OFFICE OF THE DIRECTOR OF NATIONAL INTELLIGENCE

BIO-INTELLIGENCE CHIPS (BIC)
Proposers' Day
Safe and Secure Operations

LEADING INTELLIGENCE INTEGRATION

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Deputy Office Director
15 November 2012

University of Maryland
Stamp Union
College Park, MD

Disclaimer

- This presentation is provided solely for information and planning purposes
- The Proposers' Day does not constitute a formal solicitation for proposals or proposal abstracts
- Nothing said at Proposers' Day changes the requirements set forth in a BAA
- BAA supersedes anything presented or said at the Proposers' Day by IARPA

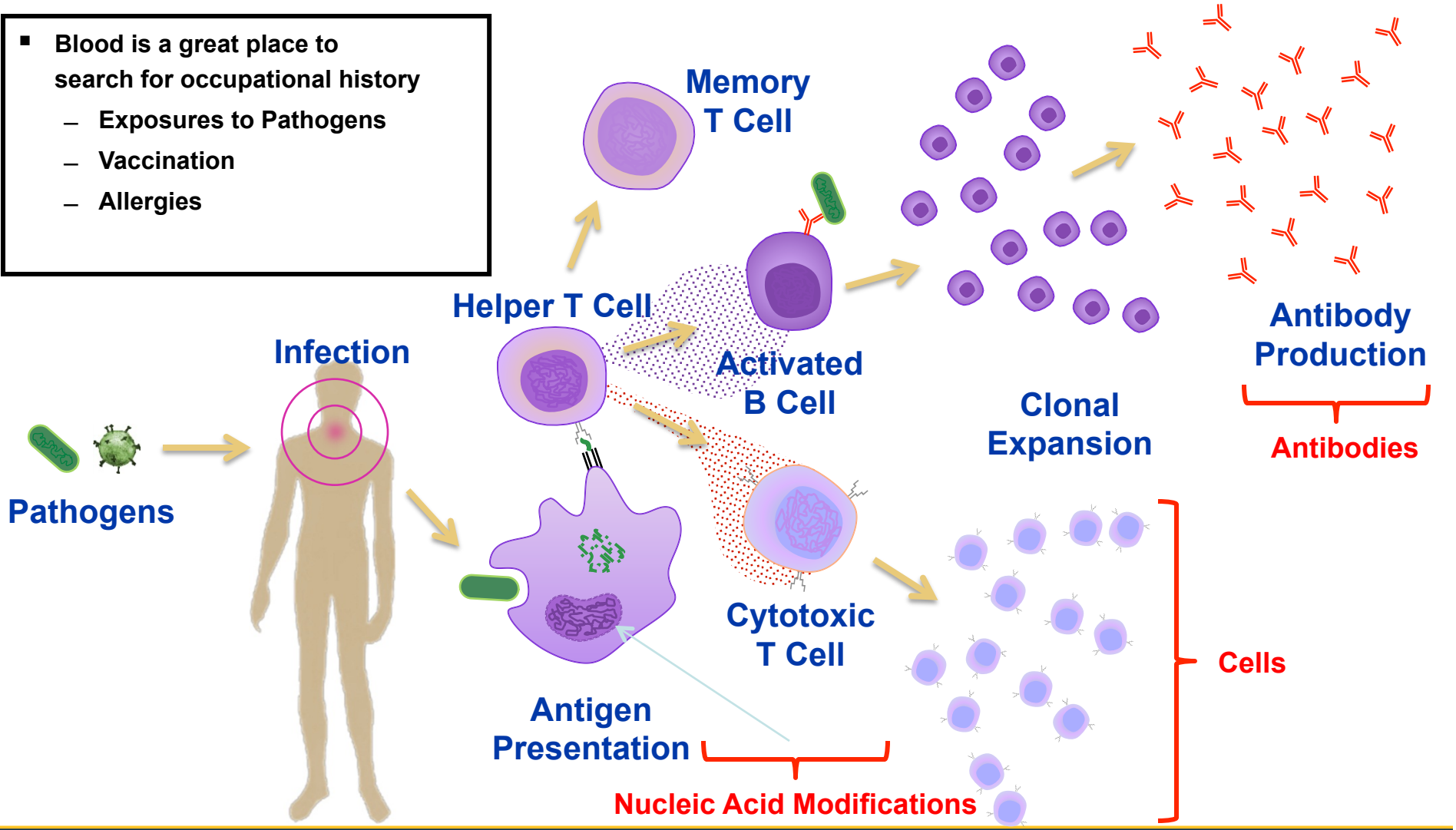
New Approach:
Detect Whether Someone Has Been Involved with Pathogens

- The human body is a great sensor which efficiently retains information associated with environmental exposure and often produces unique responses
  - BIC plans to determine whether a person has been involved with the handling or production of specific biological materials
- Existing techniques that do this are generally limited to detecting single markers, causing high false alarm rates
  - BIC will use multi-analyte processing to enable the cross-correlation of many biomarkers to create more authoritative signatures
- Current systems are bulky and operate only in a lab environment, not in the field
  - BIC will build upon advances in lab-on-a-chip technologies that can enable rapid, portable detection devices

The Human Body as a Sensor:
The Multi-analyte Approach

Human Signatures

- Blood is a great place to search for occupational history
  - Exposures to Pathogens
  - Vaccination
  - Allergies

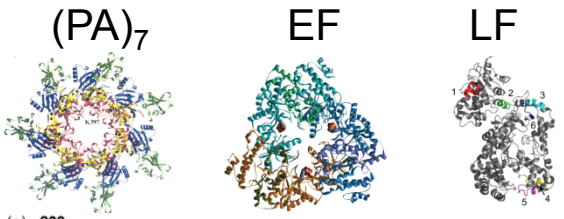


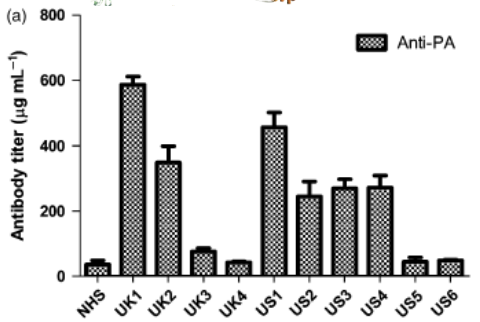
Example Bio-agents of Interest

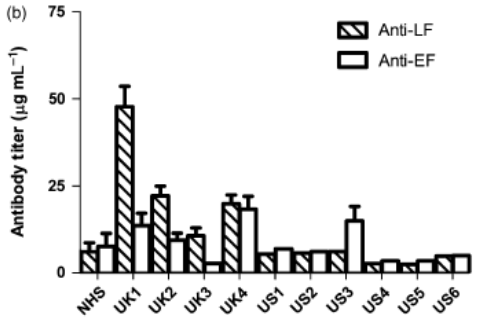
1) *Bacillus anthracis* (Anthrax)
2) Filoviruses: Ebola and Marburg
3) Engineered Pandemic Influenza (H5N1 or equivalent)
4) Ricin toxin from bean of *Ricinus communis*
5) *Burkholderia pseudomallei* (melioidosis) and *mallei* (glanders)
6) Botulinum Toxin
7) *Variola major* (Smallpox)
8) *Francisella tularensis*
9) Staphylococcal enterotoxin (SEB)
10) *Yersinia pestis* (Plague)
11) *Coxiella burnetii* (Q Fever)

