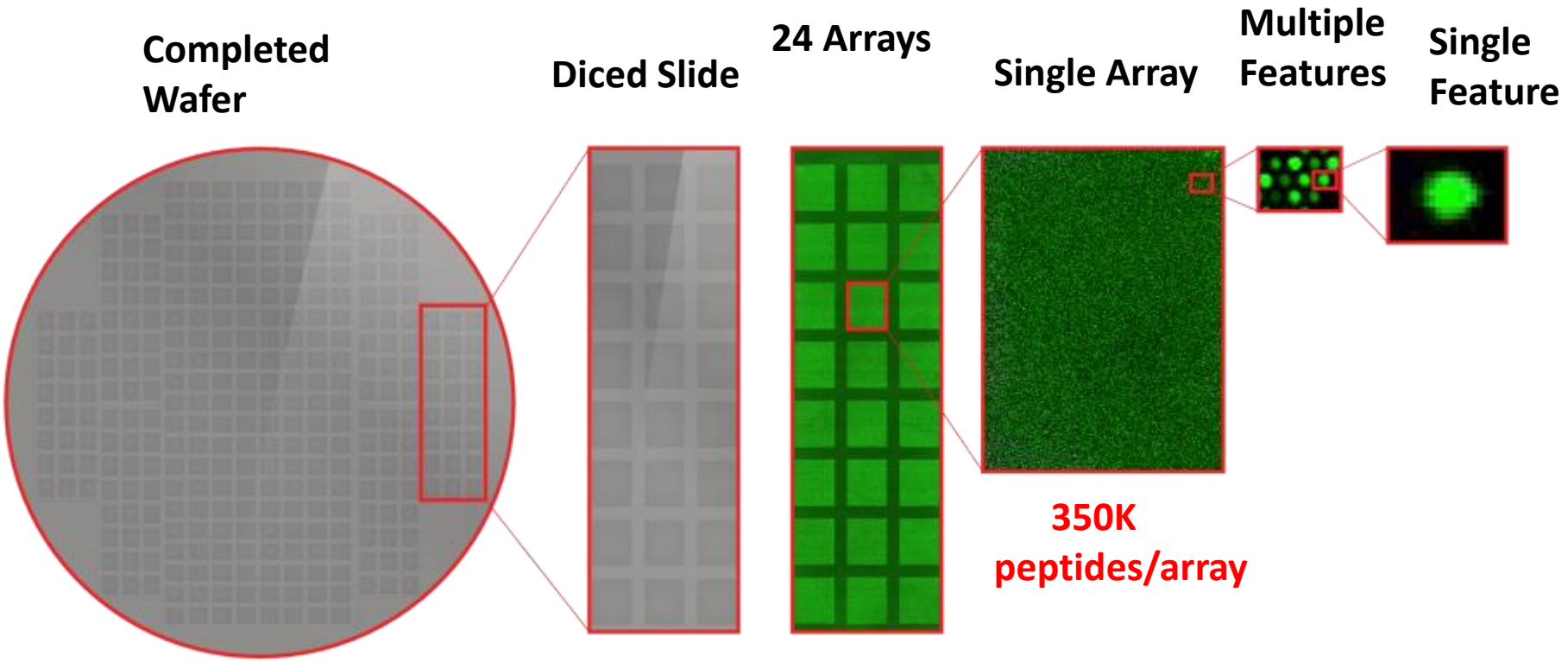


Number chip per year	Cost per chip
9,033	$290
25,135	$108
45,164	$63
90,327	$35
180,655	$21
361,309	$13

- Cost analysis includes
  - Labor cost
    - Technicians need to run the tools
  - Yield & QA cost
    - Labor + reagents
    - Each batch sample set is analyzed
  - Chemical/biochemical reagents
    - Surface prep
    - Litho & resist steps
    - Amino-acid coupling
  - Materials
    - Wafer
    - Mask set
  - Facility cost
    - Rent, unitlity, & chemical disposal
  - Tool maintenance
  - Packaging

HealthTell™

Array Production Breakdown: Wafer to Individual Spot



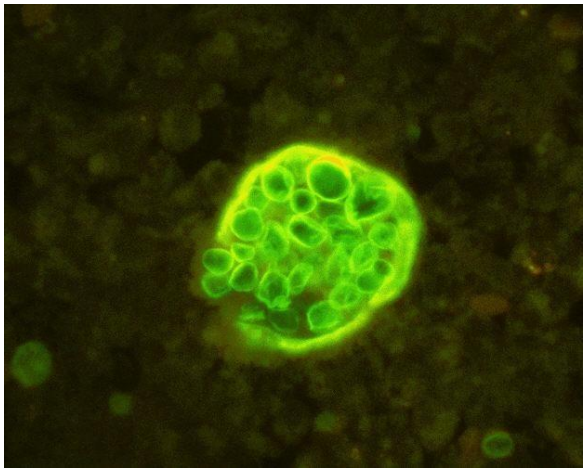
~50-Fold Improvement Over CIM10K Printed Arrays

HealthTell™

OneTest™

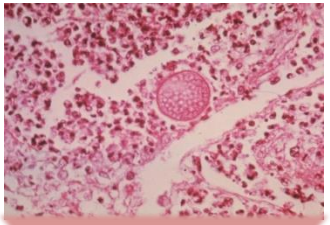
Comprehensive Health Monitoring

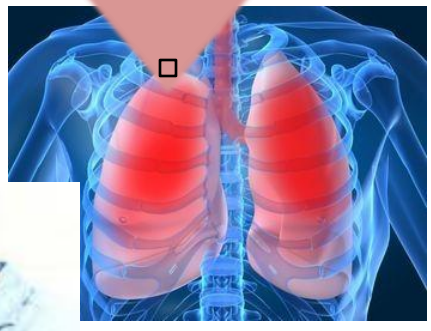
Valley Fever (Coccidiomycosis)

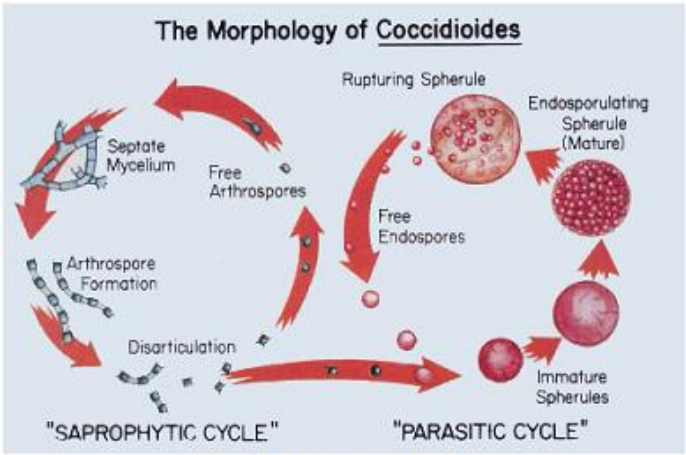


Coccidioides immitis spherule with endospores.

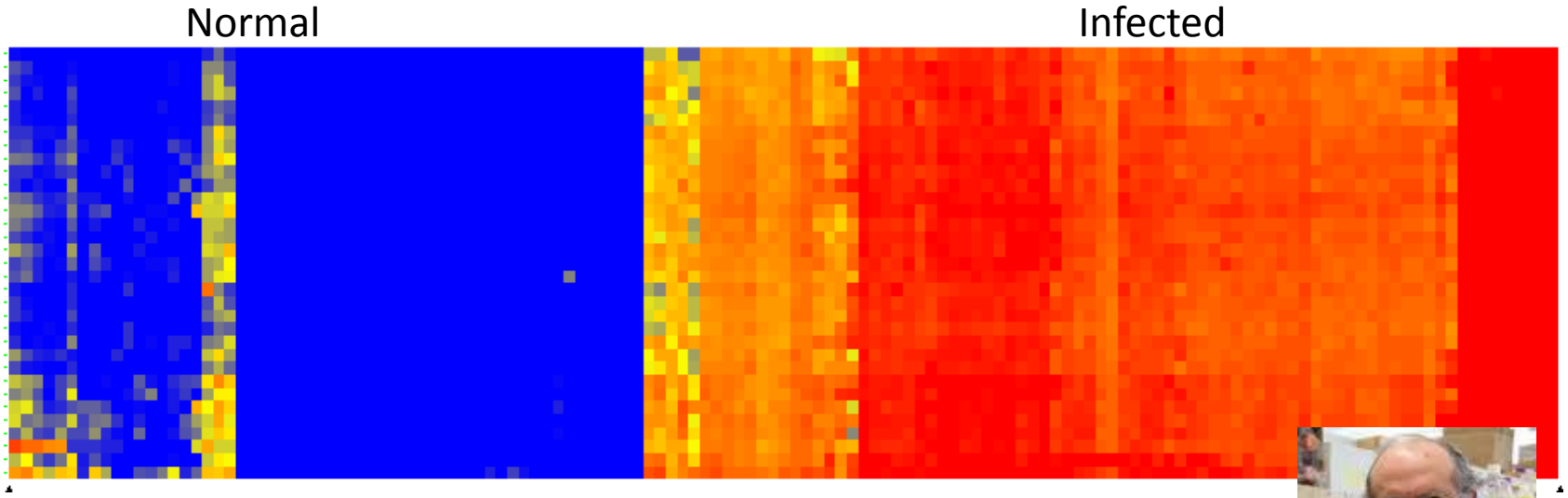
- About 30,000 reported cases annually
- Particularly prevalent the Sonoran desert
- While most cases mild, it can be life threatening
- Flu-like symptoms







