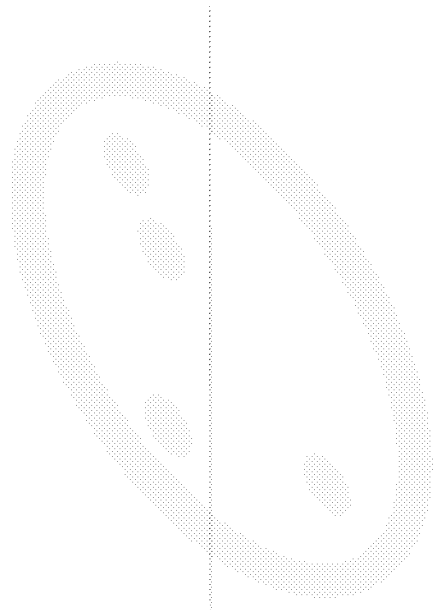
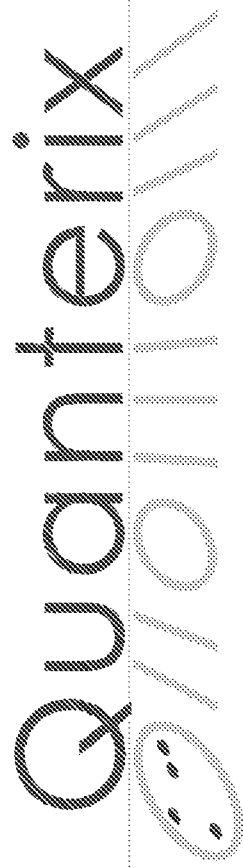




Single Molecule Arrays for Digital Detection in Complex Samples

IQT Technology Focus Day, March 25th, 2010

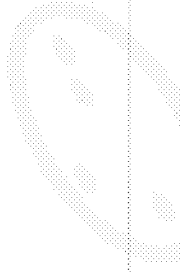
Quanterix Team

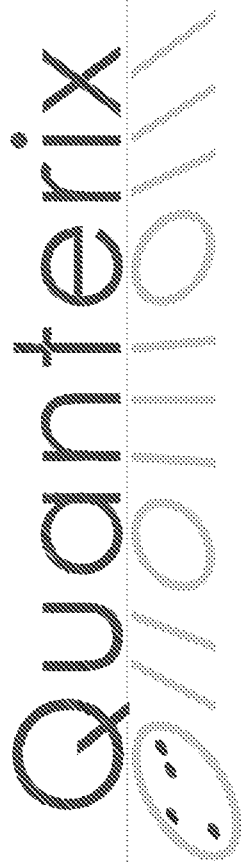
Management

- David Okrongly, PhD - President and CEO (25 years diagnostics and biotech experience with Siemens, Bayer, Applied ImmuneSciences)
- David Duffy, PhD - Senior Director, Platform Research (Surface Logix, Gamera, Harvard)
- David Hanlon, PhD - Director, Strategic Marketing (Cytoc, Proteome, Oncogene Research)
- David Fournier, MS - Principal Engineer (Illumina, Cyvera, Gerber Systems, AGFA)
- David Wilson, PhD - Senior Director, Product Development (20 years with Abbott Diagnostics)
- High performance R&D team consisting of 12 Biochemists and Engineers

Board of Directors

- Nick Naclerio, PhD - Chairman (ParAllele, Motorola Life Sciences, True Materials)
- David Walt, PhD (Tufts, Illumina)
- David Okrongly, PhD (President and CEO)
- Keith Crandell (ARCH Venture Partners)
- James Nahirny (Bain Capital Ventures)
- Doug Cole, MD (Flagship Ventures)

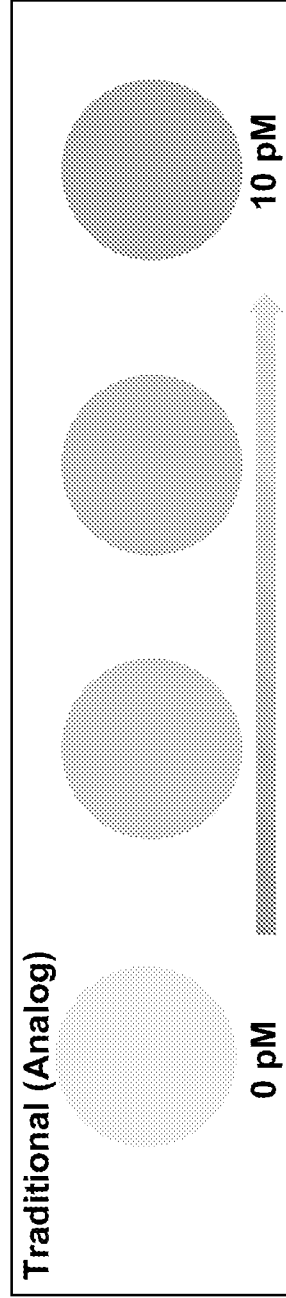




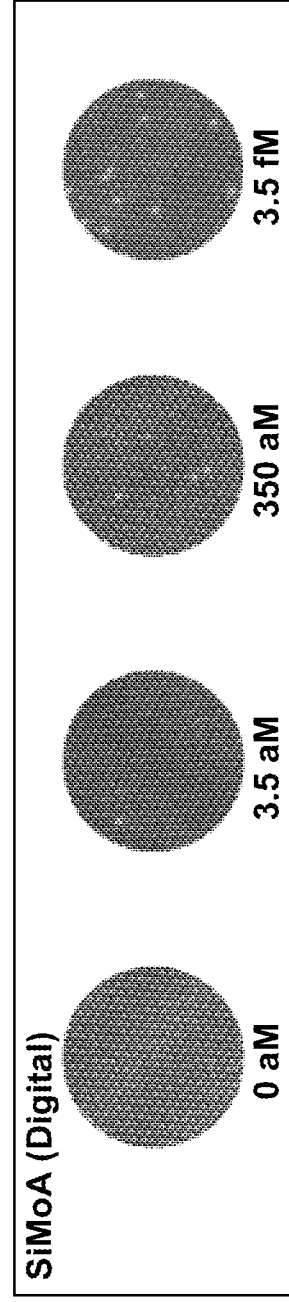
Quanterix Detection Technology: Single Molecule Array (SiMoA™)

Exclusively licensed from Tufts University and covered by 12 US
patents/applications and corresponding international filings.

SiMoA moves chemical and biological measurements from the analog world to the digital world



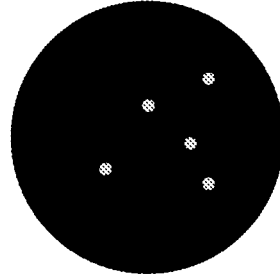
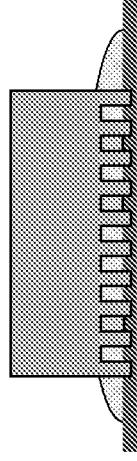
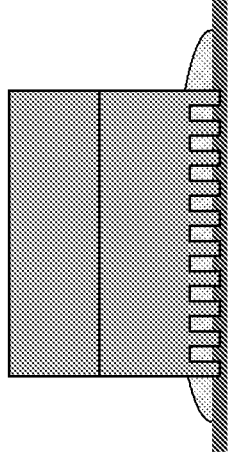
- Concentration is determined from an average signal intensity of an ensemble of molecules
- Product diluted into large volume ($>100 \mu\text{L}$)
- Sensitivity limited by high background signals



- Concentration is determined by counting discrete binary events
- Single molecules are confined in $50 \times 10^{-15} \text{ L}$ (50 femtoliters)
- Background noise below threshold does not contribute to signal

SiMoA is based on a simple three-step process

1. A sample is partitioned into an array of 50,000–500,000 femtoliter wells, so that each well contains 0 or 1 molecule
2. Catalytic chemistry generates bright optical signal in each reactor that contains at least one target molecule
3. Count the number of femtoliter wells that generate a positive signal to determine concentration



