

# New England Bioterrorism Preparedness Workshop

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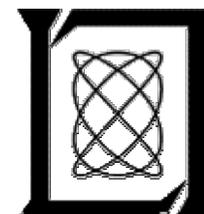
3-4 April 2002

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## Lincoln Laboratory

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

*LEXINGTON, MASSACHUSETTS*



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# Military Biological Weapons Programs

Geoffrey Forden

Security Studies Program, MIT

April 3, 2002

We now know what terrorists want from Biological Weapons, but what does a military want?

I will examine two programs we know a lot about:



Voluntarily eliminated BW program in 1969 and declassified much information



Forced to “eliminate” program and much information gained from inspectors. 1

# Estimates of Historical Weapons Effectiveness

Combatants



- Precision Guided Munitions: 0.75 Casualties/ton
- World War I Chemical Weapons: 10 Casualties/ton (0.2 deaths/ton)
- Iran-Iraq War Chemical Weapons: ~35 Casualties/ton

Civilians



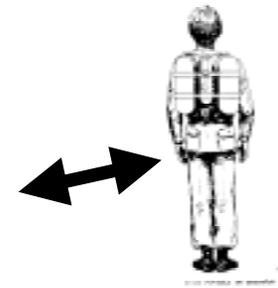
- 1995 Tokyo Sarin Attack (CW): ~2200 Deaths/ton
- Atomic Bomb (Hiroshima): ~100,000 Deaths/ton
- Anthrax Attacks (October Incident): ~1,000,000 Deaths/ton
- Thermonuclear: ?

# The U.S. Biological Weapons Program

1. Artillery shells



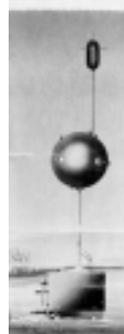
2. Stationary generator left behind by special forces.



3. Boat mounted line spray source



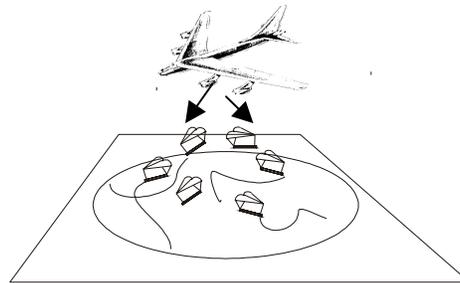
4. Naval point source



5. Self-dispersing spheres



6. Flettner rotors



7. Drone mounted line spray source.

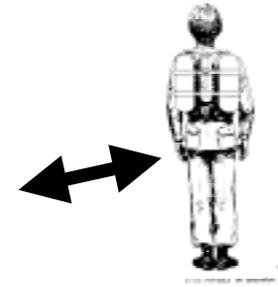


1. ~~Artillery shells~~

~~Abandoned~~



2. Stationary generator left behind by special forces.

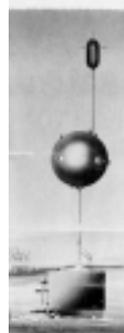


3. Boat mounted line spray source



4. ~~Naval point source~~

~~Abandoned~~



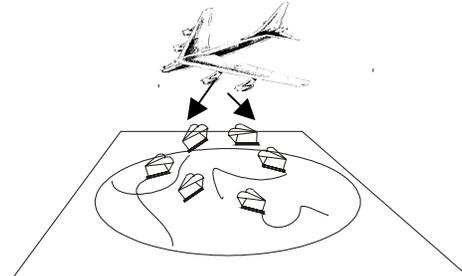
5. Self-dispersing spheres

~~Obsolete~~



6. ~~Flettner rotors~~

~~Obsolete~~



7. Drone mounted line spray source.



# Overview of Various BW weapon Systems.

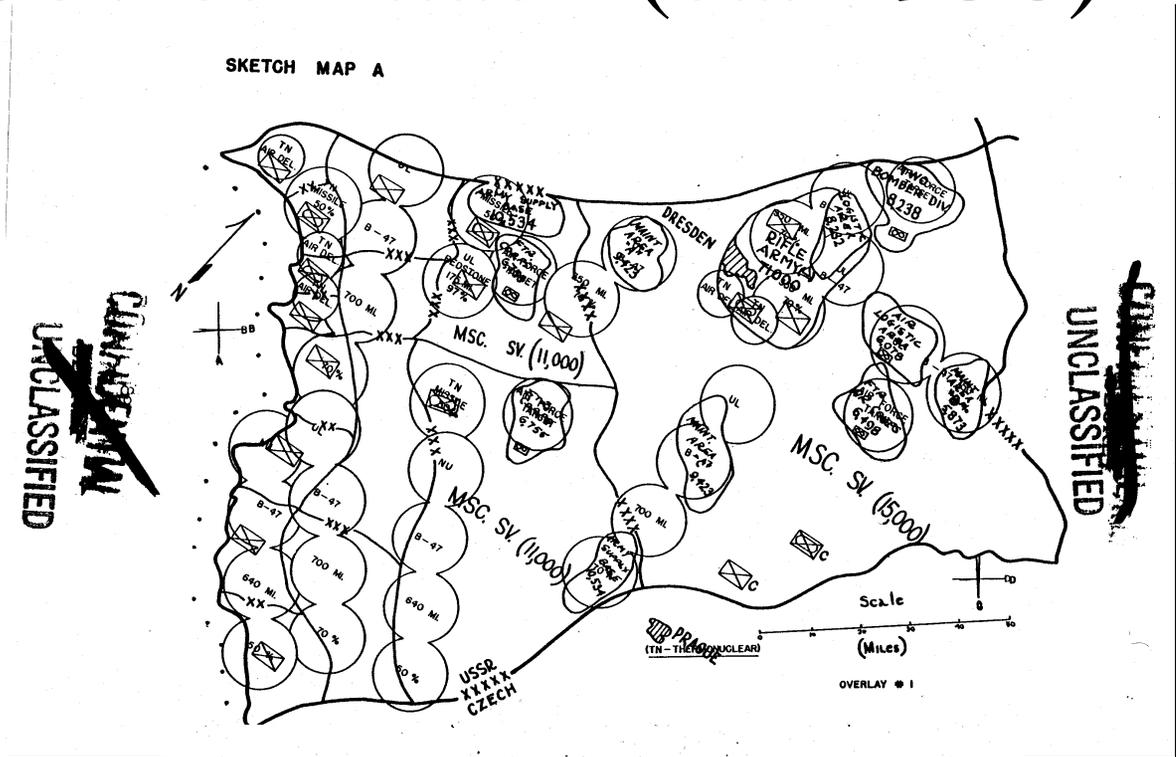


Military Uses of Biological  
Weapons:  
Serious Suggestions, Far from  
being actual war plans!



# Using Tactical BW in General War: Blunting a Soviet Attack (ca. 1958)

Resources required  
for BW mission:



	Military	Civilian
AW Killed	31,800	12,000
AW Injured	31,800	12,000
AW Total	63,600	24,000
BW Killed	39,600	46,000
BW Incapacitated	118,800	134,500
BW Total	158,400	180,500
<b>GRAND TOTAL</b>	<b>222,000</b>	<b>204,500</b>



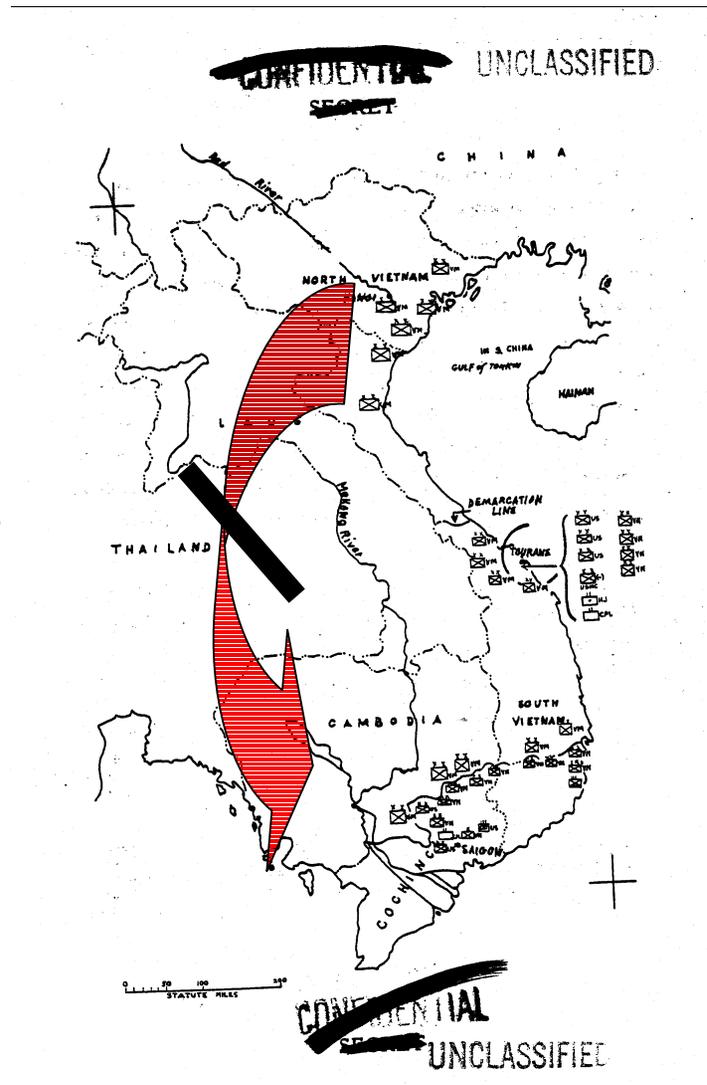
+thermonuclear weapons in the north

NOTE: Total ground troops in southern army front, 429,250.

# Using “Strategic” BW in a Peripheral War: blunting a Soviet Attack (ca. 1958)

## Goals:

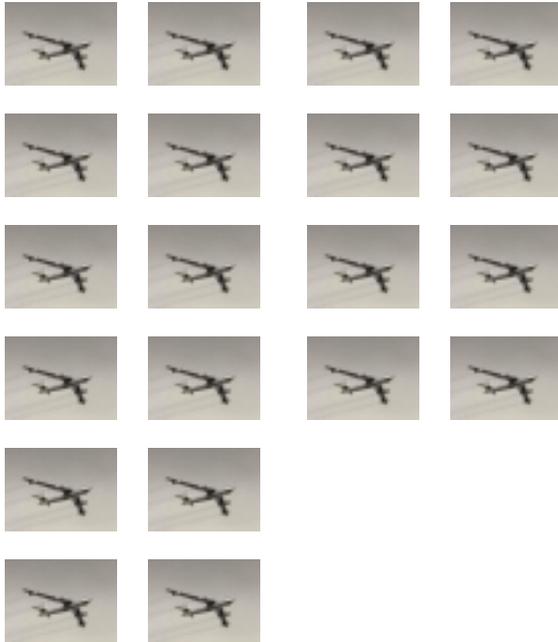
- 1) Block troop transfer from the North to the South.
- 2) Incapacitate enemy troops in the South to reduce their effectiveness at defense.



## Assumption:

US fights a static defense until all its forces are in place. It can then launch an offensive at a time of its choosing, i.e. 7 days after BW attack.

# Resources required for BW mission:



### Saigon and Tourane Perimeters

	Military	Civilian
Total in Area	137,000	243,200
Total Incapacitated	68,500	130,000
Total Lethalities	0	0
Total Casualties	68,500	130,000

### Tonkin Delta

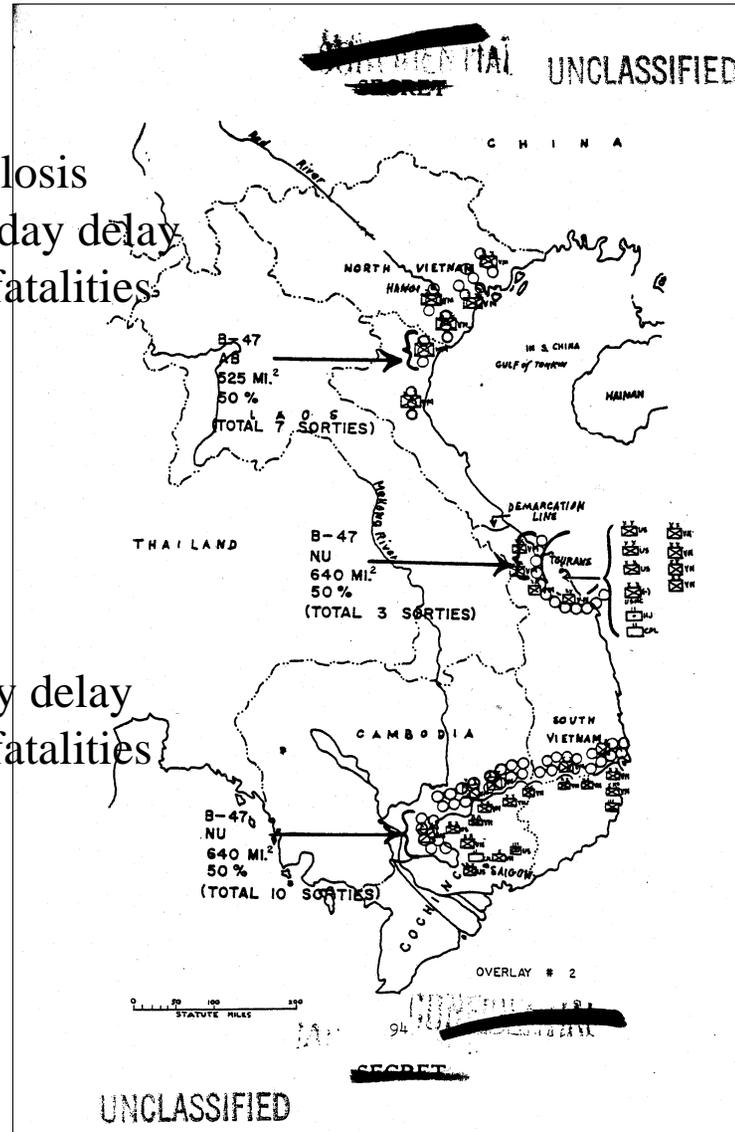
	Military	Civilian
Total in Area	84,000	1,128,000
Total Incapacitated	40,200	497,000
Total Lethalities	800	11,000
Total Casualties	41,000	508,000

~~CONFIDENTIAL~~  
~~SECRET~~

UNCLASSIFIED

Brucellosis  
12-15 day delay  
< 2% fatalities

VEE  
2-5 day delay  
< 2% fatalities

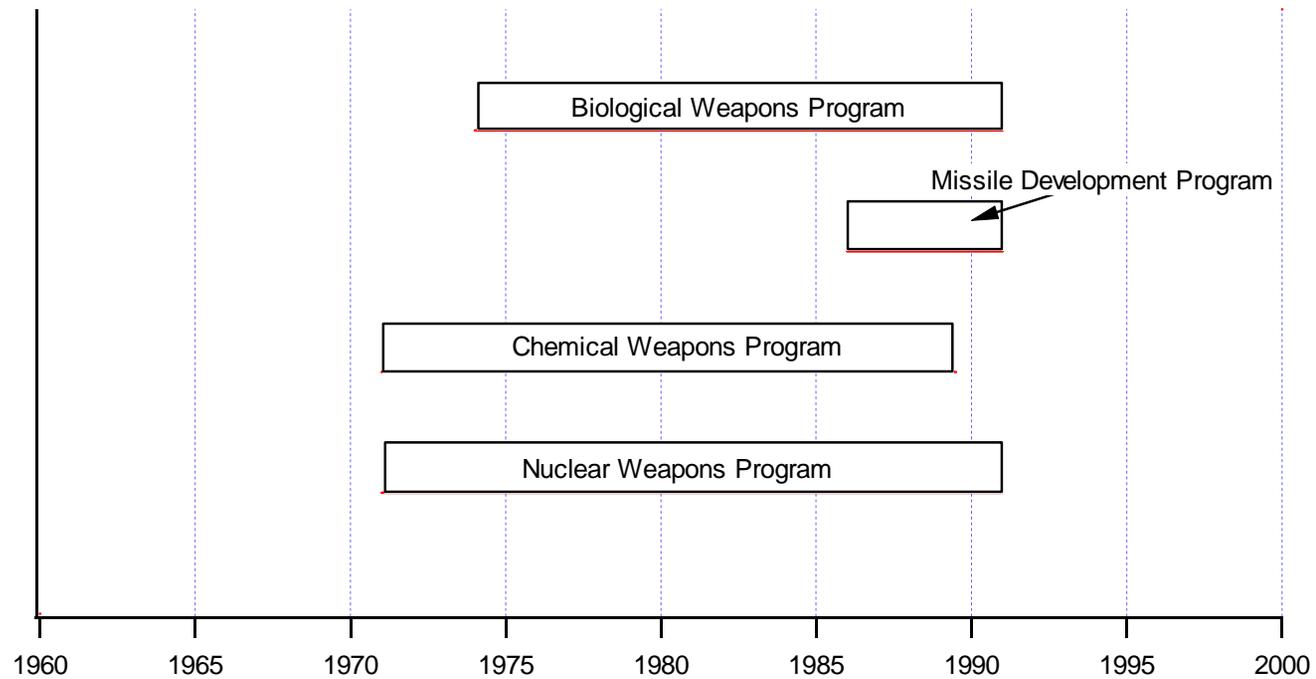


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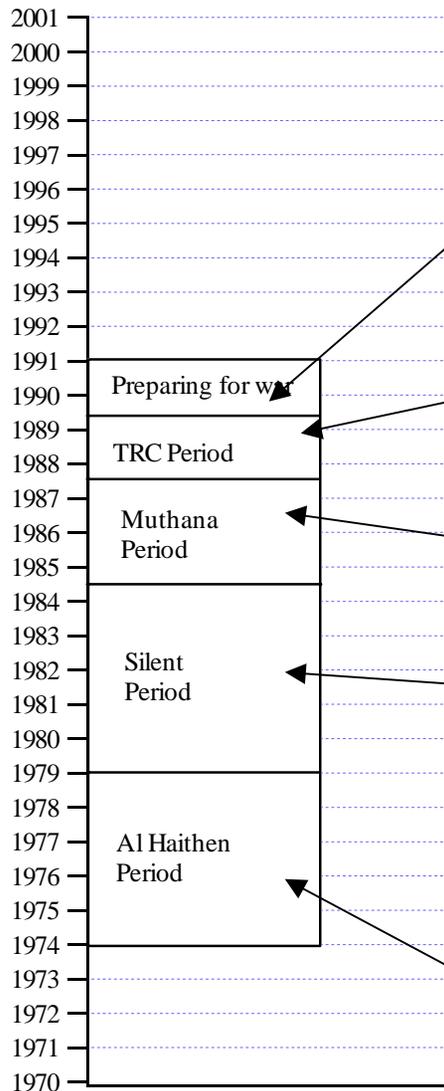
# Iraq's Biological Weapons Program

# Timeline of Iraq's WMD Programs



# The Different Periods of Iraq's

## BW Program



Concepts of operations were formed but UNSCOM does not know much about these since they are prohibited from inquiring about military matters.

A procurement network was established (for importing needed items from outside the country) and planning took place, but not much else is known about Iraq's activities.

This period was dedicated to weapons development, with some research.

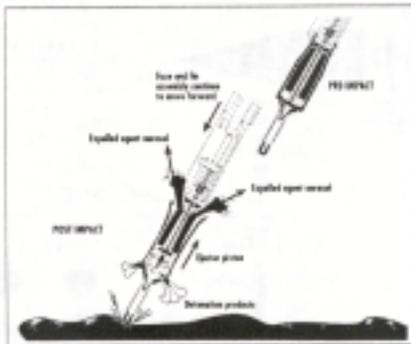
This was primarily a training period with students sent abroad for PhDs (mostly to the UK). Existing facility was closed. During this period, Iraq isolated bacteria and viruses, investigated drying processes, taught students, and built up an institute.

During this period, the goals of the program were "dirty tricks" associated with assassination of political opponents (staining shirts with ricin) and protection of the President (food poison testing). But the program failed with people being sent to jail because of fraud, for instance. However, the people who ended up leading the BW program were associated with it at this period.

# Weaponization of Iraq's BW Agents

## Biological Munitions

Example—modifying a Cardoen PM-1 submunition



Iraq tried to set up a factory for these tactical warheads that could be filled with either CW or BW. They are made to look just like normal HE 155 mm rounds, presumably to prevent Iranians from taking CBW defense measures.

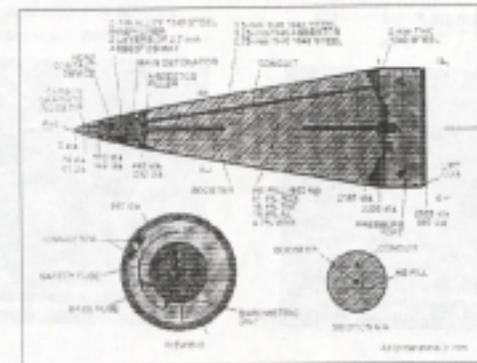
## UAV delivery of BW



Iraq tried to develop remotely controlled Mirage fighters with large drop tanks filled with BW, even into the Gulf War.

## Bio Warhead—Munitions

Bulk fill high explosive theater missile



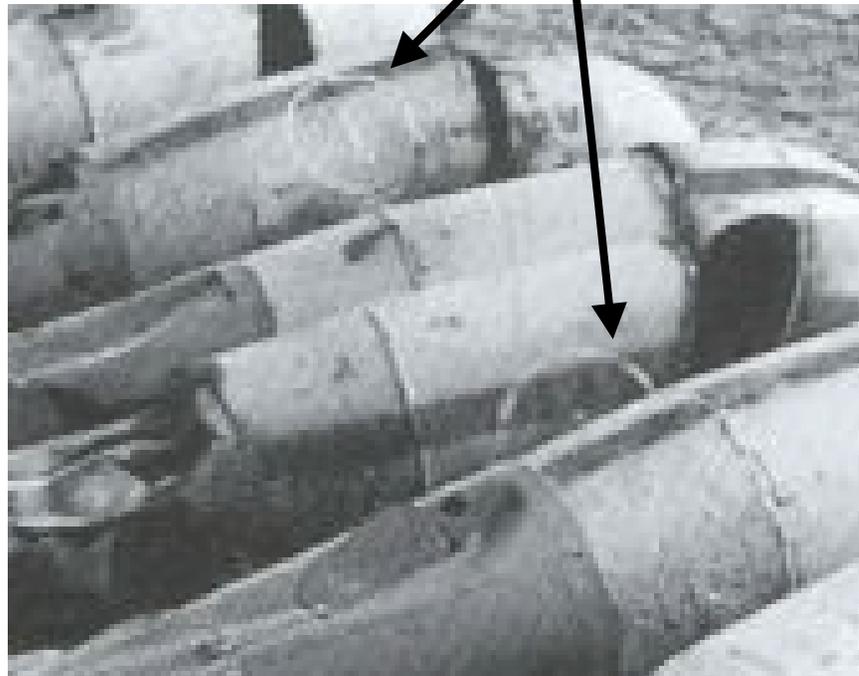
25 SCUD warheads were filled with anthrax. These were filled in the period before the 1991 Gulf War.

# Weaponization of Iraq's BW Agents (Cont')



These gravity bombs were filled with CW, as indicated by the empty circle. BW bombs had an Arabic A in side the circle.

CW markings,  
BW bombs had an Arabic  
“A”



# Supergun—A new way of getting Intercontinental Distances



A supergun (termed little Babylon), with 350 mm diameter, as actually built but apparently not tested. This version was built as a fixed direction/launch angle. But it could have been weaponized to a more mobile version.

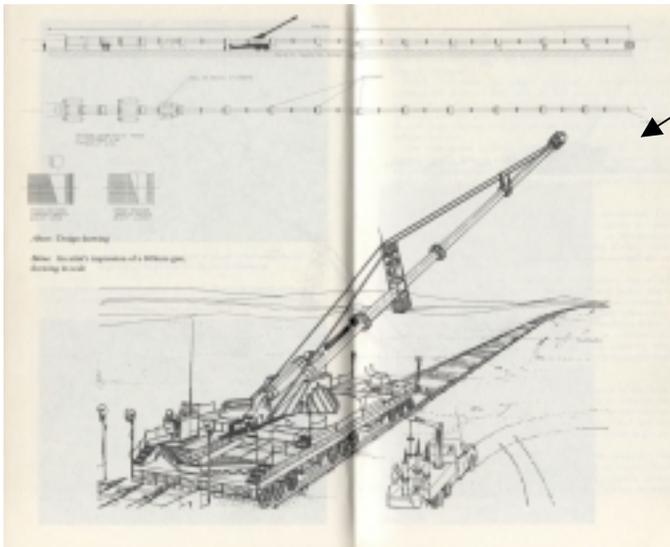


FIGURE 57 PAYLOAD CAPABILITIES OF HIGH ALTITUDE PROJECTILES

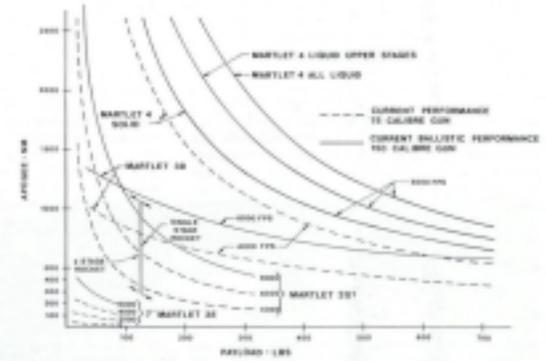
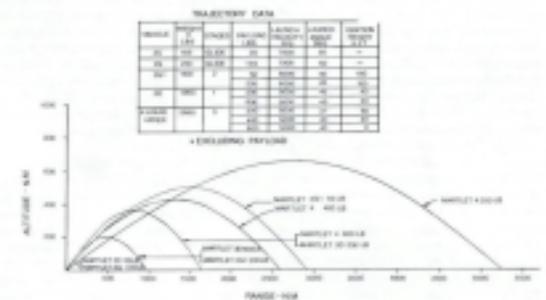


FIGURE 58 LONG RANGE PERFORMANCE, 16 INCH GUN



# Clandestine BW Dispersal



# Anthrax and Smallpox: Comparison of Two Outbreaks

Jeanne Guillemin

Senior Fellow

MIT Security Studies Program

# Anthrax and Smallpox: Comparison of Two Outbreaks

- 1979 Sverdlovsk anthrax epidemic, officially explained by consumption of infected meat; military aerosol suspected
- 1972 Yugoslavia smallpox epidemic, started by a pilgrim returning from Mecca via Baghdad, site of unreported outbreak

# Key Problem = Late Diagnosis

1. What are the political causes?
2. What are the medical/professional causes?
3. What are the public communication causes?

# 1979 Sverdlovsk Epidemic

1992-1994 investigation of an  
“unnatural” outbreak of inhalational  
anthrax

# Sources of Evidence

- KGB list of 64 victims' names and addresses
- Interviews with families/neighbors of 56 victims
- Cemetery data
- Autopsy tissue data
- Hospital records (5 survivors)
- Local hospital and factory clinic lists
- Veterinary documents/animal deaths

16-I, 19-I, в мае было выявлено 20 больных (рис.3).



Распределение больных по датам с учетом непродолжительности инкубационного периода, позволило исключить инфицирование через мясо, поступавшее на питание населения в централизованном порядке. Ясно, что в этом случае следовало ожидать взрывообразное нарастание заболеваемости. Растянутый характер вспышка приняла из-за длительного хранения мяса населением. Так, в конце апреля в семье Г., состоящей из 2-х человек пенсионеров, о наличии мяса, купленного в начале месяца, удалось узнать только в итоге продолжительной беседы. Мясо в данном случае не вызвало опасений, поскольку уже несколько раз добавлялось при варке студня. В семье учительницы С. часть жирного мяса была перетоплена для получения сала, которое использовалось в пищу. Однако из мяса, изъятых в указанных семьях, было выделено два штамма возбудителя сибирской язвы. Информация населения, передававшаяся через местное радио и печать об опасности употребления случайно купленного мяса, создала уверенность в том, что мясо все изъято и уничтожено, не сохранилось у населения, это поэтому настоятельные меры для его выявления и изъятия не принимались. Вместе с тем, наблюдения в ряде очагов показали также возможность инфицирования

Page from Soviet report,  
1988, submitted to  
US State Department.  
April 4-May 16, 1979 cases  
reported as due to eating  
infected meat over weeks.  
Fatalities 64, survivors 15.

Anna Komina  
Ceramics factory  
worker, age 54;  
resident of  
affected district

Date of onset of  
symptoms: April 4

Date of death: April 10





Valentin Petrovich Borisov  
Age 27, Soldier, Compound 32



Pyotr Pilyasov, Age 39  
Construction worker



June, 1992, Hospital 20, in Ekaterinburg's southern Chkalovsky district. Team members Martin Hugh Jones, veterinarian, Alexis Shelokov, virologist, and Matthew Meselson, biochemist and team organizer, with a university host V. A. Shpetkin, and the hospital director, Margarita Ilyenko.



Street leading towards ceramics factory (smokestack in Center) where 18 workers died of anthrax, April-May 1979



1993. Interior of pipe shop of abandoned ceramics factory. Large, third-story windows on left face northwest.

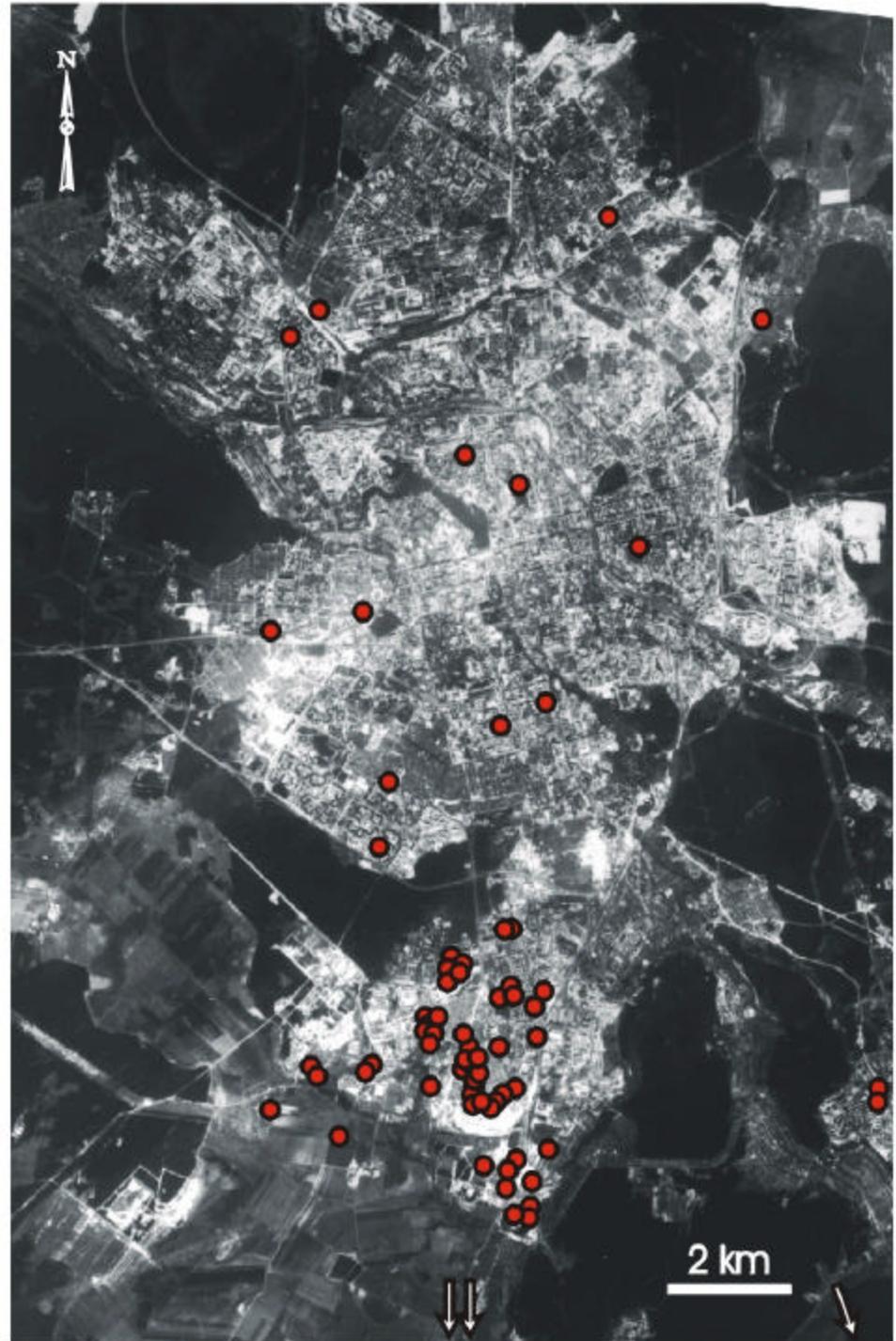


Gate of Compound 19 military base, southwest Ekaterinburg. Soldier is allowing truck to enter.



Cottage in village southeast of Ekaterinburg where animals died of anthrax in 1979, starting April 5-6, and where villagers were vaccinated and quarantined.

Sverdlovsk, c.1985  
Red dots=Nighttime  
Locations of victims.  
Addresses obtained from  
KGB and other lists.  
Southern cluster is in  
Chkalovsky rayon.  
Arrows=homes off map.