Example Vaccination Signature: Differentiating *B. anthracis* Vaccination from Natural Infection

- Three proteins compose the toxins involved in anthrax pathogenesis:
  - Protective Antigen (PA), Edema Factor (EF) & Lethal Factor (LF)
  - Immune system responds by secreting toxin-specific antibodies (Ab)
- **Hypothesis**: Relative concentrations of Ab generated in response to toxin proteins (e.g., LF, EF, PA) can discriminate naturally infected from vaccinated individuals
- **Observations**
  - Natural Infections: $[Ab_{LF}] > [Ab_{PA}]$
  - Vaccinated Subjects from US and UK: $[Ab_{LF}] < [Ab_{PA}]$
- **Limitations:**
  - Small sample size (n=17 patients, 6 controls, 10 vaccinated)
  - Response variation across subjects was high (Brenneman 2011)
  - Findings are limited to the present vaccine formulation. Changes in future vaccine formulation may alter Ab ratios
  - Querying for the presence/absence of pathway-specific enzyme (e.g. bacterial transketolase) will likely improve confidence of result (Walz 2007)







Brenneman, K.E. FEMS Immun & Med Microbiol, 2011. 62(2): p. 164-172.

Environmental Example 1: Allergies to Latex Proteins

- Study on medical gloves finds positive correlation between 4 latex allergen proteins (Hev b 1, 3, 5, 6.02) & IgE from diluted sera, n=6 (Palosuo 2007, 1998)
- Based on 12 different brands of gloves, ratio of IgE to IgG correlated with latex allergy severity, n=20 + 5 ctrls (Chen 1996)
- Microarray-based component-resolved allergy diagnostics differentiated patients with genuine allergy from sensitization, n=42 + 20 ctrls (Ebo 2010)
- Limitations:
  - Serology tests are not absolutely clinically predictive (Ebo 2012); known cross-reactivity with food allergies (Ebo 2003)
  - Potential large population of potential false alarms (e.g. healthcare worker), so the existence of the allergy is only part of the equation

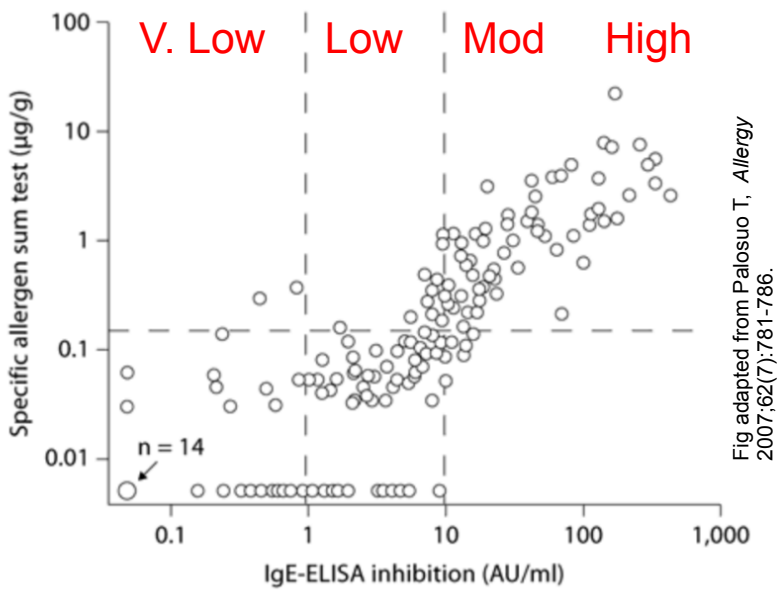


Fig adapted from Palosuo T, *Allergy* 2007;62(7):781-786.

IgE = Immunoglobulin E ; AU = Allergen unit
ELISA = Enzyme-linked Immunosorbent Assay

The horizontal dotted line marks 0.15 µg/g limit in the four-allergen sum test that delineates Low from Mod-High

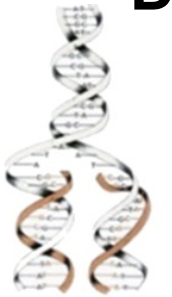
Limit of Detection
Hevea brasiliensis (Hev b) 5, 6.02 = 0.3 µg/g
Hev b 1,3 = 0.05 µg/g

Allergen-specific IgE and IgG are promising biomarkers for latex allergies

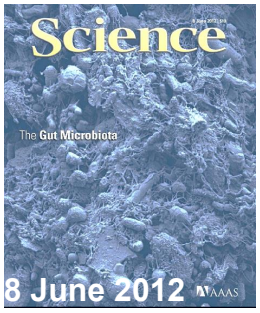
Environmental Example 2: Allergies to Laboratory Animals

- Several studies reported that >25% of laboratory workers exposed to research animals develop symptoms of allergy (Aoyama 1992, Wood 2001, Bush 2003). Mus m1 (19 kD) and albumin are known allergens from mice.

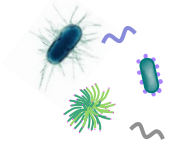
Discovering new markers of interest: *Omni-omics*