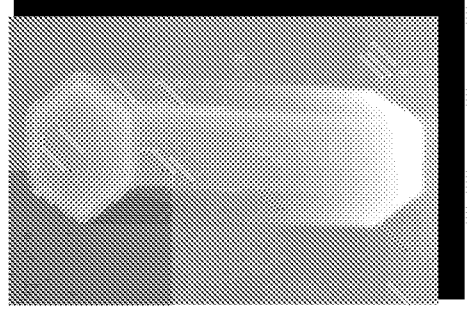
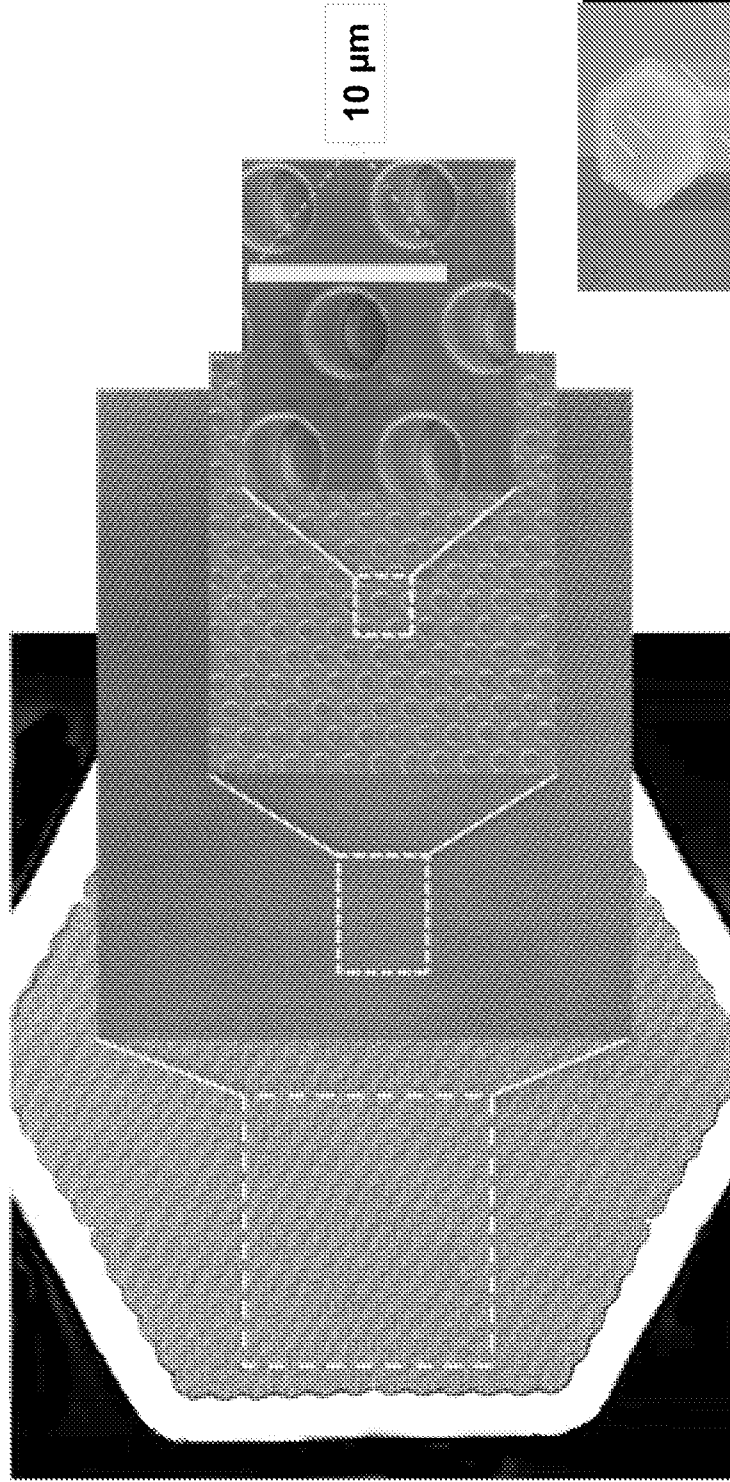
Li Z, Hayman RB, and Walt DR. *J. Am. Chem. Soc.* **2008**, 130, 12622-63
Rissin DM, Gorris HH, and Walt DR. *J. Am. Chem. Soc.* **2008**, 130, 5349-53
Rissin DM and Walt DR. *J. Am. Chem. Soc.* **2006**, 128, 6286-87
Rissin DM and Walt DR. *Nano Lett.* **2006**, 6, 520-23

Distinguishing Features of the SiMoA Approach

- Transforms chemical and biological measurements from analog to digital worlds
- Strong basis in theory that shows $>10^7$ improvement in sensitivity is possible over analog techniques
- Many single molecules are detected simultaneously, rather than serially
- Inherently scaleable and flexible detection approach; larger arrays allow switching between high multiplexing and large dynamic range
- Many analytes possible (proteins, nucleic acids, cells, small molecules)
- Technology uses low-cost excitation sources and detectors in contrast to most complex, instrumentation-intensive single molecule approaches; readily amenable to miniaturization
- Strong IP (licensed from Tufts University and developed at Quanterix) based on Poisson Statistics approach



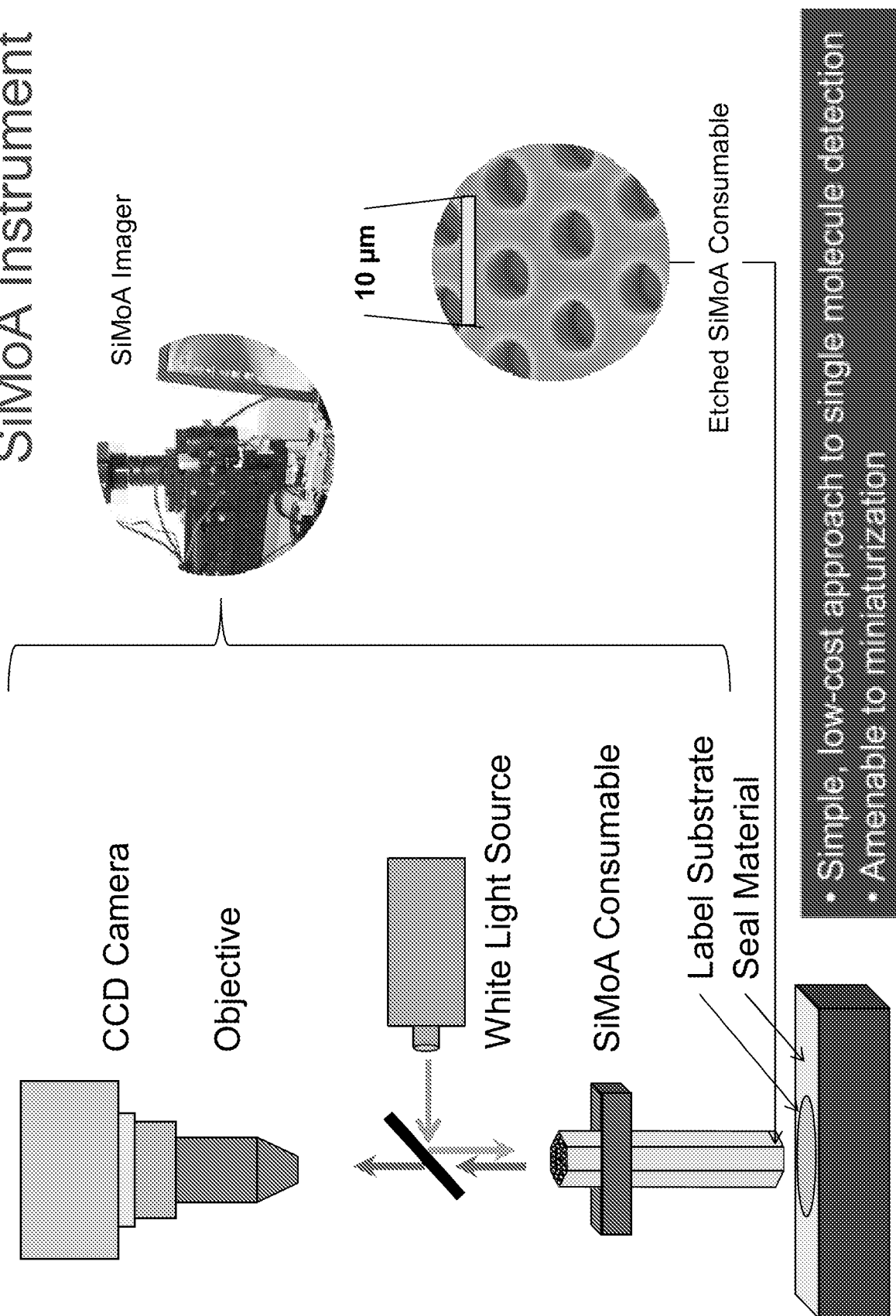
SiMoA Device



- 49,777 fibers per bundle
- Each fiber well with a defined (44 fL) volume
- Single sample loaded onto each fiber bundle