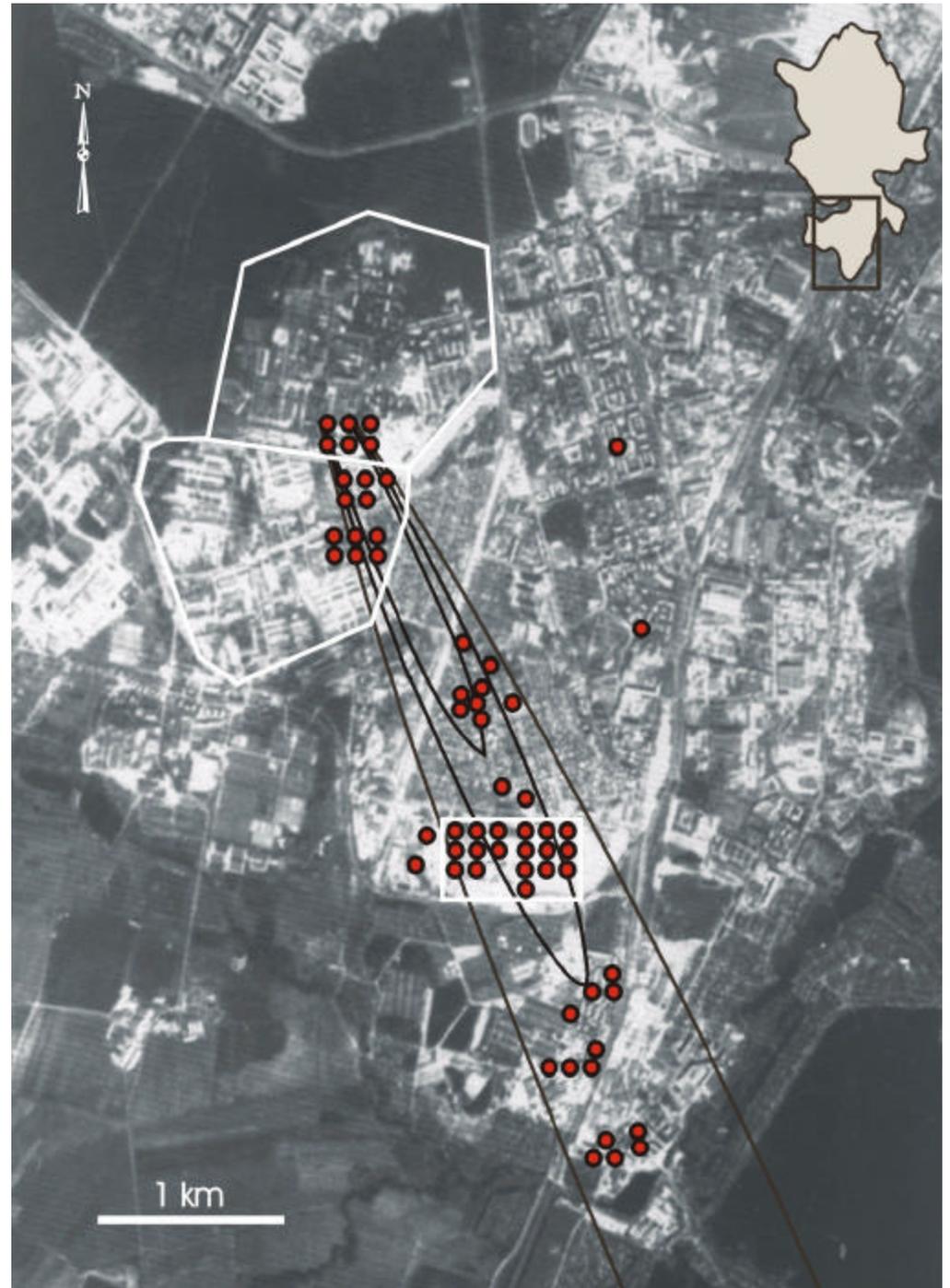


## Chkalovsky District Only (note inset of entire city)

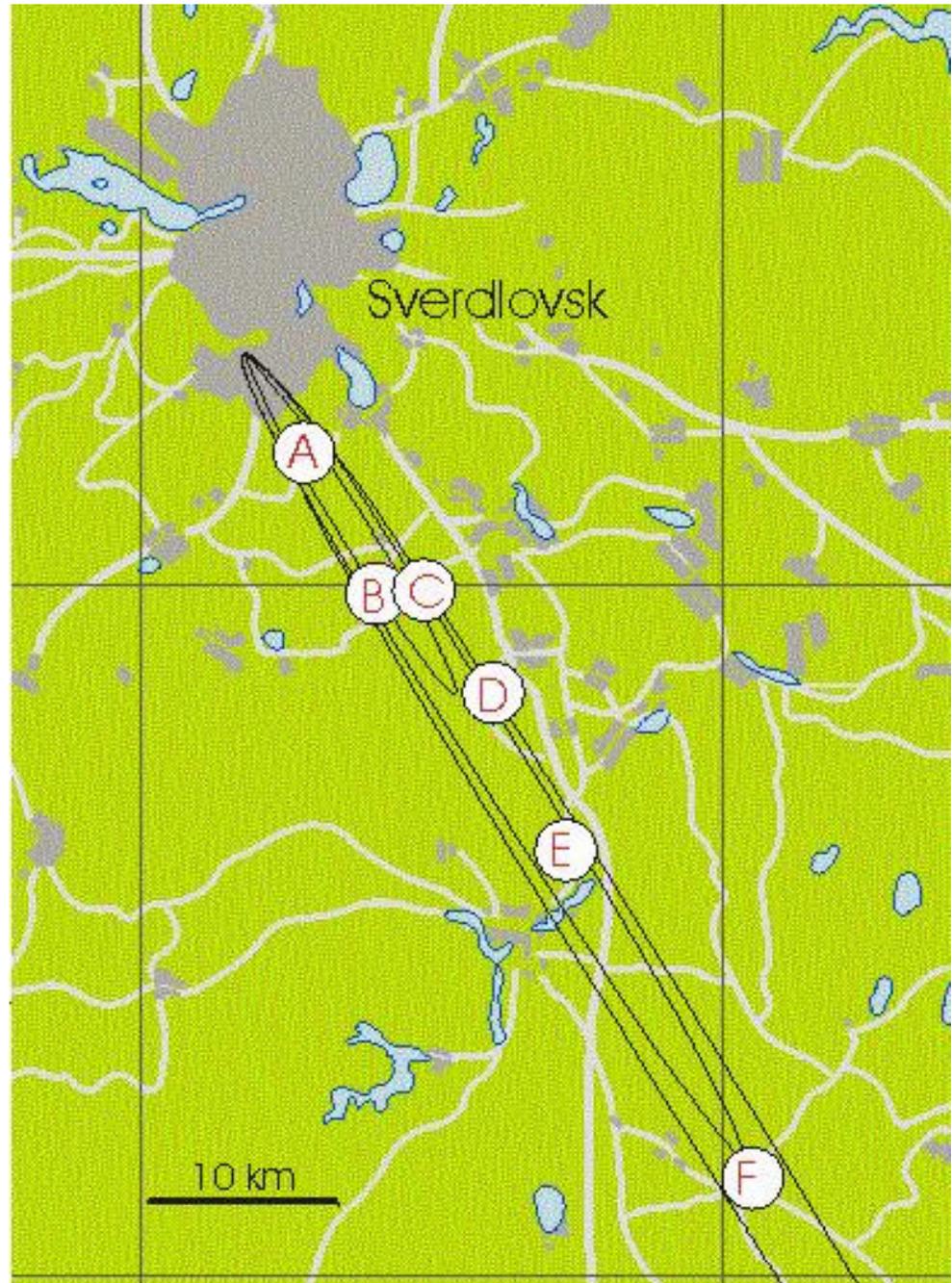
Irregular white lines show  
Compounds 19 and 32.

White rectangle indicates  
Ceramics factory.

Red dots=daytime locations of  
66 victims and 11 survivors.

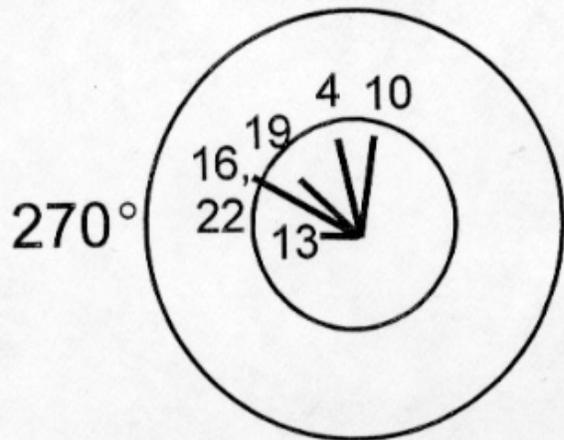


Six villages southeast of Sverdlovsk where 1979 epizootic occurred. Public health measures April through May. Interviews conducted at F, Abramovo, confirmed Veterinary documents.

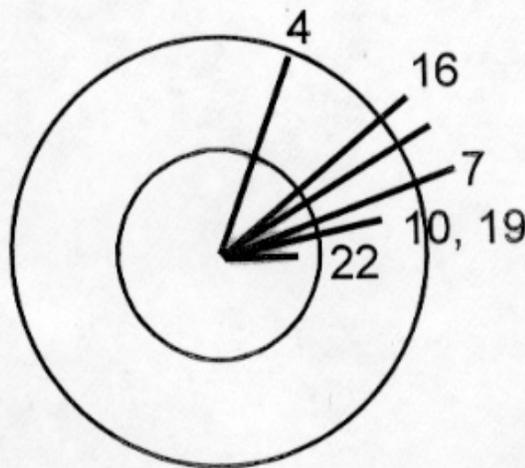


Friday March 30

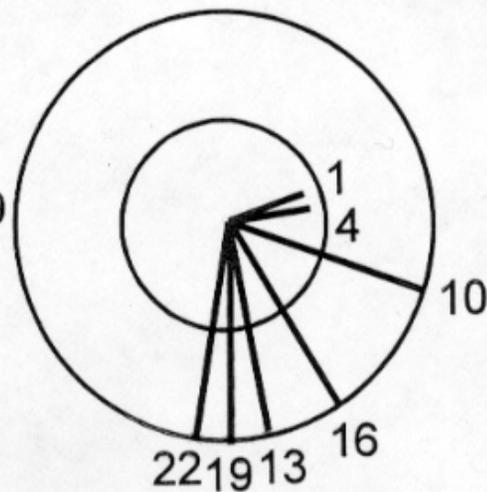
360°



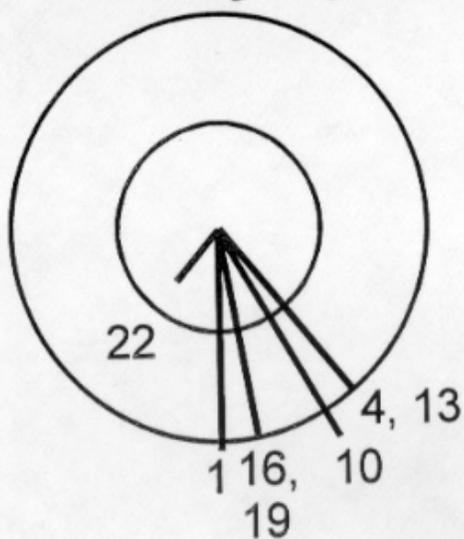
Saturday March 31



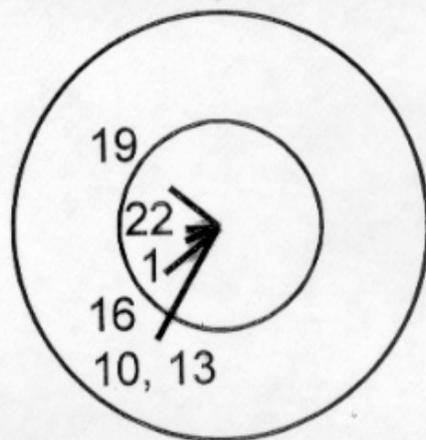
Sunday April 1



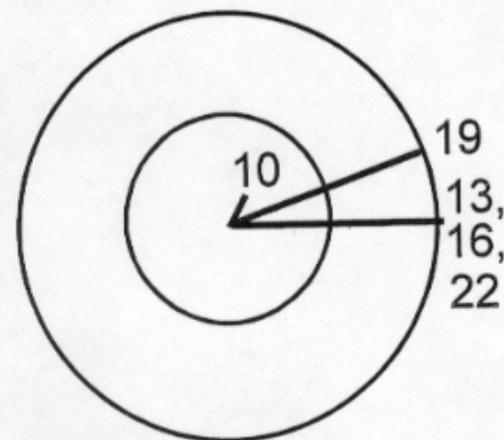
Monday April 2



Tuesday April 3



Wednesday April 4

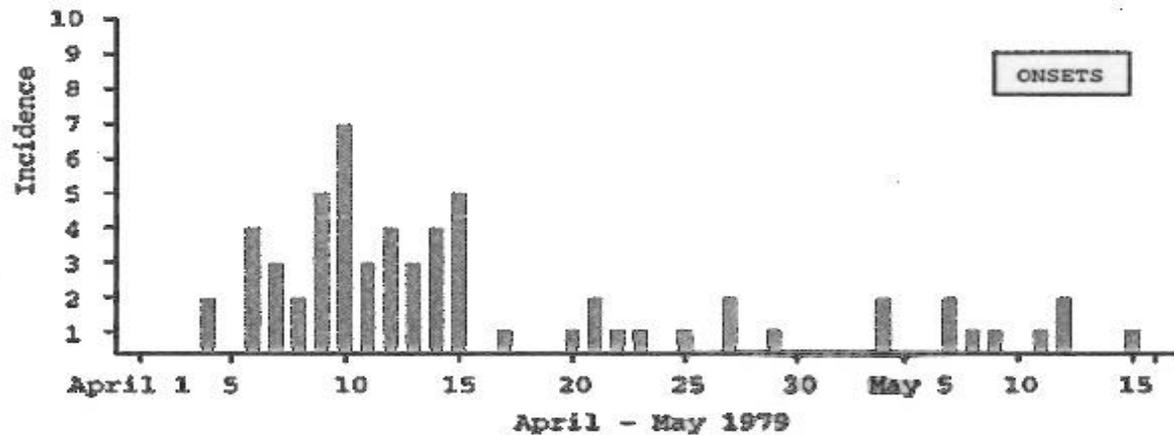


# Research Findings

- A lethal emission of anthrax spores from Compound 19 occurred during the afternoon of April 2, 1979.
- No young people under 24 or children were affected.
- Approximately 80 people (of some 5000 exposed) became infected; 11 survived with treatment.
- An estimated gram (a trillion spores) caused the fatalities; attack rate of 1-2%; fatality rate around 80% (note late diagnosis).
- Inhalation anthrax in humans can occur as long as 43 days after exposure. (First evidence in human cases)

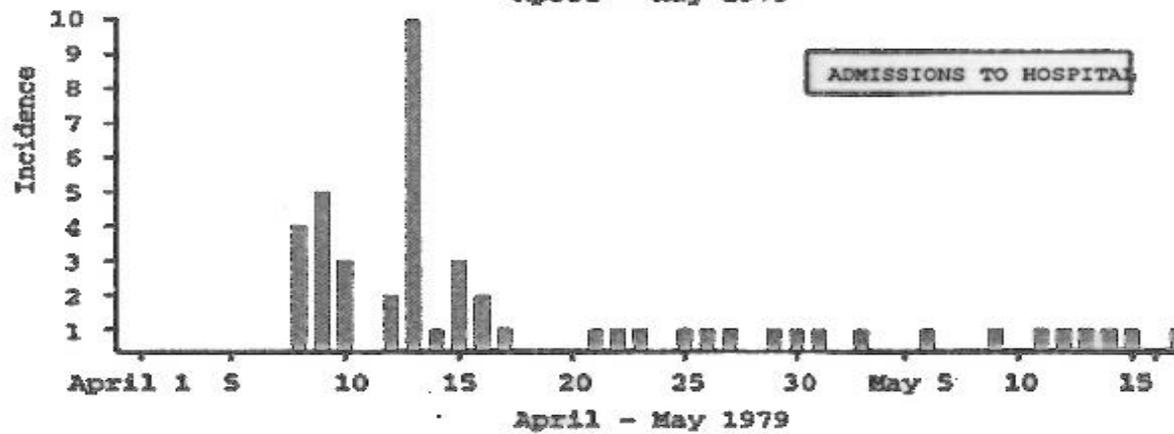
## Soviet Public Health Response

- Urban: lab diagnosis, screening for central hospital intensive care and pediatric cases, ambulance transport, autopsy team; 4000 volunteers mobilized for disinfection and distribution of antibiotics; Moscow clinical team, vaccine campaign for 50,000; building exteriors washed.
- Rural: roadblocks, carcasses burnt, enforced human vaccination, animal sheds destroyed, 3-week village quarantine.



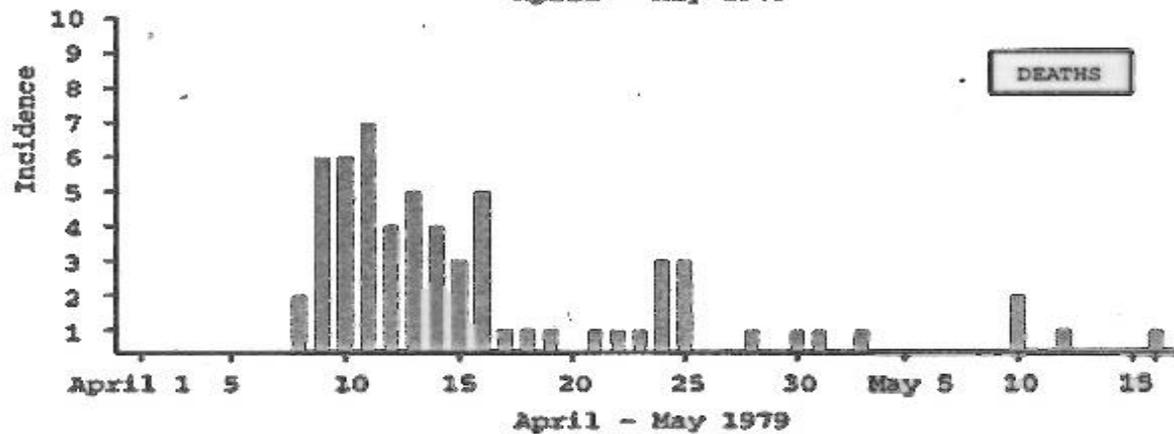
Diagnosis 9 days post  
April 2 exposure  
Total 21 deaths

Moscow doctors  
April 12 arrival.  
Total 25 deaths



17 victims die with no  
hospital care

City clean-up begun.  
30,000 vaccinated.  
April 16,  
Total 42 deaths



Last recorded death  
May 16.  
Total 66 valid cases  
11 survivors

# Smallpox Epidemic Yugoslavia, 1972

Imported Virus Contagion  
“Natural Outbreak”

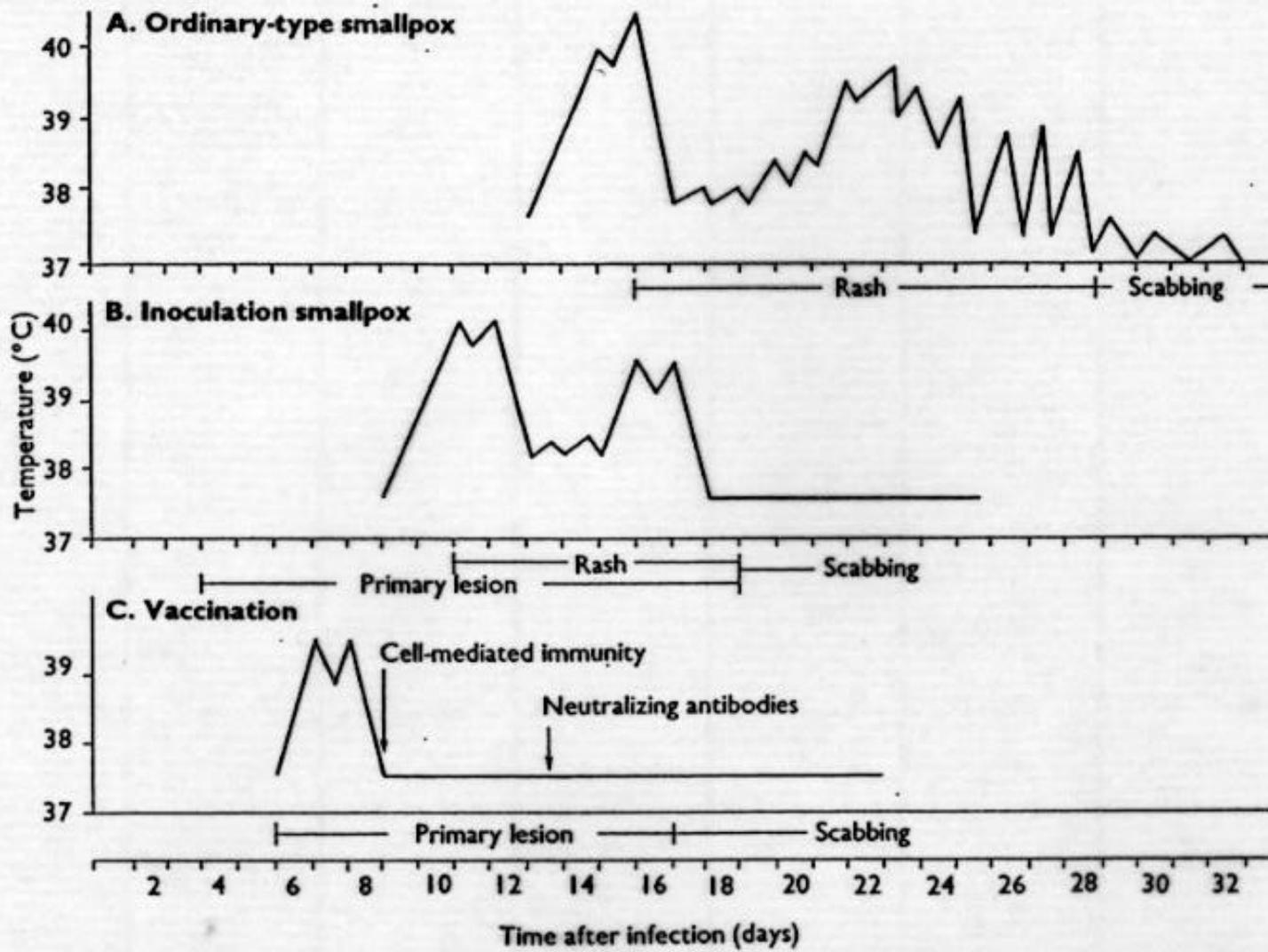


Fig. 1.3. The clinical course of moderately severe ordinary-type variola major in an unvaccinated subject (A); inoculation smallpox (variola) in an unvaccinated subject (B); and primary vaccination (C). (Temperature records from an illustration in Hime (1896) with modified wording.)

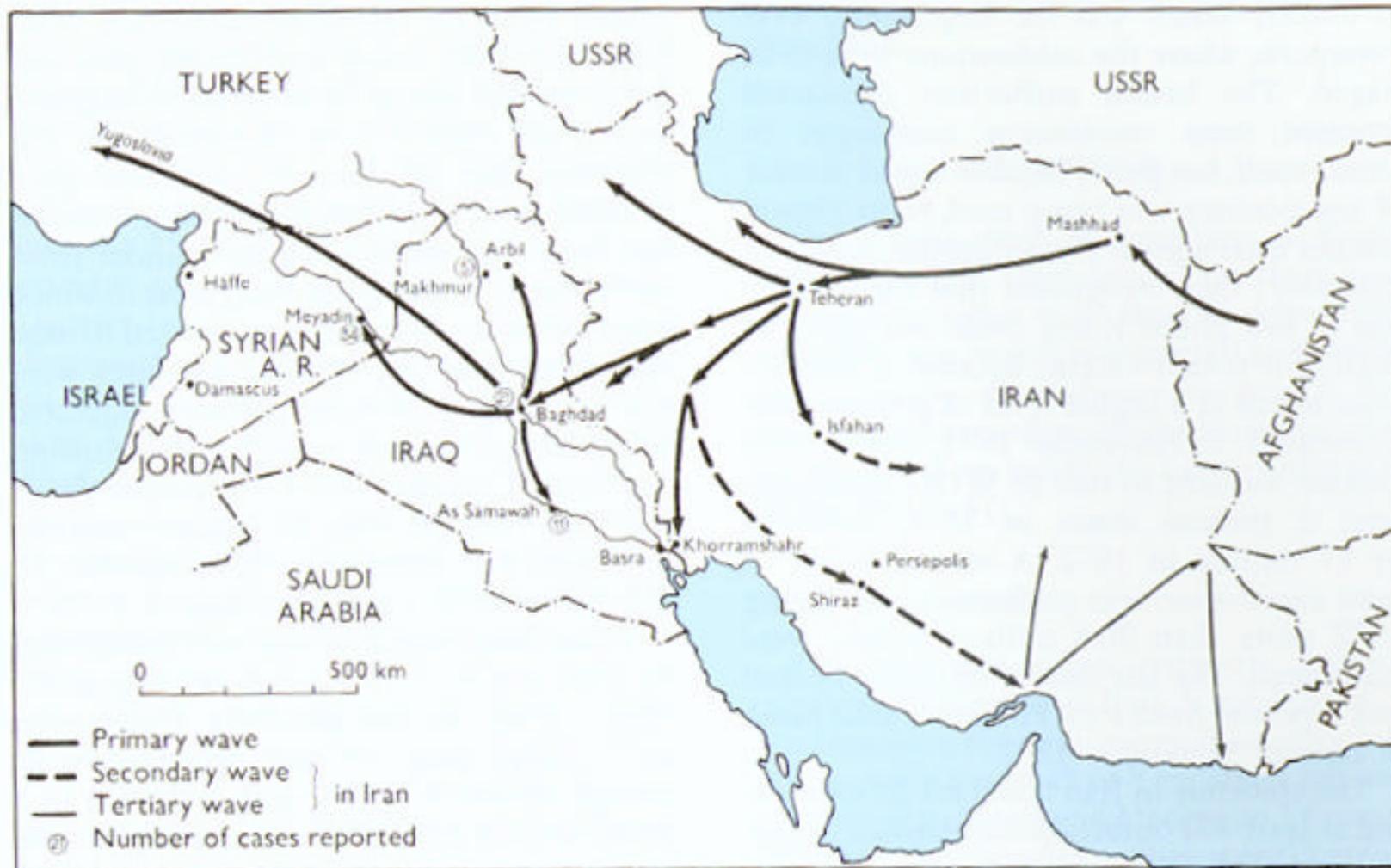
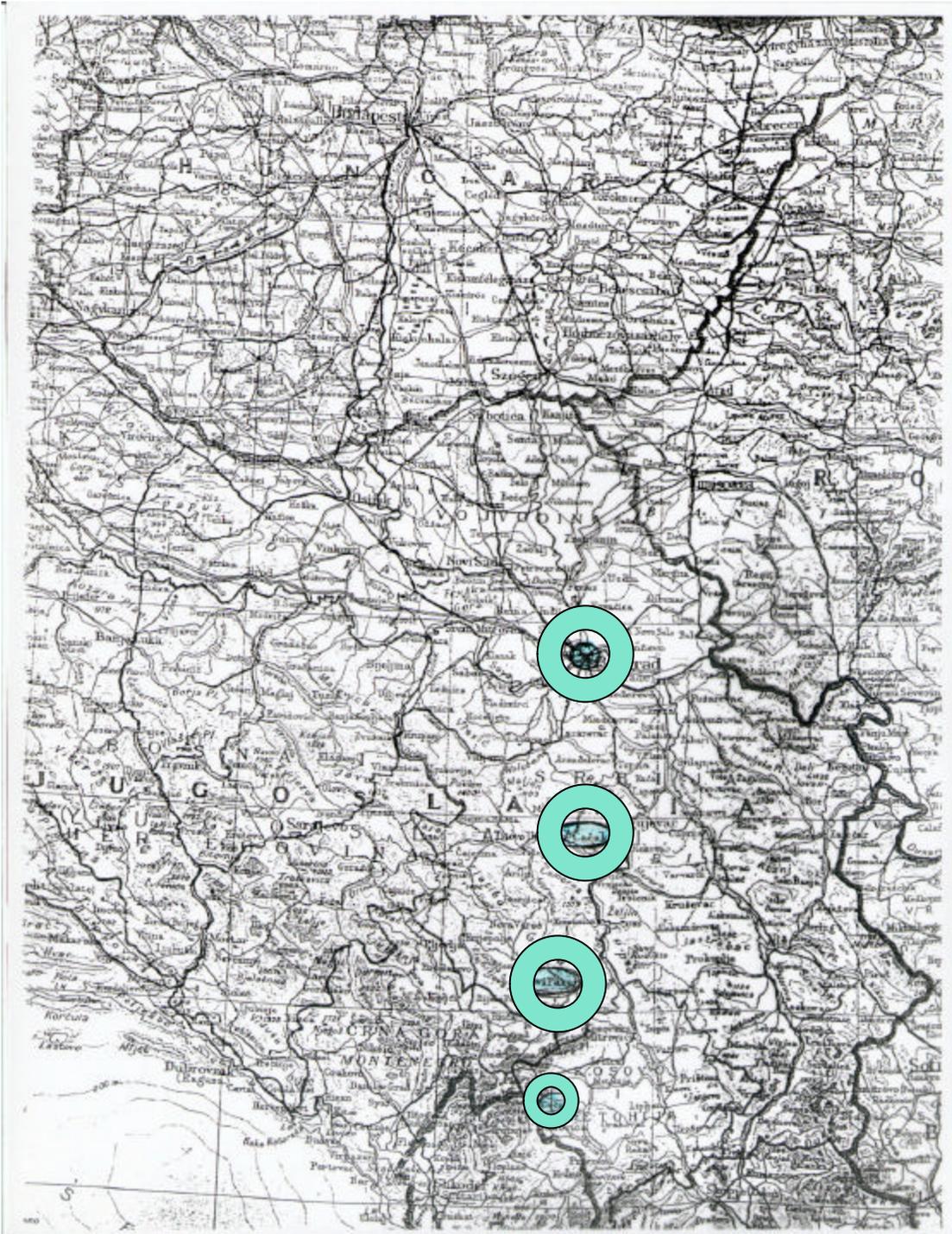


Fig. 23.5. Spread of smallpox in Iran, Iraq and the Syrian Arab Republic, 1970–1972. The disease was introduced from Afghanistan into Mashhad, Iran, in October 1970. There were three waves of dispersion through Iran, which lasted over a period of 22 months. By the end of 1971 smallpox had crossed into Iraq, where it spread north to Arbil and south to As Samawah. Transmission in Iraq was interrupted by June 1972. In February 1972, smallpox spread from Baghdad in Iraq to Meyadin in the Syrian Arab Republic, where a smaller outbreak occurred that was contained by June 1972.



Feb. 3-7 index case infected in Baghdad.

Feb.15-16 falls ill at home Danjani (Kosovo)

Mar.5 one of 11 infected by index case falls ill in Serbia

Mar.10 Serbian dies after infecting 42 in hospital

Mar.11, Serbia case total 10, Kosovo 12

Mar.13 physician in Kosovo sounds alert

**Mar.17 diagnosis and state containment initiative**

Mar.25 case total is 137

April 15 case total is 173 (123 Kosovo, 48 Serbia, 1 Vojvodina, 1 Montenegro)

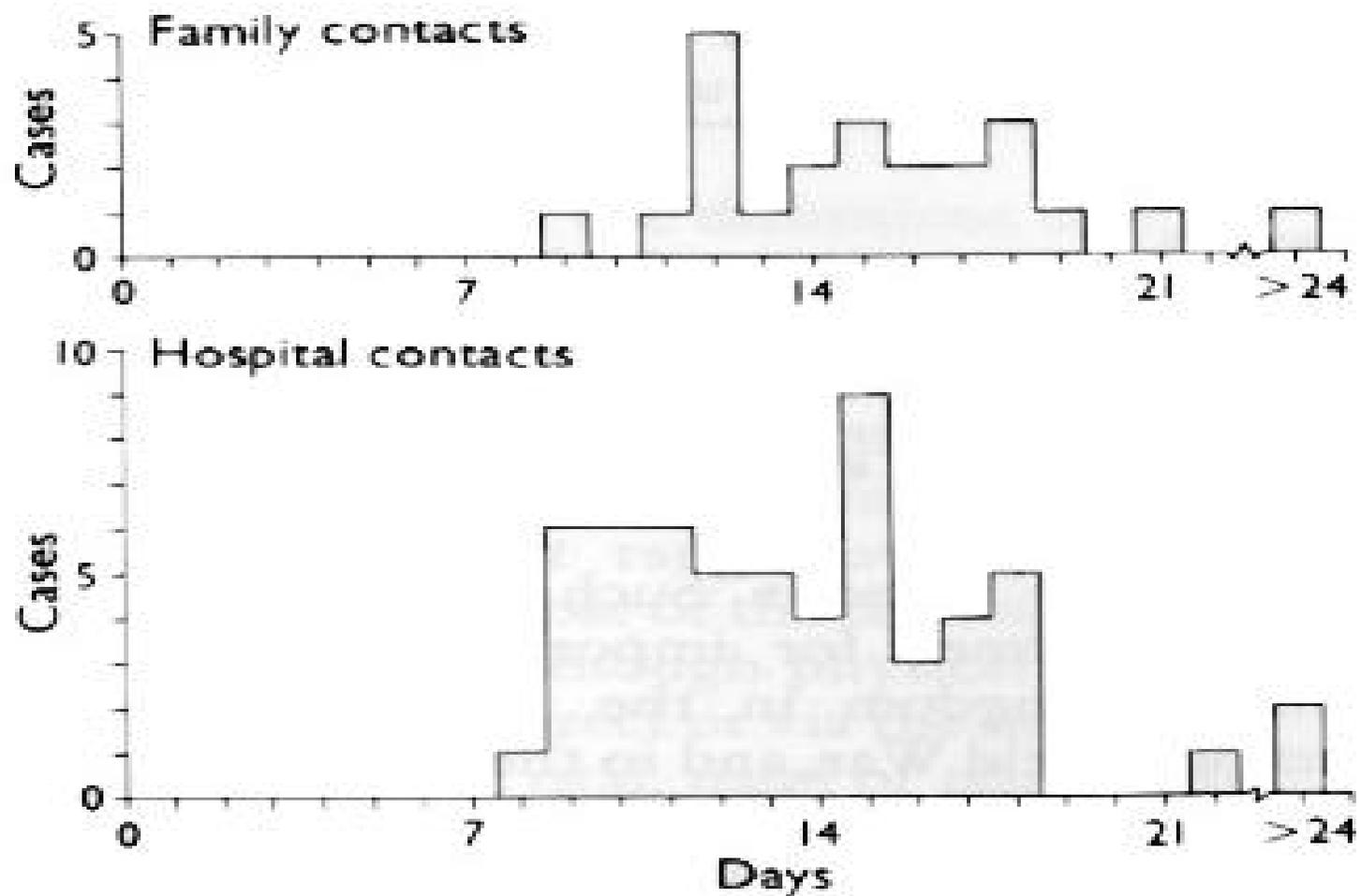


Fig. 4.8. The interval between the first possible exposure to a case of smallpox imported into Europe by air and the onset of symptoms in first generation indigenous cases, in family and hospital environments. (Based on Mack, 1972.)