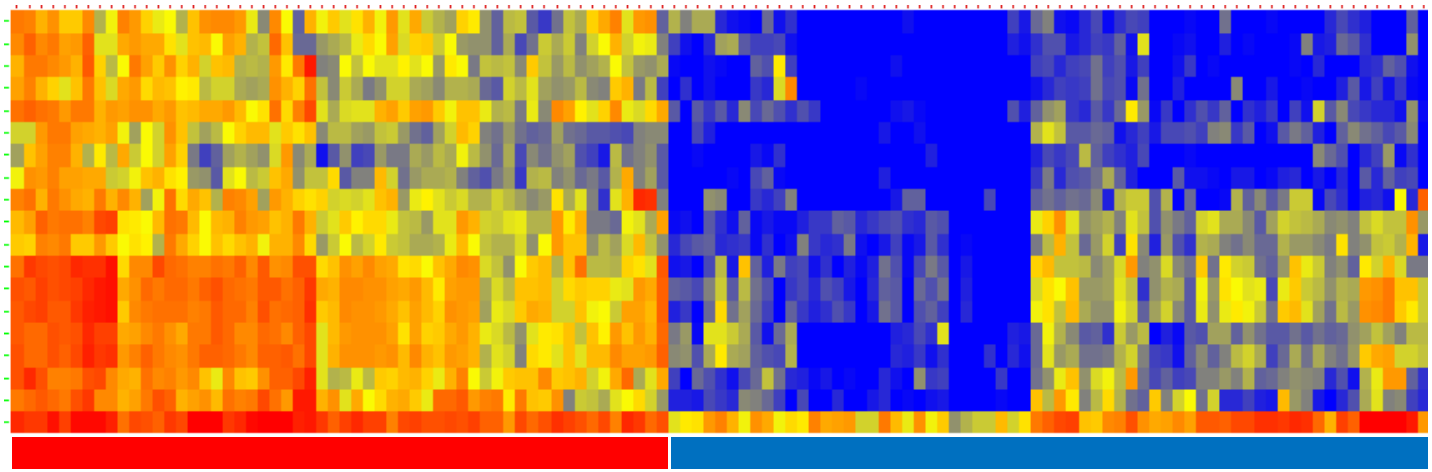
Classic Train/Test Example: Valley Fever



- 10,000 peptides on original array
- 120 patients screened and analyzed
- 100 most informative peptides selected and resynthesized
- Diagnostic array printed

John Galgiani
Univ. of Arizona

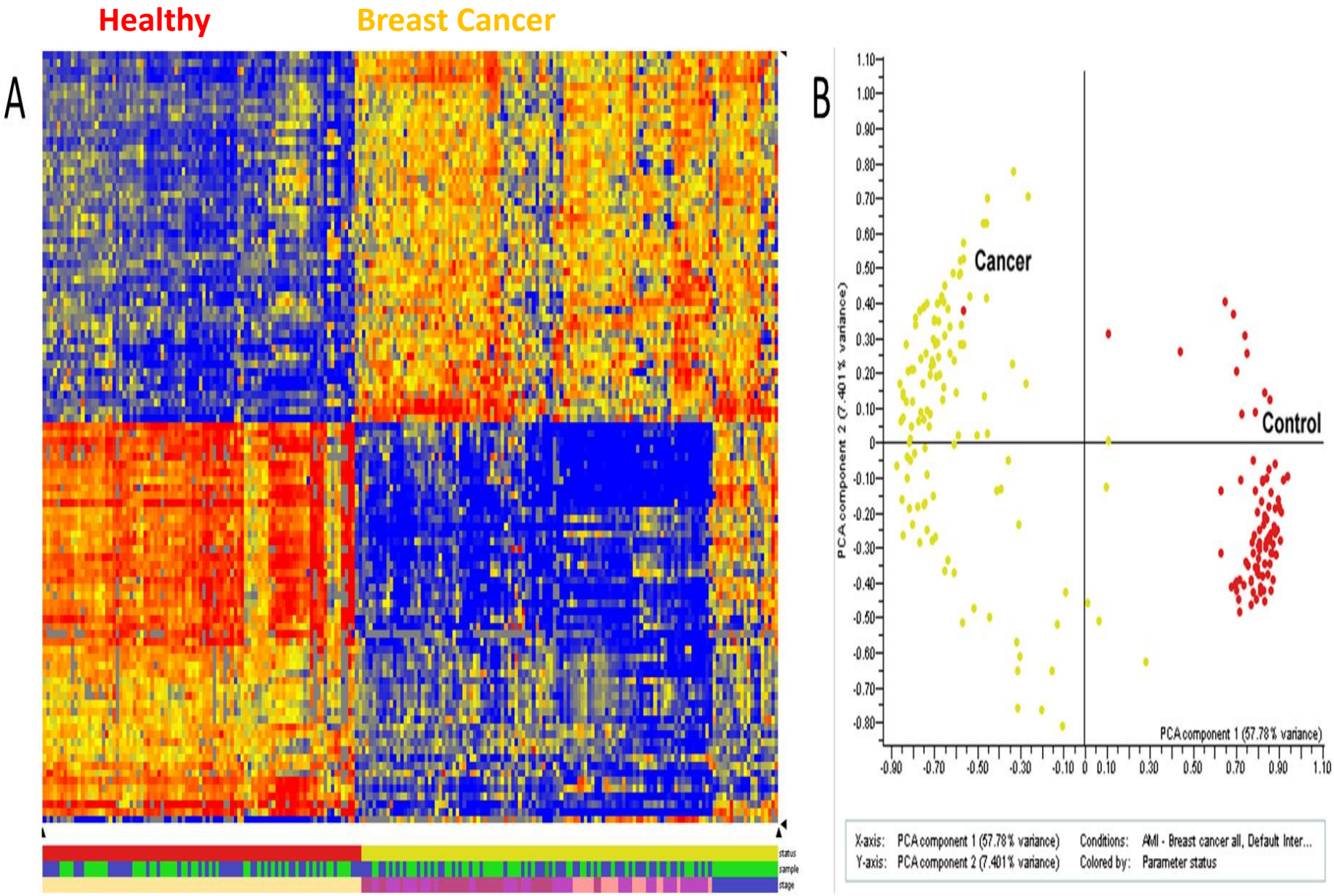
Outperforms Existing Diagnostic



- 90 blinded samples from patients presenting 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

Breast Cancer Test/Train using geographically distinct cohorts



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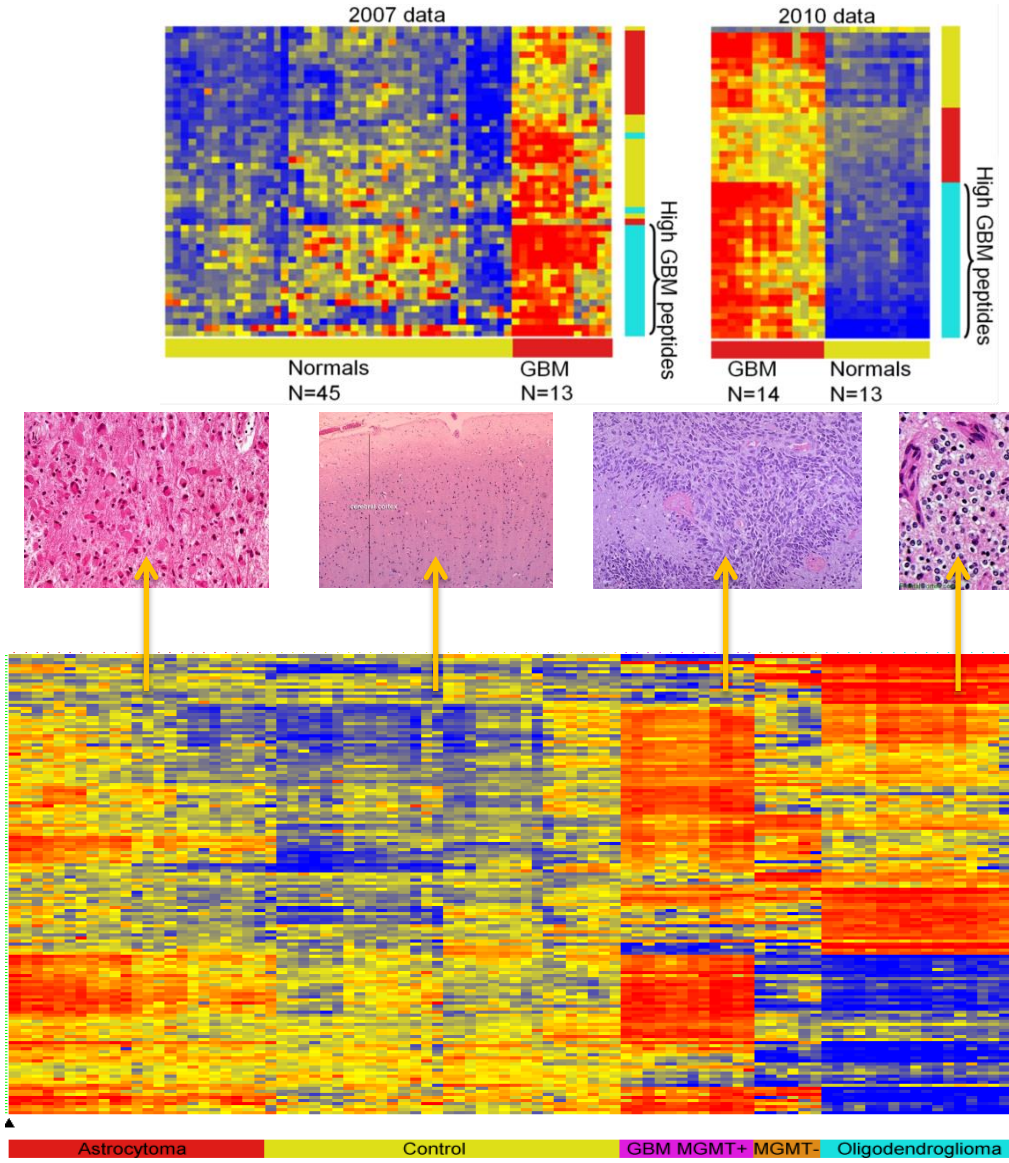
Immunosignaturing Brain Tumors