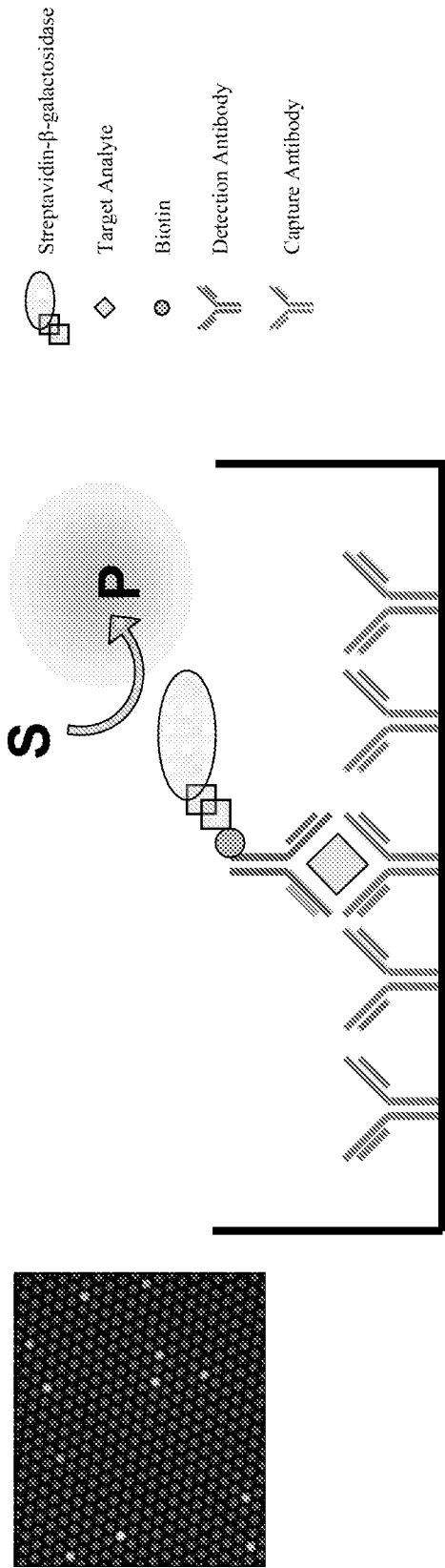
Quanterix
© 2010 Quanterix

SiMoA Instrument

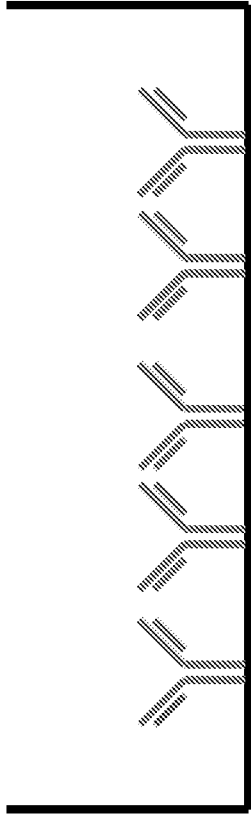


Quanterix

SiMoA enables digital ELISA detection of proteins using
conventional immunoassay reagents



Fiber surface of “ON” well (<10% of wells with analyte)



Fiber surface of “OFF” well (>90% of wells with no analyte)



Theory of Single Molecule Immunoassays

$$K_d = \frac{[Ab][L]}{[AbL]} \Rightarrow \frac{[L]}{[AbL]} = \frac{N_A V K_d}{\theta_{Ab} n_{wells}} \quad (1)$$

$$[L] + [AbL] = [L_{TOTAL}] \quad (2)$$

$$n_{AbL} = \frac{[L_{TOTAL}] V N_A}{\left(1 + \frac{N_A V K_d}{\theta_{Ab} n_{wells}}\right)} \quad (3)$$

Concentration of target, [L _{TOTAL}]	n _{AbL} for V = 100 μL	n _{AbL} for V = 10 μL	
1 pM	All	All	Typical ELISA limit of detection 
100 fM	All	All	
10 fM	86,000	38,000	
1 fM	8,600	3,800	Potential SiMoA limit of detection
100 aM	860	380	
10 aM	86	38	
1 aM	9	4	
100 zM	1	none	

- Thermodynamically, SiMoA offers tremendous sensitivity to analytes
- Techniques developed to improve kinetics to realize this potential

SiMoA Provides >1000-fold increase in Sensitivity Advantage to Enzyme Label

Analog Technology

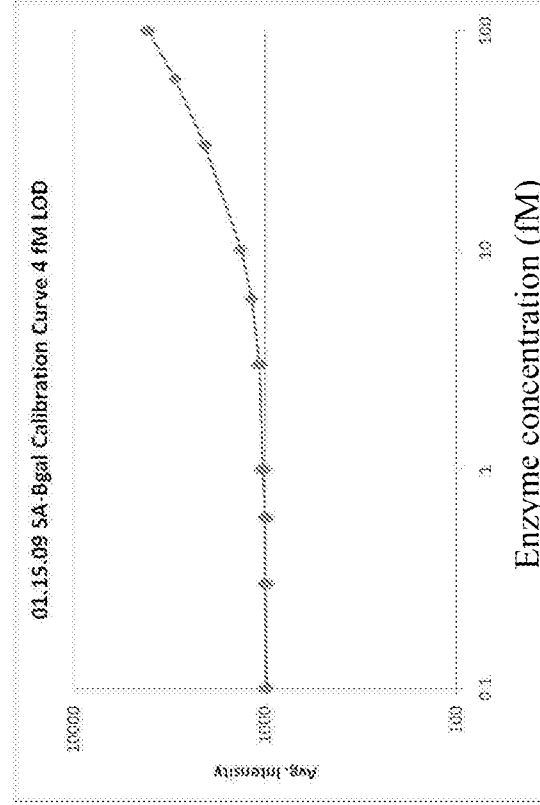
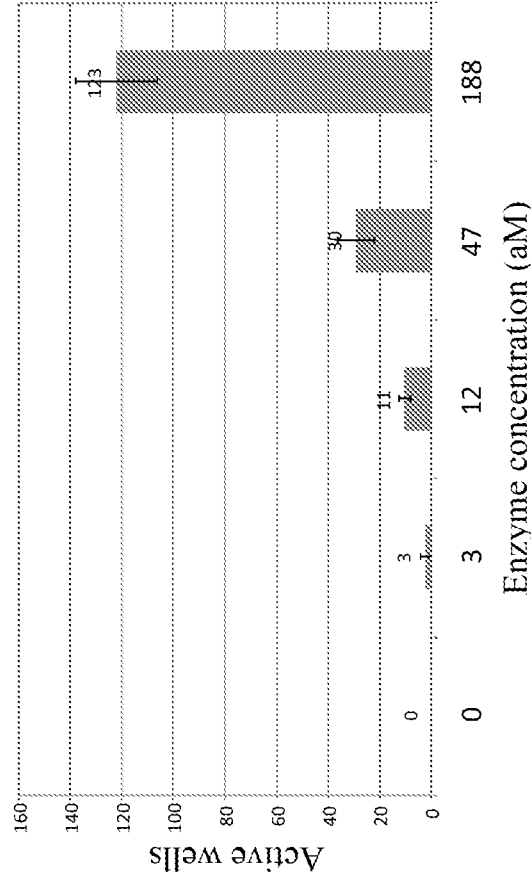


Plate reader Limit of Detection (LOD):

4 fM = 4000 aM

Digital, SiMoA Technology



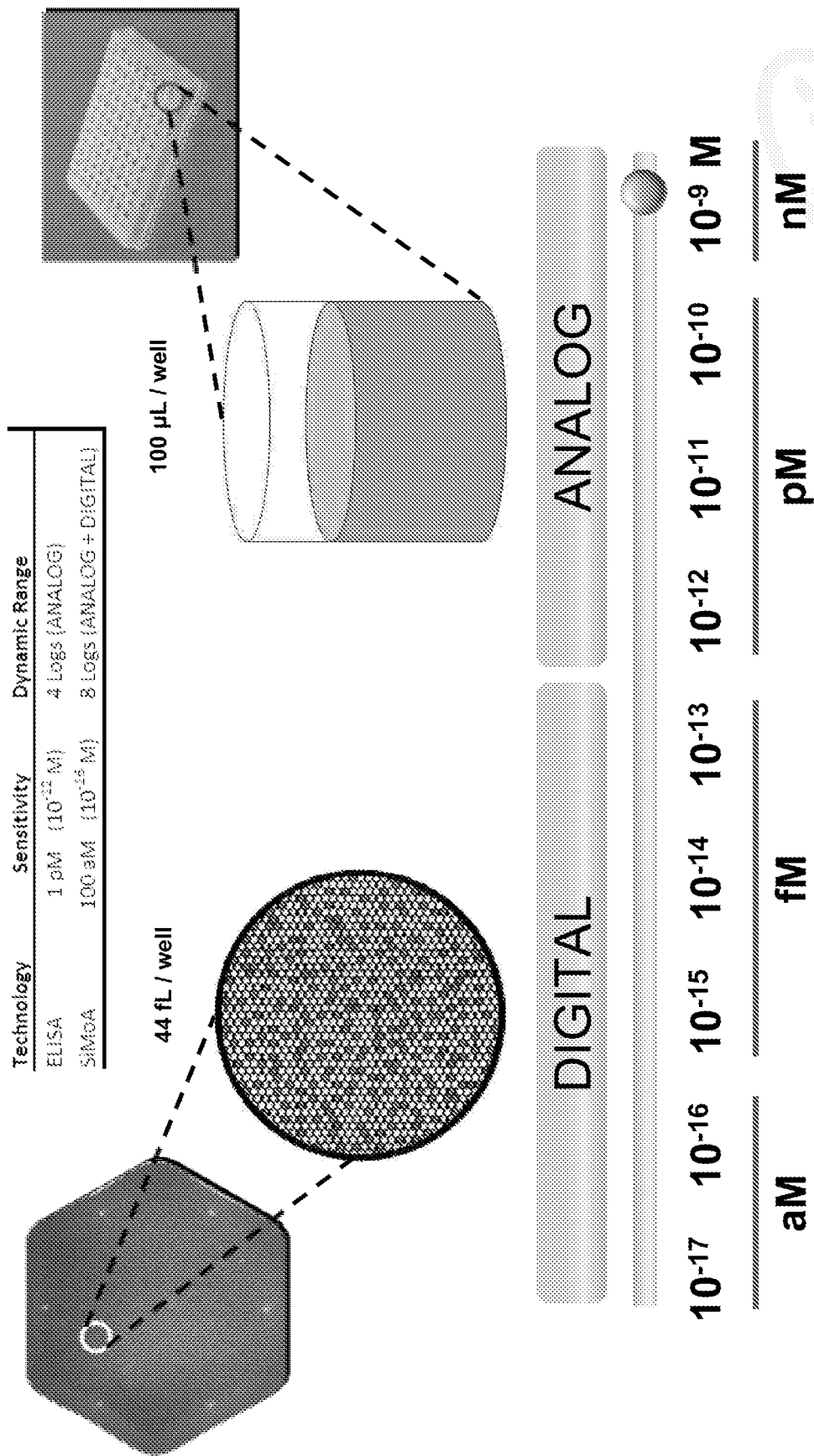
SiMoA Limit of Detection (LOD):