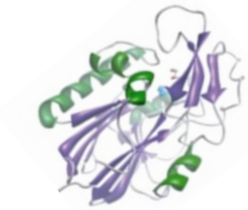
Genomics



Microbiomics

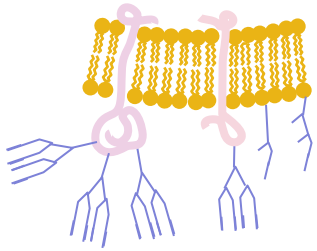


Lipidomics



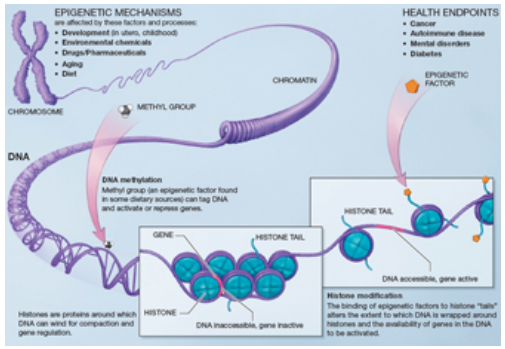
Proteomics

Immunomics



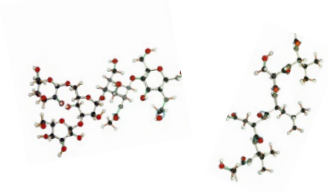
Glycomics

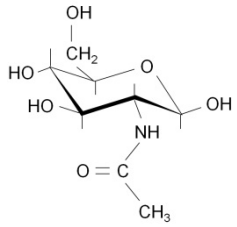
Epigenomics



Transcriptomics

Metabolomics

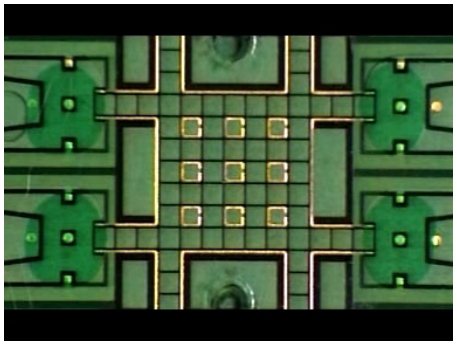




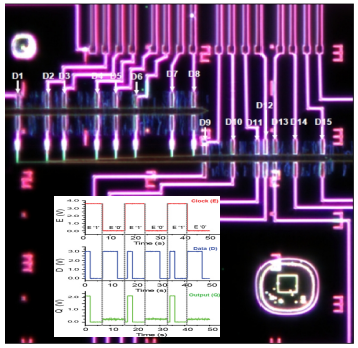
Advancements in Lab-on-a-Chip technologies

Lab-on-a-chip Emphasis

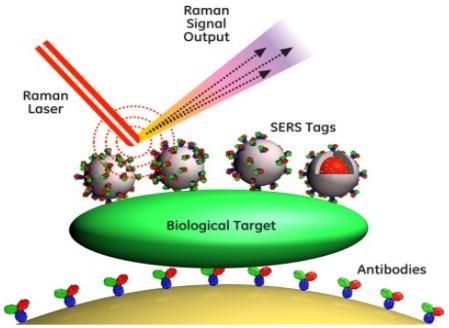
- **Advances in acoustic focusing, digital microfluidics and nanoparticles enable new biological lab-on-a-chip capabilities and platforms. These chip-scale methods include:**
  - On-chip separation, purification, mixing, dilution (Sample Pre-processing and Cleanup)
  - Individually addressable sub-pL fluid volumes (Sample Volume)
  - Rapid single-cell / molecule detection (Detection)
  - Unprecedented multiplexed analysis using multiple probe types (Multi-analyte Analysis)



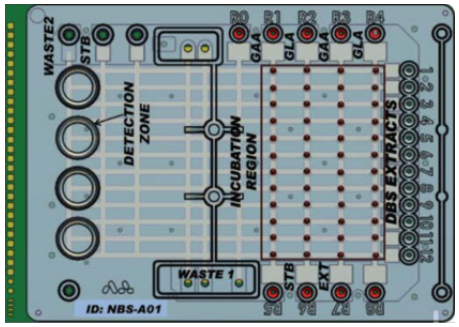
Sample clean-up and pre-processing



Automation



Detection



Multi-analyte Analysis

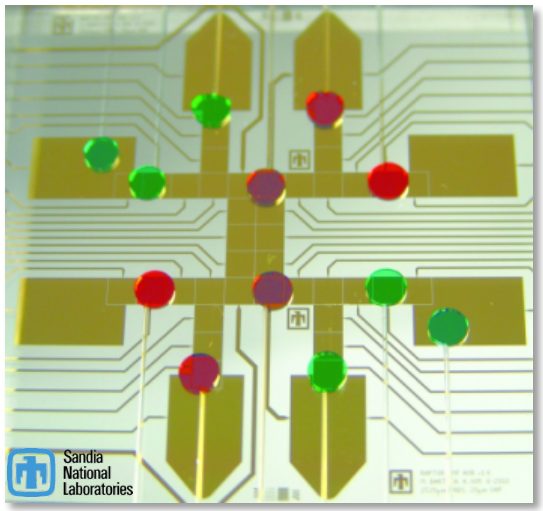
Science and Technology Trends

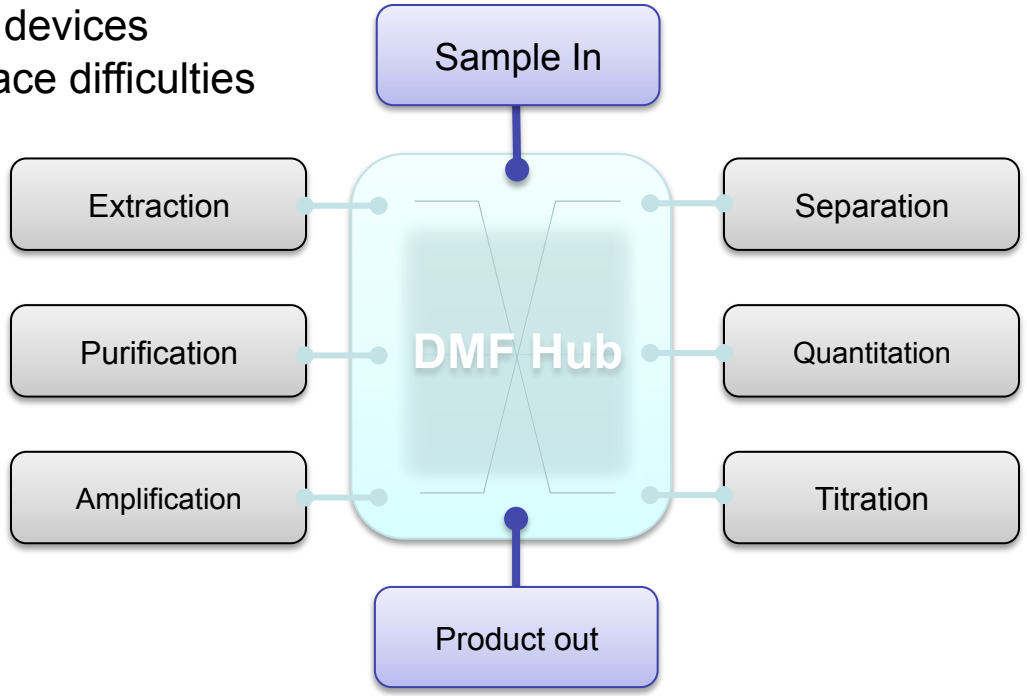
Sandia National Laboratories

Advances in "lab-on-a chip" will catalyze rapid bio-analysis

Digital Microfluidics (DMF)

- DMF as a central hub for interfacing multiple lab-on-a-chip sample processing modules through droplets
  - Advantage
    - Flexibility and spatial manipulations of droplets
    - Modularity and temporal resolution of continuous-flow microchannel devices
    - Overcome world-to-chip interface difficulties
      - Sample volume mismatch & timing