Collaborator: Adrienne C. Scheck, BNI



BARROW Neurological Institute

St. Joseph's Hospital and Medical Center. A Dignity Health Member

100% accurate detection training and testing on samples taken years apart using printed arrays

Not only can immunosignaturing detect the brain tumor, it can distinguish accurately between the common types of brain tumors

ASU Arizona State University

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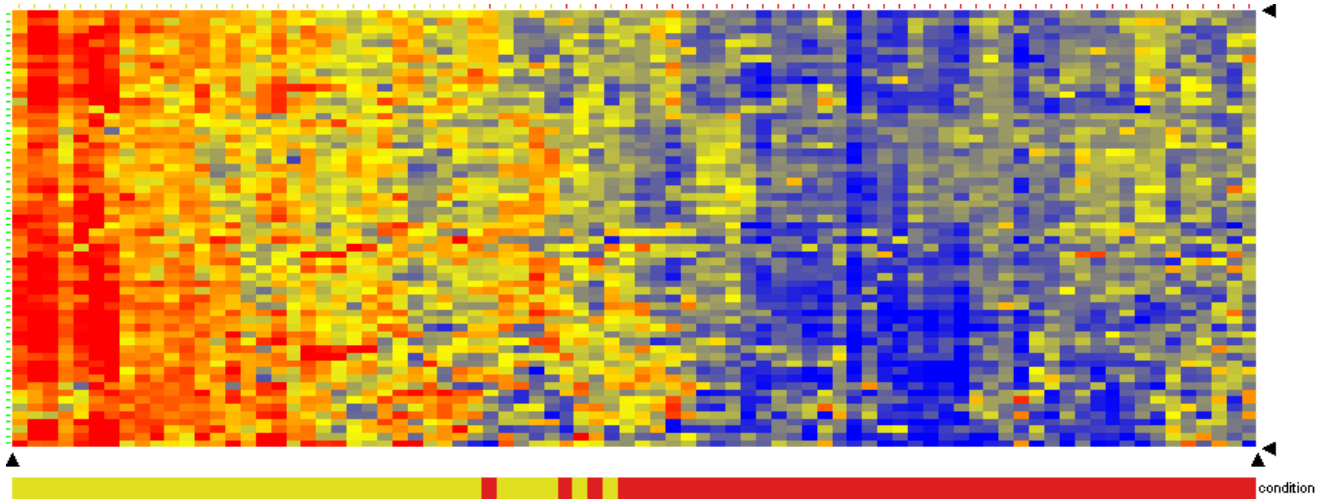
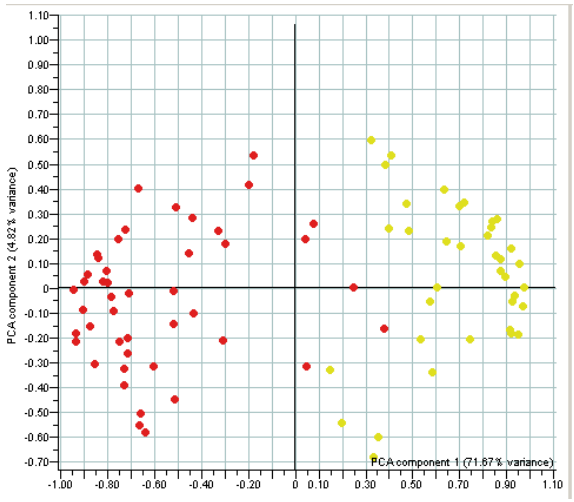
Alzheimer's:

Alzheimer's Disease Neuroimaging Initiative (10K)





Alzheimer's Disease Controls

Disease: ADNI collection of serum samples from Alzheimer's Disease (AD) and non-AD controls
Feature selection: 1 sided T-test, 50 peptides were selected
Classification: 4 samples were called normal when they were Alzheimer's (FN)
Sensitivity=89%, NPV=92%, Accuracy=95%, specificity and PPV=100%
Interpretation: AD signature blends gradually into controls with no clearly defined threshold

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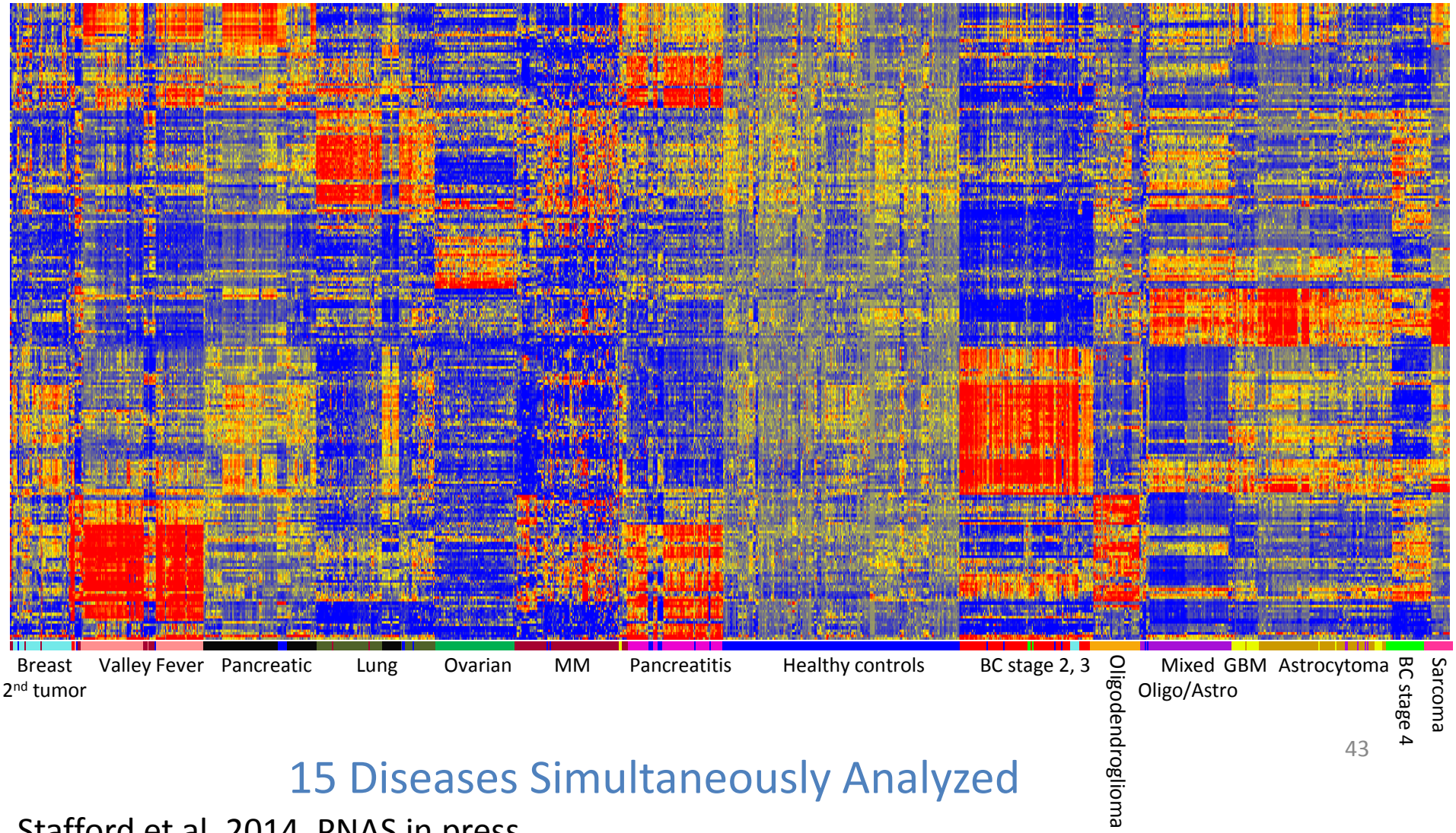
Towards Comprehensive Testing