3 aM

- >1000× absolute sensitivity improvement
- High enzyme sensitivity allows lower concentrations of labeling molecules to be used in ELISA, lowering backgrounds, and improving limits of detection

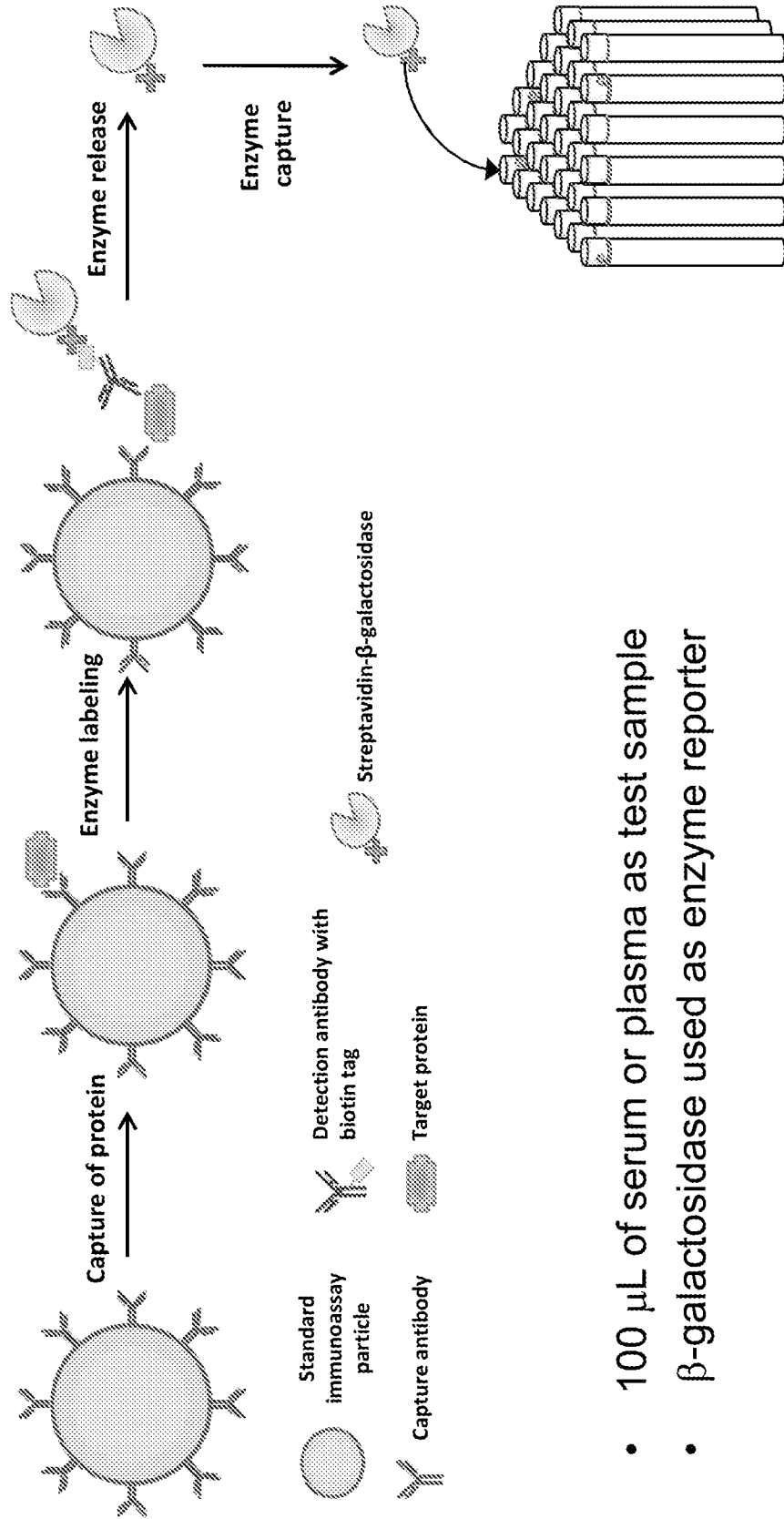
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SiMoA Technology has attomolar (aM) Sensitivity and 8 Logs of Dynamic Range



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Q77011010

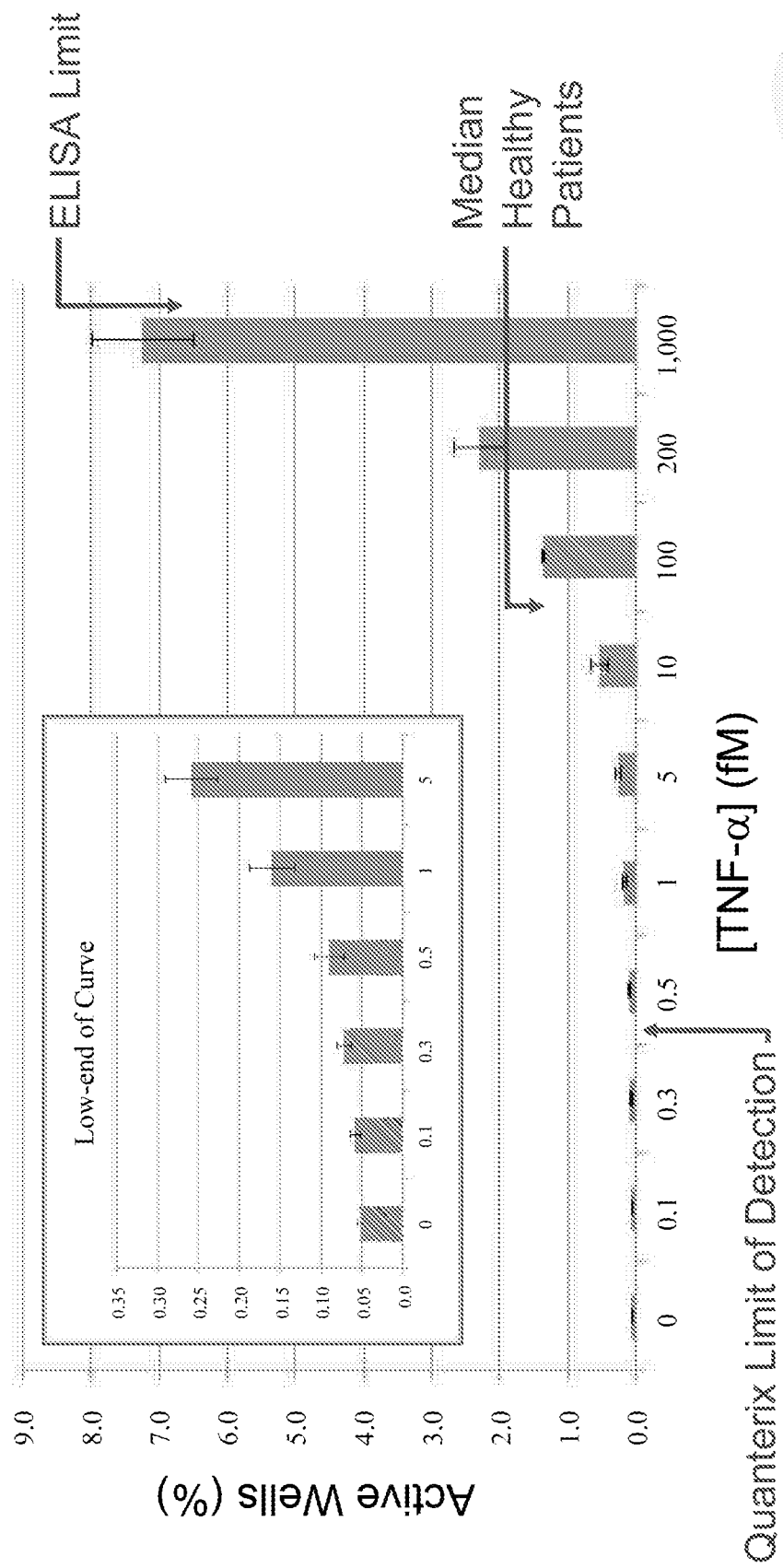
Bead-based SiMoA Immunoassay was developed to improve kinetics