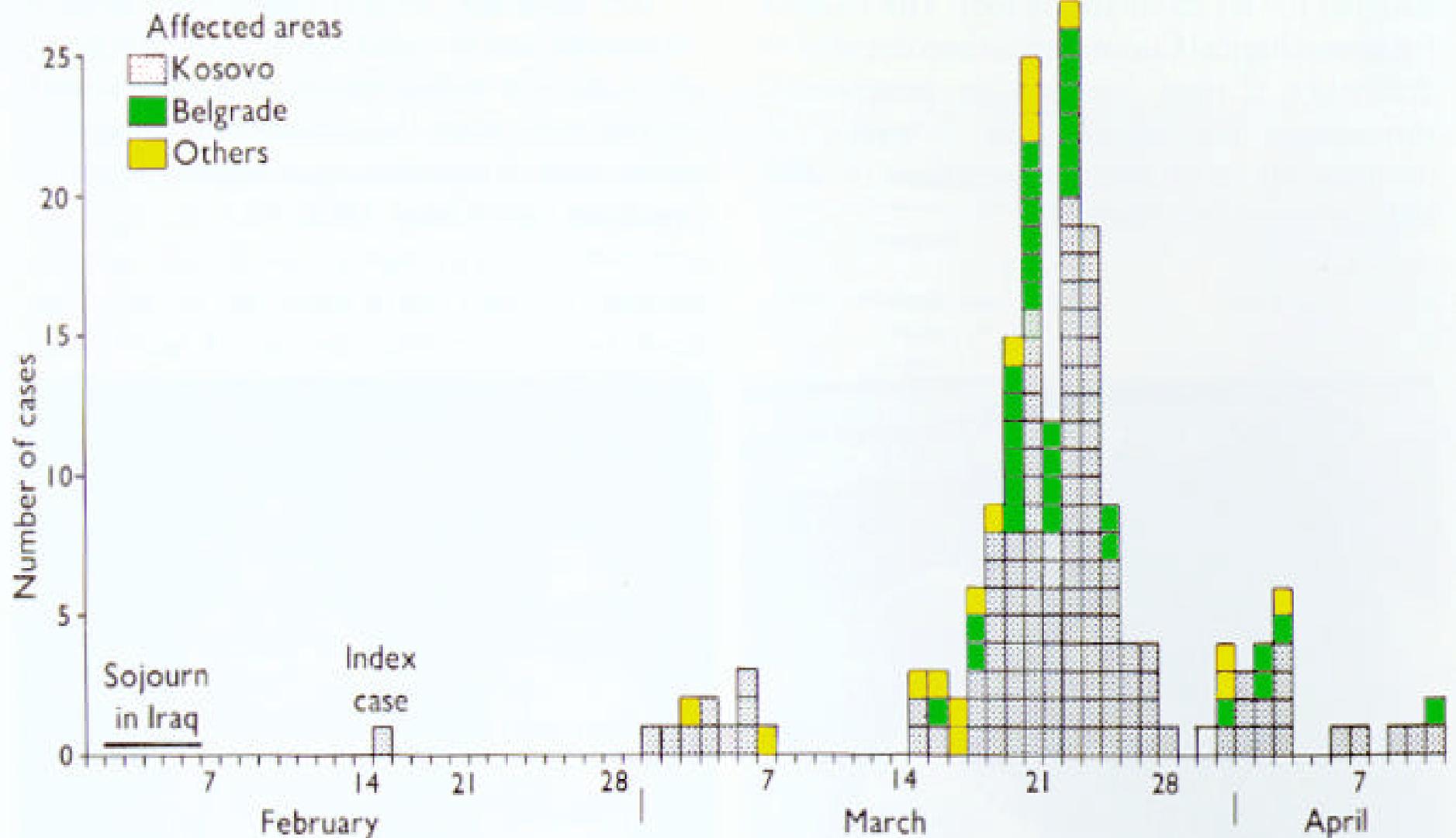
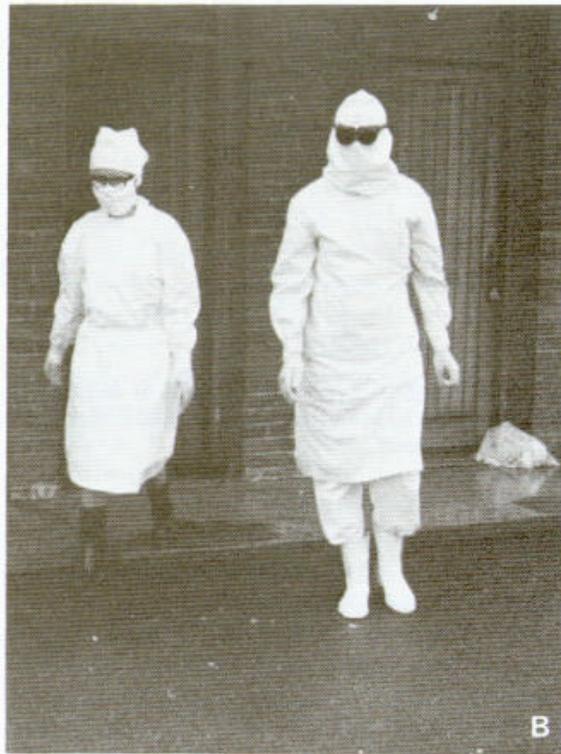


Fig. 23.7 Yugoslavia: number of cases of smallpox, by date of onset and locality, 1972. The first generation of cases occurred in Kosovo province and adjacent areas; the large second generation in Kosovo, Belgrade and some other places.



**Public Health Response**  
Mar. 15 to May 9  
Vaccine campaign,  
Quarantine, roadblocks.  
Belgrade team joins  
Kosovar local health staff  
(rural, many migrant  
workers) to begin  
concentric circles of  
Vaccinations in 25 foci,  
with family and village  
quarantine, prohibition  
of public meetings.  
18 million (of 20.8  
million citizens) were  
vaccinated in 3 weeks.  
175 cases, 35 dead (20%)  
case fatality rate. 37% of  
cases among previously  
vaccinated.

# Structural Sources of Late Diagnosis

- Political: military secrecy/religious repression
- Medical/Professional: lack of familiarity with disease (misdiagnosis)
- Communication: public uneducated about risk

# Solutions to Late Diagnosis

1. Political-public health cooperation
2. Medical technology and education
3. Accurate public communication

# US Preparedness for Biological Terrorism

Gregory Koblentz  
Security Studies Program  
MIT  
April 3, 2002

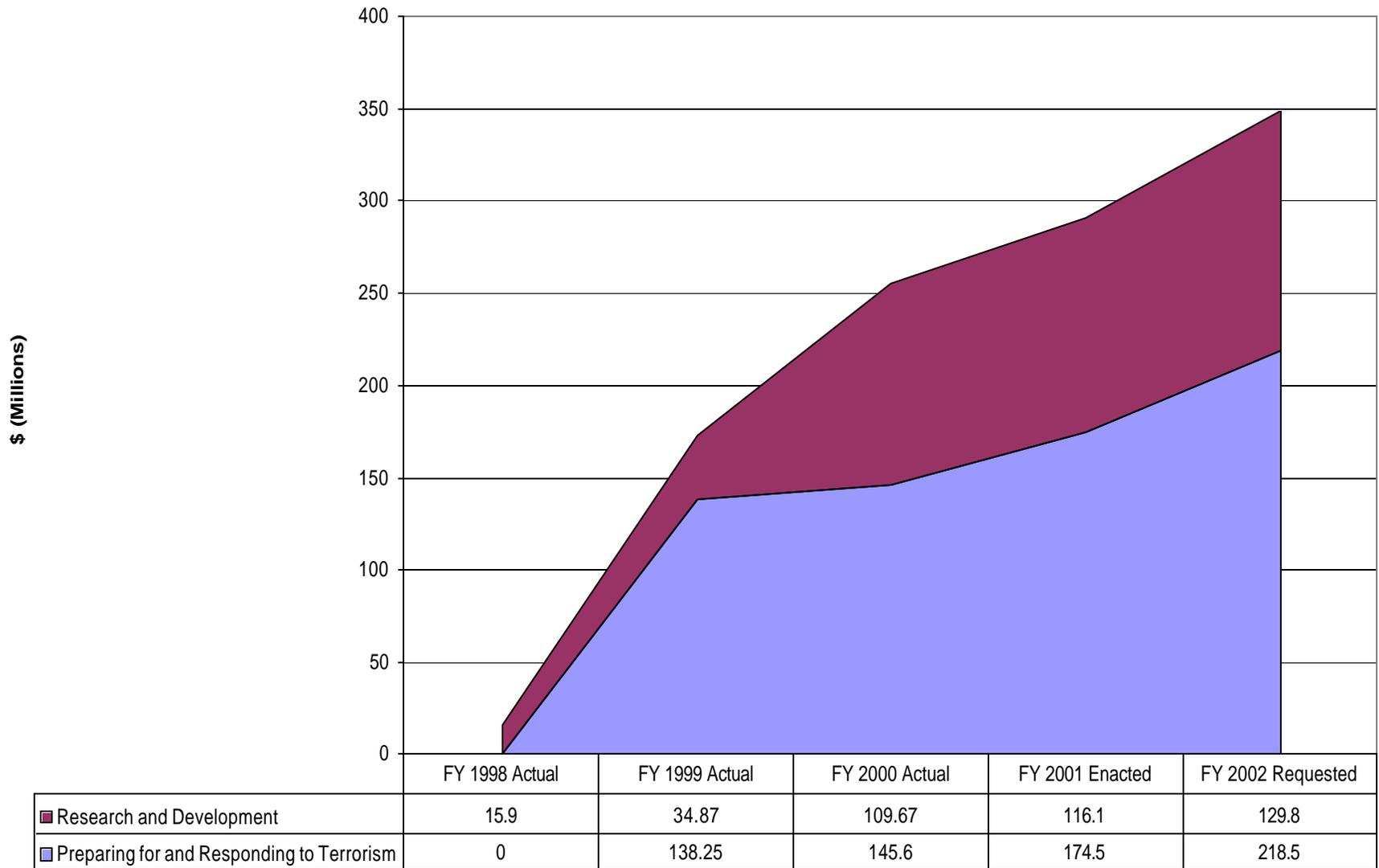
# Overview

- Background and History
- September 11 and Anthrax Letters
- Preparedness Post-September 11

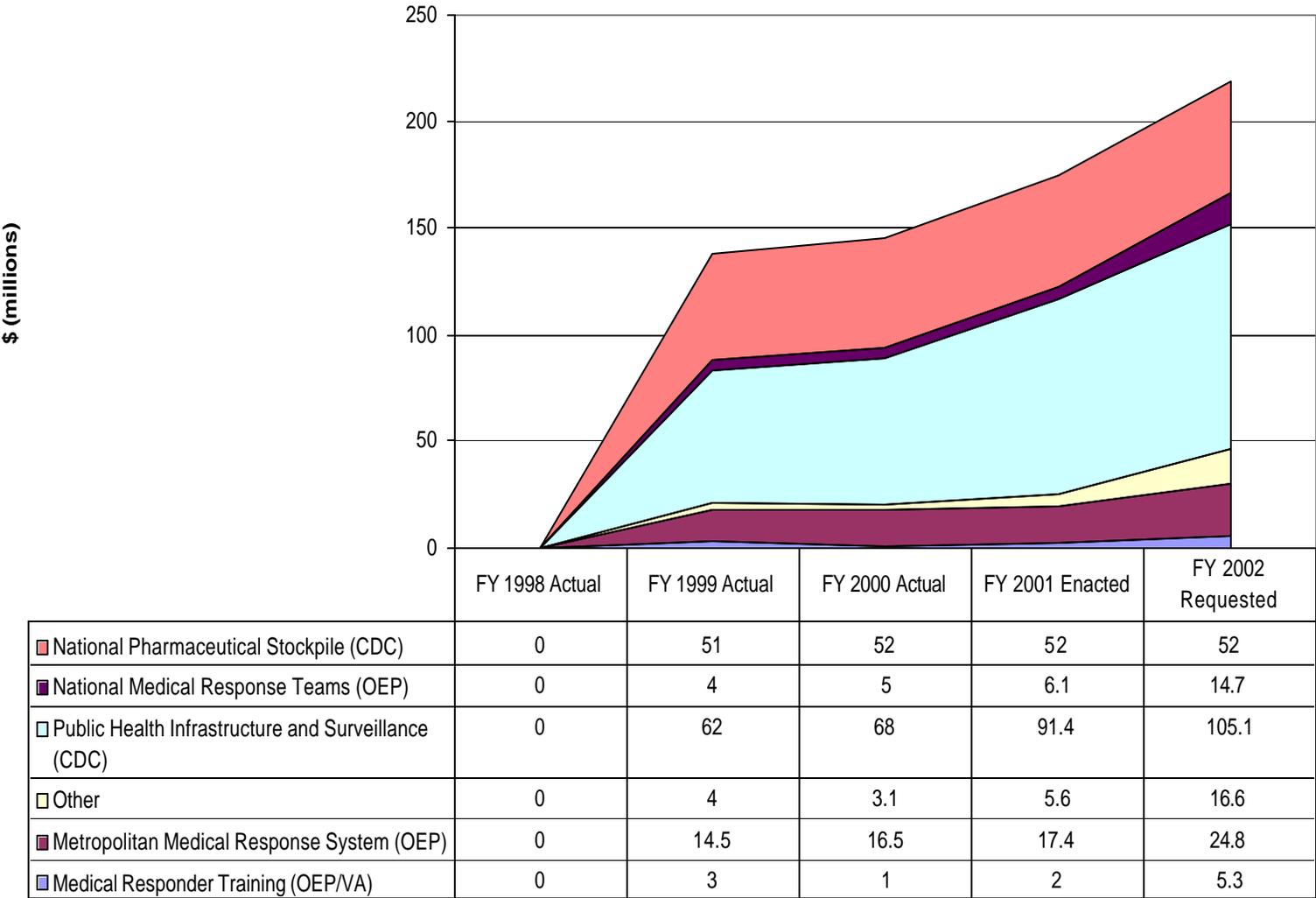
# Background and History

- 1996 Nunn-Lugar-Domenici Domestic Preparedness Program
- 1998 White House Initiative
  - Pharmaceutical Stockpile
  - Grants to State Public Health Agencies
  - Metropolitan Medical Response System
  - Research & Development

**Figure 1. HHS Spending on Bioterrorism Preparedness and Research, 1998-2002**



**Figure 2. HHS Spending on Bioterrorism Preparedness, 1998-2002**

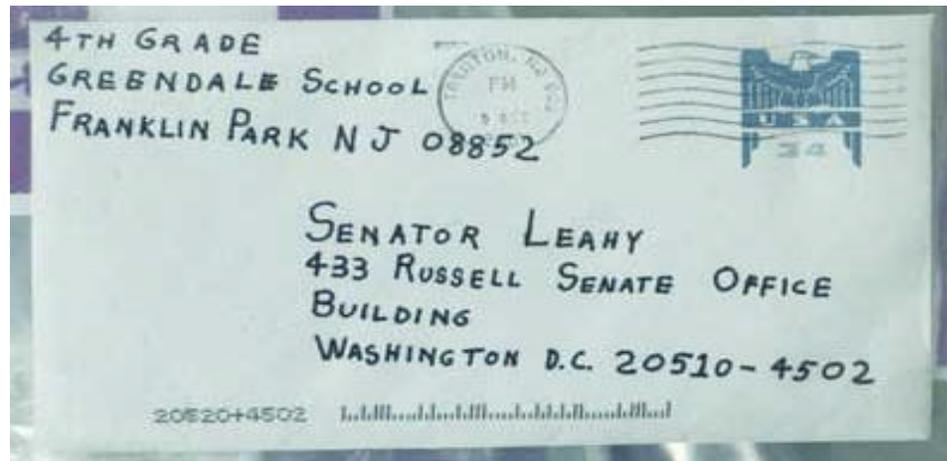


# Bioterrorism Preparedness Prior to September 11

- State and Local Laboratory, Surveillance and Epidemiological Capabilities Improving Slowly
- Pharmaceutical Stockpile in Place
- Hospitals and Healthcare Providers Neglected

# The Anthrax Letters

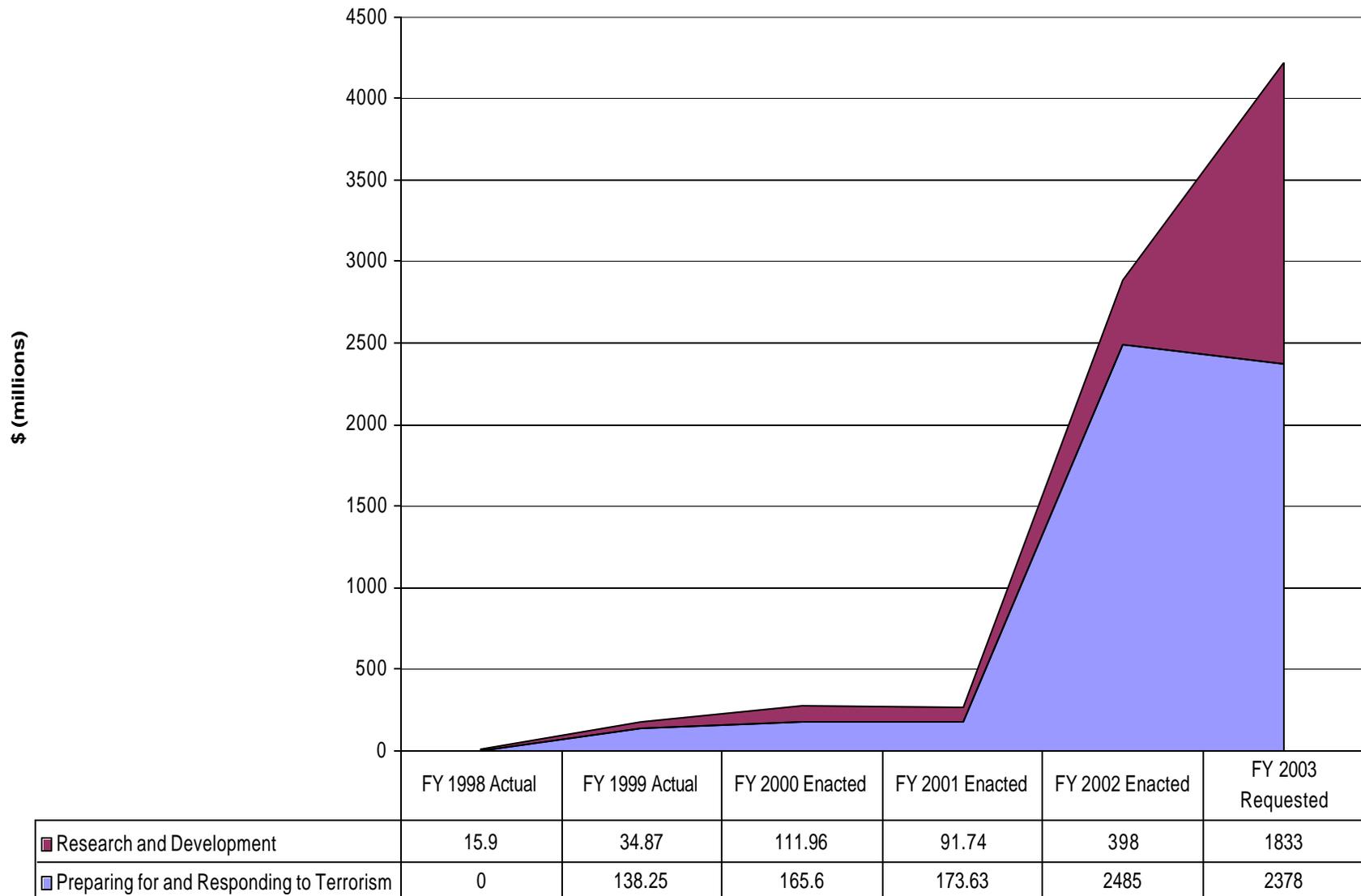
- 5 letters each with ~2 grams of anthrax
- 23 confirmed cases: 5 fatalities
- > 10,000 on antibiotic prophylaxis
- Cost of response: \$250 million



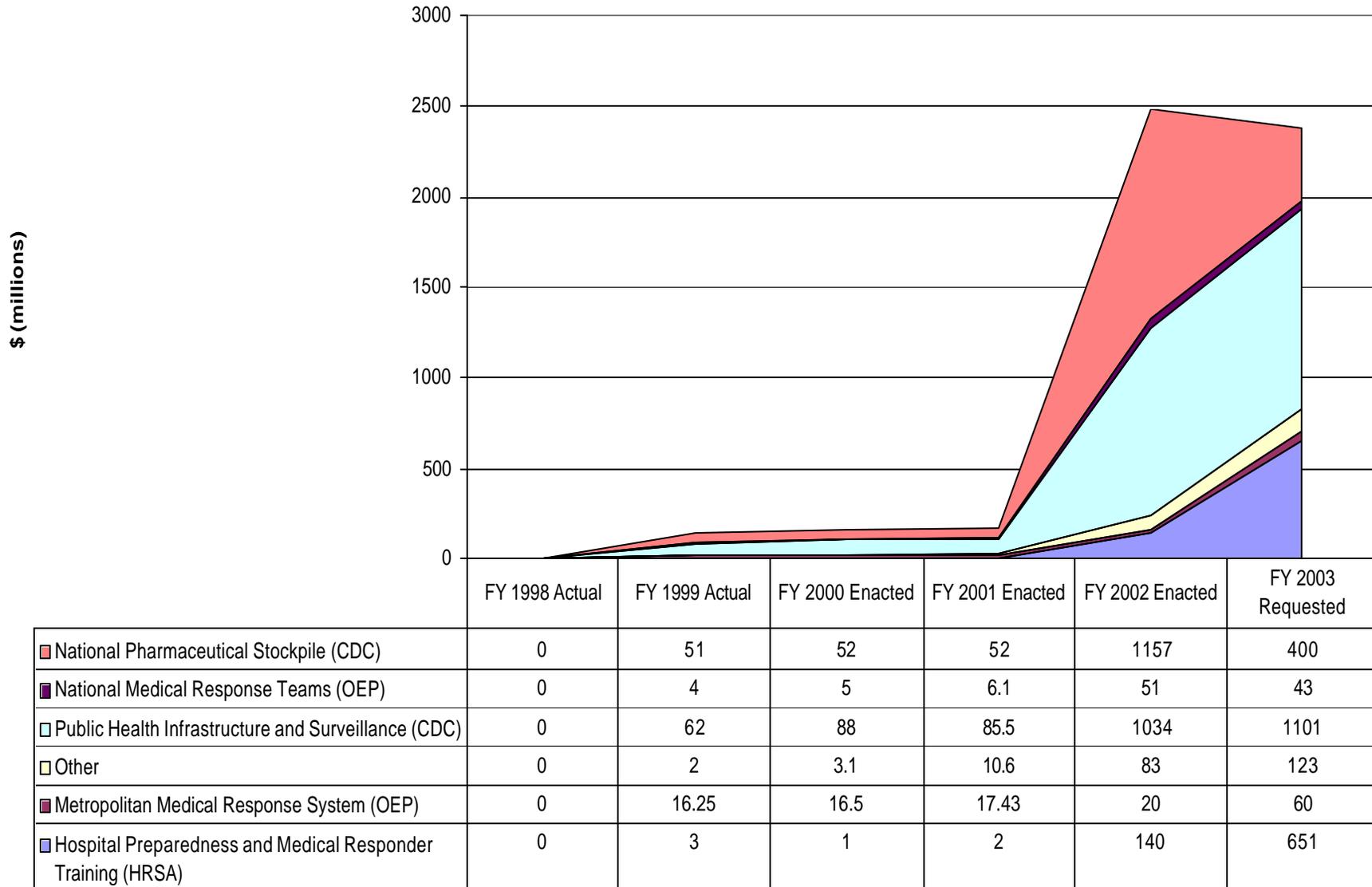
# Lessons Learned

1. Expect the Unexpected
2. Doctors are the First Line of Detection
3. Early Treatment is Key
4. Lab Capacity Needs to Be More Robust
5. Coordination and Communication Problems
6. Flawed Knowledge Assessment
7. Importance of Forensics

**Figure 3. HHS Spending on Bioterrorism Preparedness and Research, 1998-2003**



**Figure 4. HHS Spending on Bioterrorism Preparedness, 1998-2003**



# Post-September 11 Preparedness

- Existing Programs Expanded and/or Accelerated
  - Public Health Infrastructure
  - Pharmaceutical Stockpile
  - Metropolitan Medical Response System
  - Research and Development
- New Programs Established
  - Hospital Preparedness
  - Medical Responder Training

# Assessment of Post-September 11 Preparedness

- National Strategy is Required
  - Interagency
  - Intergovernmental
  - Interdisciplinary
  - Public-Private
- Bioterrorism is NOT Another Emerging Infectious Disease
  - Mother Nature vs. Bin Laden
  - Criminal and National Security Implications

# Public Health Surveillance: A local health department perspective



M. Anita Barry, MD, MPH

Director, Communicable Disease Control

Boston Public Health Commission

# Objectives

- Current public health surveillance
- Characteristics of the ideal surveillance system
- Boston's enhanced surveillance system for bioterrorism and mass casualty events
- Future plans

# Types of Surveillance

- Notifiable disease reporting
- Active surveillance
- Laboratory based surveillance
- Population based surveillance

# Notifiable Disease Reporting

- Health care providers are required by law or regulation to notify public health about:
  - Named pathogens
  - Specified diagnoses
  - Outbreaks or clusters of illness
- Usually a passive system, but can use enhanced passive technique
- Reporting requirements differ among states

# Notifiable Disease Reporting: Why it's incomplete

- Unaware of the requirement to report
- Confused about the mechanics of reporting
- Concern about confidentiality
- Someone else's job
- Unconfirmed case (wrong diagnosis, no lab)
- Forgot to do it

# Active surveillance

- Public health staff review records and other data on site (for example, at a hospital)
- Provides fairly complete data
- Very labor intensive and requires a sustained effort - resources become a problem

# Laboratory based surveillance

- Laboratories are required to report certain positive test results to public health
- Isolated laboratory data are incomplete
  - False positives, false negatives
  - Skewed testing (publicity, specific signs and symptoms)
- Molecular microbiologic techniques enhance epidemiologic investigations

# Population Based Surveillance

- Illness in closed communities (such as incarcerated populations)
- Absenteeism rates
- Insurance claims data
- Sales of specific products (such as anti-diarrheal medications)

# The Ideal Surveillance System

Fast, cheap, and easy...

# The Problem

- Traditional surveillance systems based on the reporting of specific diseases have limited potential for early detection of mass casualty events such as bioterrorism or pandemic influenza.

# Milwaukee: Cryptosporidium Infection Related to the Public Water Supply

- Estimated 400,000 people had outbreak associated diarrhea.
- 285 laboratory confirmed cases.
- Recognition of the outbreak was delayed:
  - Non-specific nature of the symptoms
  - Limited laboratory testing
  - Infrequent use of the health care system by people with diarrhea

# Identification of the Outbreak

- Shortages of over the counter anti-diarrheal medications
  - pharmaceutical sales data impacted by sales & is unlikely to detect small case numbers
- Retrospective data indicated changes in health care utilization patterns prior to identification of the outbreak

# Agents of Concern: CDC Category A

- *Bacillus anthracis* (anthrax)
- *Clostridium botulinum* toxin (botulism)
- *Yersinia pestis* (plague)
- variola major (smallpox)
- *Francisella tularensis* (tularemia)
- Viral hemorrhagic fever

# Agents of Concern: CDC Category B

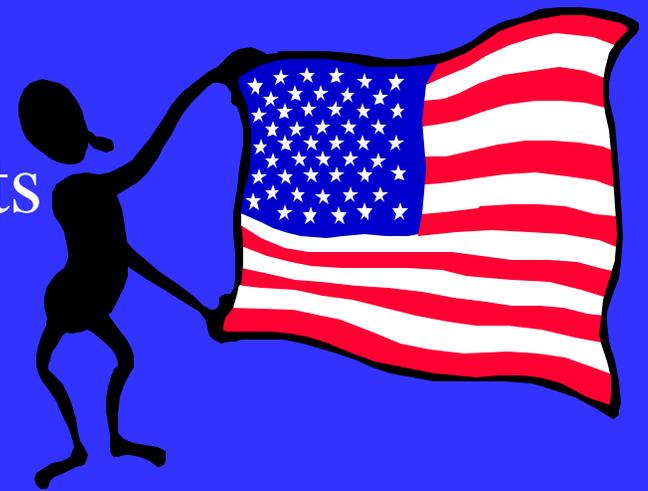
- *Coxiella burnetti* (Q fever)
- *Brucella* species (brucellosis)
- *Burkholderia mallei* (glanders)
- ricin toxin from *Ricinus communis* (castor beans)
- epsilon toxin of *Clostridium perfringens*

# Agents of Concern: CDC Category C

- Nipah virus
- hantaviruses
- tickborne hemorrhagic fever viruses
- yellow fever
- multidrug-resistant tuberculosis

# Bioterrorism Events in the United States

- 1984, The Dalles, Oregon
  - Salmonella in salad bars
  - 751 ill (45 hospitalized)
- 1996, Dallas, Texas
  - Shigella in micro-lab donuts
  - 12 ill (4 hospitalized)



# Anthrax Cases, 2001

Anthrax Among Outbreak-related Cases 2001						
Cases	FL	NYC	NJ	DC	CT	Total
Inhalational	2	1	2	5	1	11
Cutaneous						
Confirmed	0	4	3	0	0	7
Suspected	0	3	1	0	0	4
Total	2	8	7	5	1	22

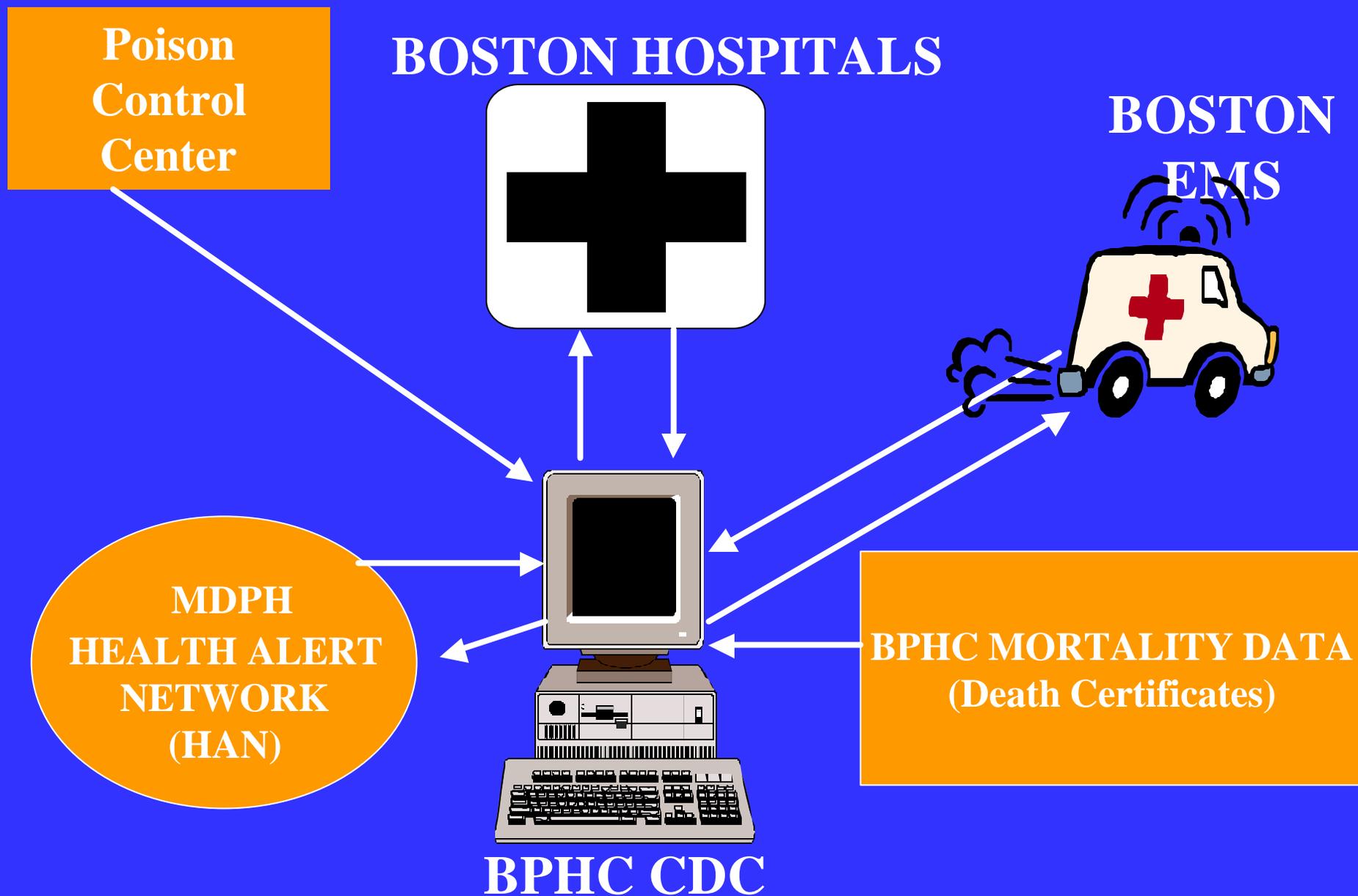
# The Ideal Surveillance System

- Sensitive (with enough specificity to make it workable)
- Timely
- Provides complete data
- Cost effective
- Linked to an effective follow-up system to interpret initial signals