15 Diseases Simultaneously Analyzed

Stafford et al. 2014, PNAS in press

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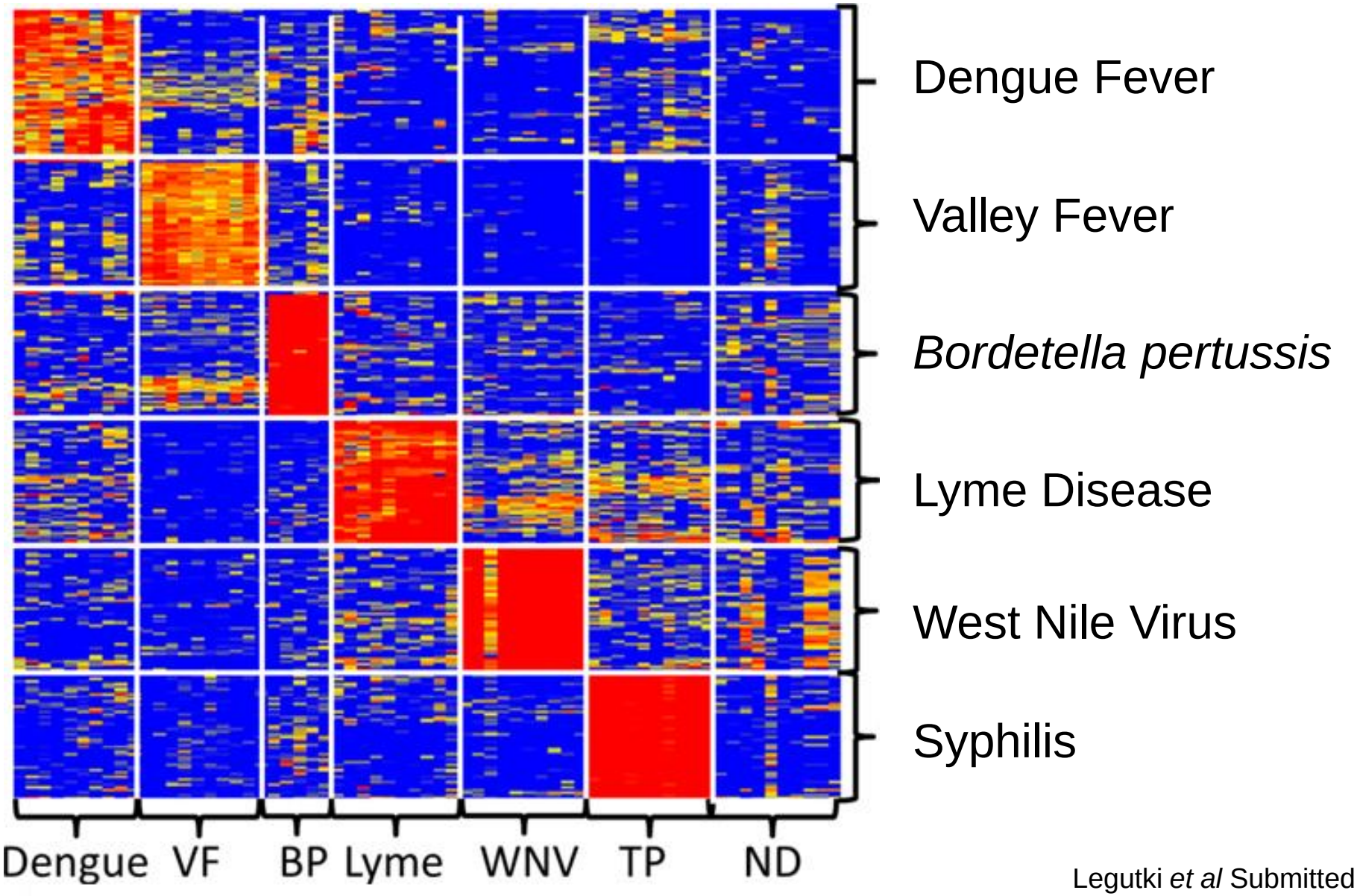
Cross Validation, 15 Diseases

disease	accuracy	Sensitivity	Specificity	PPV	NPV
2ndBC	97.8±0.14	69.1±2.82	99.21±0.1	81.05±3.46	98.48±0.11
Astro	96.93±0.17	90.1±1.3	97.82±0.17	83.79±1.11	98.73±0.18
BC	99.51±0.05	99.71±0.2	99.49±0.08	95.45±0.68	99.97±0.02
BCIVa	99.62±0.06	89.85±1.49	100±0	100±0	99.6±0.06
GBM	99.18±0.1	94.33±2	99.25±0.09	62.1±4.24	99.92±0.03
Lung	99.02±0.12	92.37±0.58	99.59±0.09	94.79±1.27	99.35±0.05
MM	98.72±0.11	100±0	98.62±0.12	85.13±1.13	100±0
ND	96.62±0.17	85.45±0.77	99.31±0.1	96.66±0.47	96.6±0.23
Oligo	99.65±0.07	92.57±1.95	99.86±0.03	95.21±1.19	99.78±0.06
OligoAstro	98.94±0.15	98.45±0.82	98.95±0.12	86.41±1.78	99.91±0.04
Ovarian	99.92±0.03	100±0	99.91±0.03	98.67±0.47	100±0
Pancreatitis	99.67±0.05	95.42±1	99.91±0.03	98.5±0.54	99.74±0.05
PC	97.69±0.11	86.61±1.39	98.79±0.08	87.22±1.19	98.67±0.12
Sarcoma	98.81±0.11	54.15±5.48	99.67±0.07	71.55±5.65	99.12±0.12
VF	99.67±0.08	100±0	99.64±0.09	96.87±0.74	100±0
total	98.77±0.04	89.87±1.32	99.33±0.08	88.89±1.59	99.33±0.07

Simultaneous Distinction of 6 Infection and Normal Sera



Legutki *et al* Submitted

10k vs 330K comparison

CIM10K KNN Classification Results using the 160 peptides

Sample	Correctly Identified	Incorrectly Identified
DTRA 1	40	0
DTRA 2	32	0
DTRA 3	40	0
DTRA 4	49	0
DTRA 5	42	1
DTRA 6	41	0
DTRA 7	33	0
DTRA 8	46	0
Local Normal	59	0
Total	382	1

HT330K KNN Classification Results using the 160 peptides

Sample	Correctly Identified	Incorrectly Identified
DTRA 1	12	0
DTRA 2	12	0
DTRA 3	12	0
DTRA 4	11	0
DTRA 5	31	0
DTRA 6	26	0
DTRA 7	27	0
DTRA 8	27	0
Local Normal	6	0
Total	164	0