- 100 μ L of serum or plasma as test sample
- β -galactosidase used as enzyme reporter

Digital ELISA is highly sensitive to proteins

Detection of TNF- α using SiMoA

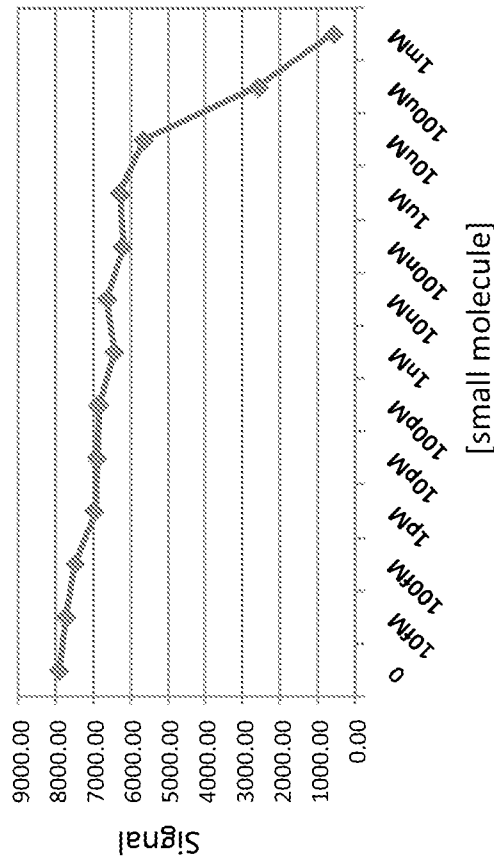
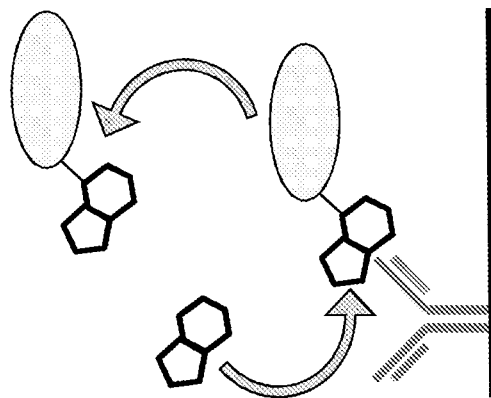


Comparison of Digital ELISA and ELISA

Protein	SiMoA LOD (fM)	ELISA LOD (fM)	Sensitivity Increase
TNF- α	0.20	1250	6250x
IL-6	0.20	400	2000x
VEGF	0.50	500	1000x
PSA	0.10	100	1000x

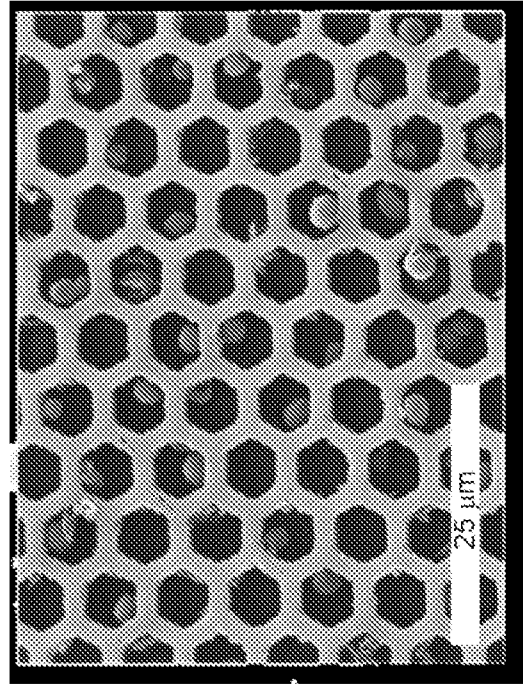
- Limit of Detection determined using SiMoA and plate reader detection using same antibody pair, enzyme label, and diluted serum matrix
- Proof-of-principle demonstrated 1000–6250 x improvement in sensitivity for digital ELISA over standard ELISA

SiMoA Detection of Small Molecules

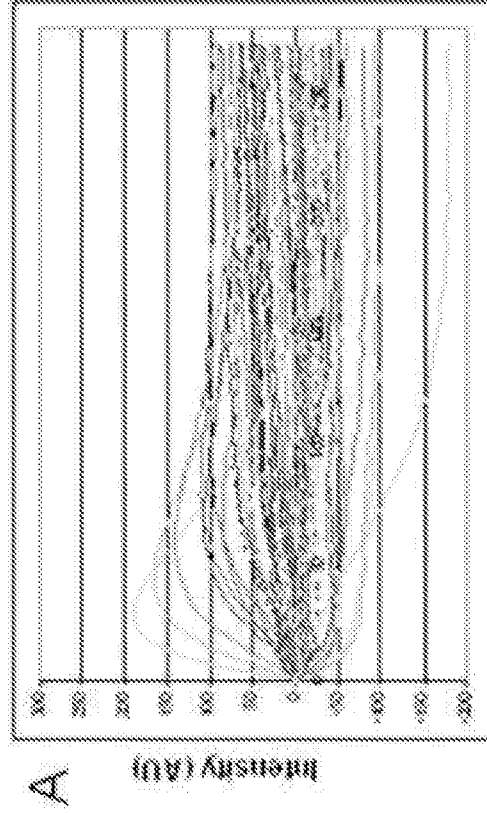


- Proof-of-principle assay for measuring small molecules (e.g., chemical weapons, explosives, hormones) using SiMoA

SiMoA can also be used for single cell assays

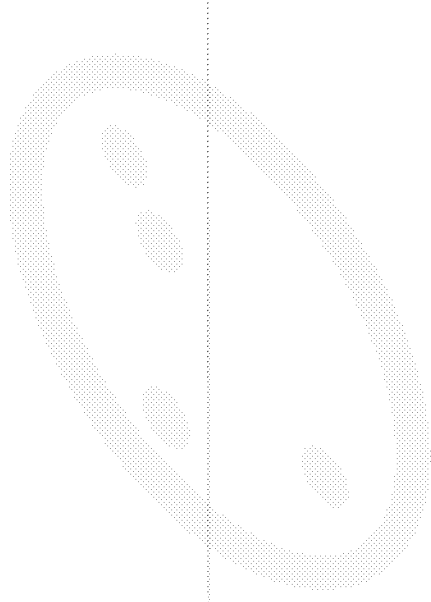
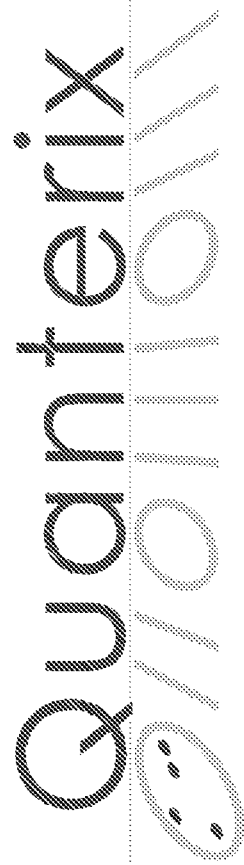


SEM of single *S. cerevisiae* cells distributed in the wells of an etched fiber bundle (Walt et al. *Anal. Chem.* **2002**, 74, 3046)



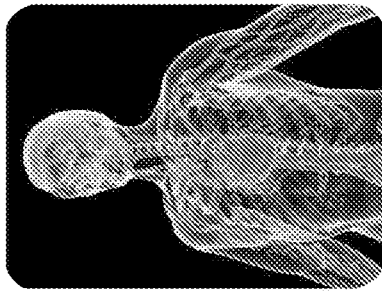
Ca²⁺ flux traces from single CHO cells expressing CCR5 stimulated with Mip1 β (Walt et al. *Anal. Chem.* **2007**, 79, 9045-9053)

- Array length-scales and detection well suited to single cell assays
- Several single cell assays possible
 - RNA and DNA detection (Walt/Quanterix SRA)
 - Calcium flux assays (Walt et al. *Anal. Chem.* **2007**, 79, 9045-53)
 - Yeast Two Hybrid screening (Walt et al. *Anal. Biochem.* **2007**, 360, 63–74)
 - Migration assays (Walt et al. *Anal. Bioanal. Chem.* **2005**, 382, 37–43)



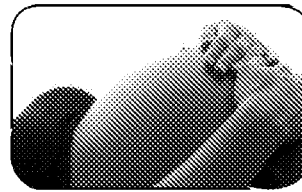
Current Applications of SiMoA

Many Opportunities Exist for High Sensitivity Diagnostics Quanterix Will Focus on Two Applications for the Quanterix Clinical Lab