# Enhanced Surveillance in Boston

- Emergency department visits
- Urgent care visits
- Boston EMS calls
- Death certificates
- Poison Control Center

# VOLUME SURVEILLANCE SYSTEM DESIGN: DATA SOURCES



# Enhanced Surveillance in Boston: Hospitals

- Every 24 hours volume data is electronically sent by SFTP to the Boston Public Health Commission (BPHC)
- Threshold data for each site based on historical data has been calculated
- If threshold is exceeded an initial assessment is automatically sent to an onsite contact

# Calculations

**Binomial distribution: adjust for month and day of the week**

**Number of events=average daily volume by month**

**n=Boston population (1990 census)**

**p= number of events/n**

**Upper CI= $p + ((1 - ?)(\sqrt{p(1-p)/(n)}))$**

**Upper threshold = Upper CI(n)**

# **Enhanced Surveillance in Boston: Hospitals (Cont'd)**

- If a cluster or any unusual cases of illness are identified on initial assessment, BPHC nurses/epidemiologists investigate further
- Data are typically available within 12 hours after the close of a 24 hour period

# Enhanced Surveillance in Boston: Other Sites

- Poison Control Center: daily volume data being sent, thresholds being adjusted
- Boston EMS: type of calls of interest selected, automatic data transfer being developed
- Death Certificates: database developed; timeliness of data input being addressed

# Enhanced Surveillance in Boston

## Preliminary Findings

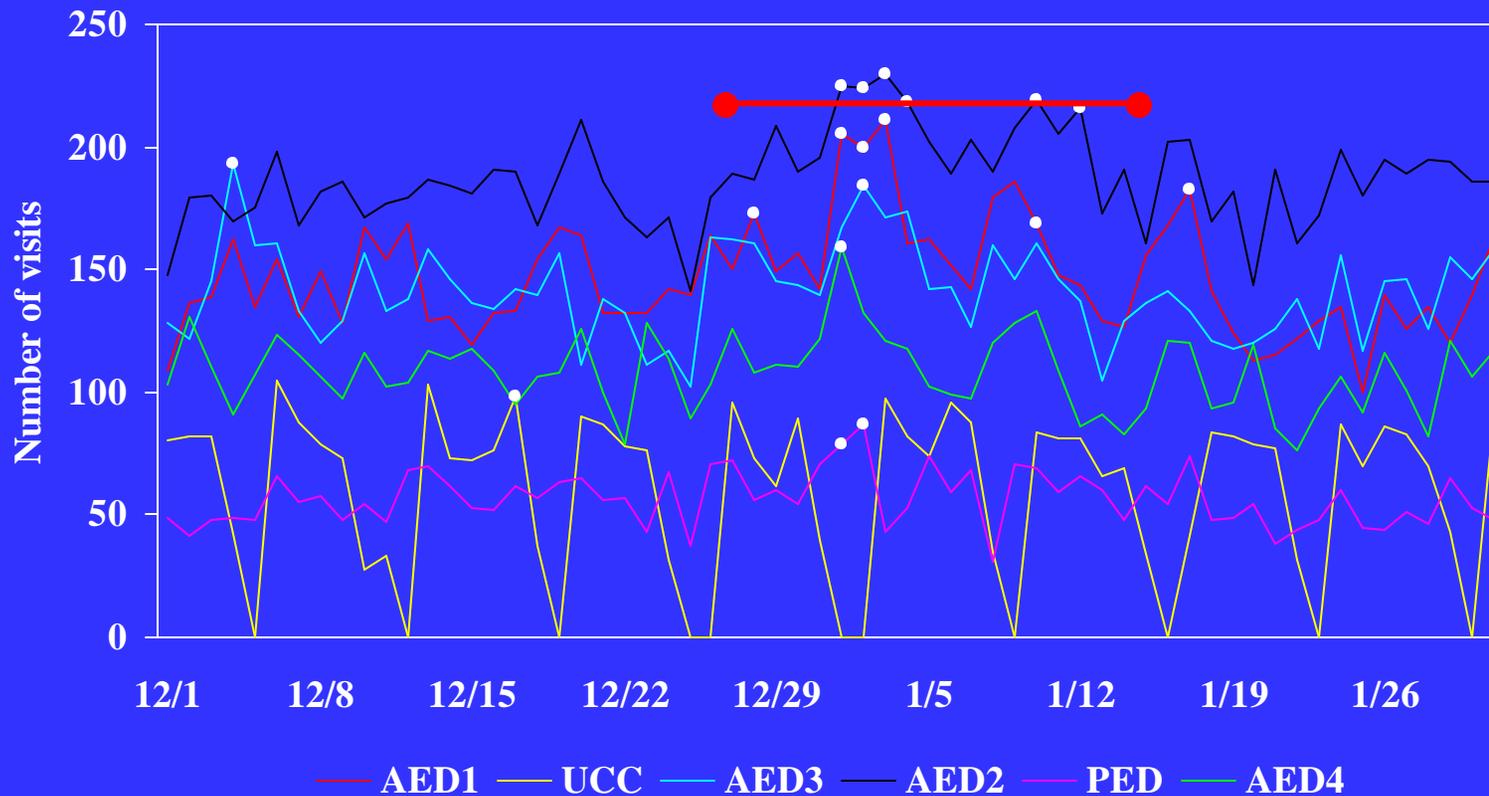
- System detected morbidity associated with a heat wave (retrospective)
- Volume data corresponded well with influenza activity in 1999 and 2000
- System identified changes in health seeking behavior post September 11

# Volume data and influenza

- In 2000 there were 103 episodes of a site exceeding threshold.
- However, 3 or more sites simultaneously exceeded threshold on only 4 days and 2 sites on 17 days.
- Most of the time (N=54), only one site exceeded threshold on a given day.

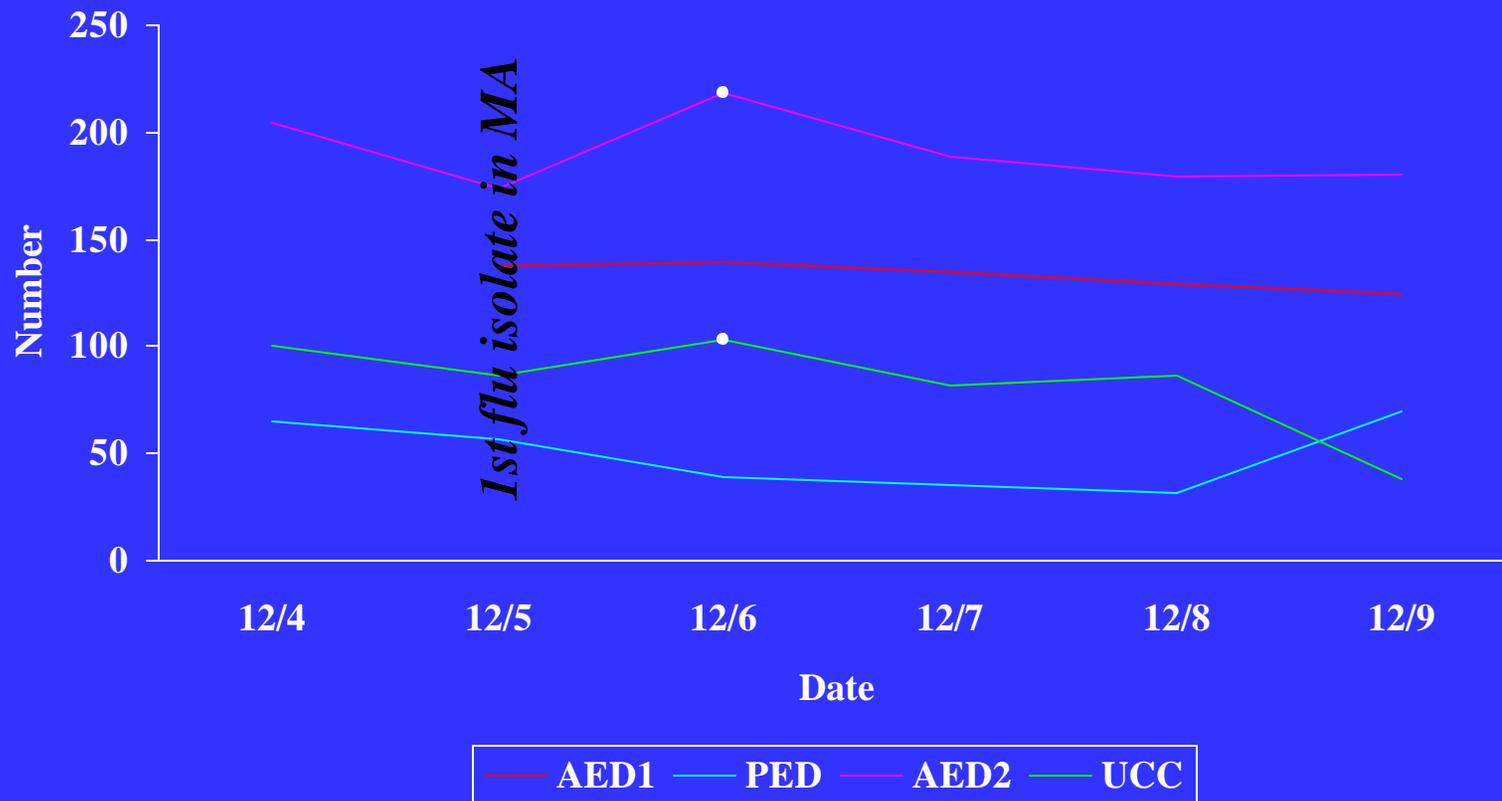
# Daily volume by site

## December 1, 1999 - January 31, 2000



- Days exceeding threshold
- Peak influenza activity in the U.S. (12/26 to 1/15/00)

# Volume Surveillance - 12/4 to 12/9/00

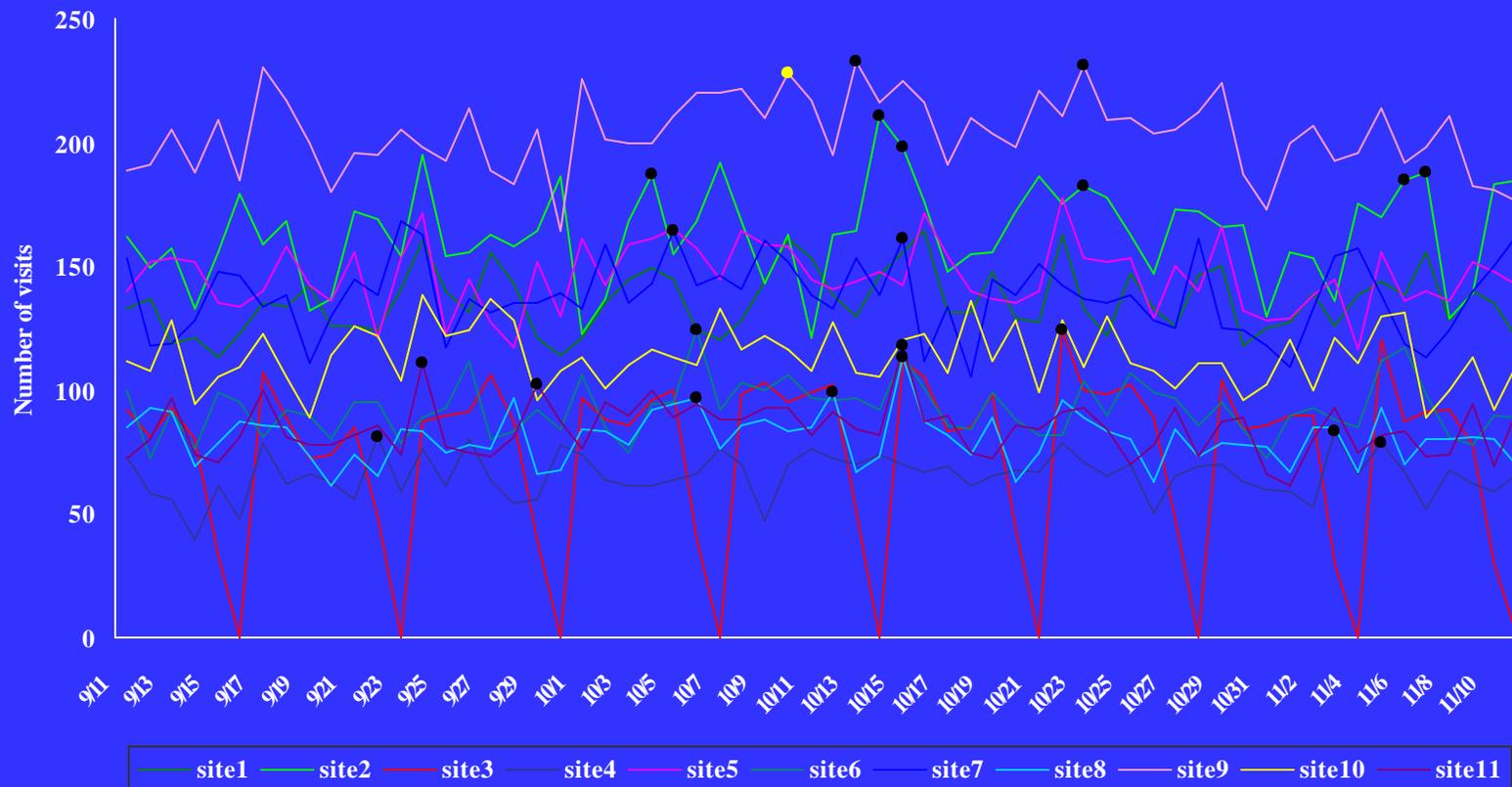


- Exceeded threshold

**Volume data: Findings from  
9/11/02 -11/11/02**

# Daily volume by site

## September 11, 2001 - November 11, 2001



?Days exceeding threshold. No infectious disease clusters identified.

## How many times did multiple sites exceed threshold on a given day?

- There were 22 episodes of a site exceeding threshold in the time period.
- For most (n=17) only a single site exceeded threshold on a given day.
- On two days, two sites simultaneously exceeded threshold.
- On one day, four sites simultaneously exceeded threshold.

# Follow-Up with sites exceeding threshold and Boston Public Health Commission's (BPHC) Response

- Persons seeking nasal swabs and antibiotics for anthrax resulted in increased activity on 10/15
- No anthrax cases or anthrax contaminated environmental specimens were identified in Massachusetts
- The BPHC posted information on anthrax including updates to BPHC's website ([www.bphc.org](http://www.bphc.org))
- Clinical advisories on anthrax were emailed to health care providers throughout the city

# Enhanced Surveillance in Boston

## *Strengths*

Adjusts for site case mix

Adjusts for seasonal changes

City wide coverage

Electronic

## *Weaknesses*

Non-specific for BT events

Changes influenced by the business of health care

# Conclusions

- Volume based surveillance is a feasible method for the early identification of a mass morbidity event
- A rapid follow-up system is a critical component to understanding initial signals
- Data from this system can be used to create educational messages for both health care providers and the public
- Additional research is needed to define the sensitivity of the individual or combined measures being used and the optimal combination to detect significant activity

# Enhanced Surveillance in Boston: Lessons Learned

- Systems must be electronic
- Add on systems will not be sustainable
- Computers system go down (even for days)
  - Develop back up plans
- Don't abandon case reporting
  - No one system is perfect
- The more complex data - the harder it will be to retrieve it manually
- Build communication networks into the surveillance system

# Enhanced Surveillance in Boston: Future Plans

- Capture more granular data
  - Chief complaint data
  - Natural language programming
  - Minimize human contact
- Add additional populations and types of health care sites
- Enhance the surveillance feedback loop
- Syndromic surveillance

# **Syndromes That May Be Associated With Bioterrorism**

- **Pulmonary**
  - **Fever**
  - **Cough**
  - **Myalgias**
  - **Hypoxia**
- **GI**
  - **Fever**
  - **Nausea/vomiting**
  - **Diarrhea (+/-bloody)**
- **Rash and fever**
  - **Vesicular**
  - **Petechial**
- **Neurologic**
  - **cranial nerve palsies, HA, fever, confusion**
- **Septic Shock**
  - **DIC**
  - **Organ failure**

# Syndromic Surveillance

- ICD-9 code data or chief complaints to identify potential BT-related syndromes
  - How much is to much
  - Follow-up is critical
  - Real time data is limited
  - Sustainability
  - Validity of chief complaint data - How do different populations describe illness

# Questions?



# Bioterrorism Preparedness - Laboratory Analysis

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# Bioterrorism Preparedness - Laboratory Analysis

An account from  
the “real world”  
of the clinical  
microbiology  
laboratory



# Clinical Laboratories - The Need for Preparation

- Agents likely to be used by terrorists
  - Unfamiliar, rarely encountered organisms
  - Potential for misidentification, mishandling of specimens, laboratory acquired infection
- Public health agency-sponsored training in the Northeast began in 1999
- Laboratory Response Network (LRN)
- Were we prepared in the autumn of 2001?



# LRN Level A Lab Preparedness

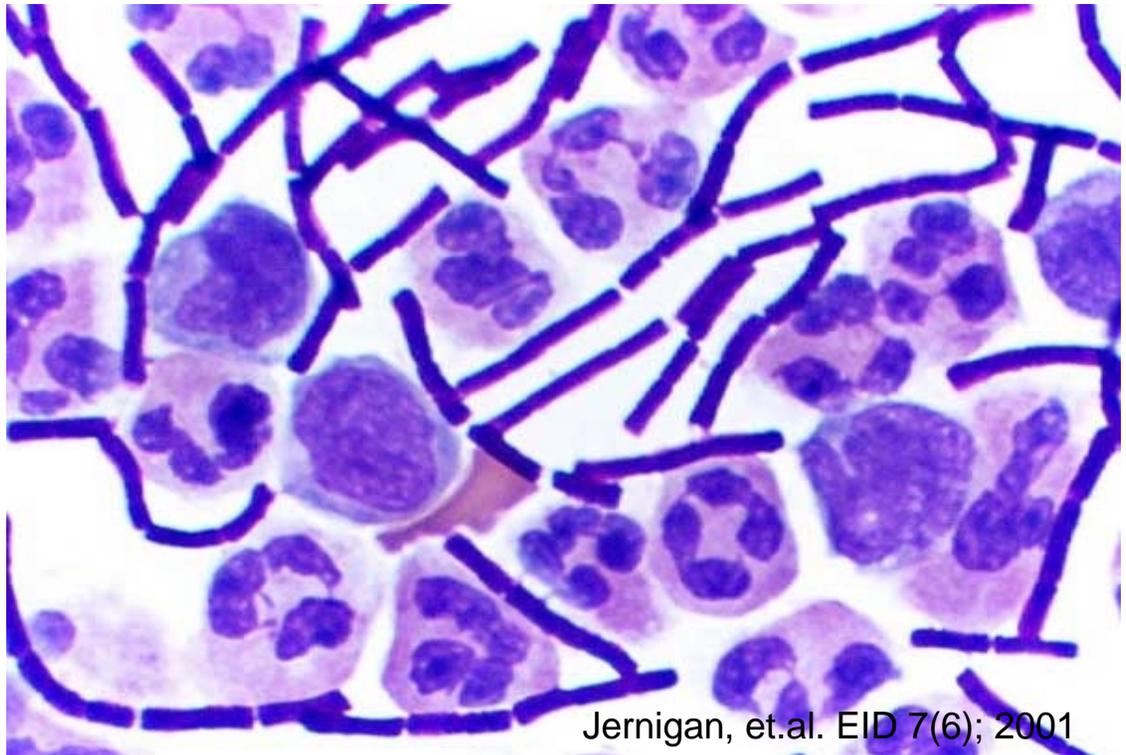
- Level A laboratory functions
  - Rule out / refer
  - Ship suspicious infectious agents to higher level labs for further study
- Level A laboratory activities
  - Formulate laboratory procedures
  - Train staff
  - Biosafety concerns
- Assistance from public health agencies

# Activities of Clinical Micro Labs

- “Average” Labs
  - Microscopic examination of specimens
  - Culture of specimens and isolation of many bacterial and fungal pathogens
  - Identification and susceptibility testing
- “Advanced” Labs
  - Viruses (culture, direct detection)
  - Mycobacteria (culture, susceptibility)
  - Certain fungi (culture and identification)
  - Molecular testing

# Level A Lab Example: *B. anthracis*

- Gram stain\* of CSF, positive blood culture or wound culture shows large gram-positive rods



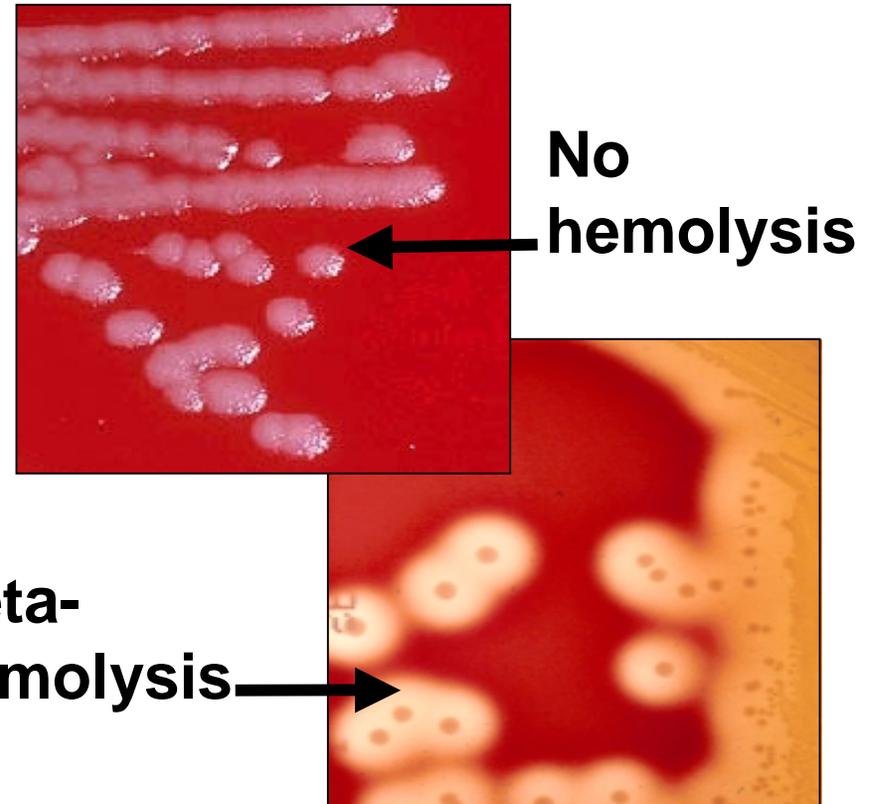
Jernigan, et.al. EID 7(6); 2001

\*Gram stain: Differential stain, not specific, but can be extremely helpful



# Level A Lab Example: *B. anthracis*

- Culture on blood agar\*. Examine for characteristic colony morphology and lack of beta-hemolysis



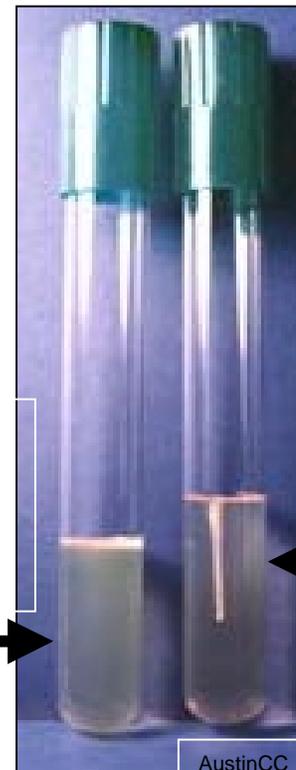
\*Agents of anthrax and plague are “easy” to grow. Agents of tularemia, brucellosis are harder to recover, may require special media



# Level A Lab Example: *B. anthracis*

- Perform identification tests. For ?*B. anthracis*, perform motility test\*

Growth throughout medium (motile)



Growth only near original inoculation stab (non-motile)

\*Minimal rule out tests (minimal manipulation of potentially dangerous cultures) are recommended for Level A labs



# Level A Lab Example: *B. anthracis*

- Ruled in?
  - *Bacillus* species with characteristic colony morphology, non-hemolytic, non-motile
- REFER
  - Contact Level B lab
  - Ship suspect isolate



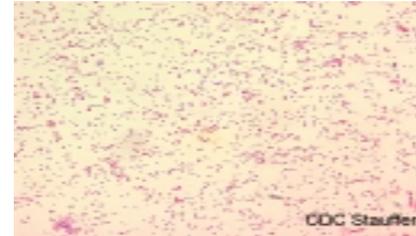
# Level A Lab Preparedness - Where Are We Now?

- Bigger seems to be “better”
  - Wider variety of pathogens encountered; personnel experienced in working with infrequently isolated agents
  - More and/or better biosafety equipment
  - Institutional support for needed resources is more likely in larger hospitals
- Small labs can still have successful preparedness programs

# Level A Lab Preparedness



**Anthrax**



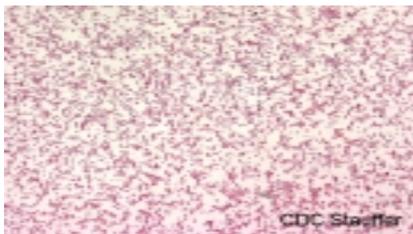
**Brucellosis**



**Plague**



**Botulism-  
Specimen processing/  
shipping only**



**Tularemia**



**Smallpox, VHF-  
More guidance needed  
for Level A labs**



**Environmental testing  
for *B. anthracis* spores**

# Clinical Lab Preparedness – Next Steps

- Extend training (category B agents)
- Enhance communication/cooperation with higher level public health labs
  - NLS
- Dissemination of some Level B procedures to select Level A labs
  - ?Rapid, specific tests/reagents
  - ?BSL3 activities in select labs
  - ?Surge capacity

# Level A Clinical Microbiology Laboratories

- Can be instrumental in early recognition
- Must be trained, alert and vigilant
- Form partnerships with public health labs for BT preparedness assistance, BT response plans, and overall improvement of the public health system

---

# Urban Testbed Initiative

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**MIT Lincoln Laboratory**  
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**3 April 2001**

MS-15433

This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.

50  
years





# Outline

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- **Thoughts on Urban Biodefense**
- **Importance of Testbeds**
- **MIT LL Urban Testbed Initial Approach**
- **MBTA subway experiments**
- **Algorithmic approach**
- **Future Work**



# Challenges Associated with Civilian Biodefense

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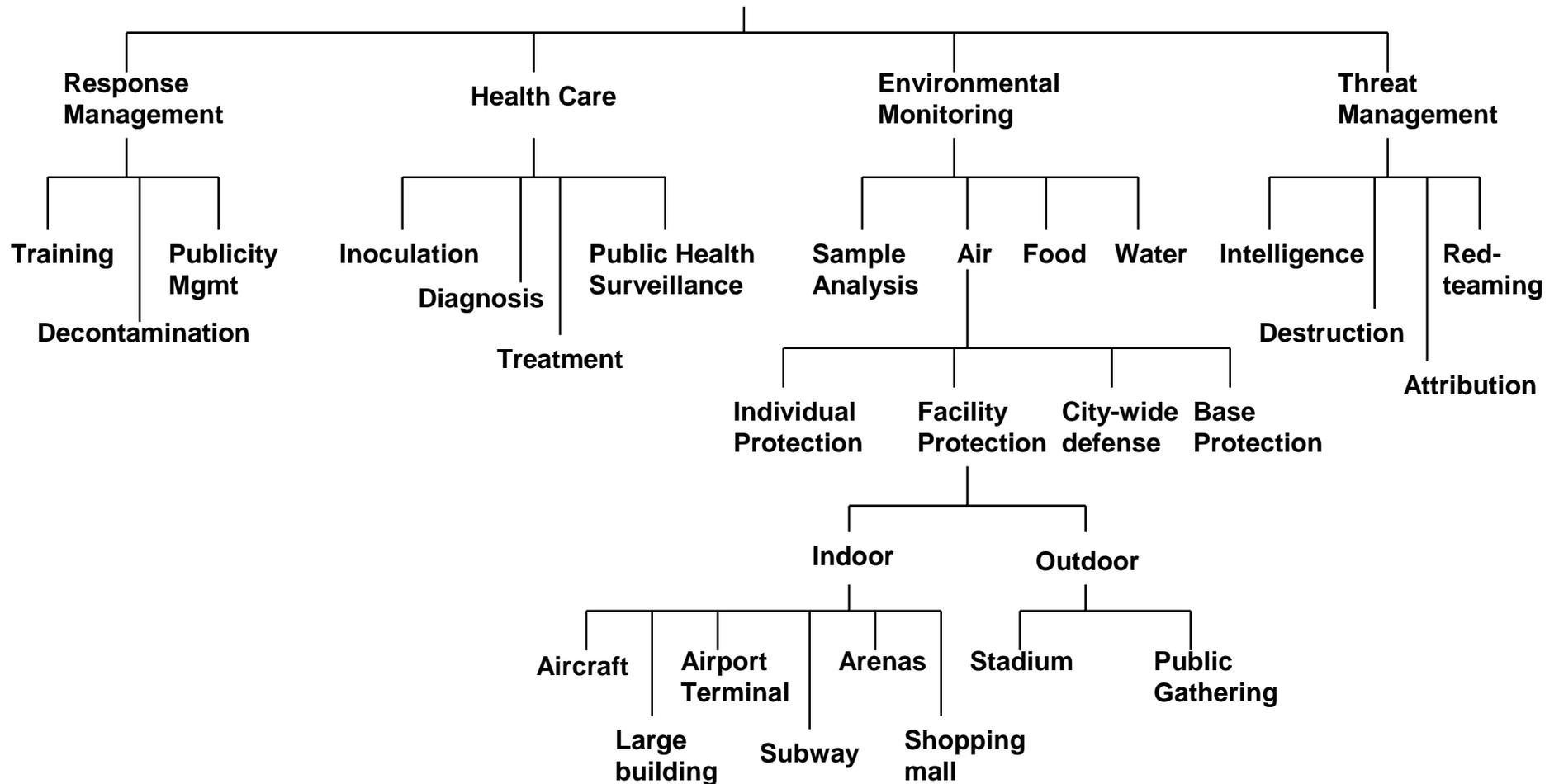
- **Any high-density site (city, airport, facility, building) represents a potential target**
- **Population to be protected is diverse (age, mobility, health)**
- **No environmental sensing systems will be tolerated that have high false negative or false positive rates**
  - **If they alarm too much or miss events, they will be ignored**
- **Current clinical diagnostic technologies and medical infrastructure are not suited to rapid detection of bioagent events**
  - **Advanced diagnostics (e.g., PCR) use is rare, even in large city hospitals**
  - **No medical reporting systems are in use that have real-time detection of infectious disease patterns as their objective**



# Biodefense Components

## Homeland Biodefense

*Numerous relationships between portions of this hierarchy are not shown.*



**Biodefense development must be multi-faceted.**



# Needed Biodefense Investments

---

- **Point-of-care and public health not well integrated**
  - Health care system is the current detector
- **System (multi-sensor) environmental monitoring development**
  - Focus has been on basic technology and devices
- **Characterization of environments of high-threat facilities**
  - Sensor technology not universally applicable
  - Helps to set requirements
- **Large-scale urban protection**
  - Sparse sampling/sensing
  - Low probability event with catastrophic consequences (akin to nuclear detonation)
- **Red-teaming**



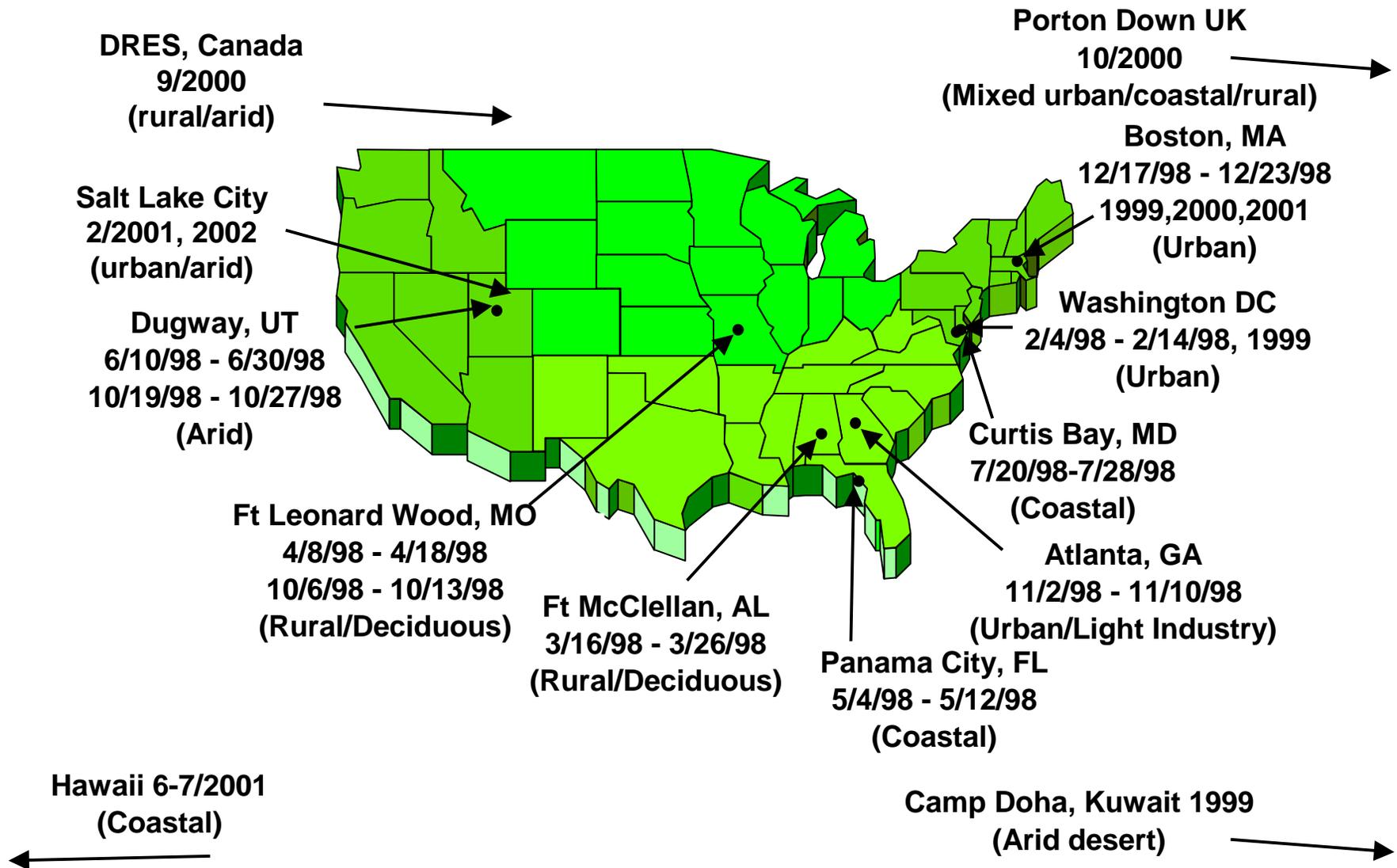
# Environmental Monitors

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- **DoD environmental monitors designed for outdoor force protection**
  - High sensitivity preference
  - Current cost prohibits mass-production
  - Unproven performance in urban or indoor areas where air is filled with interferents
  