Minimal P-Value

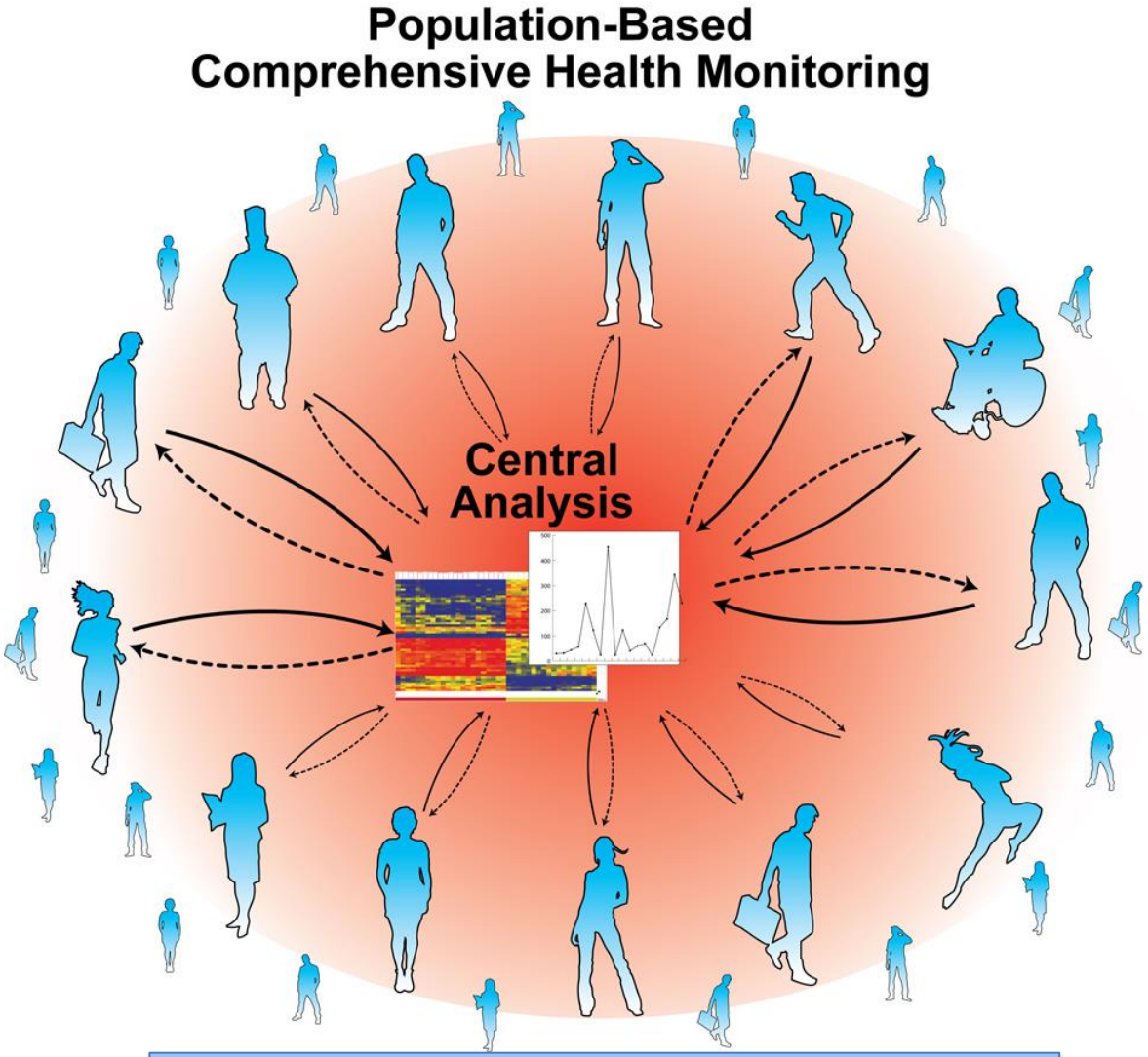
2.7×10^{-13}

6.2×10^{-38}

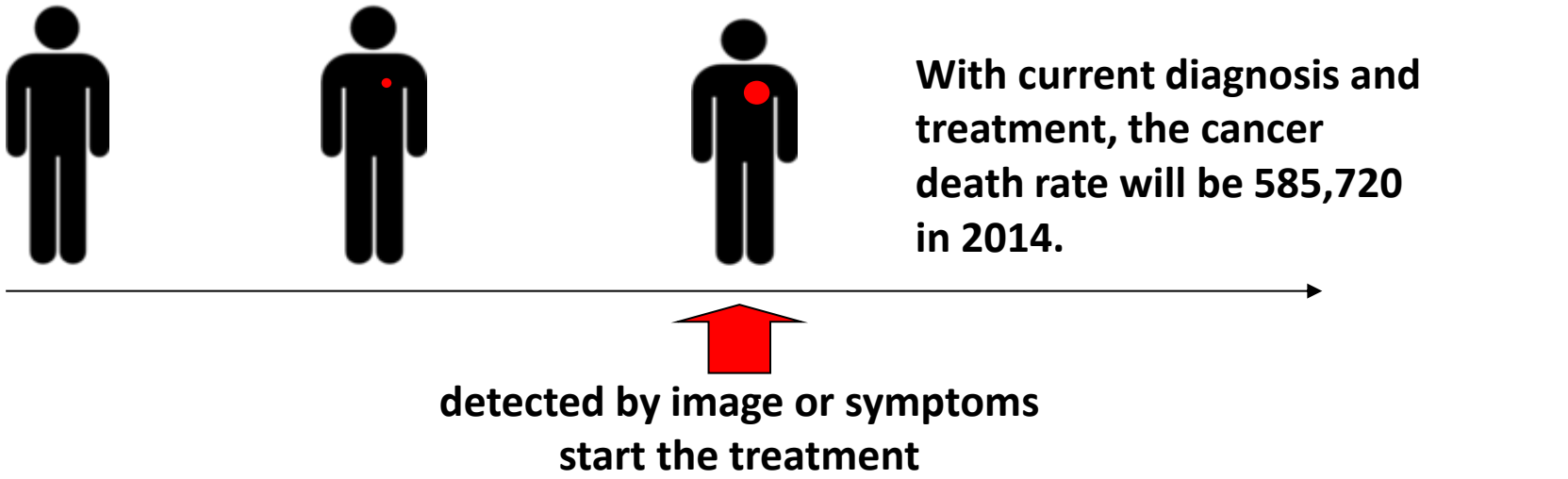
Transition from Post- to Pre-Symptomatic Medicine Requires System to Continuously Monitor Health of Well People

Specifications:

- Comprehensive
- Sensitive – Early Detection
- Simple
- Inexpensive
- Specificity – What is Wrong?

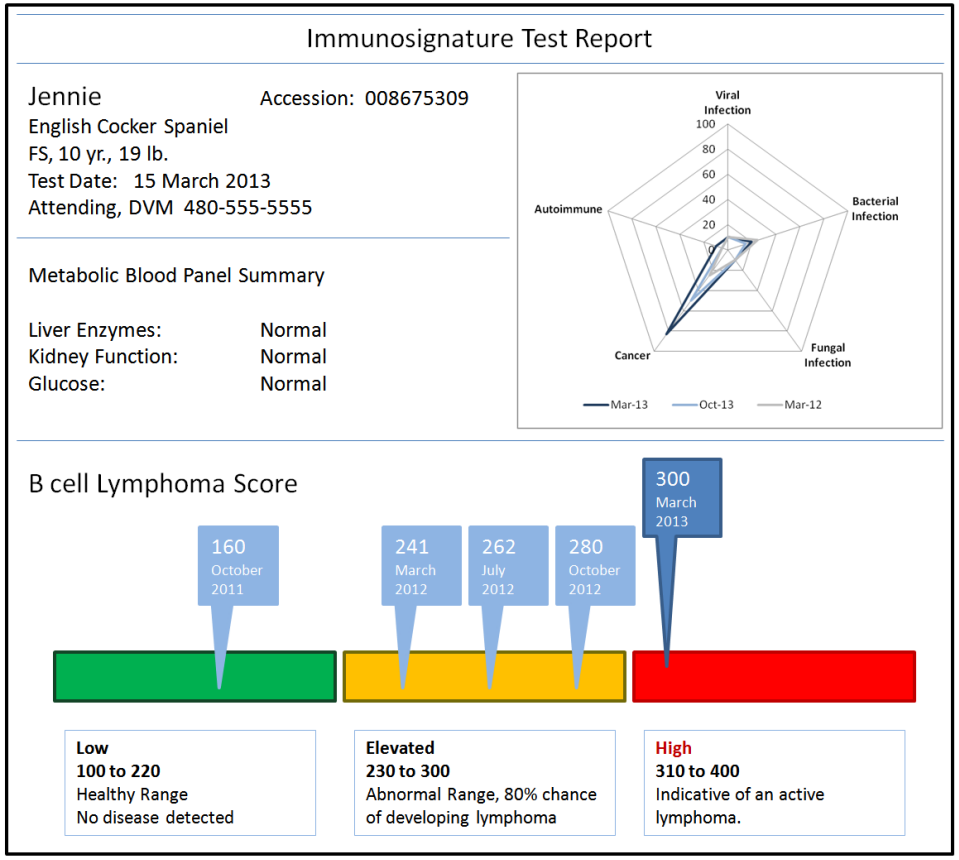

Population-Based
Comprehensive Health Monitoring
Central
Analysis
Direct Mail-in of Samples