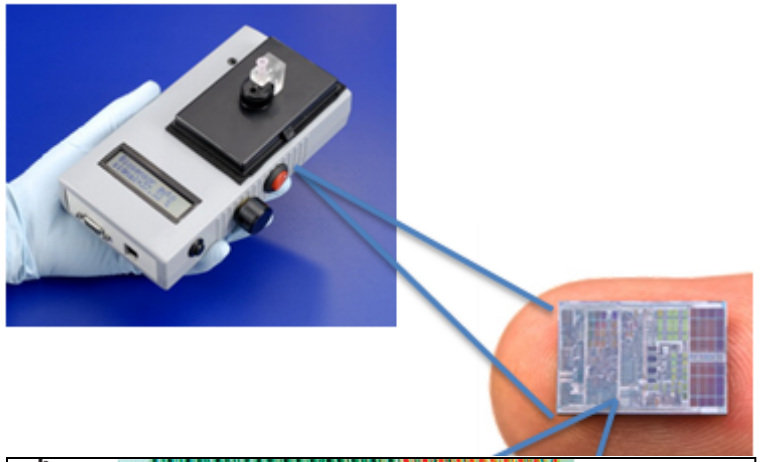




Bio-intelligence Chips (BIC) Summary

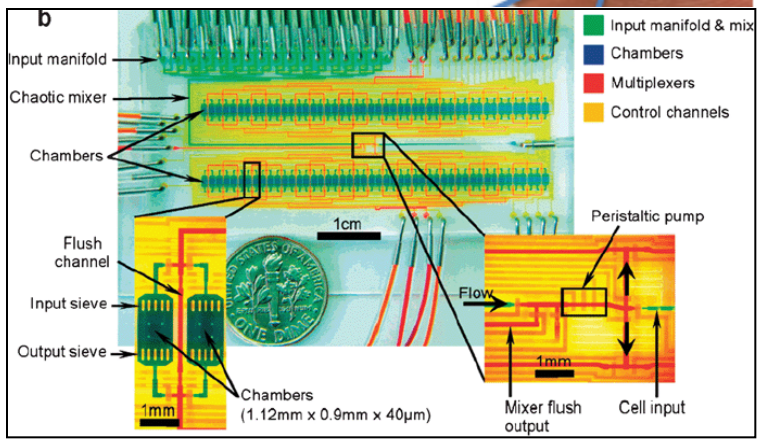
Vision:

- Rapidly determine human exposure to biological pathogens and associated production activities
- Build an agile, dynamically reconfigurable database of assays to enable field programmable test methodologies
- Develop hand-portable instrument capable of analyzing biological analytes from human secretions with single-molecule resolution in less than 10 min
- Leverage developments in *omni-omics* to generate a multi-dimensional serumprint of every person-of-interest



Key Technical Challenges:

- On-chip sample separation from small molecules to whole cells
- Individually addressable sub-pL automated fluidics control including the ability to recycle analytes
- Rapid single-molecule / single-cell detection using a variety of modalities: different probe-types and phenomenologies
- Multiplexing



Bio-intelligence Chips – Program Details

BIC Program Objective

- **Develop a deployable instrument to do rapid multiplexed bioanalysis of human biomarkers in the field, e.g.**
  - Isolation and detection of antibodies
  - Detection of antigen-specific memory cells
  - Finding modifications in nucleic acids

- **Develop new capabilities that could potentially enable the identification of the potential bioweapons maker/handler through rapid analysis of human biomarkers found in blood to identify signatures of interest**

  - Obtain serology fingerprints (serum prints) through the cross-correlation of diverse bioassays

BIC Program Plan and Example Metrics

Phase 1A	Phase 1B	Phase 2	Phase 3
12 months	12 months	18 months	18 months

Discovery Platforms

Chip-Scale Methods

- Processing
- Automation
- Detection
- Bioinformatics

Bioassays (1 organism or 1-plex)
Sample volume: <100 uL
Sensitivity: > 95 %
Specificity: > 95 %
Time: < 30 min

Bioassays (2-plex)
10 uL
sens > 95 %
spec > 95 %
Time: < 20 min

Bioassays (10-plex)
1 uL
sens > 95 %
spec > 95 %
Time: < 10 min

Each plex refers to a family of analytes required to assess the status of the immune response for one organism (inc assoc. toxins)

SWaP: 9V DC, 250g, 4x4x1 cm

Reagent shelf-life: > 12 months

Example Program Aspects

Phase 1A	Phase 1B	Phase 2	Phase 3
12 months	12 months	18 months	18 months

Discovery Platforms
LoD = 100 ng/mL

Each plex refers to a family of analytes required to assess the status of the immune response for one organism (inc assoc. toxins)

- Determine bioassay signatures of program interest in a 2-plex format. Simultaneous, 2-plex discovery is required while the identification of only 1-plex is mandatory for the on-chip bioassay which will be evaluated and down-selected in Phase 1B
- The limit of detection for proposed biomarker must be commensurate with physiological analyte concentrations
- Example deliverables: a listing of analytes and specific epitopes that identifies organism accompanied by dose response curves (include error bars) or Receiver Operating Characteristic (ROC) curves

Example Program Aspects

Phase 1A	Phase 1B	Phase 2	Phase 3
12 months	12 months	18 months	18 months

Each plex refers to a family of analytes required to assess the status of the immune response for one organism (inc assoc. toxins)

Chip-Scale Methods

- **Processing**
- **Automation**
- **Detection**
- **Informatics**

Bioassays (1 organism or 1-plex)
Sample volume: <100 uL
Sensitivity: >95 %
Specificity: >95 %
Time: < 30 min

Reagent shelf life: > 12 months

- Phase 1B proposers may choose to develop technologies for any of the chip-scale methods and/or propose an integrated bioassay
- Proposers shall specify their own component-level metrics based on state-of-the-art
- Proposers who choose to develop a chip-scale bioassay shall
  - Apply knowledge of Phase 1A and develop an on-chip 1-plex assay using whole blood
  - Example deliverables: a listing of analytes and specific epitopes that identifies organism accompanied by dose response curves and/or ROC curves
  - A portable brass-board is desirable to support independent test and evaluation (T&E)

Example Program Aspects

Phase 1A	Phase 1B	Phase 2	Phase 3
12 months	12 months	18 months	18 months

- Develop on-chip 2-plex assay of relevance to human exposure to biological pathogens
- The assay shall use ~10 μL of whole blood & produce an answer end-to-end in < 20 min. The assay shall exceed > 95% sensitivity and specificity
- Example deliverables: a listing of analytes and specific epitopes that distinguishes vaccination from natural infection for at least 2-plex accompanied by dose response curves and/or ROC curves
- A portable breadboard is mandatory to support independent T&E

Informatics

Bioassays (2-plex)
10 uL
sens > 95 %
spec > 95 %
Time: < 20 min

Each plex refers to a family of analytes required to assess the status of the immune response for one organism (inc assoc. toxins)