- **Urban Civil Protection has markedly different requirements from military use**
  - Low false alert rate and low cost a priority
    - › Lower sensitivity partial solution may be preferred
  - Wide variation in environments (e.g. stadium vs. subway)
    - › Densely populated areas add to natural biological interferents
    - › Airflow, HVAC are important design considerations

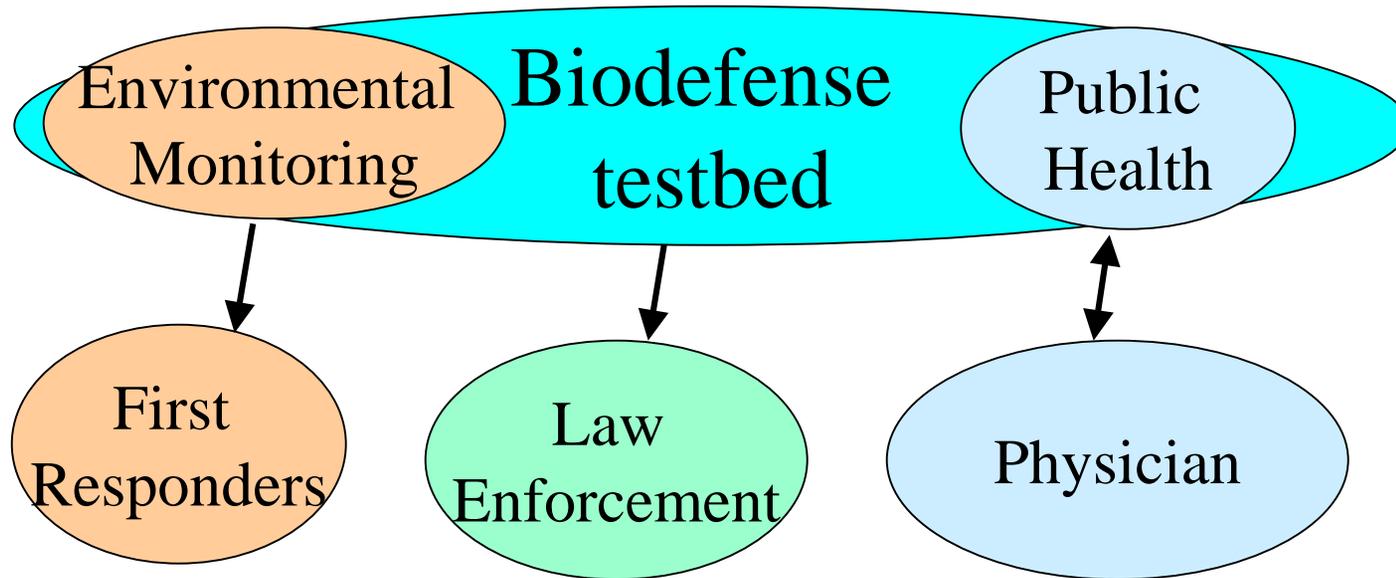


# BAWS III Background Measurement Campaign





# Testbeds as an Important Development Tool

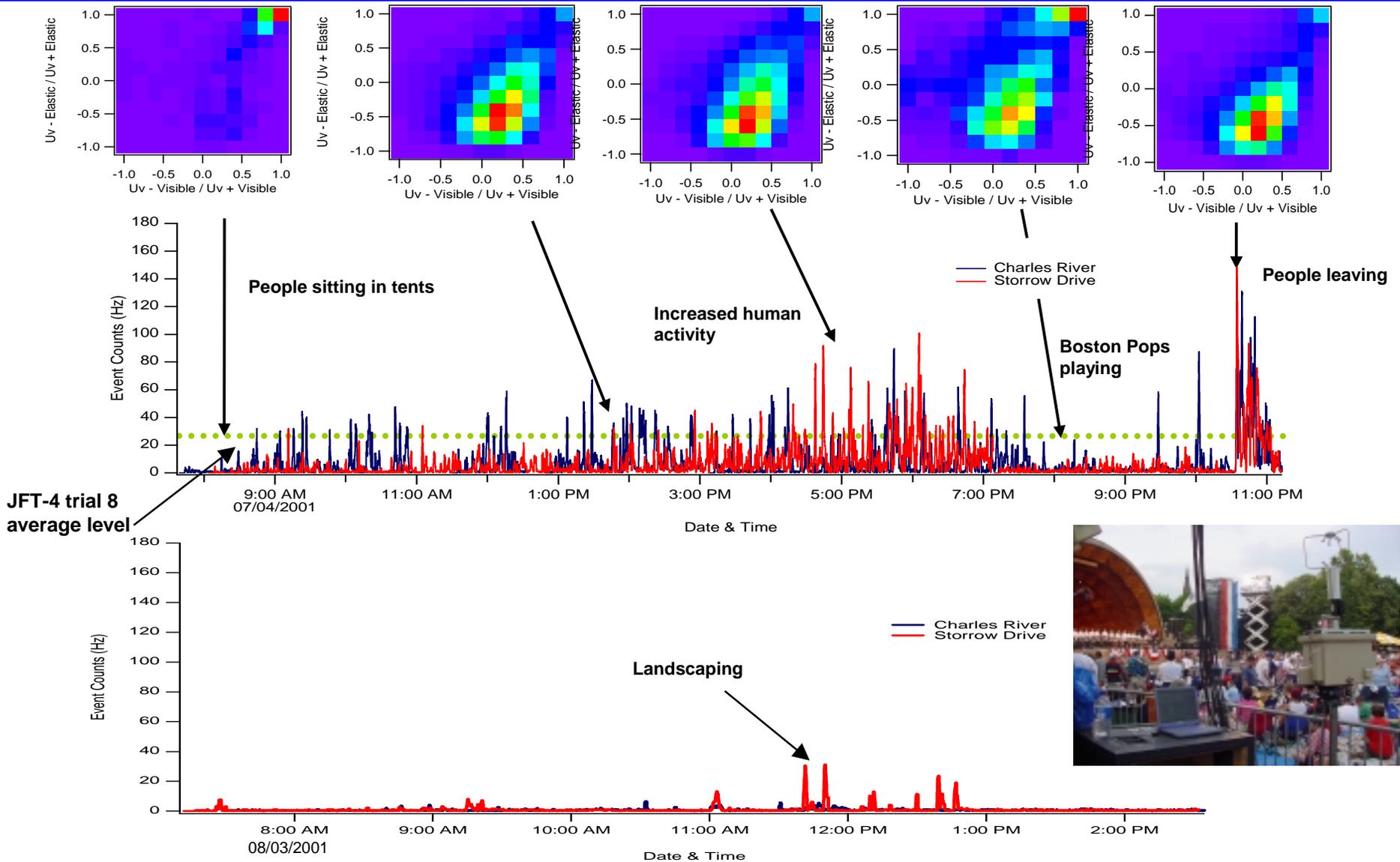


## Testbeds are needed for both public health and environmental monitoring systems

- Understand the problem and set system requirements
- Improve training
- Infuse emerging technologies in realistic settings
- Understand unique environments of various facility types



# BAWS July 4, 2001 Esplanade Measurement





# MIT/LL Urban Testbed Project Goals

---

- **Define a system architecture for facility defense using environmental monitors**
- **Understand the natural air composition and the response of existing instruments in those facilities**
- **Develop decision logic methodology that is extensible to other urban defense problems**



# Urban Testbed Status

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- **Project funding began in June, 2001**
- **Coordination with Boston-area authorities for the past 1-2 years**
  - **MA Bay Transportation Authority (MBTA), Boston Emergency Management Authority (BEMA), MA Emergency Management Authority (MEMA), MA Dept of Public Health, National Guard, Logan airport, others**
- **BAWS measurements at Boston Marathon, July 4<sup>th</sup> celebration**
- **Measurements in MBTA subway station; sensors being installed in a station.**
  - **Particle counters, airflow, temperature, humidity, train motion.**
  - **Periodic measurements in other locations or with sensors that cannot be installed for long periods.**
- **Develop alerting algorithm approach**
- **Controlled chamber releases**
- **Discussing measurements in other Boston locations**



# Subway Protection Considerations

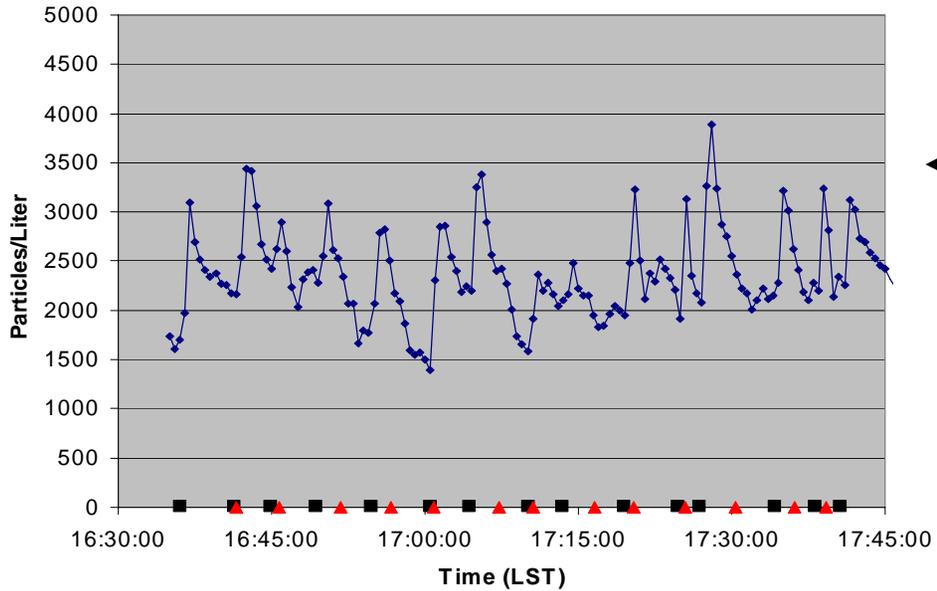
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- **Threat has been established**
  - Aum Shinrikyo Tokyo Sarin gas release
  - Numerous entry points and hiding places
  - Train “piston effect” moves air through the system
- **System is spatially distributed**
  - Many low cost sensors preferred over few high cost sensors
  - Release point cannot be anticipated apriori
- **Important to find dual-use applications for system**
- **Principal response actions**
  - Stop trains (plug tunnels?)
  - Activate vent fans?
  - Evacuate and prevent additional access

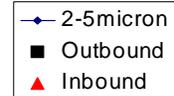


# Station Particle Counts

Particle Counts for 2-5 micron region

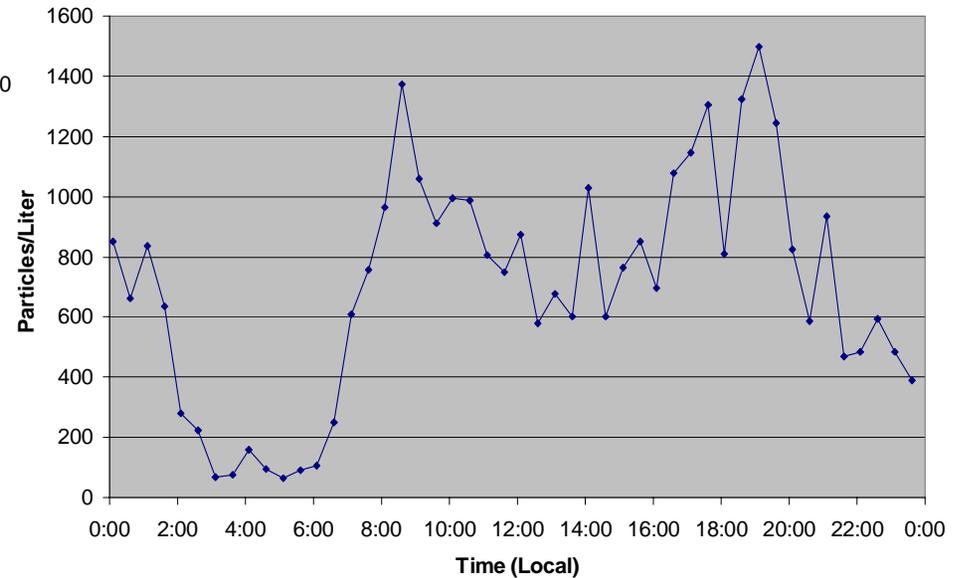


**Train Traffic significantly alters particle counts**



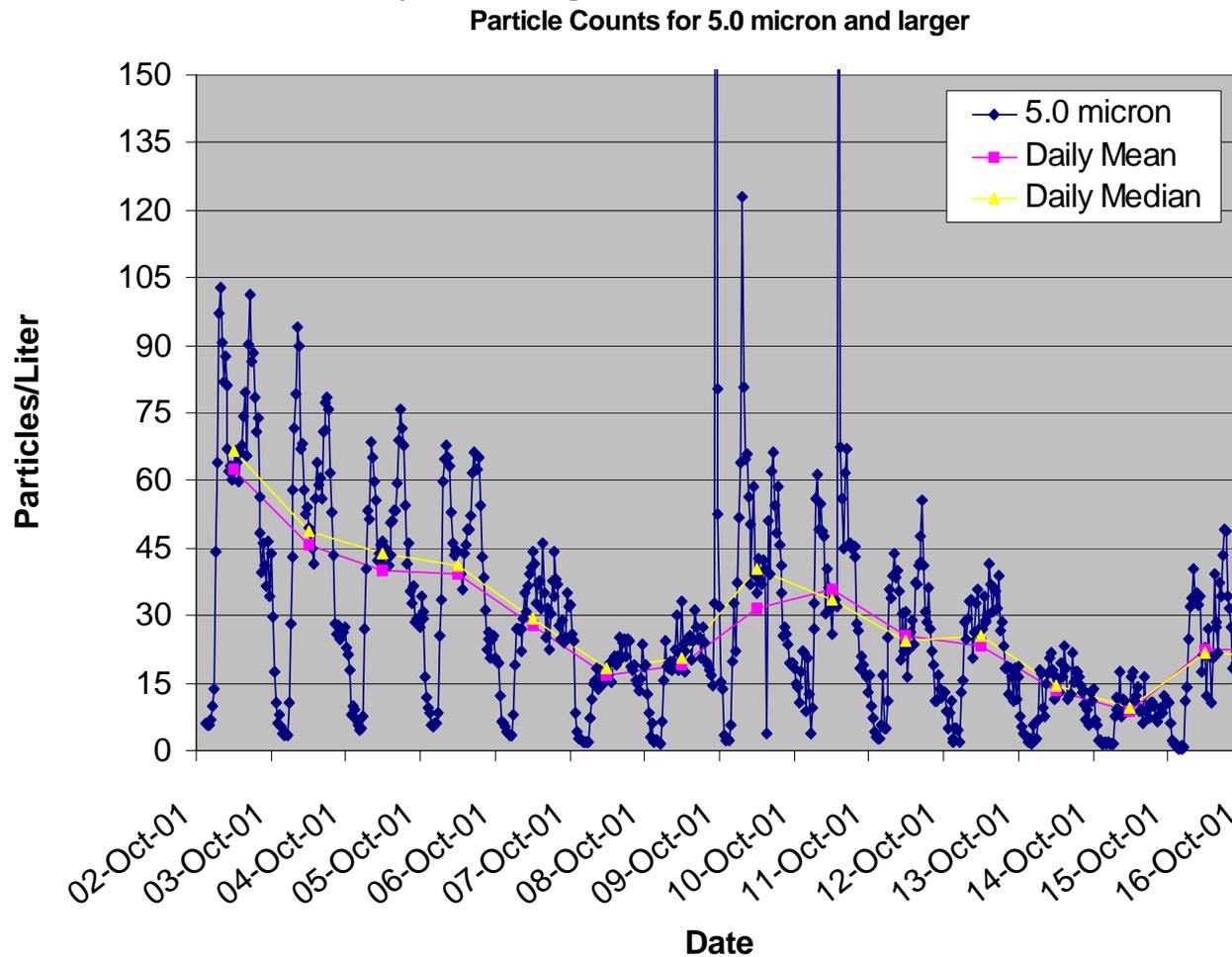
**Diurnal Cycle significantly alters particle counts**

Particle Counts for 2-5 micron region





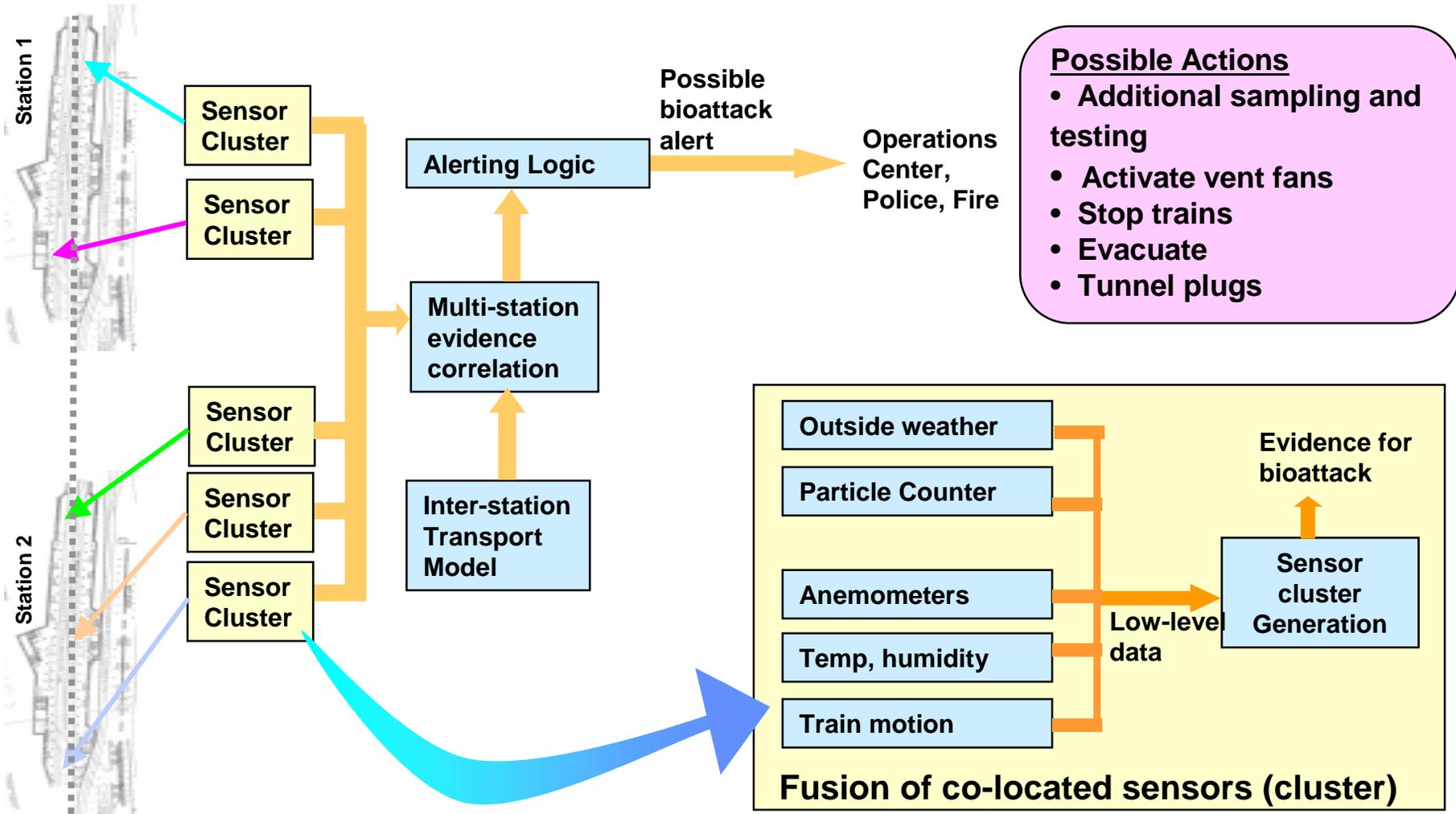
# Subway effects on Particle Counter Sensors



**Particle counter sensors degrade quickly due to laser optics contamination. Full instrument sensitivity regained after cleaning.**



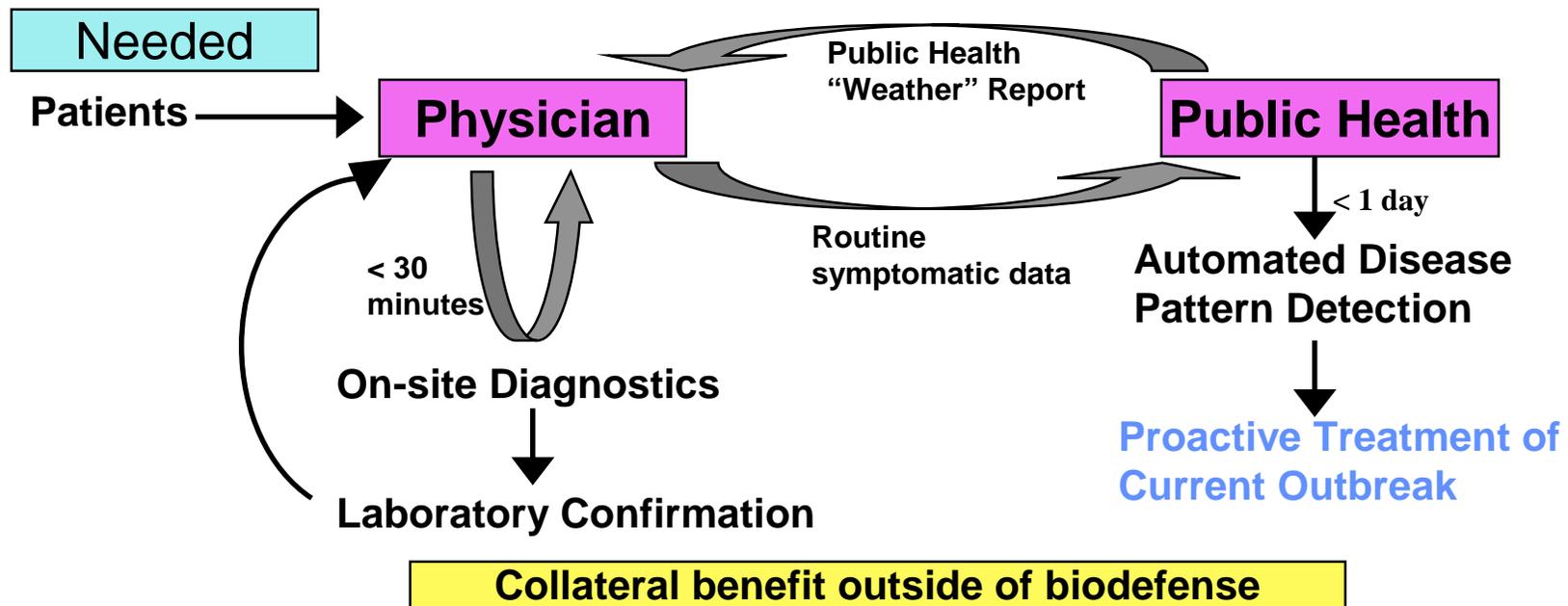
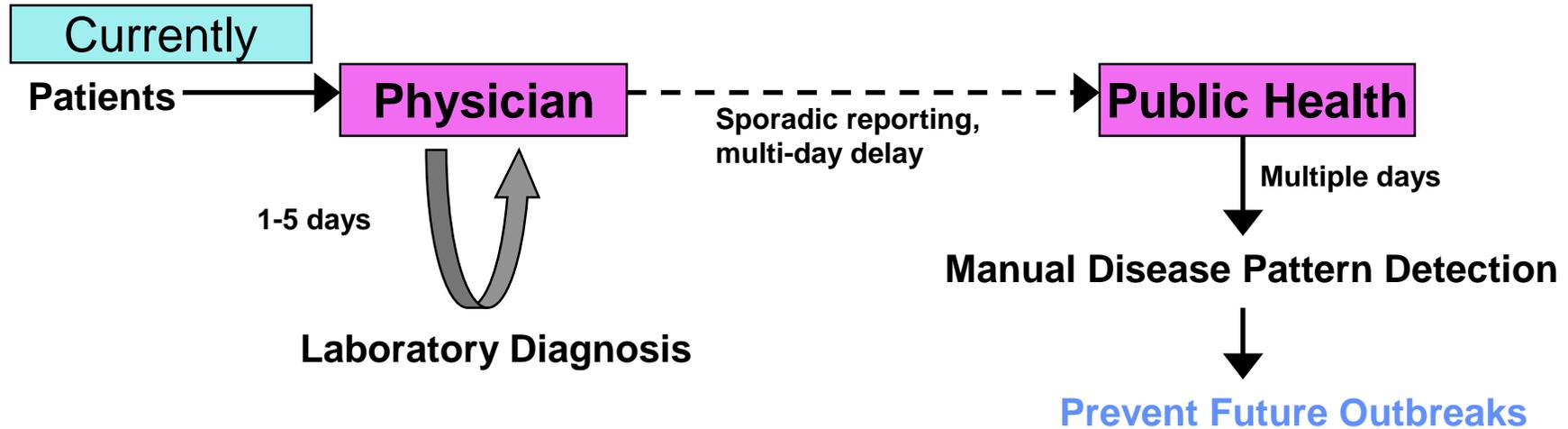
# Subway Alerting Algorithm Architecture



**Multiple sensors required to agree and sensitivity reduced to reduce risk of false alert.**



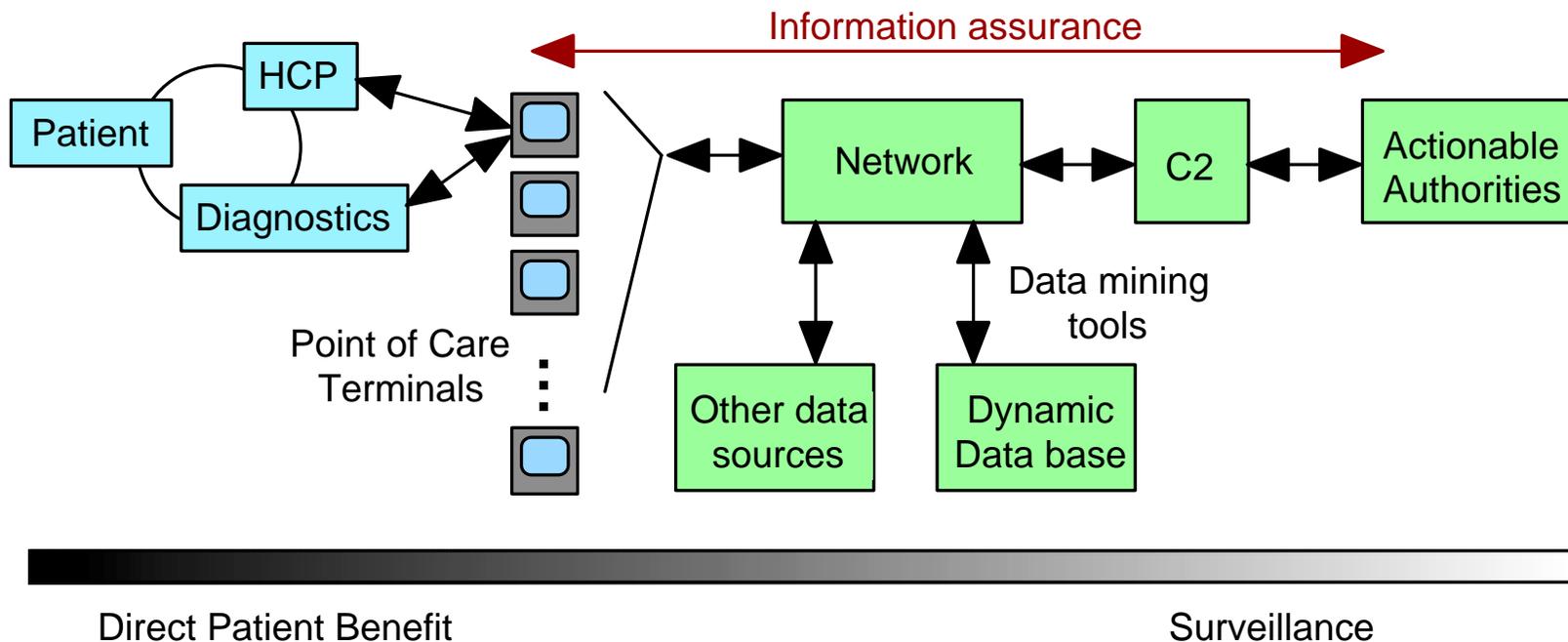
# Health Care Provider and Public Health Integration





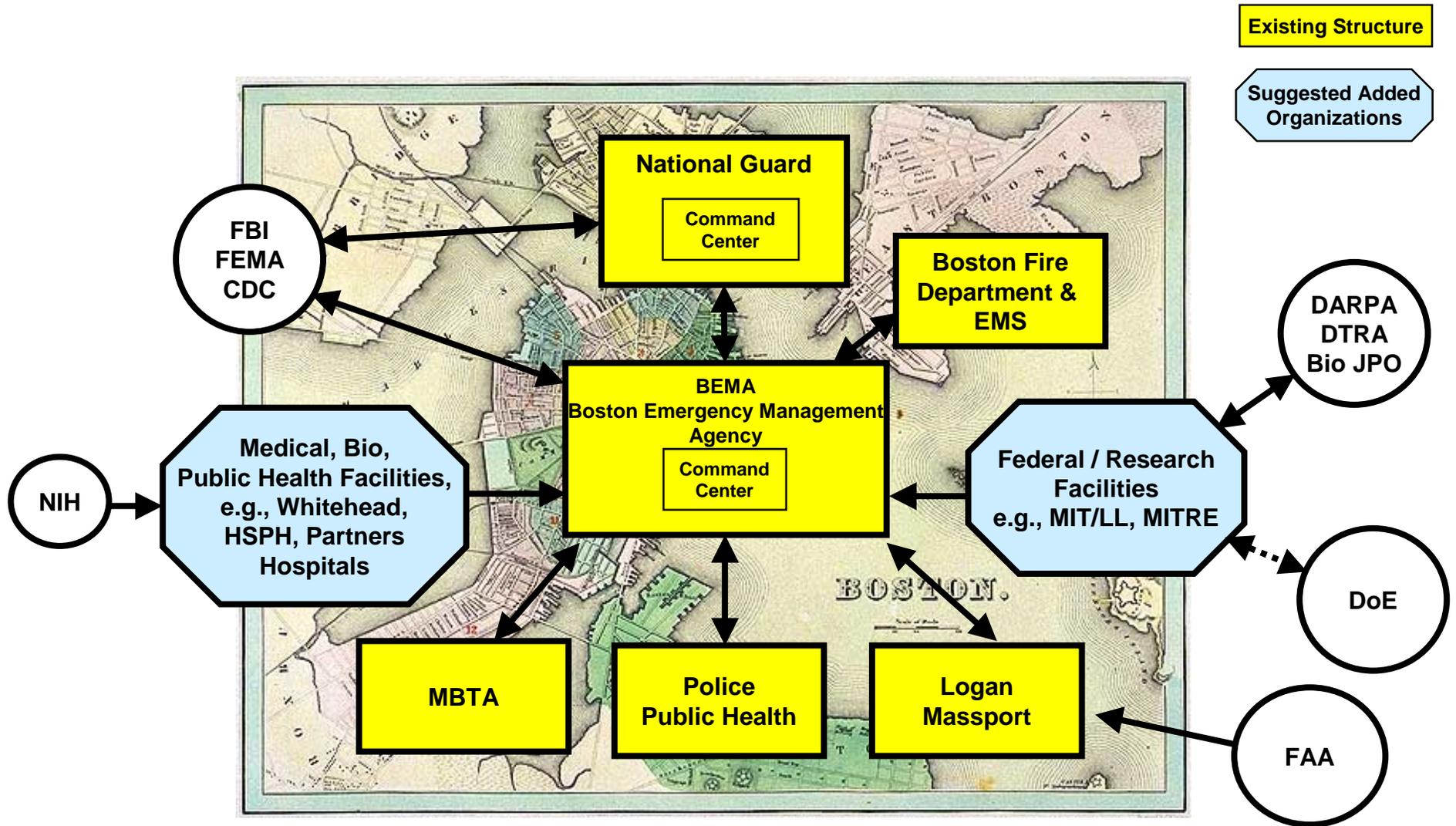
# Merging of Health Care with Defense Against Biological Weapons