Vision: *Eradicating Cancer by Immunosignature Monitoring of Health*



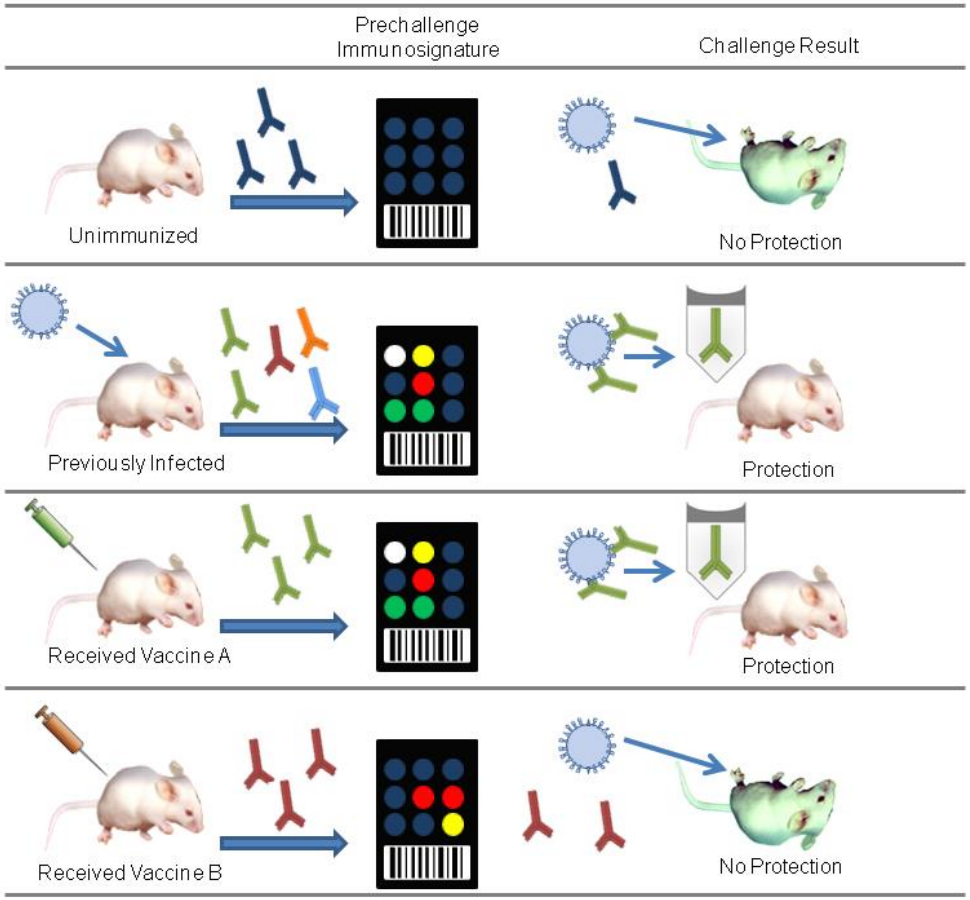
Calviri, LLC

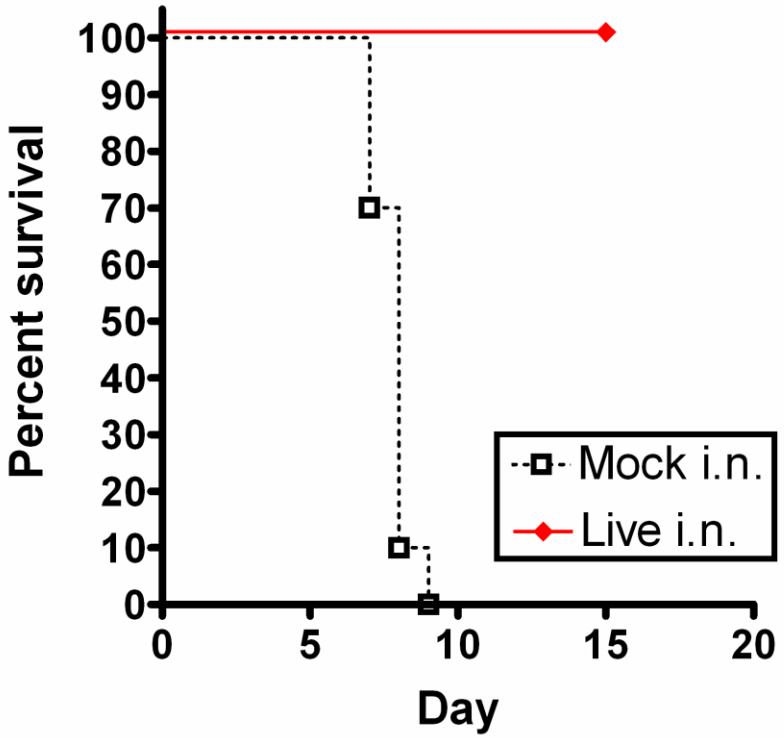
Goal: Complete Annual Wellness Test for Dogs



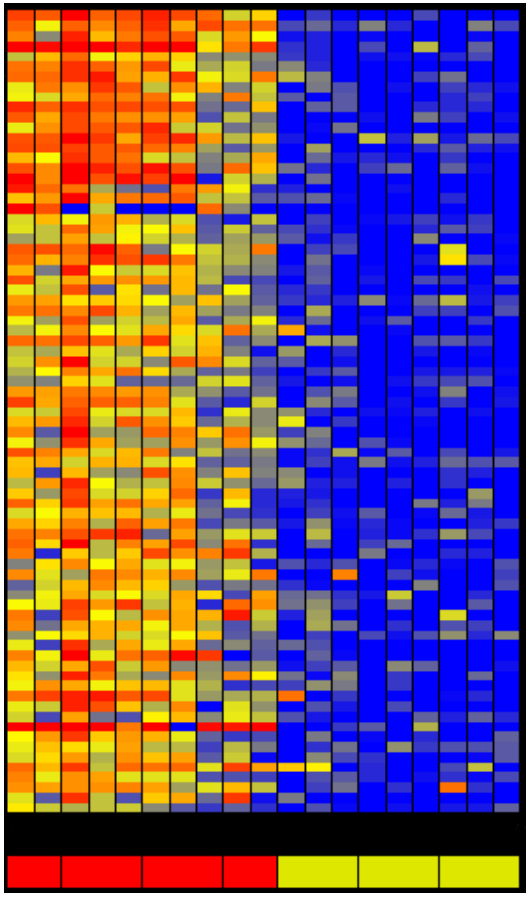
- A comprehensive assessment of health
- Indicative of Health Status
- Derived from a single, simple to use, low cost test

Which Vaccine is Protective?

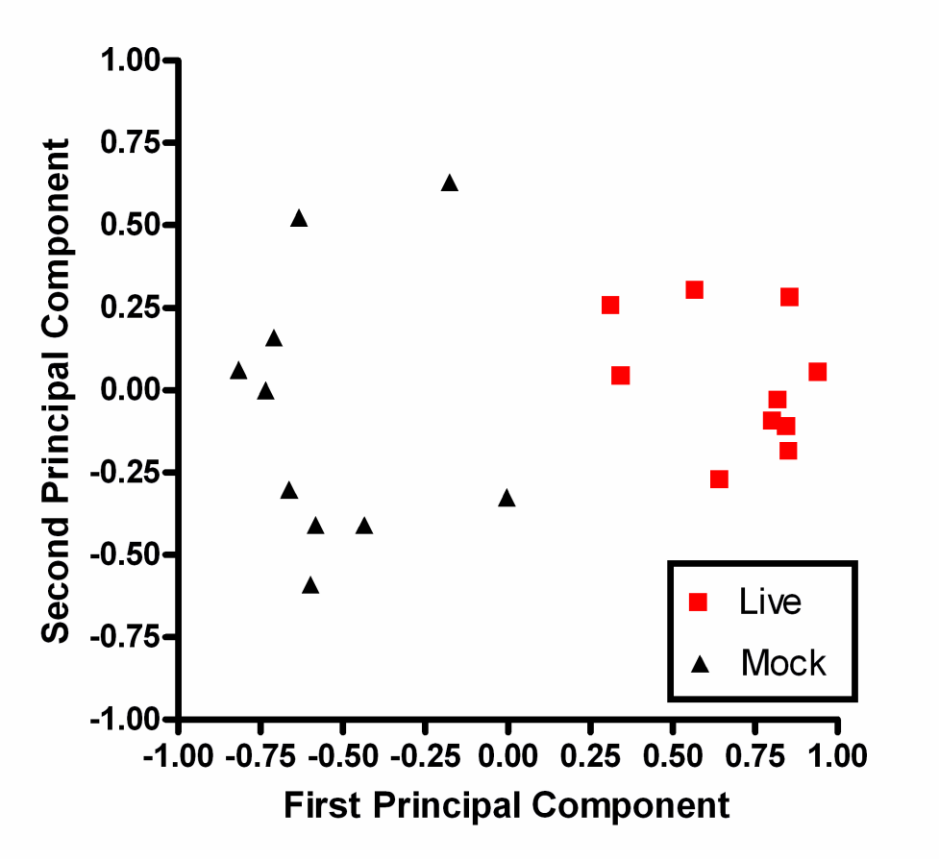




The Immunosignature Distinguishes Infected from Mock Infected Mice

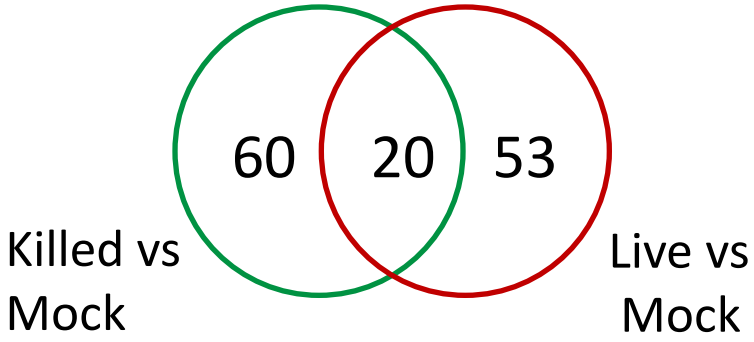


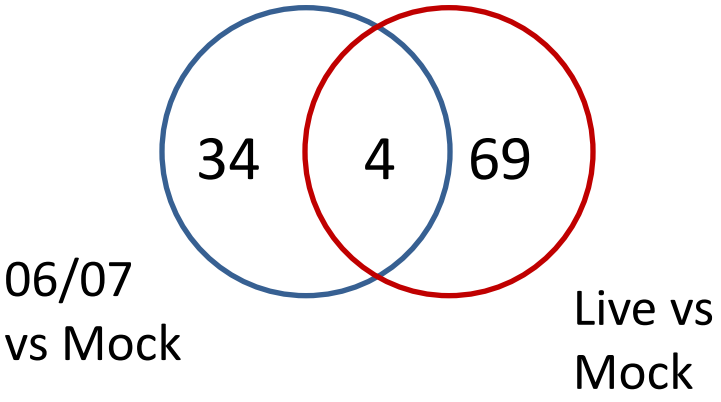
Live PR8 Mock (PBS)