SWaP: 9V DC, 250g, 4x4x1 cm

Reagent shelf life: > 12 months

Example Program Aspects

Phase 1A	Phase 1B	Phase 2	Phase 3
12 months	12 months	18 months	18 months

- Develop on-chip 10-plex bioassay based on at least two orthogonal modes (e.g., mass spectroscopy and fluorescence)
- The assay shall use ~1 μL of whole blood & produce an answer end-to-end in < 10 min. The assay shall exceed > 95% sensitivity and specificity
- Example deliverables: for each detection mode, a listing of analytes and specific epitopes for at least 10-plex accompanied by dose response curves and/or ROC curves
- A portable prototype is mandatory to support independent T&E

Informatics

Each plex refers to a family of analytes required to assess the status of the immune response for one organism (inc assoc. toxins)

Bioassays (10-plex)
1 uL
sens > 95 %
spec > 95 %
Time: < 10 min

SWaP: 9V DC, 250g, 4x4x1 cm

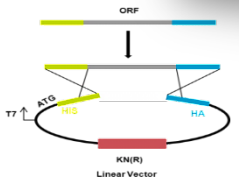
Reagent shelf life: > 12 months

Challenges for Multi-Bioassay Design: Discovery Platforms

Proteins, peptoids, peptides, aptamers, etc. can be explored

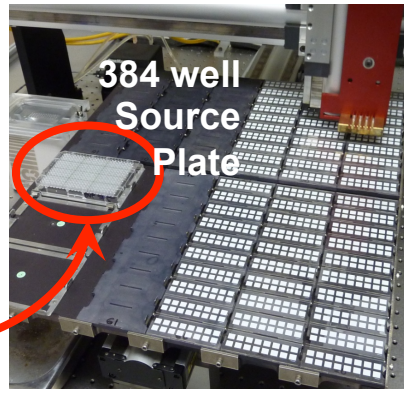
HT PCR Cloning

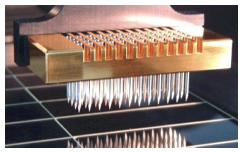
Genomic DNA

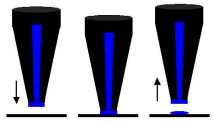


2,000+ genes per week

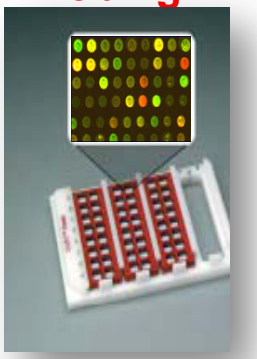
Printing







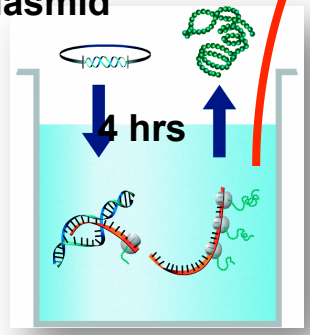
Probing



Cell Free *in vitro* Expression

Input Plasmid

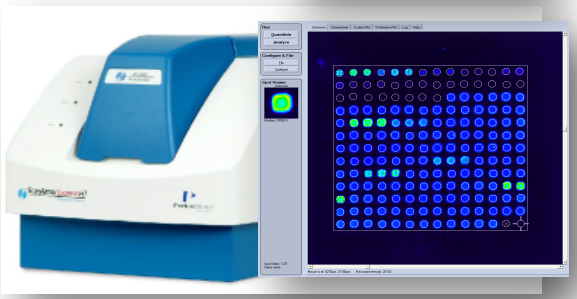
Output Protein



Performance Goals

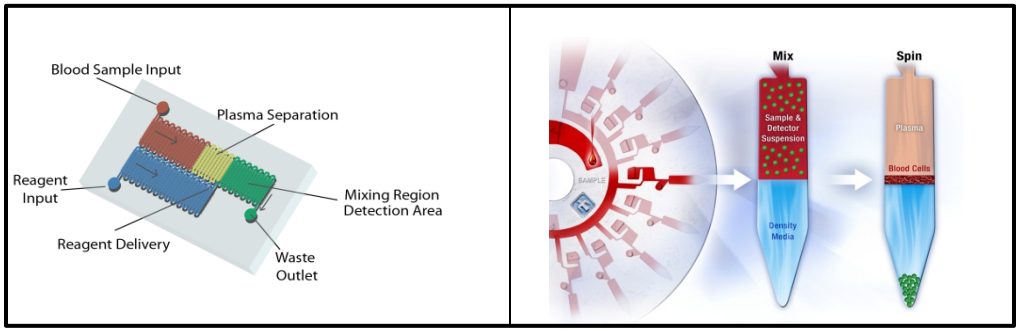
Limit of Detection: < 100 ng /mL
LoD Dynamic Range: ≥ 3 logs
Sample type: whole blood
Number of simultaneous organisms to target: ≥ 2

Analysis



Challenges for Chip-scale Methods: Sample Preprocessing, Automation, Detection

Pre-Processing



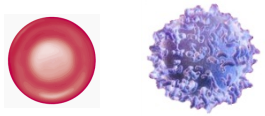
Isotachophoresis

1 nm DNA | 5 nm Interleukin | 12 nm Antibody

Dielectrophoresis

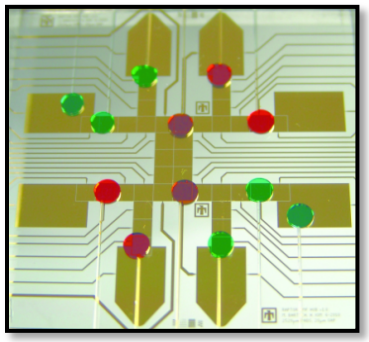
1 μm Bacteria | 5 μm Chromosome

Acoustic-focusing

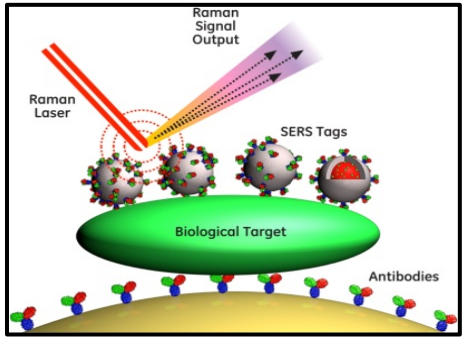


6-8 Erythrocyte | 8-21 μm Lymphocyte

Automation



Detection

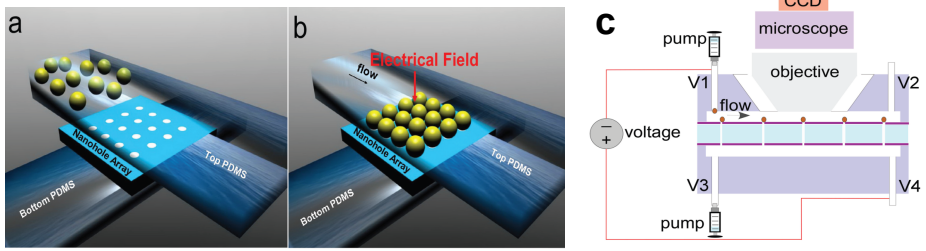


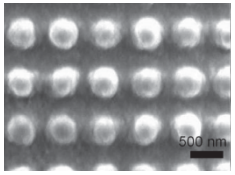
Initial Performance Goals:
Analyte extraction efficiency > 90%
Detection SNR > 10
Time: ≤ 30 minutes