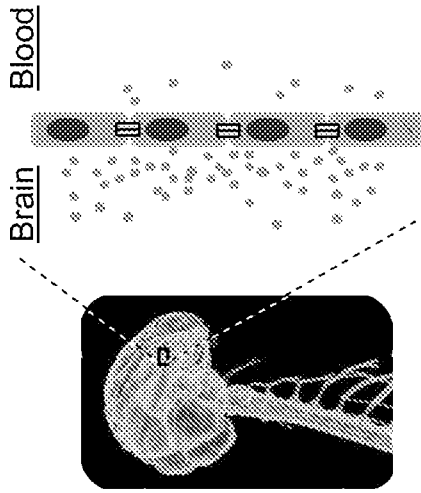


Cardiovascular Disease

- Early MI detection / rule-out
- Risk stratification
- Stress test reduction



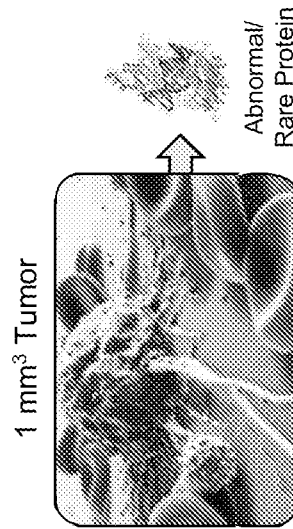
Prenatal Screening



Neurological Disease

Alzheimer's Disease

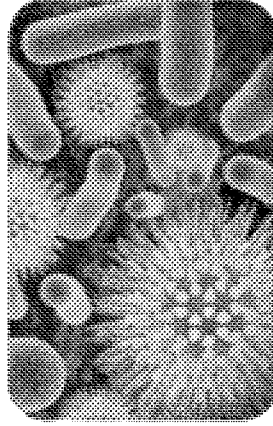
Endothelial Cells



1 mm³ Tumor

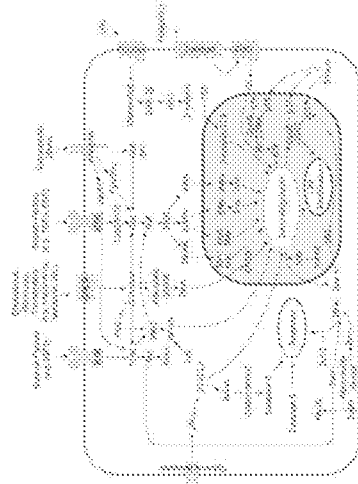
Oncology

Prostate Cancer Recurrence
 Monitoring



Infectious Disease

- Blood products screening
- Antigen based Dx



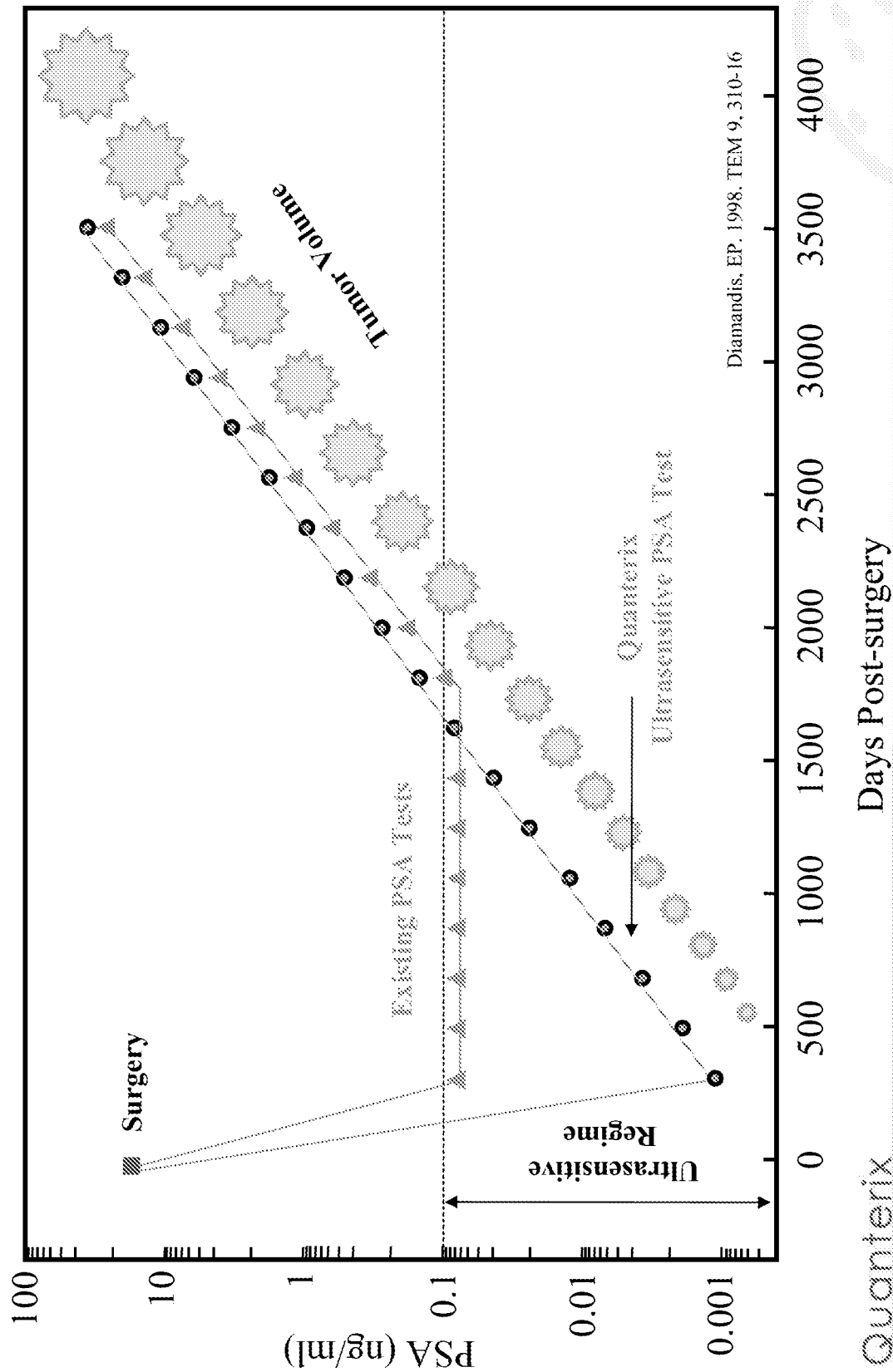
Inflammatory Disease

- Theranostics

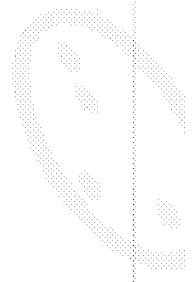
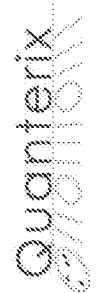
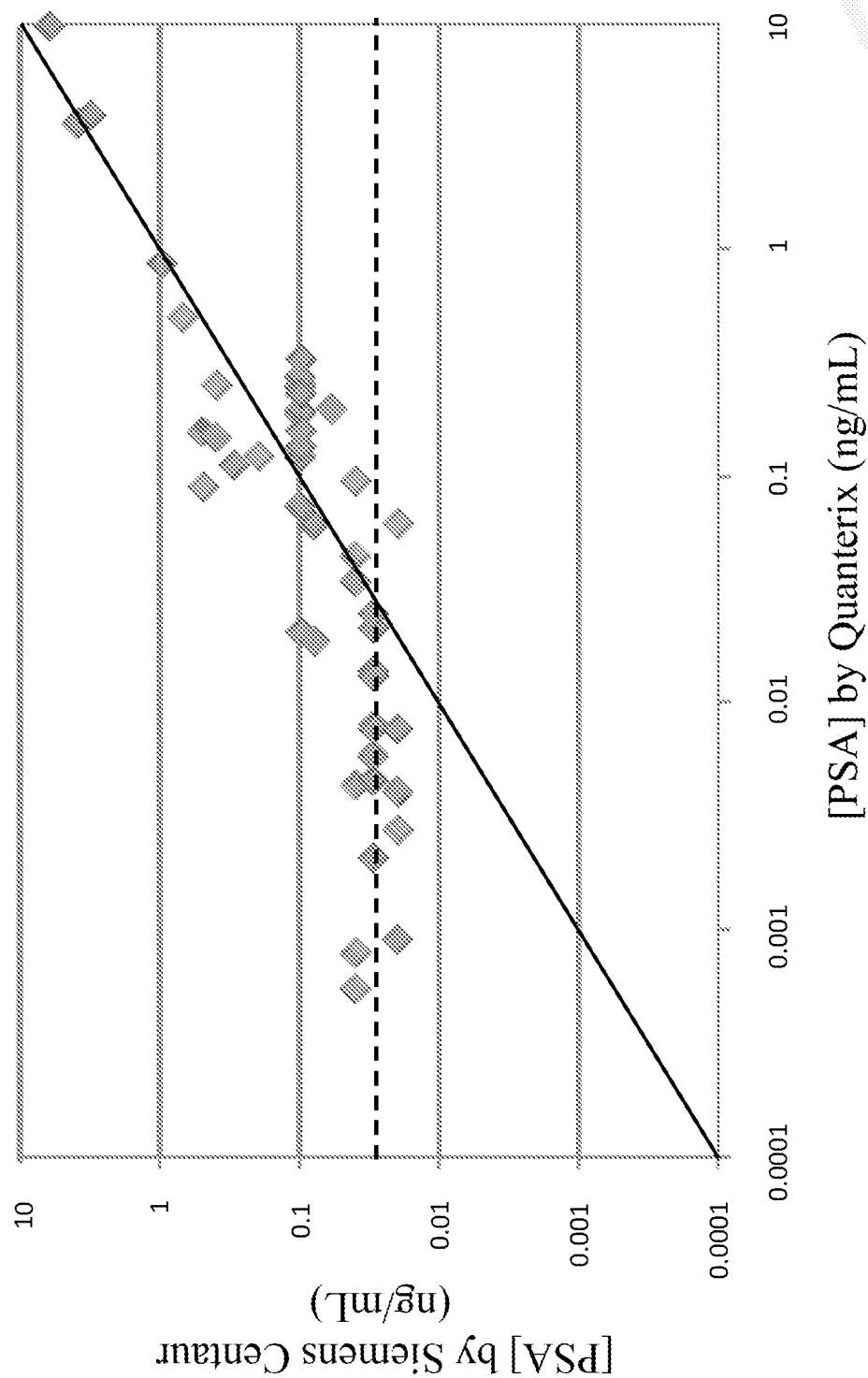
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Prostate Cancer Surveillance