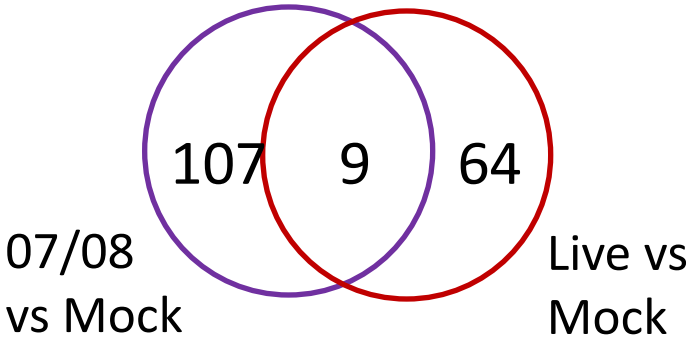


73 Peptides Selected Using expression profile mapping.

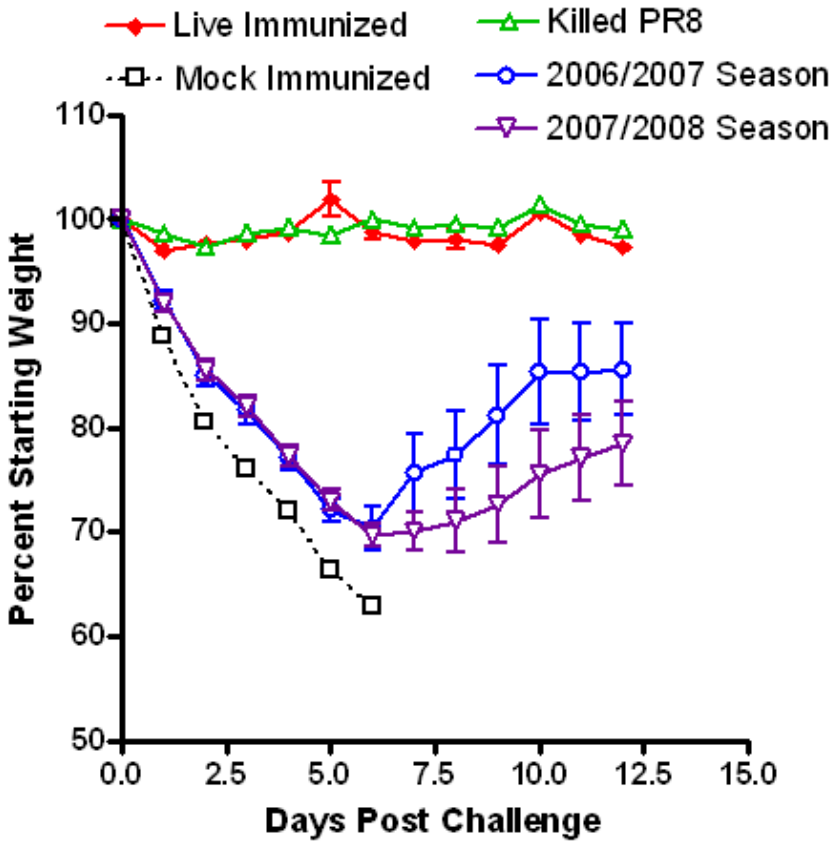
Killed PR8 Predicted as More Effective Than Seasonal Trivalent Vaccines

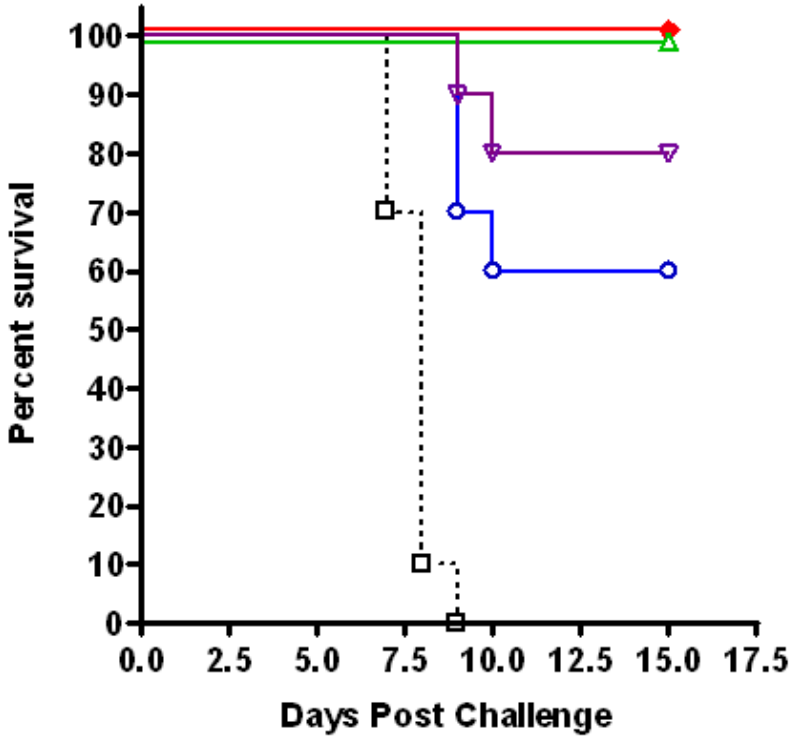






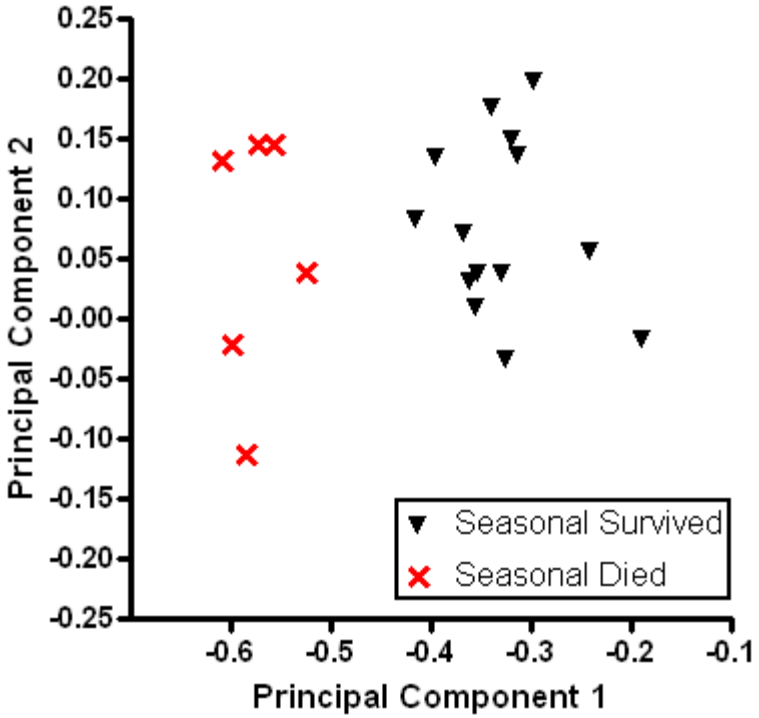
Challenge Results

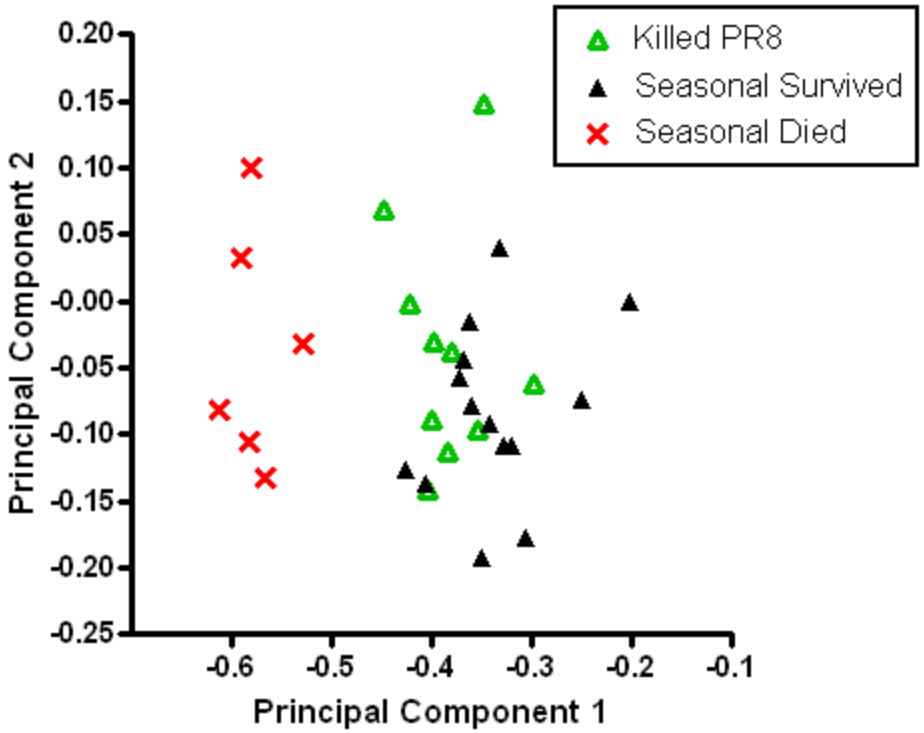




Legutki and Johnston, 2013, PNAS (Embargoed In Press)