Challenges: Detection

- For detectors that employ a maximal E-field with localization of analytes
- Multi-layer fluidic platform with both microfluidic and nanofluidic channels
  - Electrokinetic forces to control the flow of nanoparticles in a nanochannel
  - Nanoparticles can be captured, assembled, and released, forming a real-time nanophotonic structure

H. M. Chen, L. Pang, M. S. Gordon, Y. Fainman, Small, 7, 2750-2757 (2011).



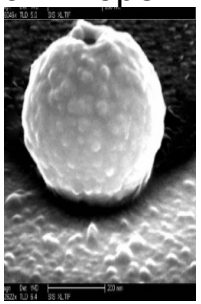


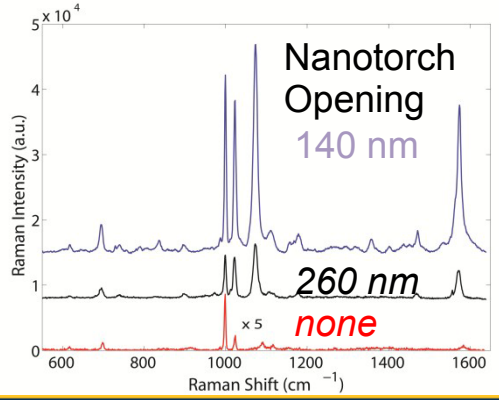
Ultrafast and Nanoscale Optics Group
UNIVERSITY OF CALIFORNIA, SAN DIEGO

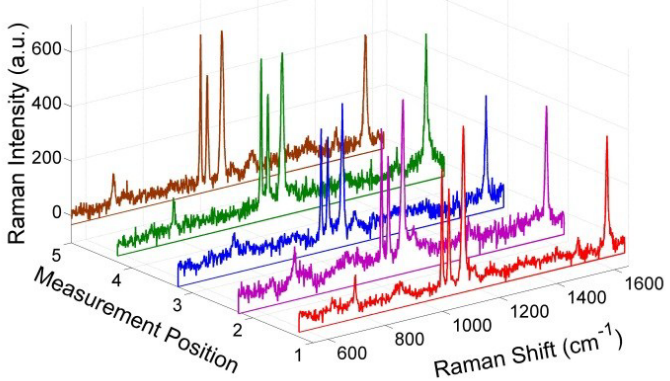
- Single-molecule SERS
  - *Advanced structures*
  - *>10^8 Enhancement Factor*

>80% reproducibility from 5 different nanotorches within same substrate

Nanotorch w\
50 nm opening







H. M. Chen, L. Pang, A. *King, G. M. Hwang, Y. Fainman, Nanoscale (2012)*

Challenges for Chip-Scale Methods: Sample Volume

- What is the minimum sample volume required to minimize false negatives and maximize detection probability?

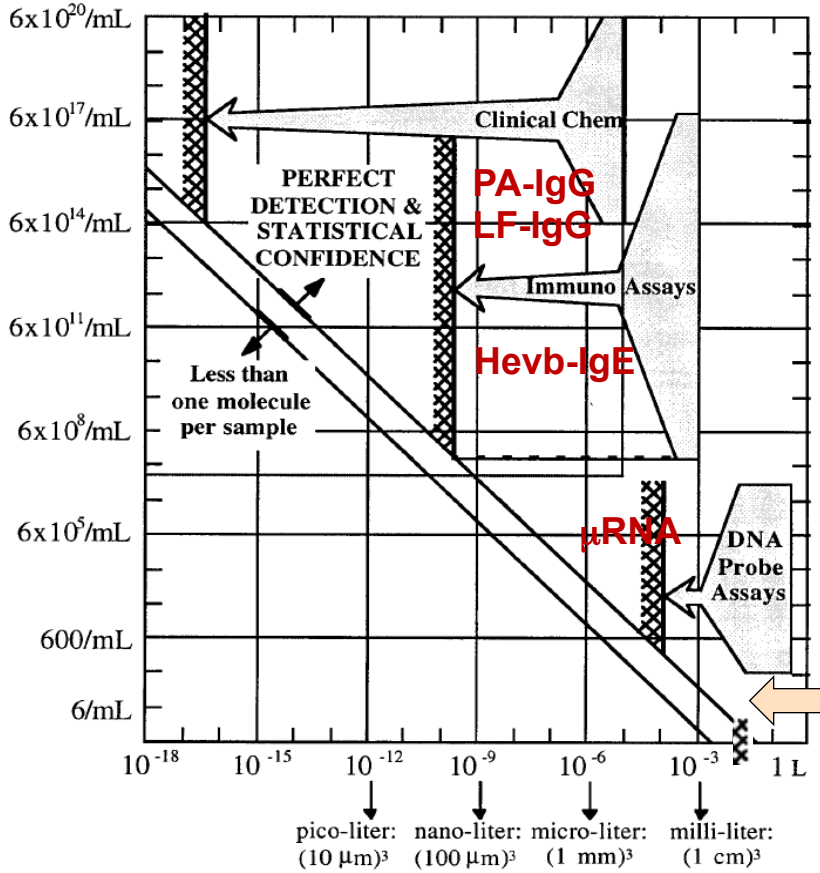


Fig adapted from Petersen KE (1998) *Biomedical Microdevices* 1(1):71-79

- The minimal sample volume required for an assay is dependent on the physiological concentration of the analyte.

  Given: 12 antigen-specific B cells/mL of blood
  Assume: Perfect collector/separator and single molecule detector.
  Result: Minimum sample volume = 1/12th mL

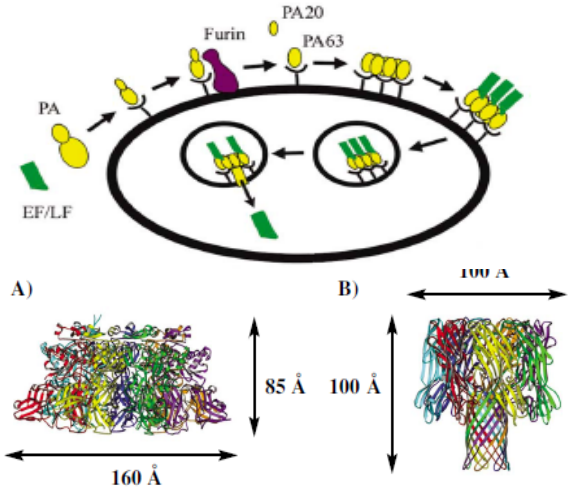
- Reality: > 10 mL of blood required based on state-of-the-art limitation largely due to preservation challenges & poor detection limit

[Antigen-specific B cells] ~ 12/ml (Nduati 2010)
[Antigen-specific CD4+ cells] ~ 8/ml (Alanio 2010)

Example Analytes:
PA/LF-IgG – antibodies specific to anthrax proteins
Hevb-IgE – antibodies specific to latex protein allergens

Challenges: Bioassay Design for Exposure Status

Figs from Nguyen TL (2004) J Biomolecular Structure and Dynamics 22: 253.