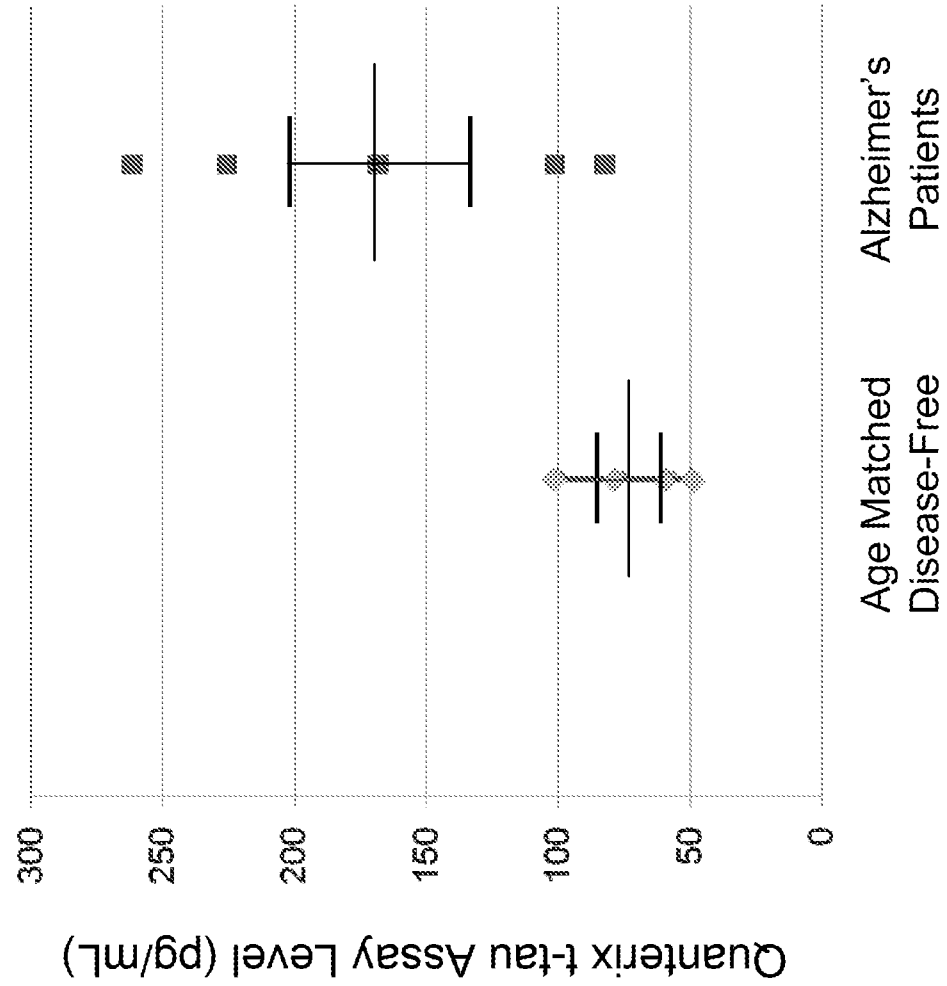
Example of Post-Operative Patient Following Radical Prostatectomy

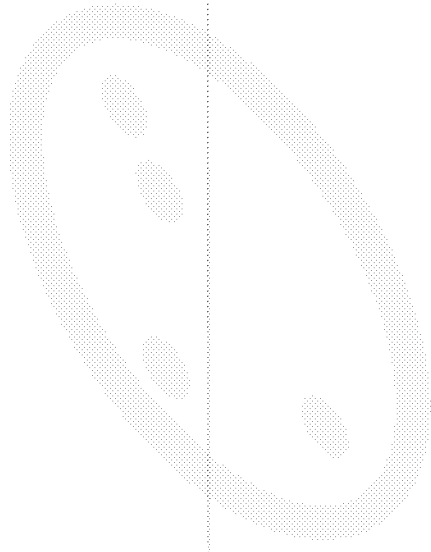
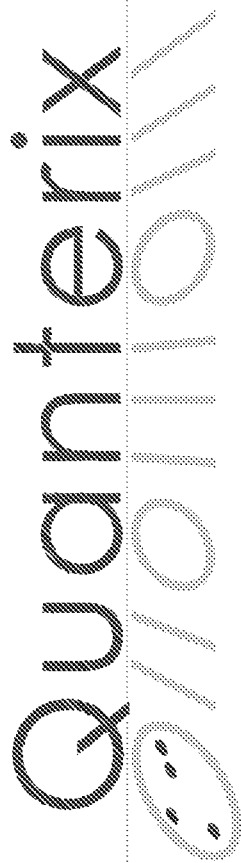


Quanterix's assay can detect PSA in serum at concentrations >1000-fold lower than standard



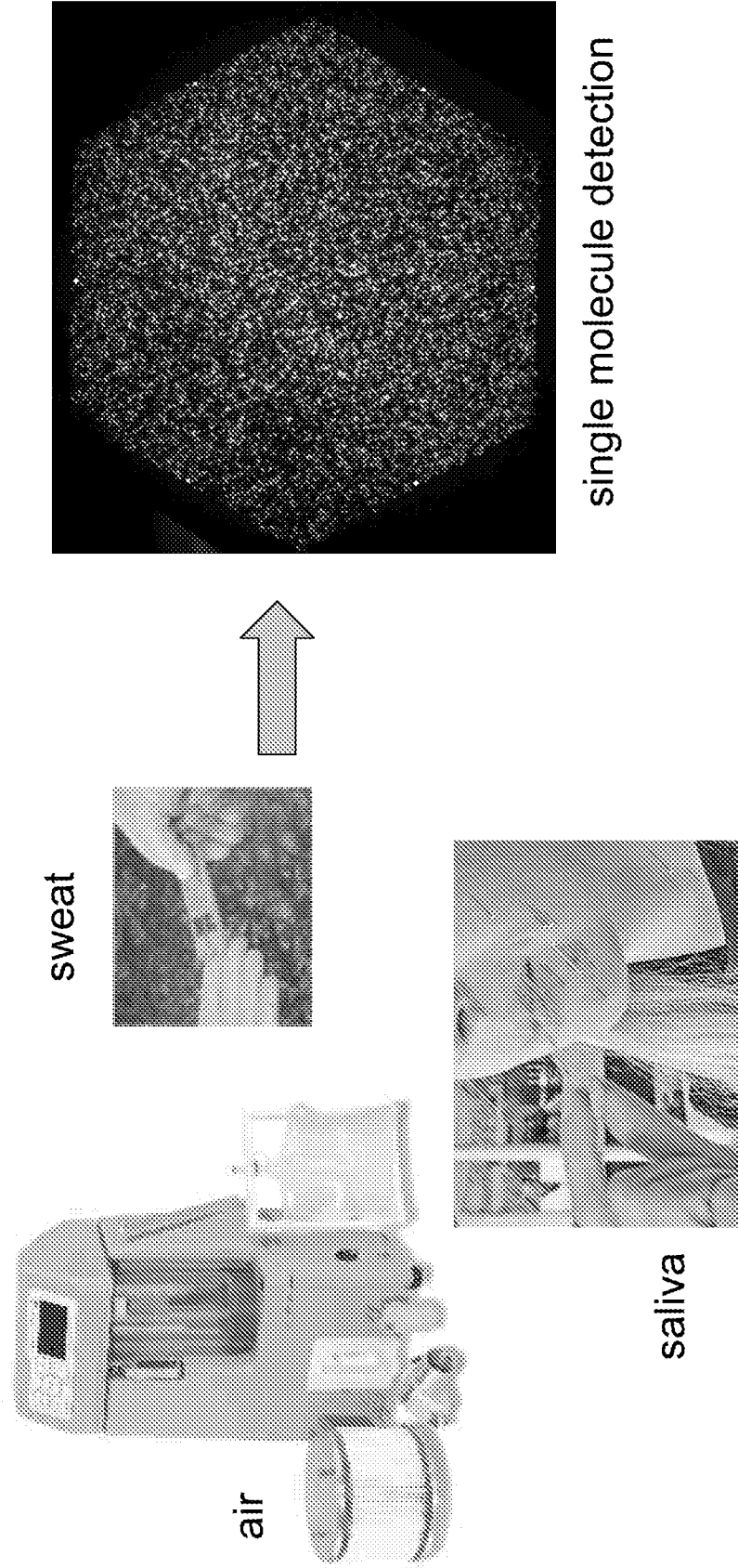
Quanterix t-tau Assay is the First to Measure t-tau Protein in Blood of AD Patients and Age-Matched Controls