Immunosignature of Seasonal TIV Survivors Groups Survivors with The Killed PR8 Immunized

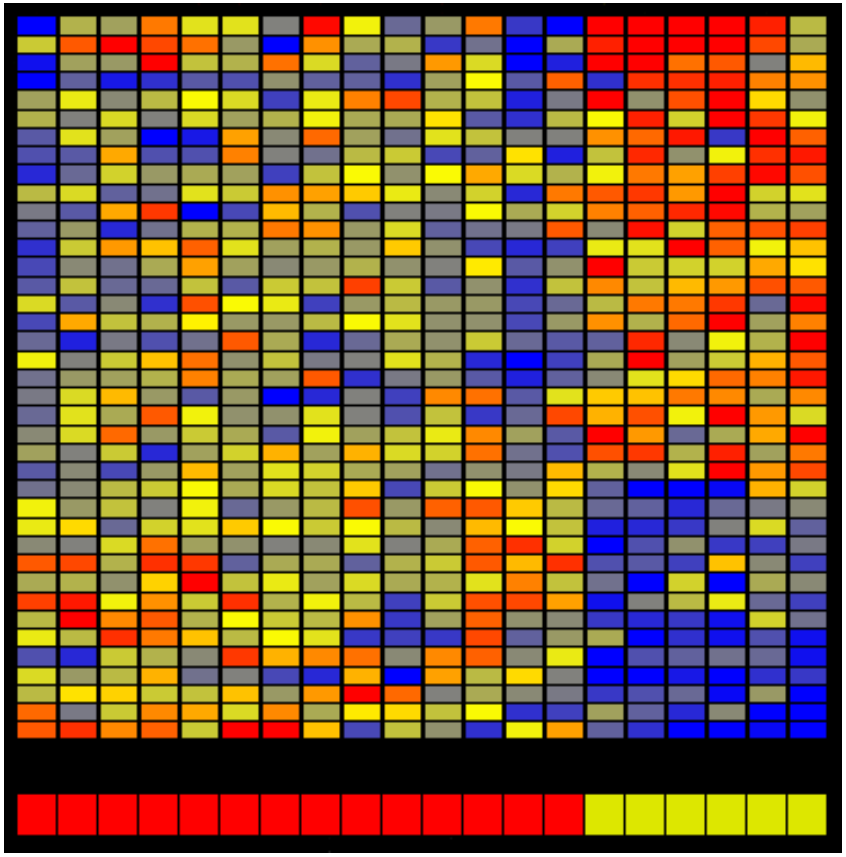




38 Peptides Selected using a T test with $p < 0.05$
Benjamani and Hochberg MTC and >1.3x Fold Change

Legutki and Johnston, 2013, PNAS (Embargoed In Press)

TIV Recipients Who Died Lack Specific Reactivity

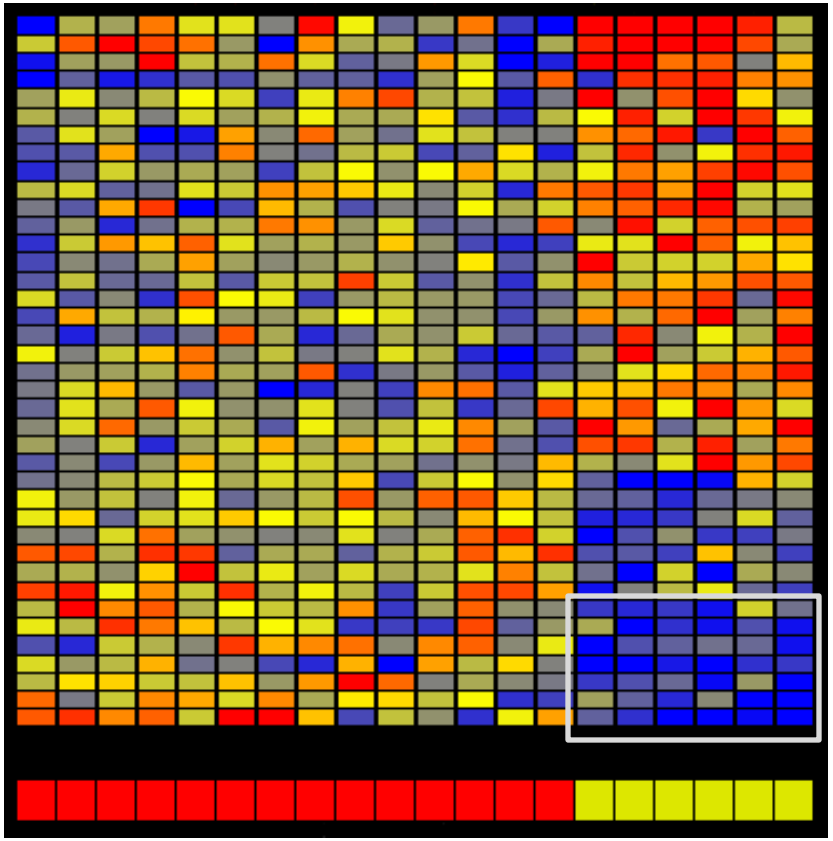


Survived Died

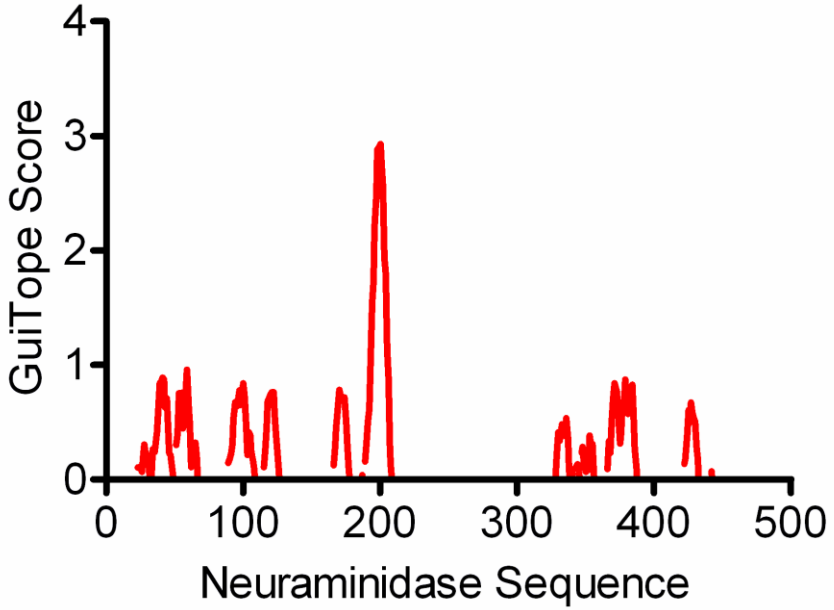
Whole PR8 Virus ELISA in 2006-2007
TIV Recipients

Legutki and Johnston, 2013, PNAS (Embargoed In Press)

Missing Reactivity Aligns to NA195-219



Survived Died



Legutki, JB & Johnston, SA: PNAS (Embargoed In Press)
GuiTope: Halperin, R. 2012. BMC Bioinformatics 13:1

Summary

- **Immunosignature Technology is a Universal Diagnostic Platform**
- **It is Simple, Sensitive and Potentially Inexpensive**
- **It Also Can Be Employed as a Discovery Tool**

Russian-American Collaboration

ASU BIODESIGN INSTITUTE
ARIZONA STATE UNIVERSITY

Российско-Американский ПротивоРаковый Центр
РАПРЦ
RAACC
Russian-American Anti-Cancer Center

Altai State University

HealthTell™

Professor Andrei Chapoval, Director

Acknowledgements

- Innovations In Medicine
  - Neal Woodbury, co-director
  - Chris Diehnelt
  - John Lainson
  - Zbigniew Cichacz
  - Phillip Stafford
  - Zhan-Gong Zhao
  - Donnie Shepard
  - Bart Legutki
  - Andrey Loskutov
  - Penny Gwynne
  - Loren Howell
  - Douglas Daniel
  - Rebecca Halperin
  - Lucas Restrepo
  - Luhui Shen
  - Hu Duan
  - Debra Hansen
  - Pattie Madjidi
- HealthTell, Inc.
  - Bill Colston
  - Kathryn Sykes
  - David Smith
  - Fabrication Team
- NextVal, Inc.
  - Matthew Greving
- Complex Adaptive Systems Initiative
  - George Poste
- Other Collaborators
  - John Galgiani, U. of Arizona
  - Hoda Anton-Culver, UC Irvine
  - Sam Hanash, Fred Hutchinson Cancer Center
  - Adi Gazdar, UT Southwestern
  - Adrienne Scheck, Mayo Clinic
  - Dawn E. Jaroszewki, Mayo Clinic

$Funding: The Biodesign Institute at ASU
HealthTell, Inc.
DTRA, NSF, DARPA, ARO