Are there any EF-, LF-, PA-specific IgG or IgM?

No → Subject is neither vaccinated nor infected

Yes

Is ratio of PA/LF > 10 ?

No

Yes

PA80-specific IgG or IgM detectable?

No

Yes

PA63-specific IgG or IgM detectable?

Yes

No

Bacterial transketolase activity? (Walz 2007)

Yes

DNA / RNA detectable?

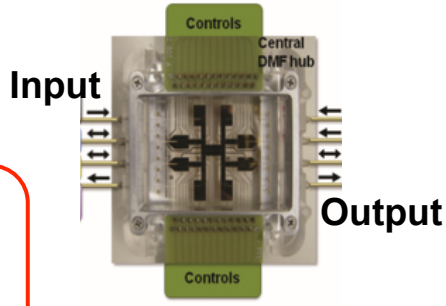
Yes → Subject is infected

No

Subject is not vaccinated but previously infected

No

Subject is vaccinated

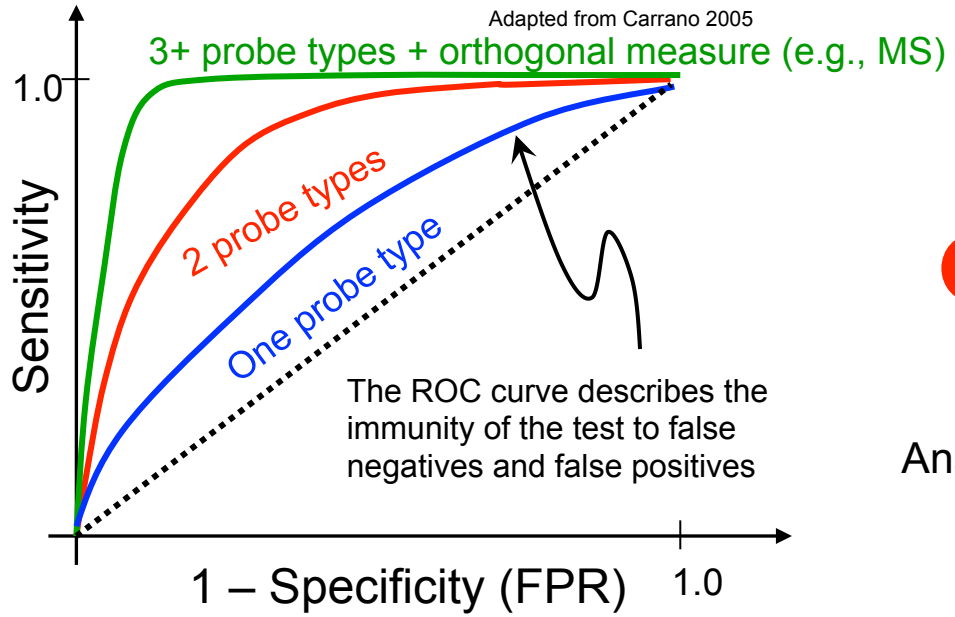


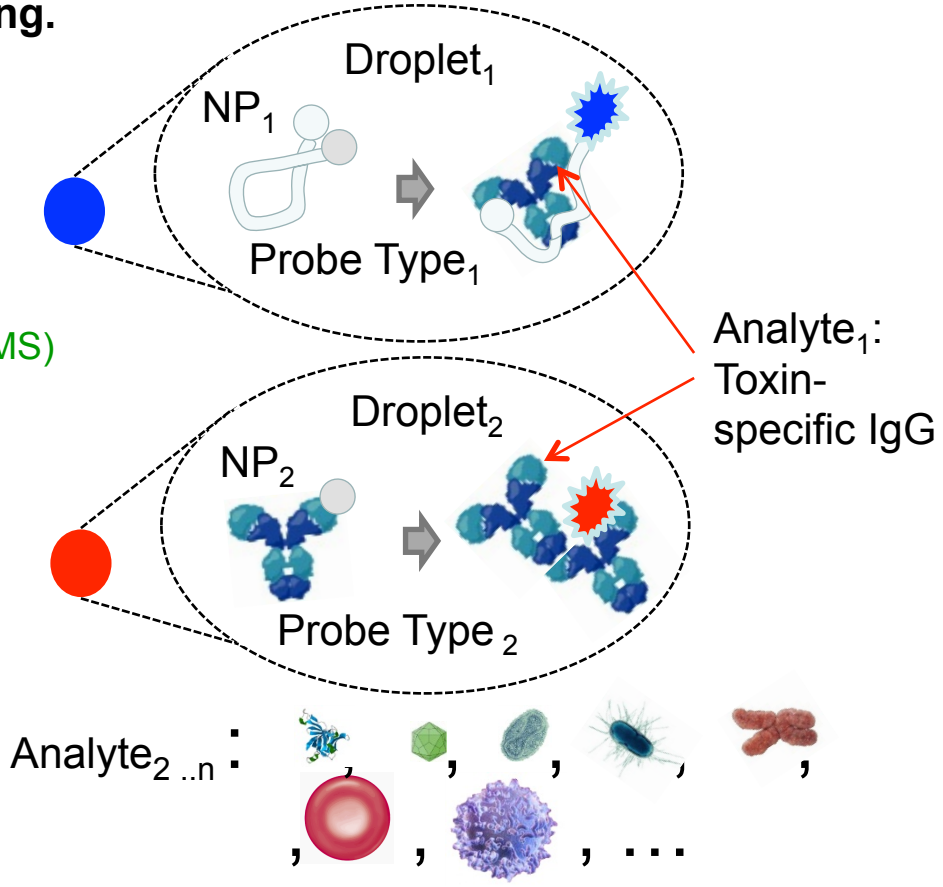
Challenges: Multi-analyte Analysis

Multiplexed assays (protein-based or nucleic acid-based). Multiplexed readout strategies for diverse assay types have, to date, not been integrated on a common substrate, in part due to non-specific binding.

Approach:

Nanoparticle (NP) based assays enable single-substrate platform for acquiring relevant biological data *en masse* rapidly.





Independent reactions co-occur in different droplets, reducing false positive interpretations

Challenges: Bioinformatics

- BIC will leverage the findings of *omni-omics* research community data worldwide (e.g., protein markers, metabolites)
- And optimize and integrate component-level developments in on-chip sample-prep, automation, and nanoparticle substrates
- The rapidity and multiplexity in BIC enable the concomitant consideration of diverse assay results

Guidance on Phases

Phases 1A&1B

Organizations with innovative approaches can propose to one or more of the areas sought in the discovery platform, chip-scale methods (sample-preprocessing, automation, detection, multiplex analysis, algorithms development), or bioassay development emphases. Please apply respective phase-specific performance metrics to your proposal.