- **Concept:**
  - Implement advanced point-of-care diagnostics (including but not limited to gene-chips), into IT networked system
  - Enables rapid determination of biological attack
  - Benefits natural infectious disease diagnosis, effective treatment





# Boston Area Agencies with Biodefense Responsibilities





# Summary

---

- **Civilian bioterrorism defense requires that the environment of high-threat locations be well understood**
  - Environment drives sensor & system design
- **Initial testbed being installed at Boston subway station**
- **Measurements to date point out deficiencies of current sensors & software**
- **Modern recognition/data fusion techniques being applied to data**
- **Measurements at additional Boston threat locations under discussion**

---

# Facility Defense Against Aerosol Attack

**Dan Cousins**  
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**cousins@ll.mit.edu**

**3 April 2002**

**MS-15434**

**This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.**

**50**  
years



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**MIT Lincoln Laboratory**



# Outline

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- **Facilities and attack scenarios**
- **Sensing an attack**
- **Facility protection techniques**



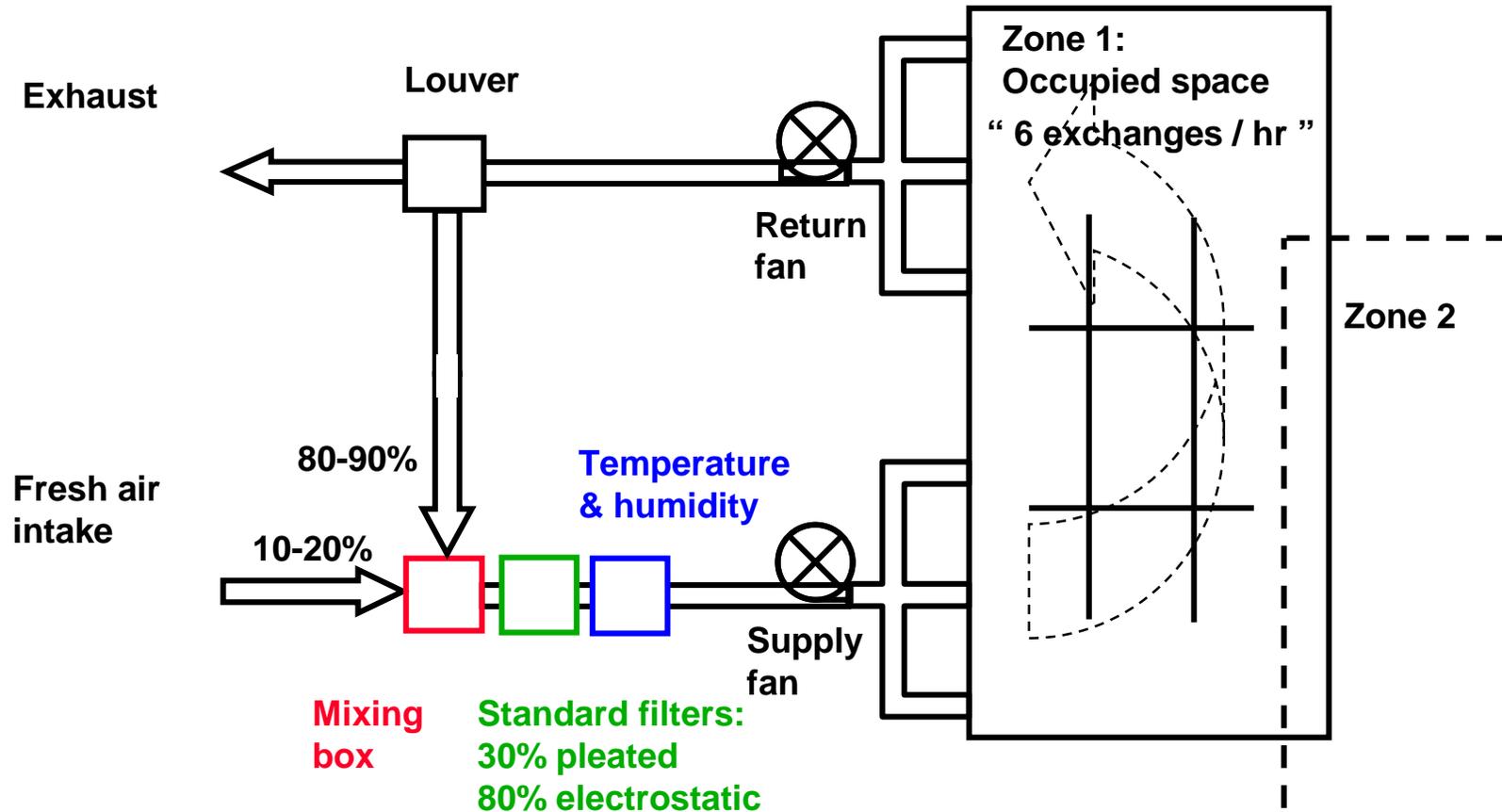
# Types of facilities

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- **Simple structures**
  - Residences, barracks
- **Buildings with ventilation system**
  - **Multiroom office building**
  - Large open space (arena, terminal, ...)
- **Subway**
- **Outdoor sites**
  - Stadium
  - Public gathering
  - Military operations



# Simplified Ventilating System





# Types of Attacks

---

- **External attacks**
  - Nearby cloud release
  - Burst release into air intake
- **Internal attacks**
  - Burst release into air return
  - Burst release into a large open space
  - Low level continuous release
- **Small amounts of agent are substantial threats**

1 gram bioagent uniformly dispersed into  $10^8$  liter building (100m x 100m x 10m);

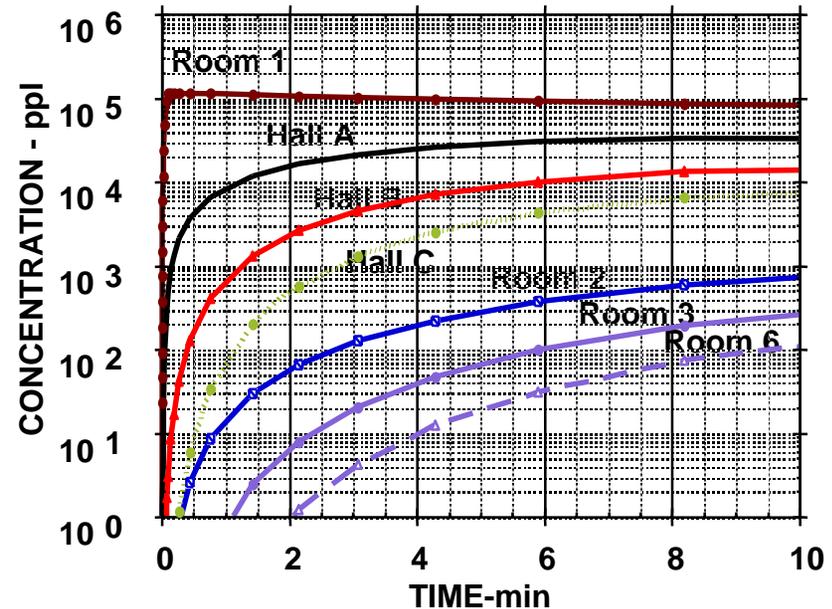
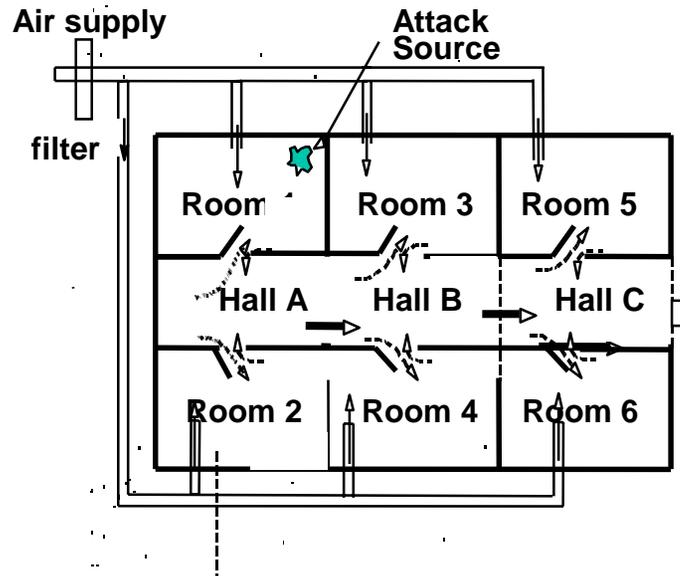
Corresponds to lethal exposure (100 ppl x 10 liter/min x 10 min;  $10^{10}$  particles /gram)



# Modeling an Attack

## Burst release in an interior room

- Bioagent - 15 grams over 5 sec
- Room-Hall coupling - 10%



- Lumped parameter models are well established  
instantaneous and uniform concentration within each room
- Initial particle dispersal and deposition are more complicated to model.



# Emergency Management Measures

---

- **Information**

- **Observing suspicious activity**
- **Knowing who to treat**
  - › **Primarily, but not exclusively, bio agents**
  - › **Records of access (badge swipes, tickets,...)**
  - › **Voluntary response to public announcement**
  - › **Physical examination**
- **Preserving forensic evidence**

- **Plan of action**

- **HVAC emergency management decision tree**
  - › **Suspicious event near air intake -> shut down intake**
  - › **Suspicious event inside building -> full fresh air**
- **Communication channels**
- **Evacuation plan**
  - › **Orderly movement to controlled safe area, avoid cross contamination**



# Outline

---

- Facilities and attack scenarios
- Sensing an attack
- Facility protection techniques



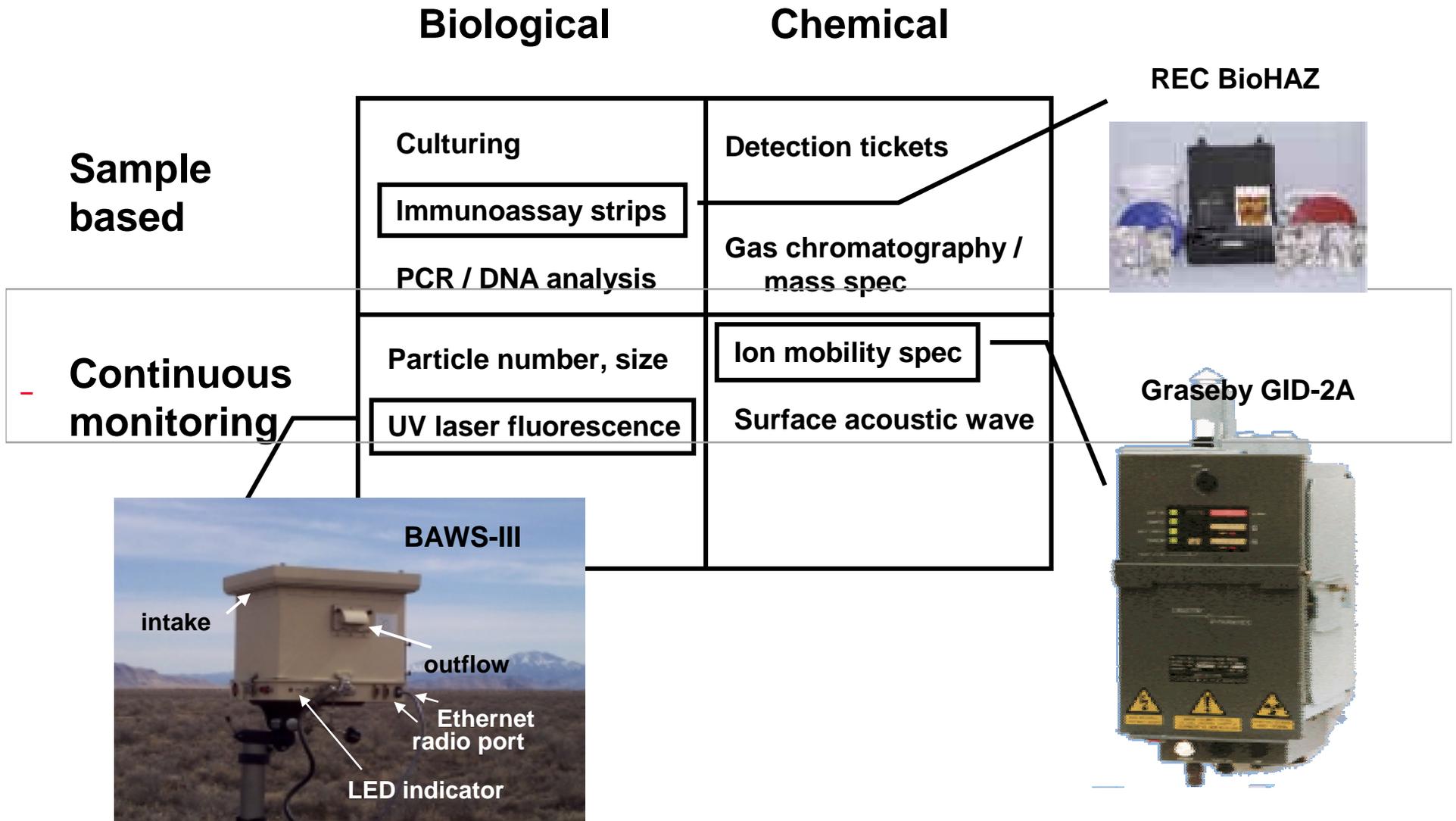
# Rationale for Sensing

---

- **Issue alarm**
  - initiate facility response
  - high  $\text{Prob}_{\text{detection}}$  ; low  $\text{Prob}_{\text{false alarm}}$  ; wide range of agents
- **Identification of agent**
  - initiate medical treatment
- **Mapping of contamination zone**
- **Assessing decontamination (“all-clear”)**

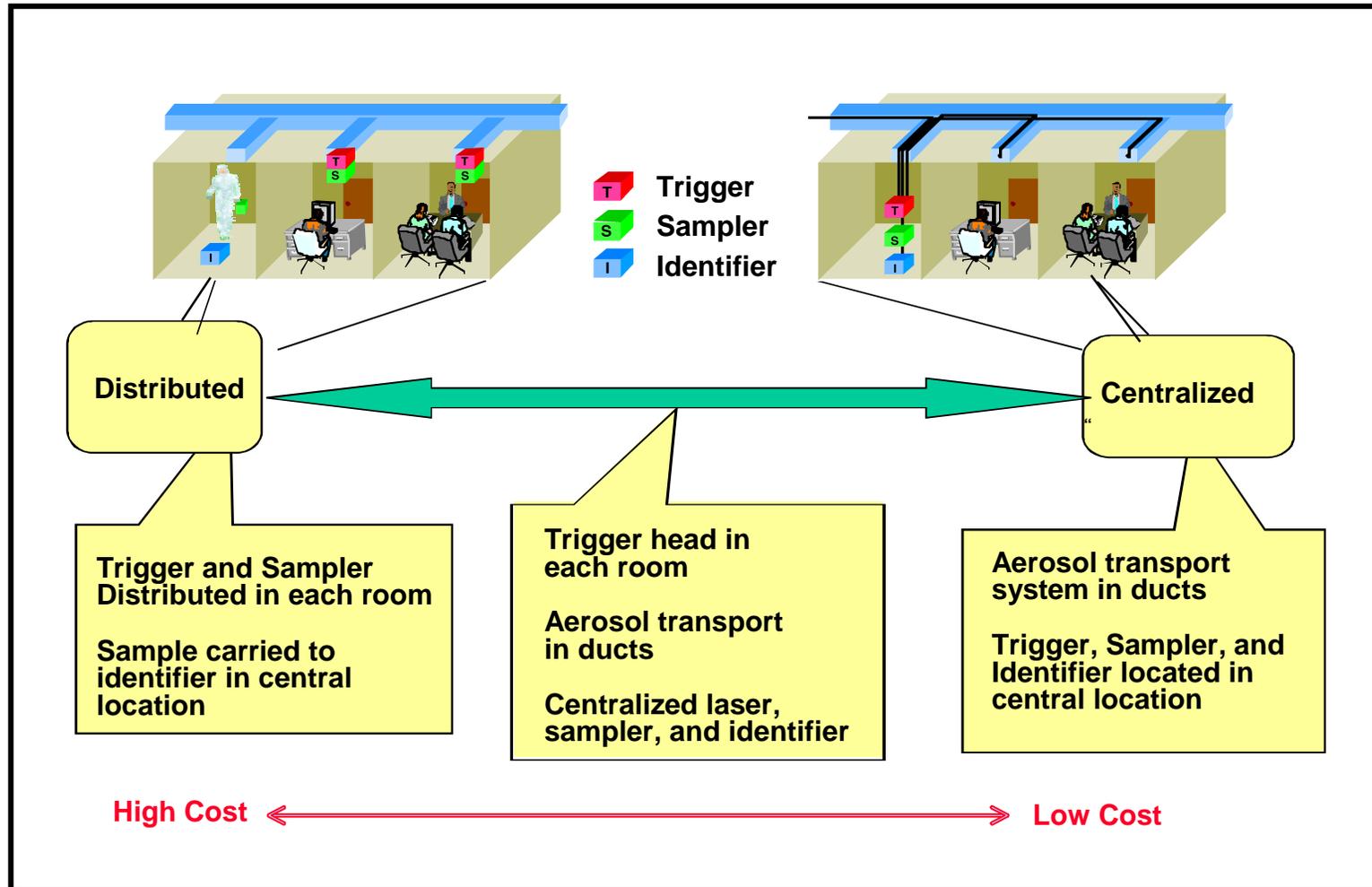


# State-of-the-Art Bio / Chem Sensors



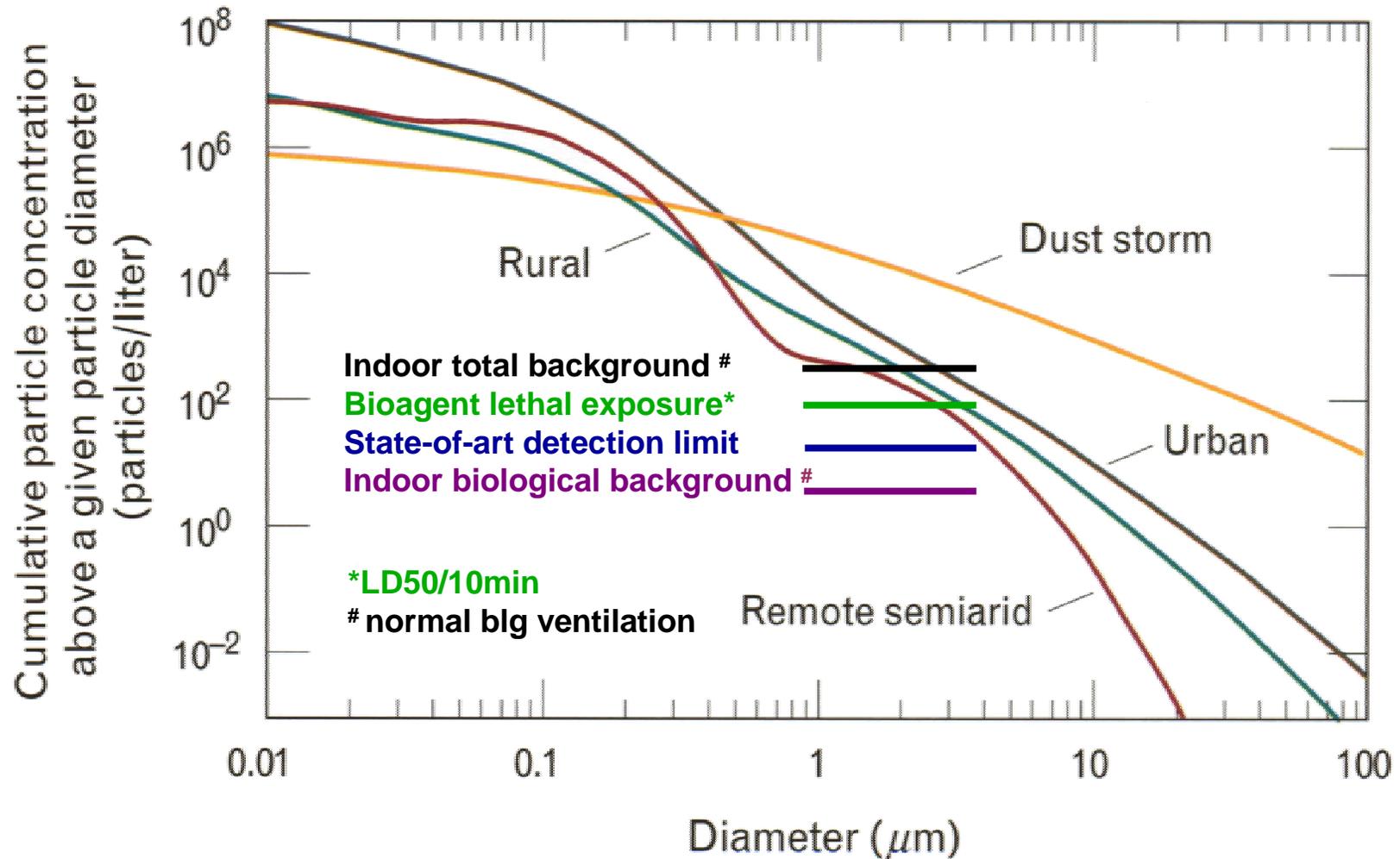


# Sensor Architectures for Building Defense





# Atmospheric Aerosol Content

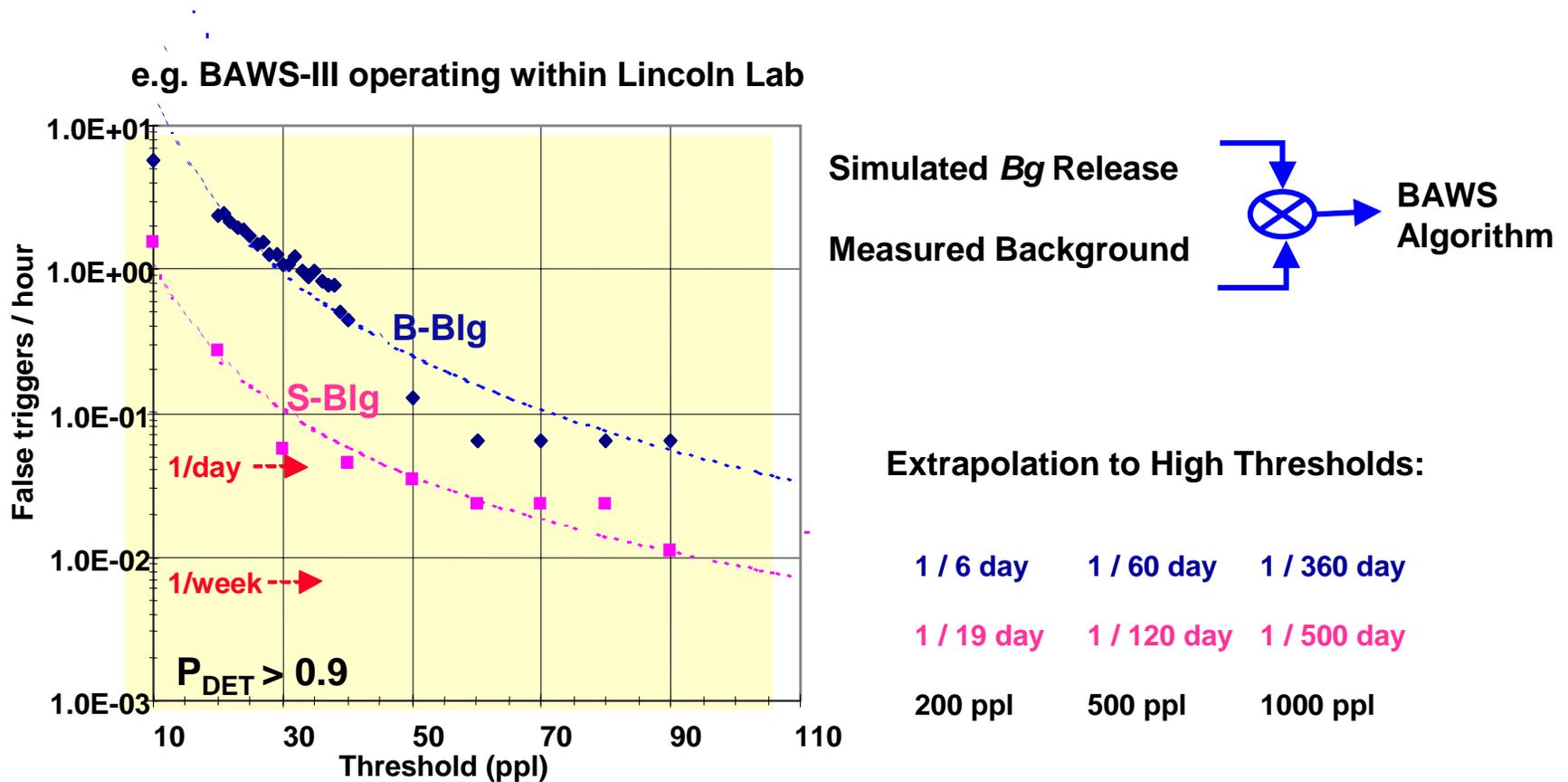


After R. Jaenicke in Aerosol-Cloud-Climate Interactions, P. Hobbs editor (1993).



# False Trigger Rate

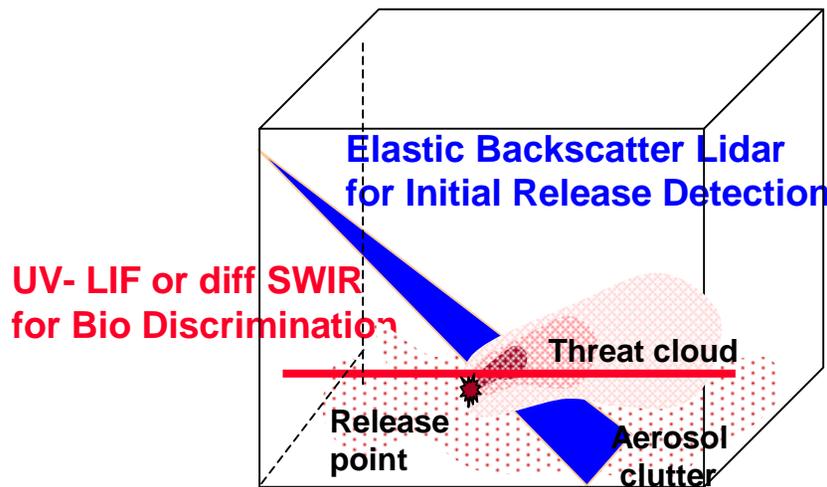
- Sensor will trigger less frequently when operated at higher threshold.





# Indoor Standoff Aerosol Detection

- Any point sensor is limited by aerosol transport in large open space.
- Need to detect the release promptly at a specific point
- Bio sensor concept:



Minimum for detecting 1000ppl threat

	Dwell time	Range cell
Elastic	0.1 sec	1 m
UV LIF	10 sec	3 m
Diff SWIR	10 sec	2 m

50m range, eyesafe laser; 100 lux lighting



# Outline

---

- Facilities and attack scenarios
- Sensing an attack
- Facility protection techniques



# Facility Protection Measures

---

- **Physical security**
  - **Protect fresh air intakes (location, access, surveillance)**
  - **Personal screening (may be difficult in civil defense)**
- **Ventilation system protection**
  - **Passive air filtration**
    - › **Upgrade filters (best ASHRAE filters > 95%)**
    - › **Overhauling the system (HEPA / carbon)**
  - **Positive pressure to overcome infiltration**
  - **Sensor triggered airflow control**



# Passive Air Filtration

---

- **In-line passive filtration is well established**
  - HEPA filters remove >99.97% suspended particles > 0.3  $\mu\text{m}$ .
  - Activated carbon filters adsorb most chemical vapors
- **Substantial cost to overhaul existing ventilation system**
  - Purchase and replacement of filters
  - Increased blower motors for higher pressure drop
  - Reinforced ductwork
  - Very little infiltration is allowable (gasket seals, overpressure)
  - Increased energy costs
- **Research topics**
  - Low pressure drop filter structures
  - In-line sterilization (UV, radiation, thermal,...)



# Facility Defense Effectiveness

Estimated exposure reduction  
to external bio attack

- |   |                  |
|---|------------------|
| • <b>“Unprotected” building</b>   | <b>1</b>         |
| • <b>Upgraded standard filters<br/>(or in-room HEPA)</b>                              | <b>10-100</b>    |
| • <b>In-line HEPA filters</b>   | <b>100-1000</b>  |
| • <b>In-line HEPA filters<br/>with overpressure<br/>and triggered airflow control</b> | <b>&gt; 1000</b> |



# Summary

---

- **Most buildings with ventilation systems are vulnerable to aerosol attack via a number of scenarios.**
- **Without deployed sensors, an attack may go undetected resulting in higher exposure and lack of treatment to exposed occupants.**
- **There are some simple measures that can be used to increase situational awareness and provide limited protection.**
- **A substantial degree of protection can be achieved at substantial cost with sensor triggered airflow control and HEPA/carbon filters. In this case, sensors may be operated at higher thresholds.**

---

# Aerosol Triggers

Thomas H. Jeys

New England Bioterrorism Preparedness Workshop

3-4 April 2002

MS-15436

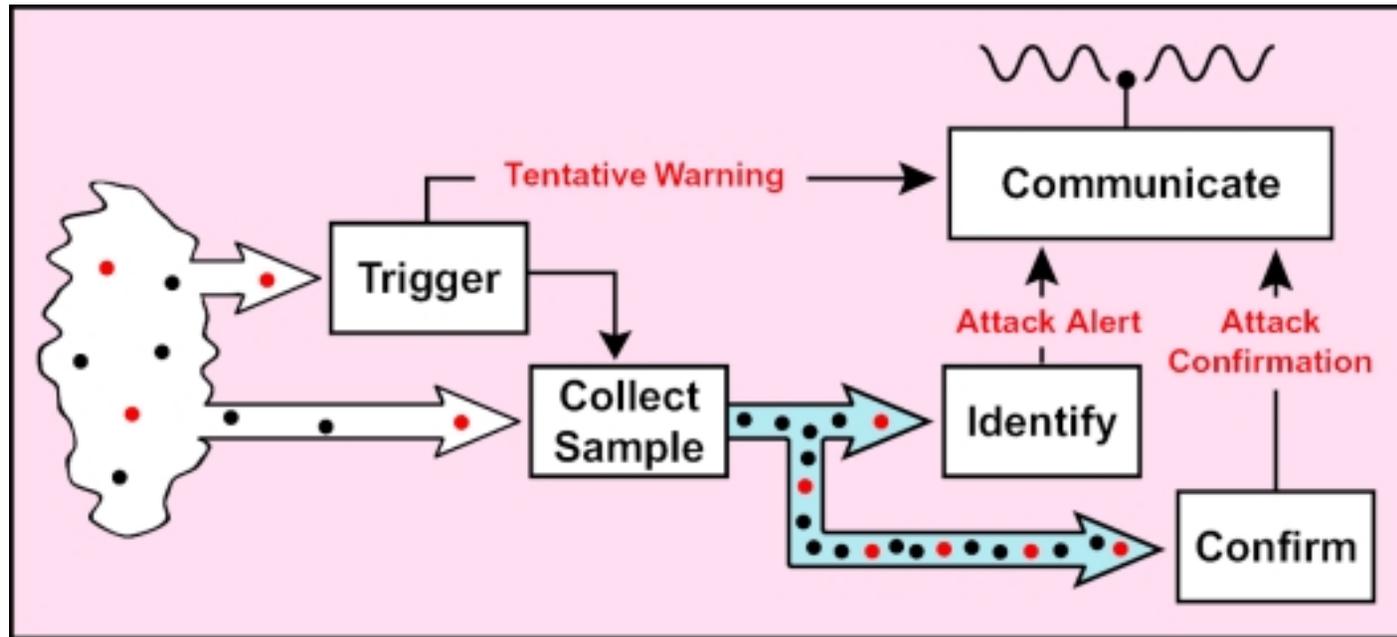
This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.

50  
years

 MIT  
LINCOLN LAB



# Biosensor Architecture



- **Trigger (< 60 s)**
  - Continuous operation
  - Alert of potential threat aerosol
- **Collector (5 min)**
  - Activated by trigger
  - Provide sample of aerosol particles

- **Identification (15 min)**
  - Preliminary identification of agent
- **Confirmation (4 – 24 hr)**
  - Final identification of agent
  - “Gold Standard” tests
  - Performed in laboratory (TAML)



# Bio-Aerosol Triggers

---

- **Raw Particle Counters**
  - Small, low cost
  - Nondiscriminatory - very high false trigger rates
  
- **Fluorescent Particle Counters**
  - **Ultra Violet Aerodynamic Particle Sizer (UVAPS)**  
Trigger for Biological Integrated Detection System (BIDS)  
Manufactured by TSI Inc. (St. Paul, MN)  
**Fluorescence Aerodynamic Particle Sizer (FLAPS)**  
Different trigger algorithm than UVAPS  
Trigger for Canadian Integrated Biological Agent Detection System (CIBADS)
  
  - **Biological Agent Detection Sensor (BAWS)**  
Trigger for Joint Biological Point Detection System  
Manufactured by Intellitec (Deland, FL)



# Biological Agent Warning Sensor (BAWS)

- **Army Advanced Technology Demonstration**
  - Began BAWS development in 1996
- **Four design generations developed**
- **Extensively tested**
  - Performance
  - Environmental
- **Integrated into the Joint Biological Point Detection System**
  - Development transitioned to JBPDS in 1999.