Potential Applications for SiMoA in the Defense and Intelligence Communities

SiMoA would interface with sampling methods and allow detection of low protein concentrations



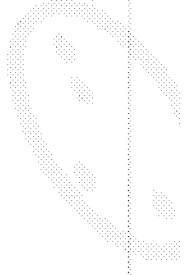
- Bridge gap between microsampling technologies and ultra-sensitive detection of proteins

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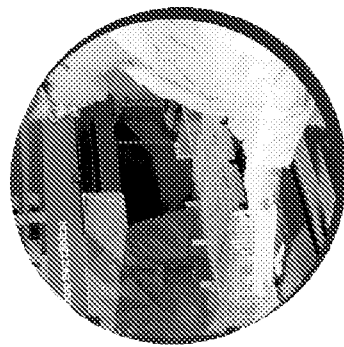
Ultra-Sensitive Detection of Pathogens and Biomarkers for Chemical Exposure

- Detection of nucleic acids (NA) using PCR is the cornerstone of sensitive detection of biological threats
 - Nucleic acids can be spoofed, leading to false positives
 - Not suitable for detecting non-NA analytes (e.g., proteins, small molecules)
 - Does not provide functional information of organism detected
- SiMoA could provide complementary information to PCR for ultra-sensitive detection
 - Pathogen detection via protein markers
 - Biomarkers indicating exposure to chemicals required to make explosives
 - Functional information as well as indicating presence/absence
 - Wide variety of analytes possible to be detected

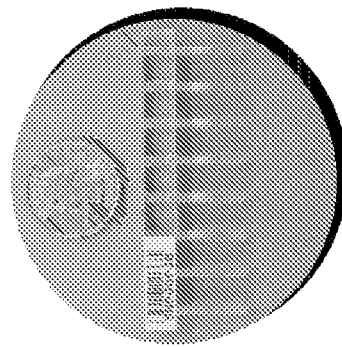


SiMoA is amenable to miniaturization and automation for a number of testing environments

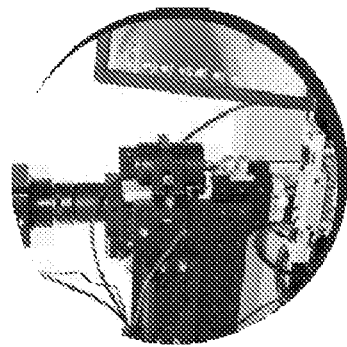
Today: Internal CLIA Lab



Semi-Automated
Sample Handling

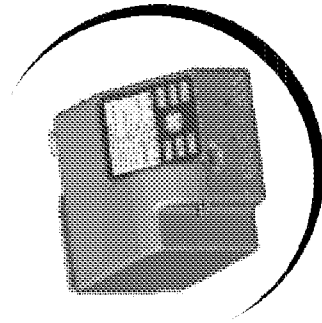


12 x 8 Glass
50K Array Format
Manual Loading



Semi-Automated Sealing
Automated Image Analysis

Future: External Laboratories & Field

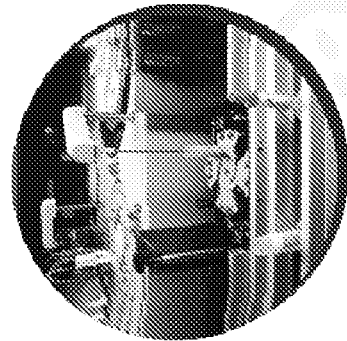


Automated Sealer / Imager
COGS <\$20K

In Development



Low Cost Plastic Consumable
500K Array



Leading to a
Fully Automated,
Track-Ready System

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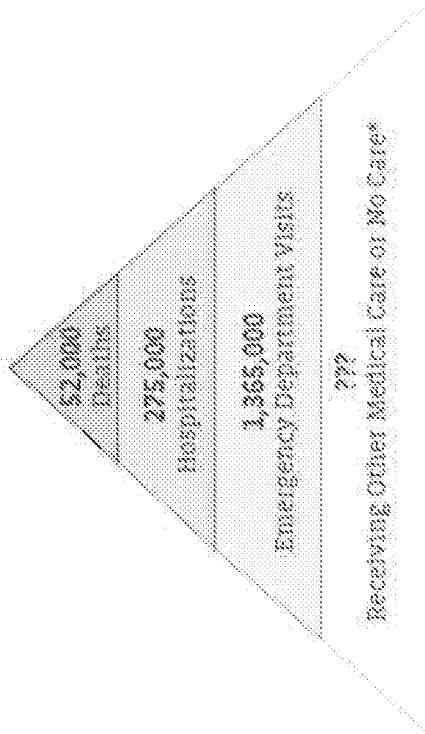
Concept for Hand-held Single Molecule Detection

- Portable SiMoA detection unit, ultra-low analyte concentrations
 - Self contained reagent cartridge
 - Capable of supplying multiple samples
 - Self contained reaction/detection cartridge
 - Serves as sample input location and disposal container
 - Small form factor instrumentation readout unit
- Ultra-low Analyte detection at the point of interest

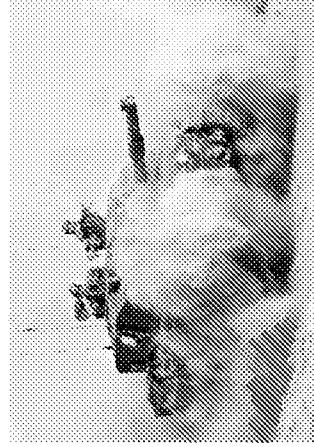
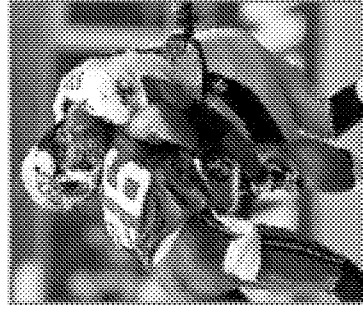
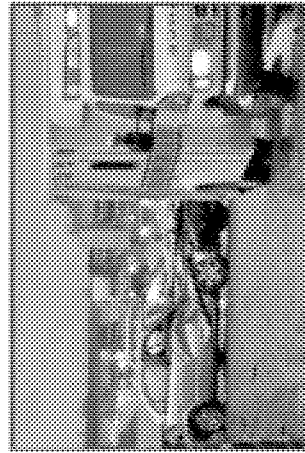


Traumatic Brain Injury (TBI)

- TBI severity ranges from “mild” (brief change in mental status/consciousness) to “severe” (extended period of unconsciousness or amnesia)
- TBI contributes to 30% of all injury-related deaths¹
- Direct and indirect costs exceed \$60B²



An estimated 1.7 M TBIs occur in the US annually



CTE; 19-fold increase in memory disorders

31% of evacuated US military personnel had TBI (DVBIC)

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¹ Faul M et al. Centers for Disease Control and Prevention, 2010
² Finkelstein E et al. Oxford University Press, 2006

A Rapid Blood-Based Test for TBI Would Have Significant Value

Ideal cost-effective diagnostic tools needed

- Replace imaging and improve accuracy

Levels of brain specific proteins altered in cerebrospinal fluid (CSF)

- S100B, GFAP, NSE, Tau
- Correlation with injury severity/outcome

CSF collection is invasive and exposes patients to additional risk

- Drainage influences intracranial pressure (ICP)

Alternative non-invasive sources for identification of low abundance neuronal specific proteins desired

- Detection of mild injury that cannot be observed with CT/MRI
- Predict secondary pathologies (e.g. elevated ICP, ischemia)
- Real-time assessment of neuroworsening neurologic injury

"You want a nice serum marker for TBI, you do it through Tau. That is where you may have a use for Tau because the blood brain barrier is damaged. Then you may be able to get some assessment of the degree of injury following a TBI by looking at Tau in the plasma."
Dr. Rudolph Tanzi, Massachusetts General Hospital

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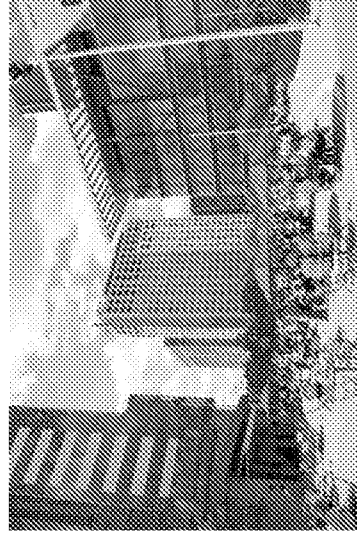
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11,000 sq. ft. laboratory/office space



Contact

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