Phases 2 & 3

Phases 2 and 3 will build on the successes of Phase 1; deliverables will focus on entire systems, not individual areas. Proposals should therefore describe their multidisciplinary capabilities, which could include lab-on-a-chip designers, bioassay development, diagnostic instrumentation/ equipment designers, bio-technologists, immunologists and others needed to support the proposed approach.

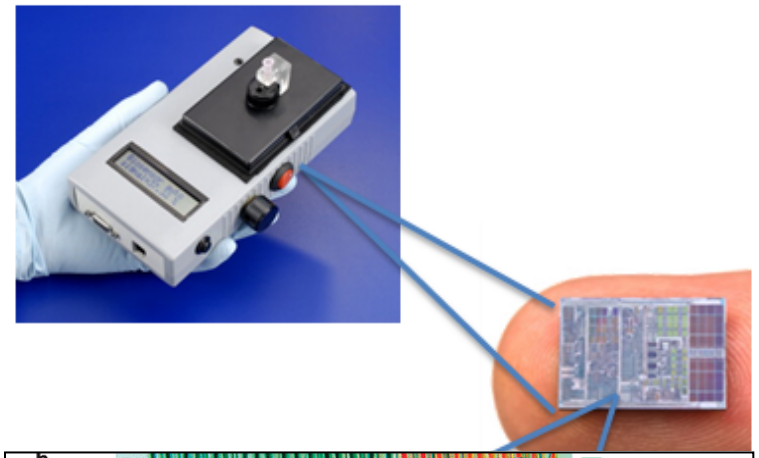
Bio-intelligence Chips Summary

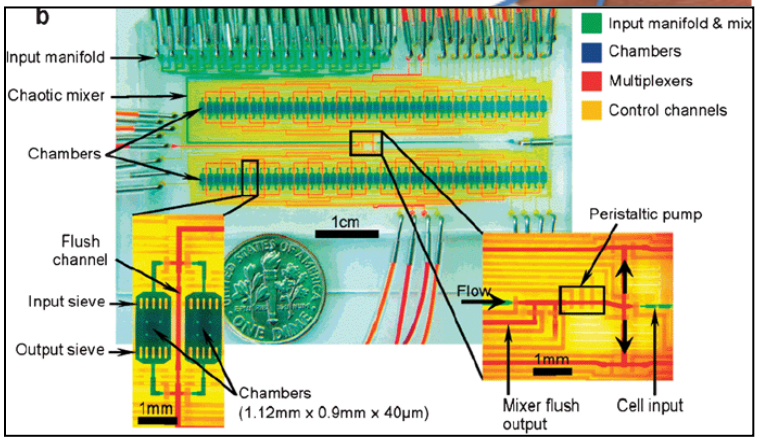
Vision:

- Rapidly determine human exposure to biological pathogens and associated production activities
- Build an agile, dynamically reconfigurable database of assays to enable field programmable test methodologies
- Develop hand-portable instrument capable of analyzing biological analytes from human secretions with single-molecule resolution in less than 10 min
- Leverage developments in *omni-omics* to generate a multi-dimensional serumprint of every person-of-interest

Key Technical Challenges:

- On-chip sample separation from small molecules to whole cells
- Individually addressable sub-pL automated fluidics control including the ability to recycle analytes
- Rapid single-molecule / single-cell detection using a variety of modalities: different probe-types and phenomenologies
- Multiplexing





OFFICE OF THE DIRECTOR OF NATIONAL INTELLIGENCE

Intelligence Advanced Research Projects Activity (IARPA)
Proposer's Day Briefing
Bio Intelligence Chips (BIC)

LEADING INTELLIGENCE INTEGRATION

15 Nov 2012

Recurring questions

- Questions and Answers
- Intellectual Property
- Preparing the Proposal (BAA Section 4)
  - Electronic Proposal Delivery
  - Organizational Conflicts of Interest
- Streamlining the Award Process
  - Accounting system
  - Key Personnel
  - Statements of work
- IARPA Funding

Responding to Q&As

- Please read entire BAA before submitting questions
- Pay attention to Section 4 (Application & Submission Info)
- Read Frequently Asked Questions, IARPA web site @ http://www.iarpa.gov/faq.html
- Send your questions as soon as possible
  - Write questions as clearly as possible
  - Do NOT include proprietary information

Intellectual Property

- Should no proprietary claims be made, Government rights for data first produced under IARPA contracts will be unlimited.
- The default is usually Government Purpose rights for data developed with mixed funding – if you do not specify, this is the minimum that we expect
- You should state in the proposal any restrictions on deliverables relating to existing materials (data, software, tools, etc.)
- If selected for negotiations, you must provide the terms relating to any restricted data or software, to the Contracting Agent

Preparing the Proposal

- Note restrictions in BAA Section 4 on proposal submissions
  - Interested Offerors must register electronically IAW instructions on: **https://iarpa-ideas.gov**
  - Interested Offerors are strongly encouraged to register in IDEAS at least 1 week prior to proposal "Due Date"
  - Classified proposals – Contact IARPA Chief of Security
- BAA format is established to answer most questions
- Check FBO for amendments & IARPA website for Q&As
- BAA Section 5 – Read Evaluation Criteria carefully
  - e.g. "The technical approach is credible, and includes a clear assessment of primary risks and a means to address them"

Preparing the Proposal (BAA Sect 4)

- Note IARPA's OCI policy – see http://www.iarpa.gov/IARPA OCI_081809.pdf
  - See also eligibility restrictions on use of Federally Funded Research and Development Centers, University Affiliated Research Centers, and other similar organizations that have a special relationship with the Government
  - Focus on possible OCIs of your institution as well as the personnel on your team
  - See Section 4: It specifies the non-Government (e.g., SETA, FFRDC, UARC, etc.) support we will be using. If you have a potential or *perceived* conflict, request waiver as soon as possible
- Cost Proposal – we only need what we ask for in BAA

Streamlining the Award Process

- Approved accounting system needed for Cost Reimbursable contracts
  - Must be able to accumulate costs on job-order basis
  - DCAA (or cognizant auditor) must approve system
  - See http://www.dcaa.mil, Information for Contractors under "Publications"
- Statements of Work (format) may need to be revised
- Key Personnel
  - Expectations of time, note the Evaluation Criteria requiring relevant experience and expertise
- Following selection, Contracting Agent may request your review of subcontractor proposals

IARPA Funding

- IARPA funds applied research for the Intelligence Community
  - IARPA cannot waive the requirements of Export Administrative Regulation (EAR) or International Traffic in Arms Regulation (ITAR)
  - Not subject to DoD funding restrictions for R&D related to overhead rates

- IARPA is not DOD

Disclaimer

- BAA is being developed
  - Following issuance, look for amendments and Q&As
- There are likely to be changes
- Content of BAA will be specific to program
- Nothing said here will supersede BAA