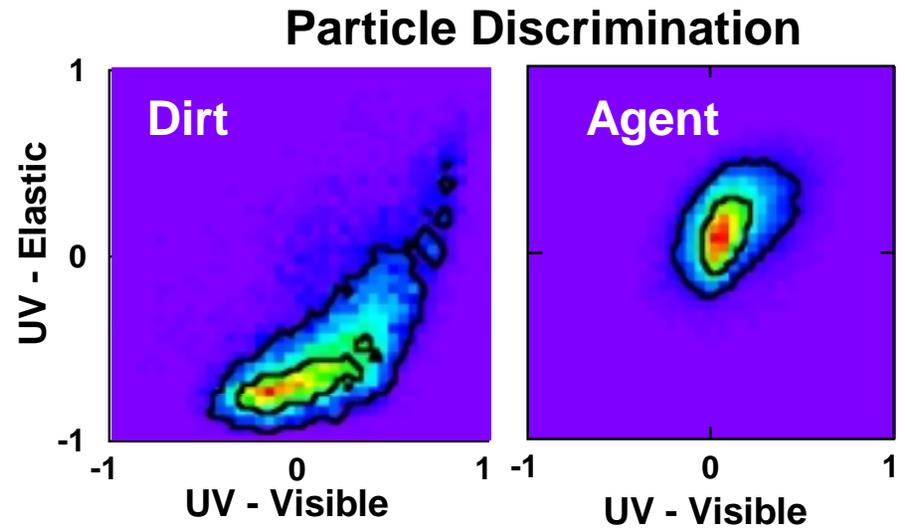
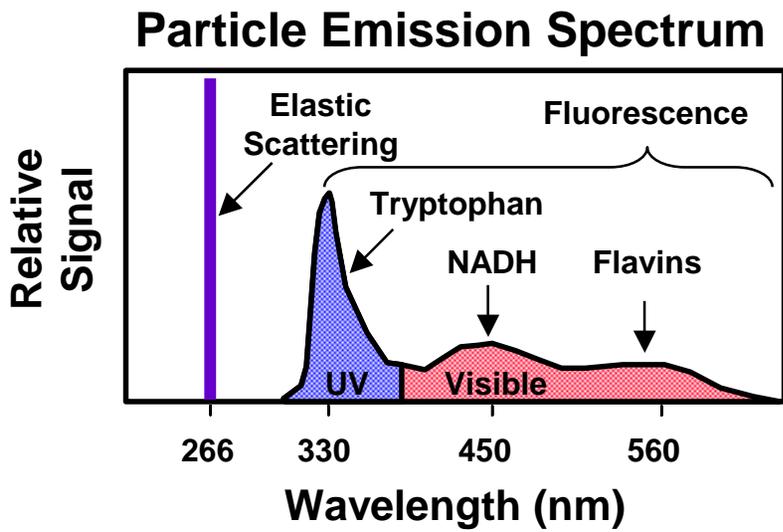
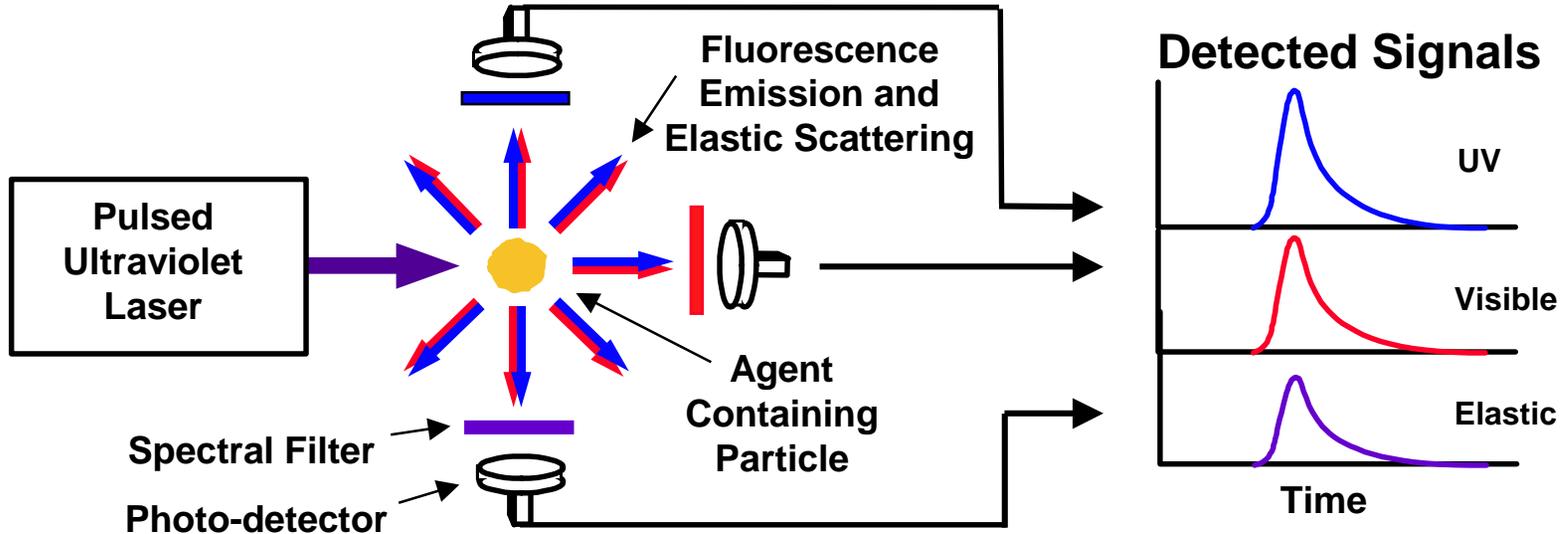
## BAWS III



Size	0.8 ft <sup>3</sup>
Weight	19 lbs
Power	35 W



# BAWS Concept





# Joint Biological Point Detection System



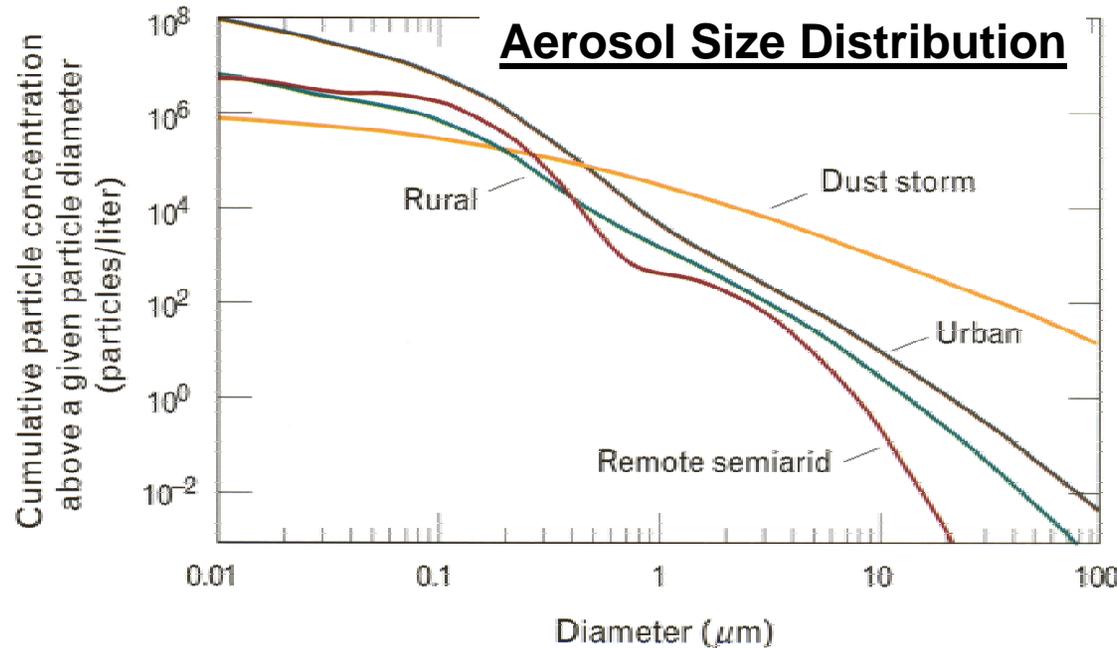
- Automated suite of sensors for detection and identification of biological attacks
  - Trigger – BAWS
  - Collector – Wetted Wall Cyclone
  - Identifier – Immunoassay
  - Confirmatory Samples



**BAWS**



# The Atmospheric Aerosol Composition



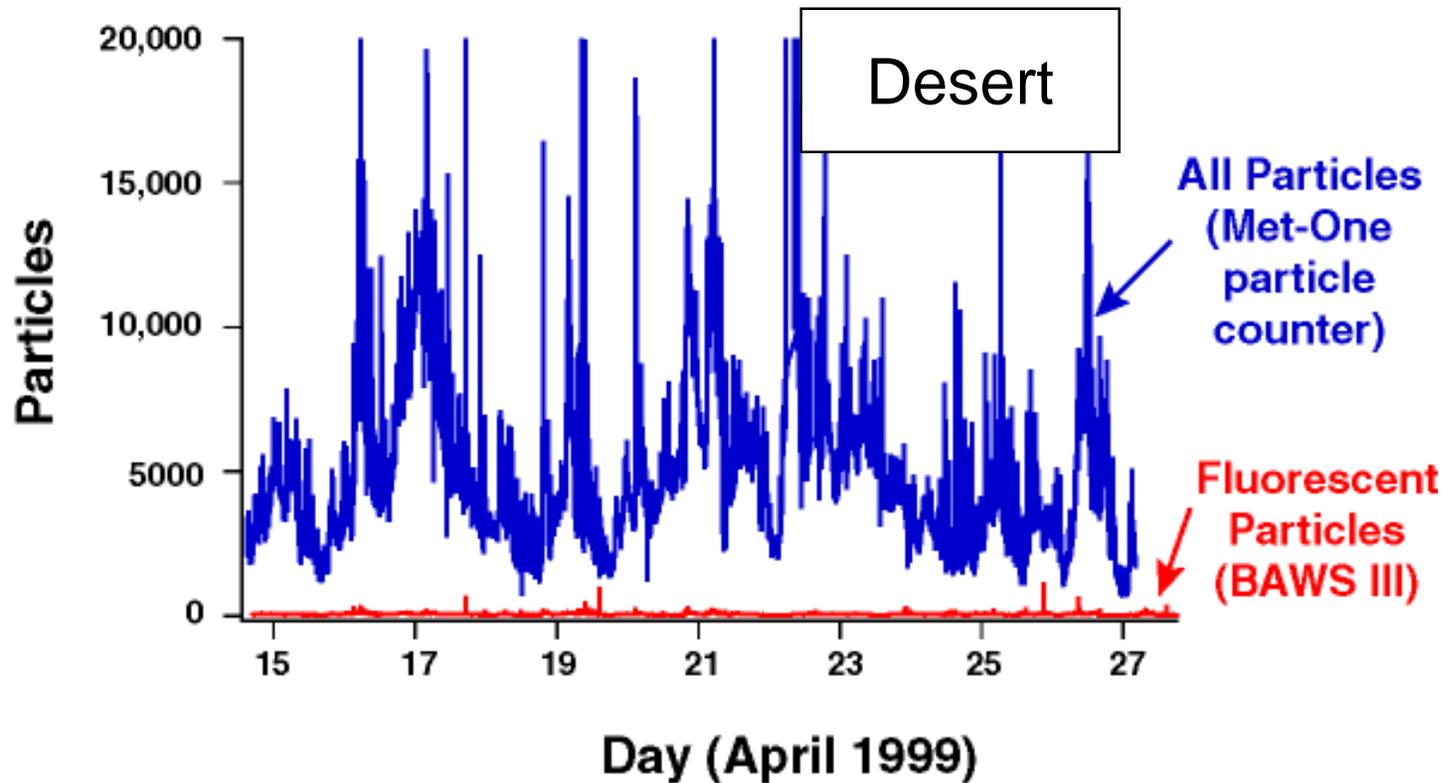
\*After R. Jaenicke in Aerosol-Cloud-Climate Interactions, P. Hobbs editor (1993).

## Composition of Coarse (>1 micron) Aerosol

Organic Aerosols	Particles per Liter		Inorganic Aerosols
Man Made	0 – 2000	100 – 10,000	Clays, Sands, Composites
Fungi	0 – 100		
Bacteria (culturable)	0 – 1		
Pollen	0 – 1		



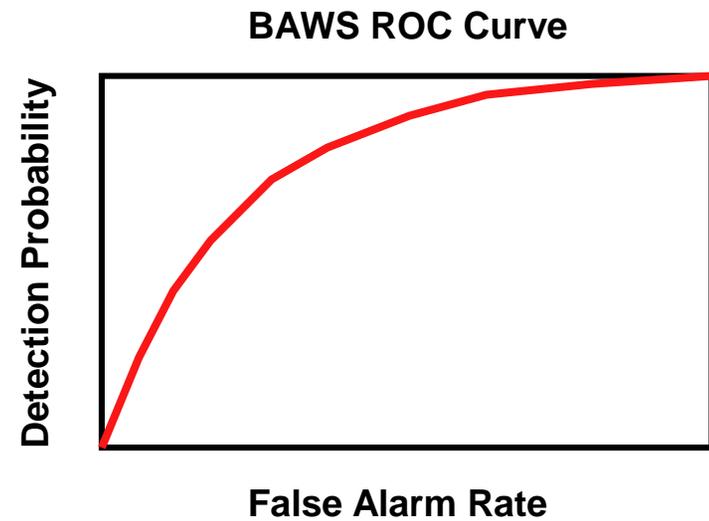
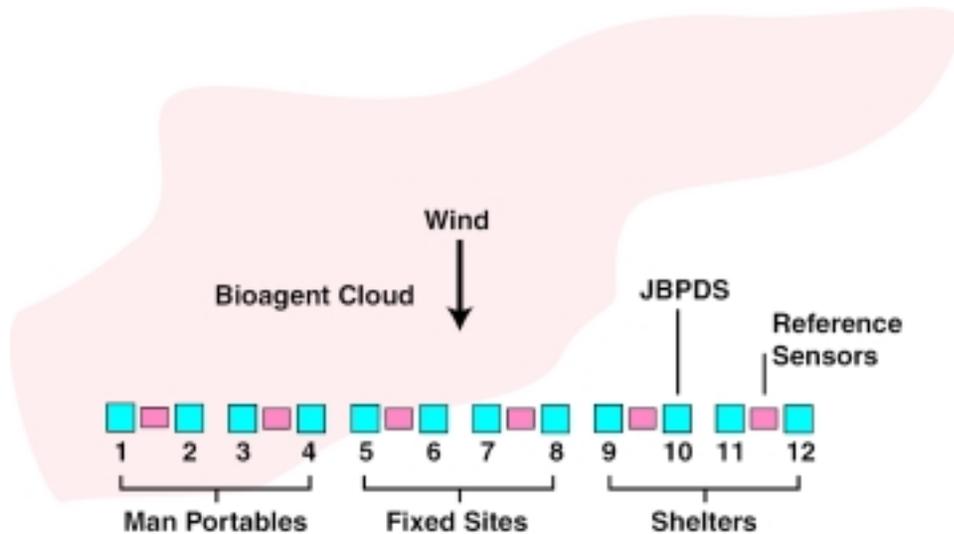
# Total vs. Fluorescent Particles



- Most sand particles do not fluoresce and are “invisible” to BAWS

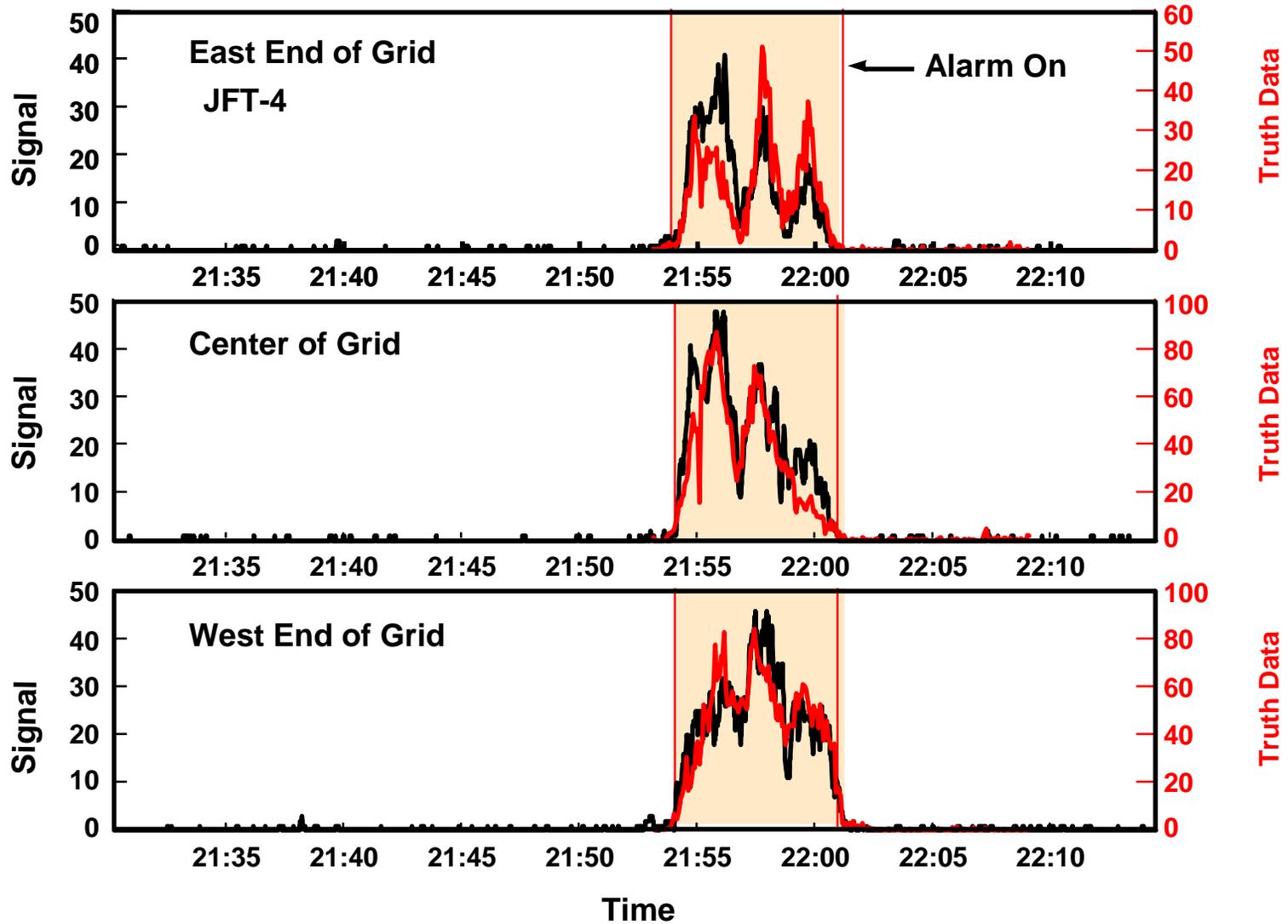


# Field Trials





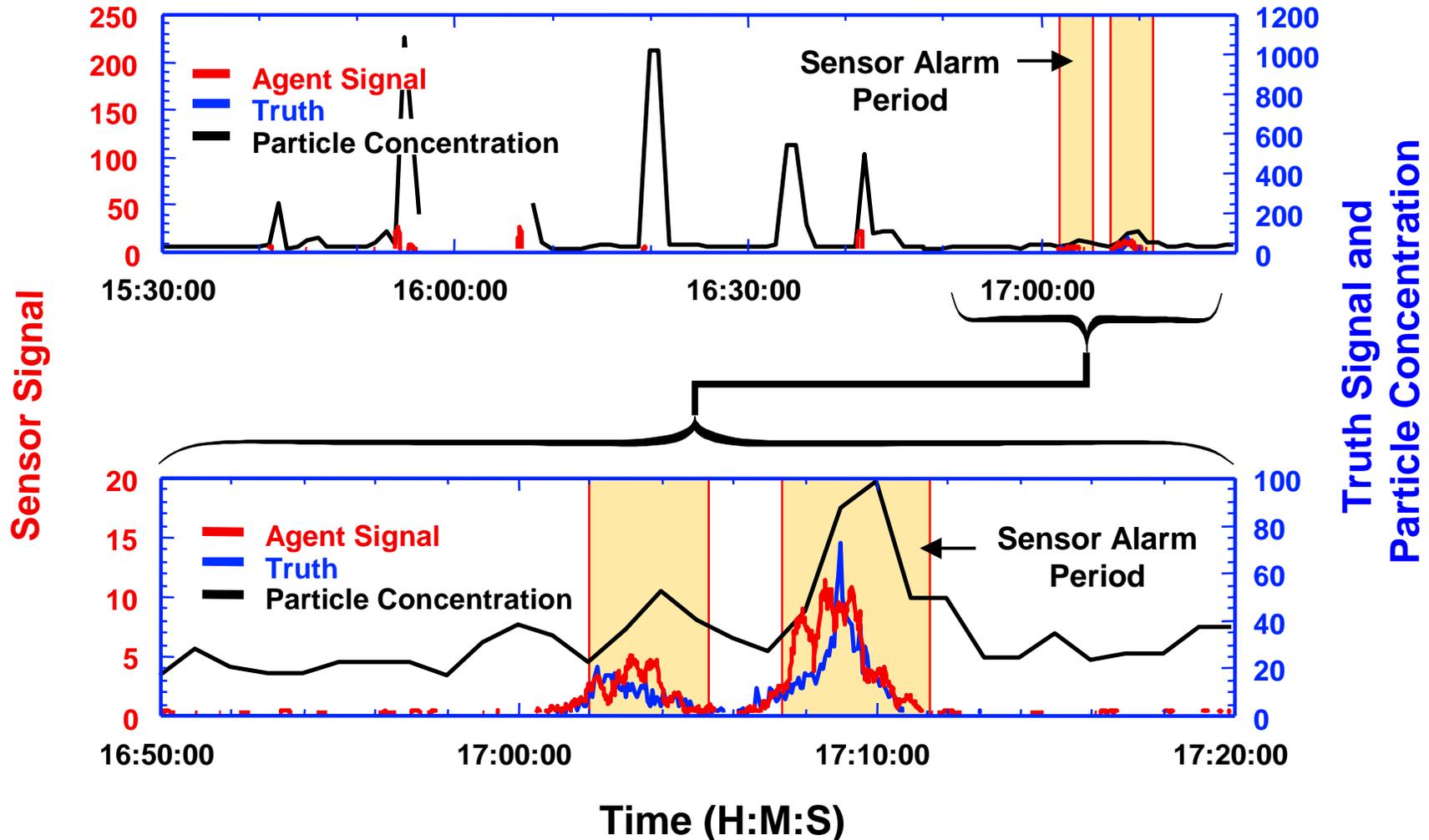
# Response of BAWS Array to Agent Aerosol





# Response of BAWs to Interferent and Agent Aerosol

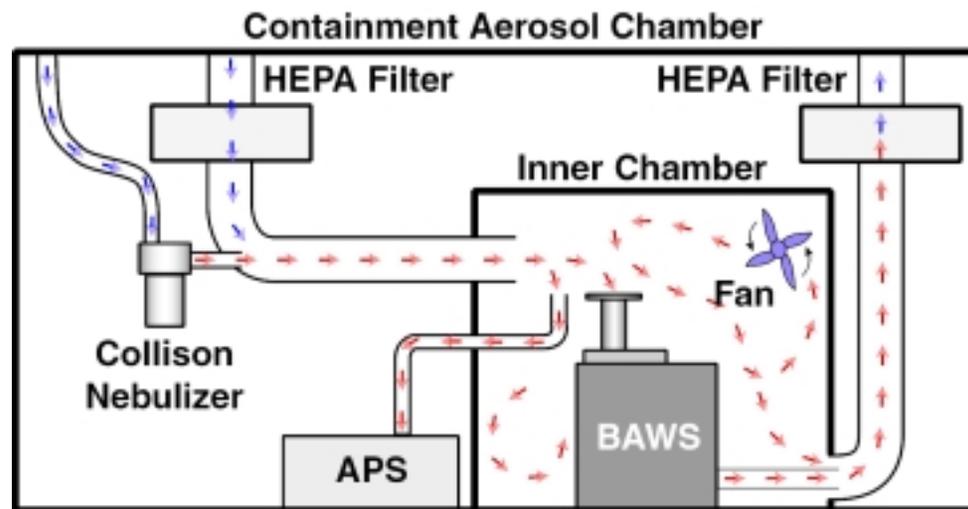
## Sensor at East End of Grid





# Live Agent Tests of BAWS

- **Comparison of BAWS response to real agents and simulant agents**
  - Simulant Agents; BG, *Erwinia herbicola*, Ovalbumin, MS2
  - Three Real agents



- **Results: BAWS detects live agents as well as, or better than, simulant agents**
  - Equivalent sensitivity
  - Equivalent discrimination



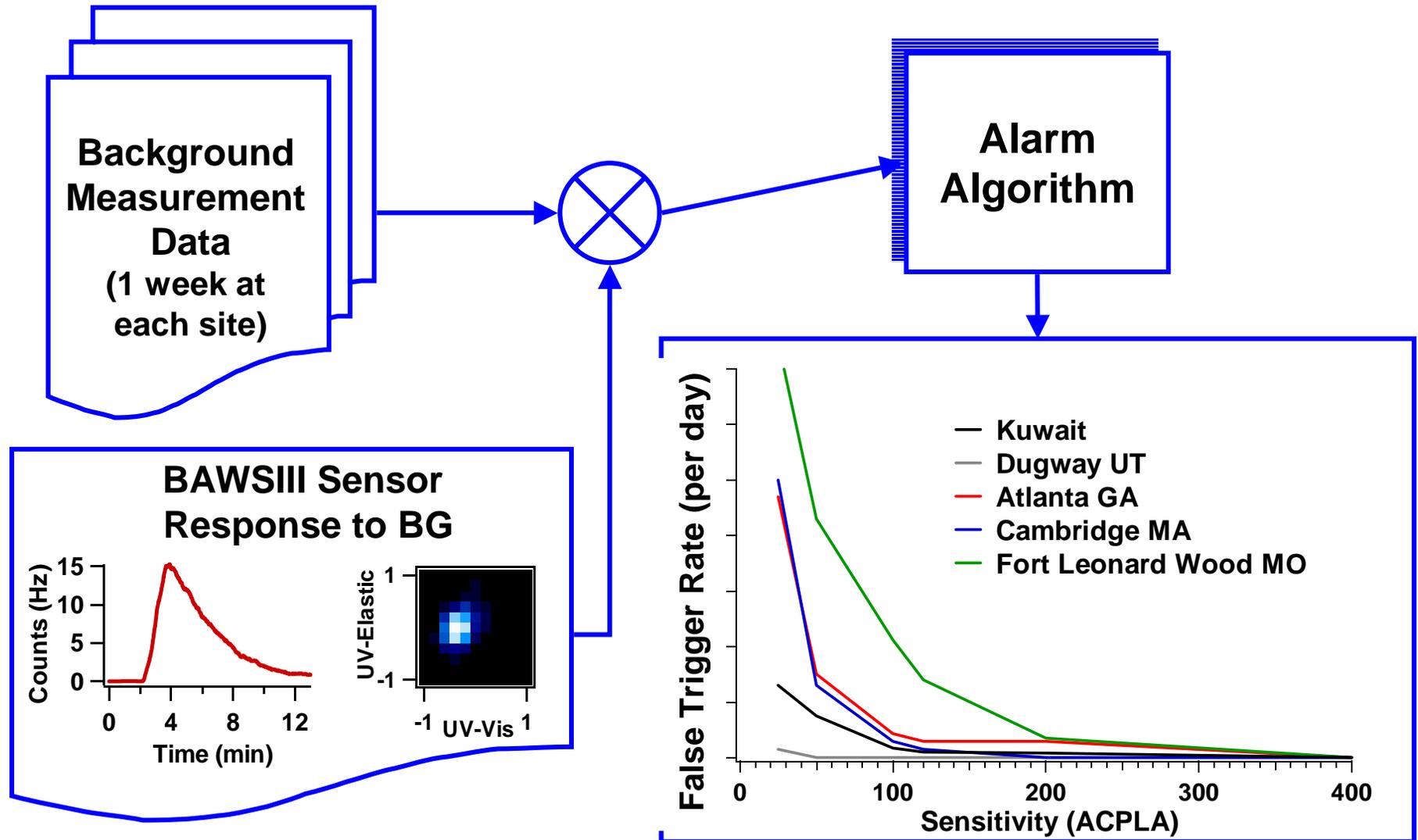
# BAWS Performance Testing

---

- **Joint Field Trials**
  - JFT 3, Dugway Fall '96
  - JFT 4, Dugway Fall '97
  - JFT 4.5, Dugway Spring '98
  - JFT 6, DRES Canada Fall '00
- **Army ATD Field Trials** Spring '99
- **Joint Biological Point Detection System Field Trials**
  - Mini Field Trials Fall '99
  - Gamma-Killed Bio-Agents Spring '99
  - PPQT Spring '00
  - Live Agents Summer '00
  - Porton Down, UK Fall '00
  - Ambient Breeze Tunnel, Battelle Spring '01
  - Operational Assessment 2 Fall '01
- **Background Measurements**
  - USA tour '98 – '99
  - Kuwait Spring '99
  - Altitude study Fall '00
  - Salt Lake City Spring '01
  - Hawaii Summer '01



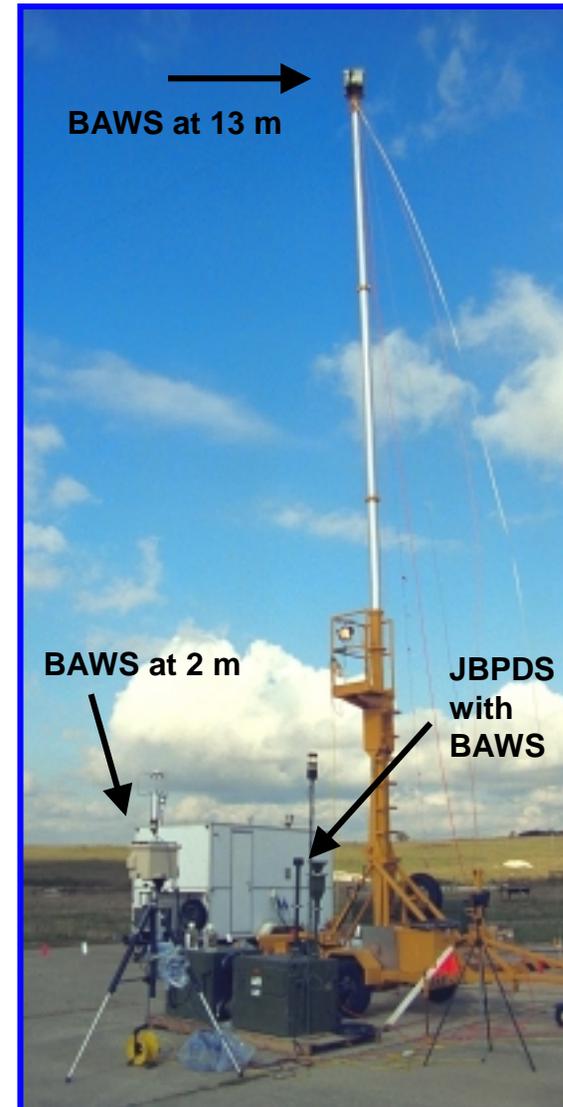
# Simulation of BAWS Response to Agent Attacks in Different Environments





# Detector Position vs. False Trigger Rate

- **England (Sep '00)**
  - One week of measurements
  - 21 agent simulant challenges
  - 8 interferent challenges
- **Sensor Performance vs. sensor height**
  - BAWS at 2-m and 13-m height
  - Ten times fewer false triggers at 13-m height





# Summary

---

- **BAWS developed for early warning of a biological agent attack**
  - continuously operating point detector
  - small size, low weight, low power consumption
- **Generic detection (not identification) of threat aerosol**
  - Individual detection of aerosol particles
  - Discrimination of threat particles from non-threat particles
  - Sensitive, low false alarm rate, fast response
- **Subjected to extensive testing**
  - Performance
  - environmental
- **BAWS integrated into JBPDS**



---

# Emerging Technologies in Sample Analysis

4 April 2002

New England Bioterrorism Preparedness Workshop

MIT Lincoln Laboratory

**Bernadette Johnson**

**(781)981-1902**

**bernadette@ll.mit.edu**

MS-15453

This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.

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MIT Lincoln Laboratory



# Outline

---

- **Current techniques in sample analysis**
  - Clinical (subject of yesterday's talk)
  - Environmental
- **Challenges associated with environmental sampling**
- **Examples of technologies in use and in development**



# **CDC's Sample Analysis Guidelines**

## **(example: *B. Anthracis*)**

---

- **Persons suspected of exposure/infection**
  - Cultures of blood and spinal fluid
  - Cultures of tissues or fluids from affected areas
  - Microscopic examination
  - PCR
  - Nasal swab (occasionally for exposure, but not for diagnosis)
  - Antibody testing (exposure, not validated for diagnosis)
  
- **Environmental contamination**
  - Cultures of air samples, surface swabs, suspicious powders
  - Microscopic examination of suspect material
  - Evaluation of growth properties of suspect agent
  - PCR
  - DFA (direct fluorescent assay) to detect key bacterial proteins
  - Specialized tests, such as immunoassays (SMART)



# How Do These Techniques Compare?



**Rapid ID**

**Immunoassays**

Bioagent  
Antibody  
Labeled Antibody  
Substrate

- Selectivity from high affinity binding of antibody to agent-specific structures

~15 minutes

**Orthogonal ID Confirmation Technologies**

**Polymerase Chain Reaction (PCR)**

Chemical multiplication of DNA  
(x10<sup>6</sup>)

- Selectivity from sequence-specific DNA/RNA recognition
- Enzymatic amplification provides superb sensitivity

1- 4 hrs

**Culture-based assays**

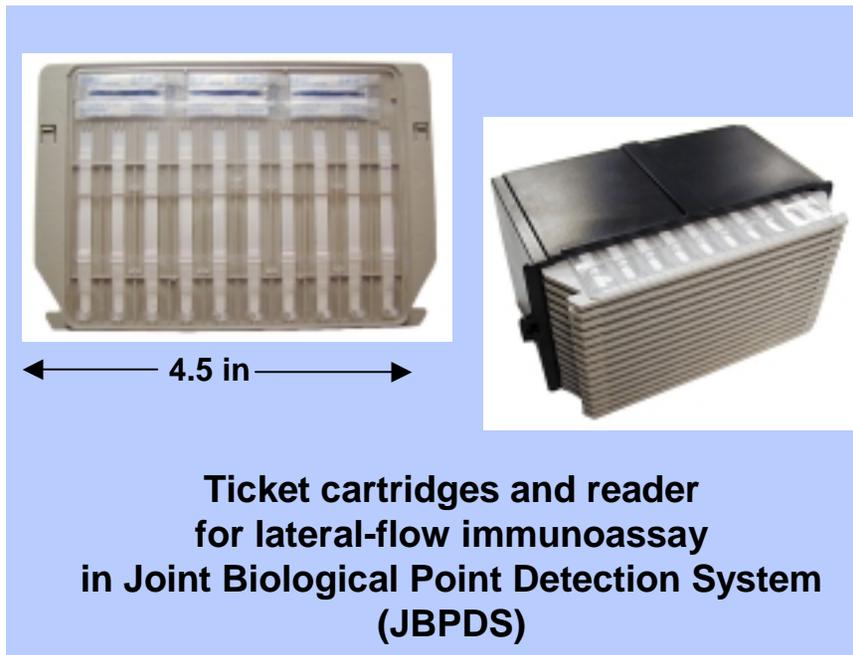
- Traditional method since Pasteur – still “gold standard” for ID
- Viable organisms replicated in culture and identified using biochemical assays and microscopy

1-3 days





# Examples of In-use and **Developmental** Immunoassay Devices



**Response Equipment Co.  
Bio-HAZ Biodetector**



**Dendrimer-Based Alert Ticket (ARL)**



**Upconverting Phosphors (SRI)**



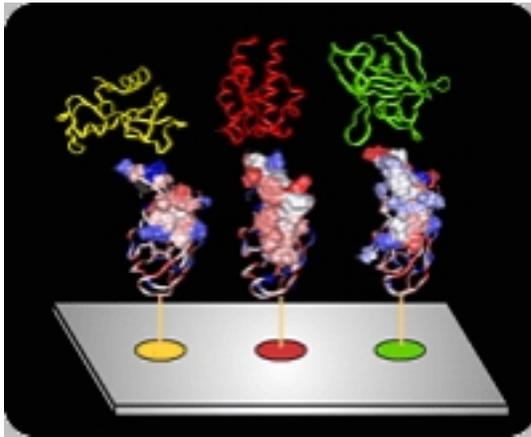
# Features of Immunoassay Analysis

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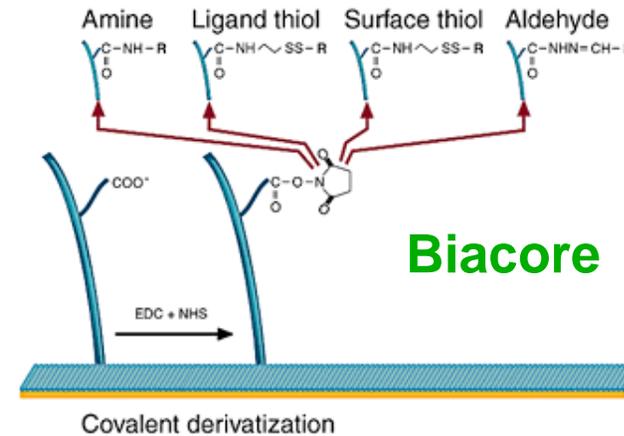
- **Can be used on environmental samples with little or no preparation**
- **Readout is fast (~ 15 minutes) and simple (colorimetric or fluorimetric)**
- **Sensitivity modest (~10,000 - 100,000 particles)**
  - **Depends on antibody-antigen binding affinity and readout scheme**
- **Specificity reasonably good**
  - **Depends on antibody construct and antigen specificity**
- **Current IAs are not multiplexed; development of protein microarrays may lead to sensitive, multi-assay analysis tools**



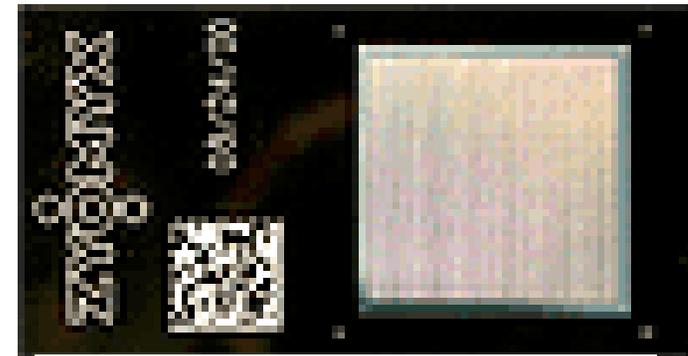
# Examples of Existing Protein Microarrays



Phylos (2000 element)



Ciphergen (multiple classes of proteins)



Zyomyx (10,000/cm<sup>2</sup>)

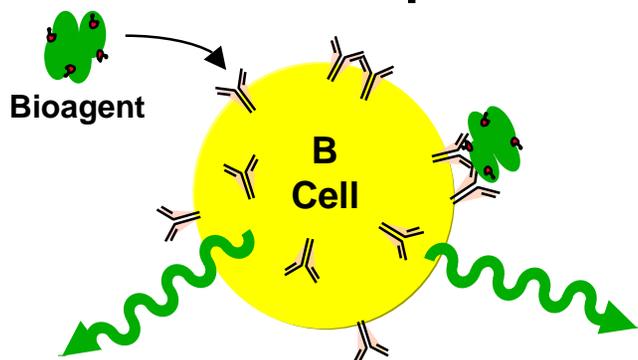
- Protein microarray technology development driven by drug screening and disease-marker investigations
  - Diagnostics (clinical and environmental) still developmental

MIT Lincoln Laboratory



# Developmental Antibody-Based Sensor: CANARY

## Concept



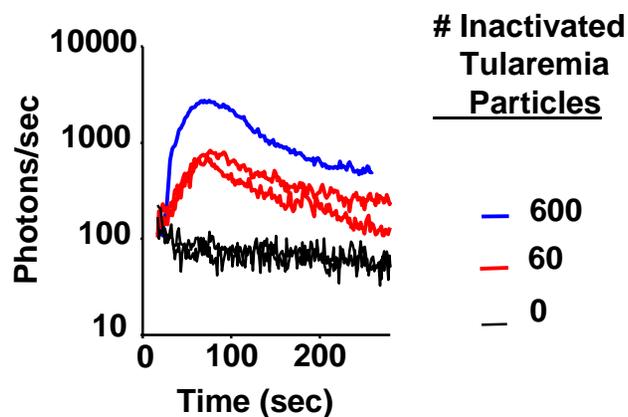
B cell emits ~200 photons within  
30 seconds after bioagent binding

## Prototype microcentrifuge device



## Tests Against Killed Tularemia

(Collab. with NMRC)



## Status of B-Cell Lines

### Complete

FMDV  
VEE  
*Vibrio cholera*  
Orthopox viruses  
*Yersinia pestis*  
*Brucella spp*  
*Francisella tularensis*

### In development

*Coxiella burnetti*  
*Bacillus anthracis*  
*E. coli* O157:H7



# PCR-Based Analysis Tools

- **Systems being developed (and deployed) that provide agent ID within 30 minutes of introduction of prepared sample**

Semi-automated field-portable  
PCR devices



*RAPID - Idaho Technologies*



*SmartCycler  
XC System - Cepheid*

Example of handheld PCR  
device

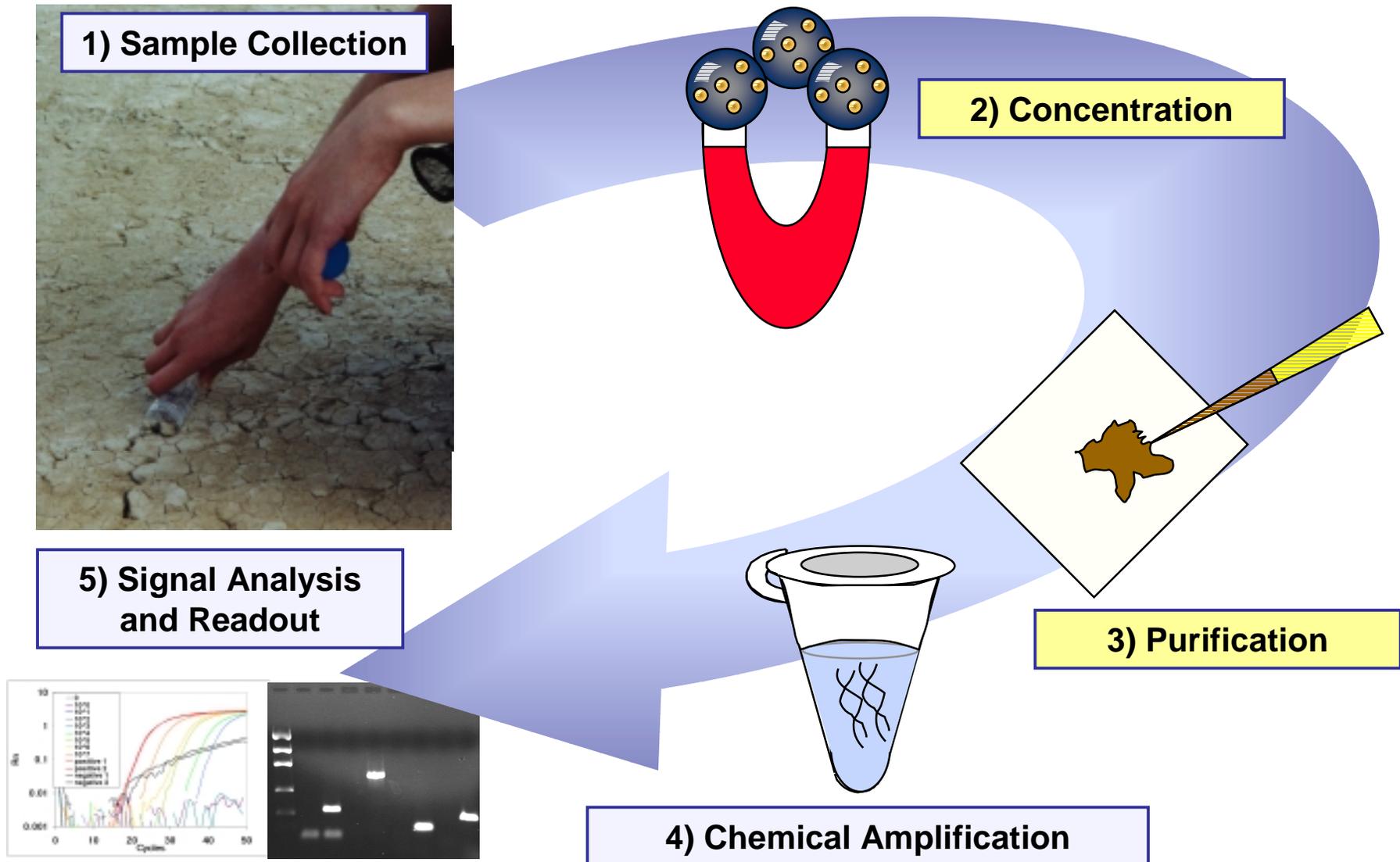


*HANAA - Handheld Nucleic  
Acid Analyzer, developed by  
LLNL, Cepheid, and ETG, Inc.*

- **Challenge remains in automating sample preparation and analysis**
  - Pathogen cells or spores must be ruptured to liberate the DNA/RNA
  - DNA/RNA must be separated from protein debris/environmental impurities

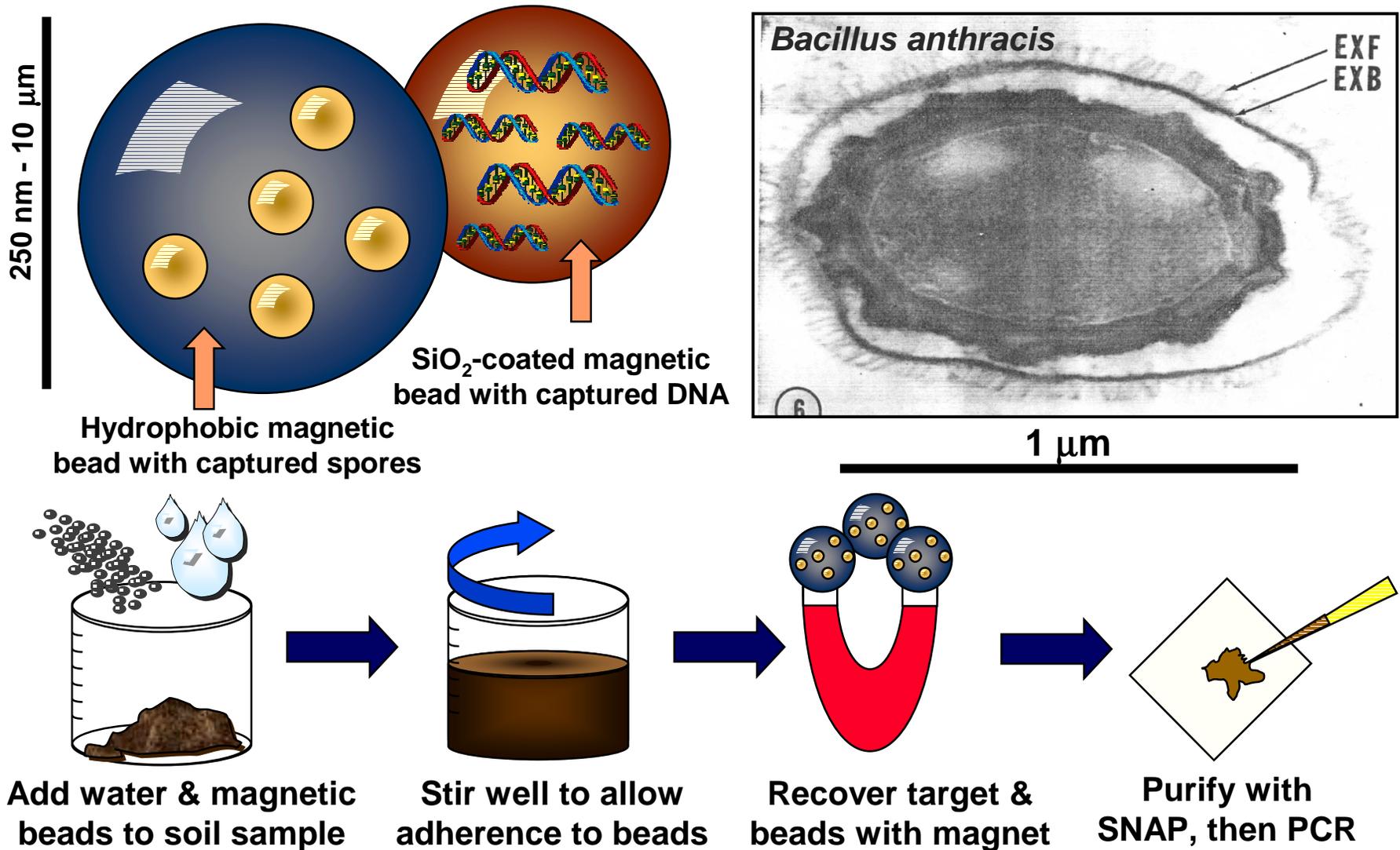


# Overview of Sample Preparation





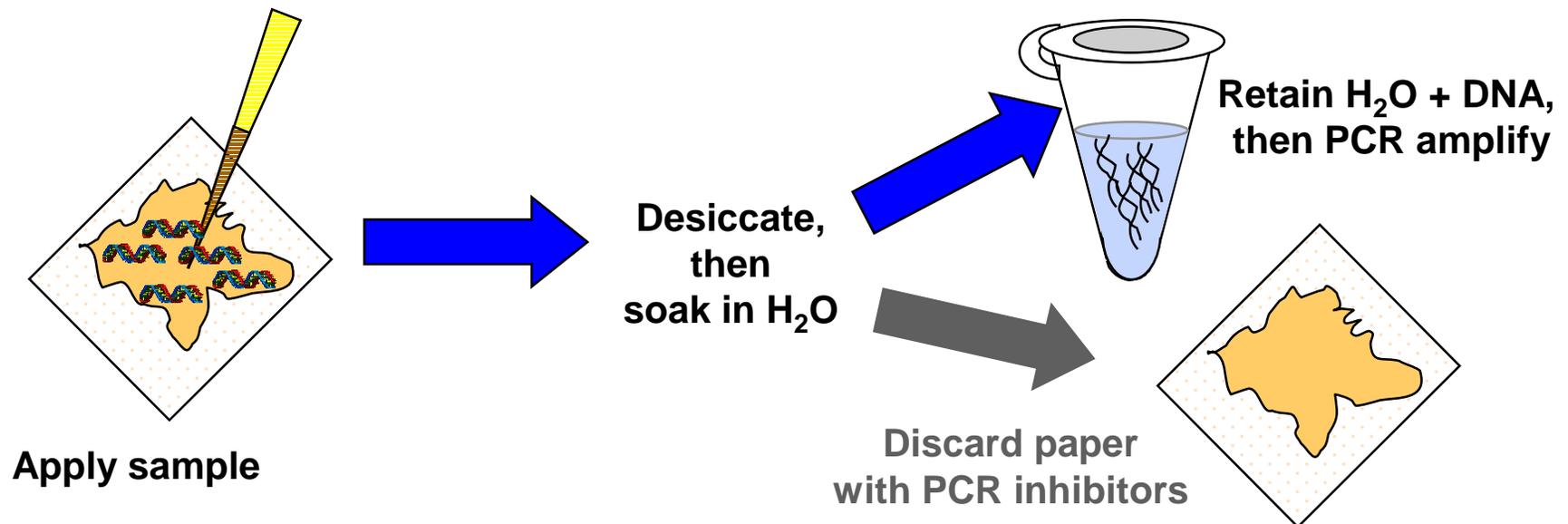
# Target Concentration: Affinity Magnet Protocol





# DNA Purification: Simple Nucleic Acid Prep (SNAP)

- Chemically treated paper is the key component of SNAP
- Lyses cells, binds PCR-assay inhibitors, and purifies DNA
- **Advantages:**
  - Fast and easy (1/5th the time of other published protocols)
  - Water is only added reagent (no phenol, chloroform, or alcohol)
  - Lightweight, compact, enables archiving
  - On-site fixation: preserves DNA & kills pathogenic organisms

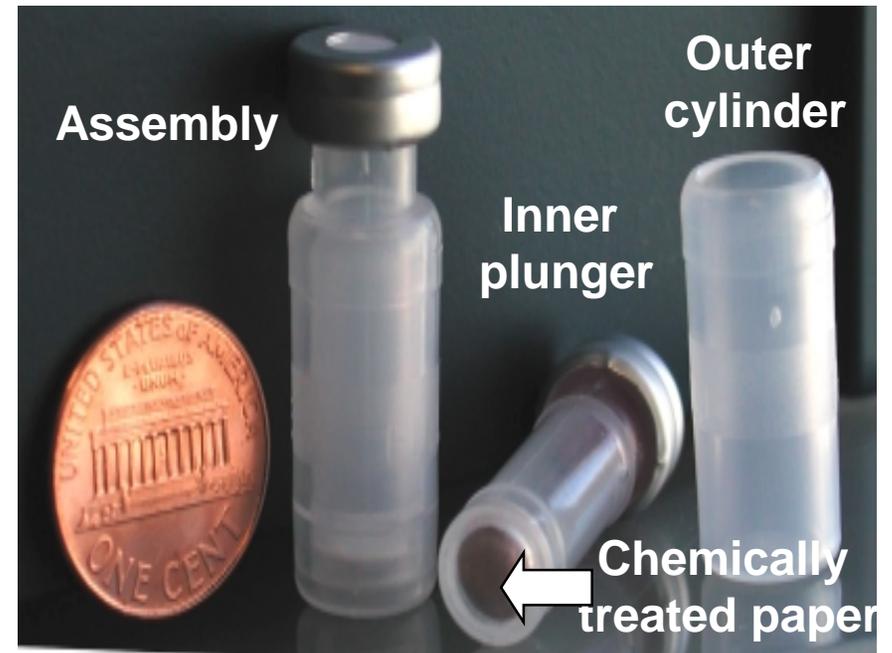




# Lincoln Interim Nucleic-acid Kit (LINK) (Developed in response to October 2001 events)

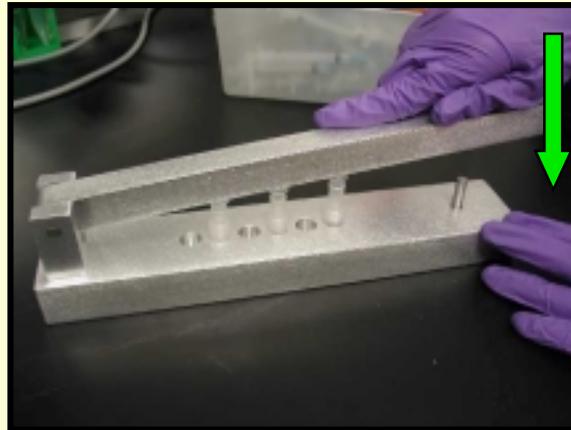
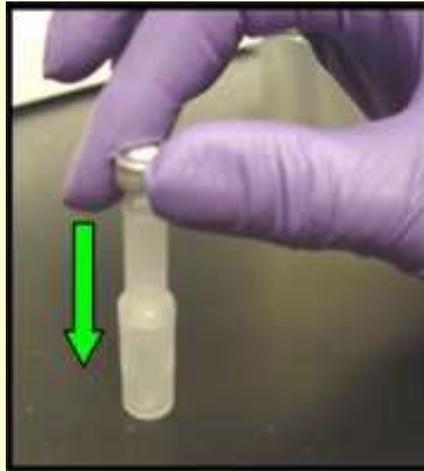
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- **LINK as a solution:**
  - Incorporates SNAP paper but in a more user-friendly format
  - Faster processing than basic SNAP
  - Easier to sample, handle, and process
  - Enables on-site fixation
  - Outside can be decontaminated
  - 6 minute processing time
  - Single-step processing
  - Results equal to or better than basic SNAP





# How to Use LINK



1) Apply sample  
Sit for 5 minutes

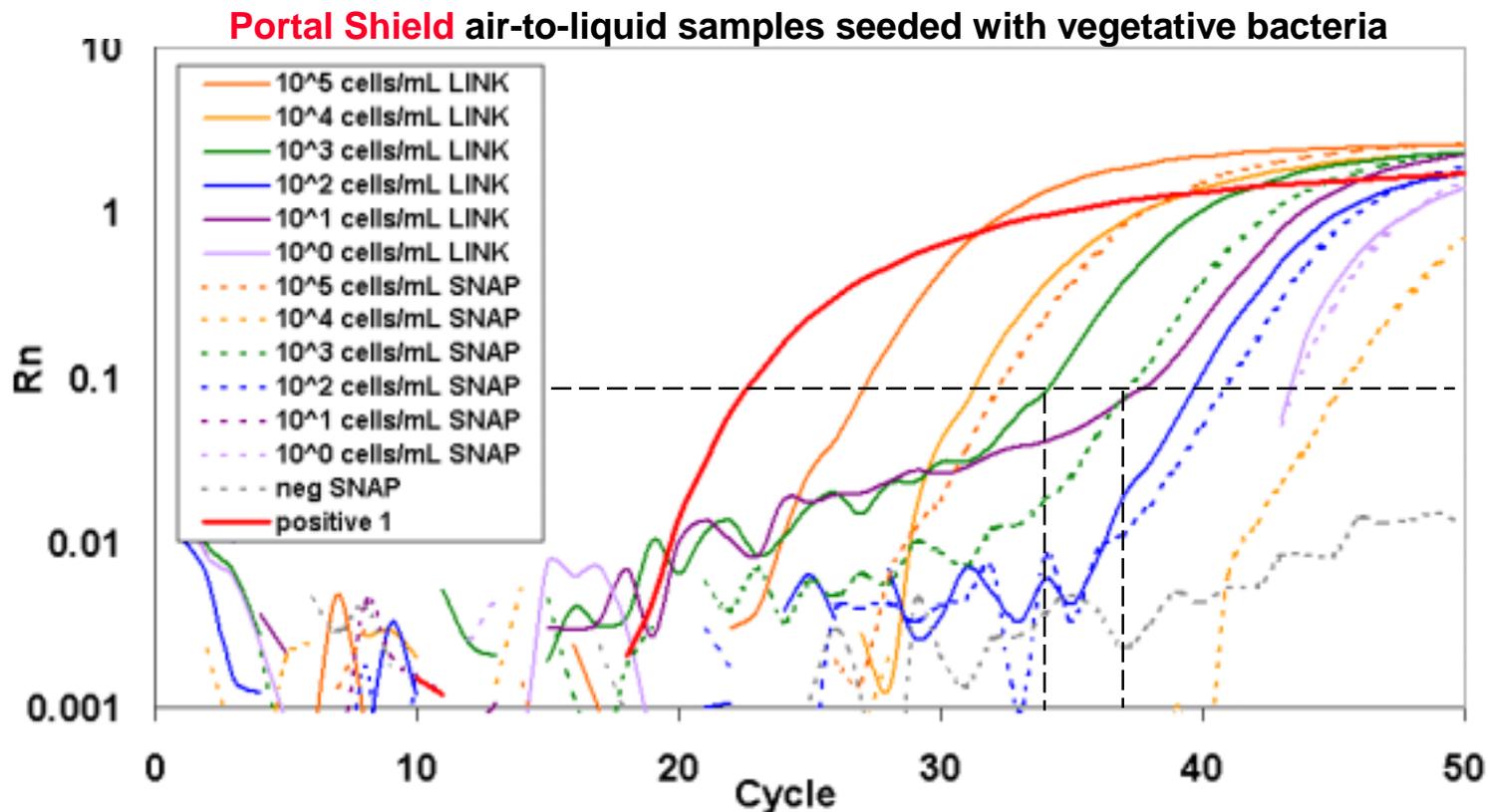
2) Process in **one step**

3) Remove DNA  
**Total time ~6 minutes!**



# LINK Cartridge Works with Varied Samples

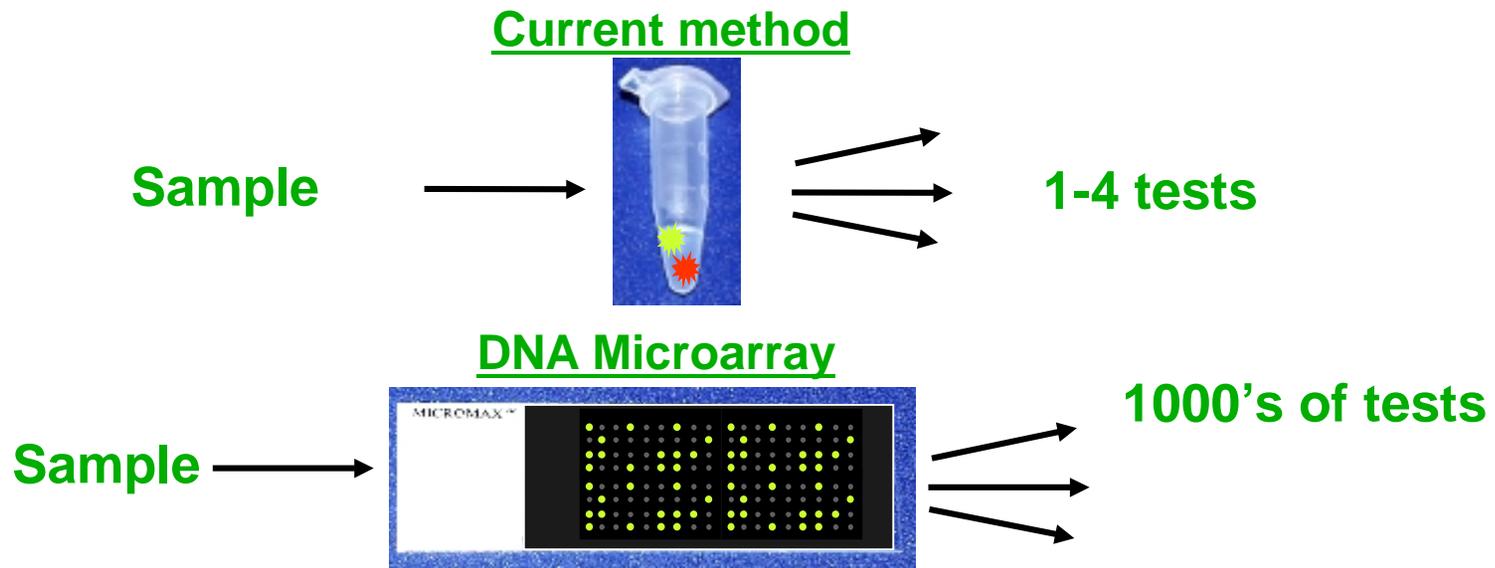
- LINK detection from:
  - **Portal Shield** air-to-liquid samples seeded with vegetative bacteria
  - Untreated domestic sewage (Boston) seeded with vegetative bacteria
  - Paper, envelopes, skin seeded with bacterial spores
  - Air impaction with dry bacterial spores





# What About DNA Microarrays?

- DNA Microarray: Any 2D or 3D substrate having many ( $\sim 10^2$ - $10^5$ ) different nucleic-acid capture sites (probes)
- Can identify both strain and drug resistance of pathogens
- Can offer highly multiplexed assay capability





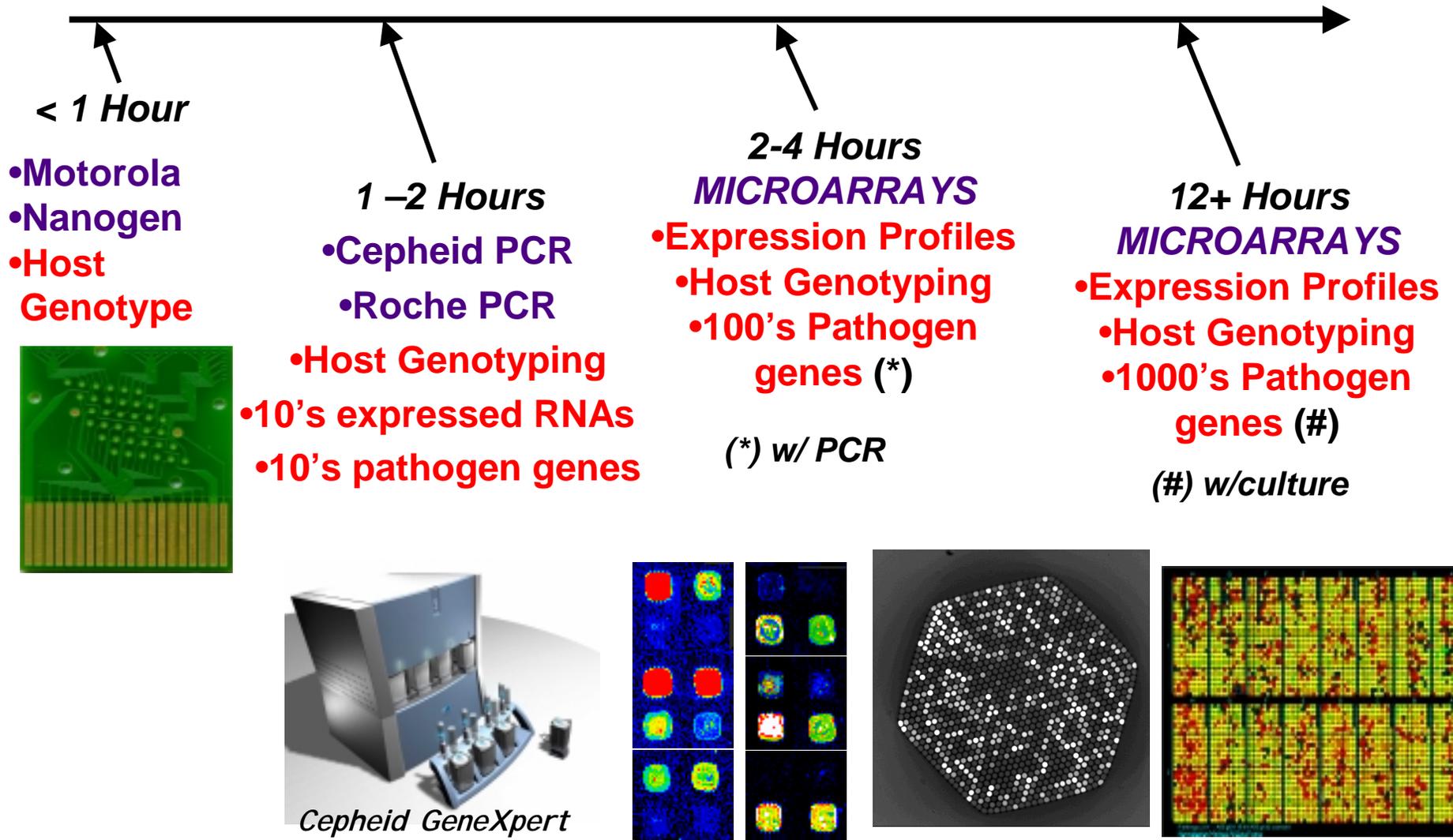
# Pathogen Identification via DNA Microarray

---

- **Detect small amounts (<100 copies per ml) of pathogen-specific nucleic acids in environmental sample**
- **Arrays might provide log orders more information than current PCR-based approaches (e.g. TaqMan)**
- **Challenges for diagnostic applications:**
  - **Never demonstrated for environmental (or clinical) samples**
  - **Amplification may be necessary before micro-array assay**
  - **Sample preparation required (as in PCR techniques)**



# Assay Times for Current and Emerging PCR/DNA Systems





# Summary

---

- **Environmental sample analysis parallels methodology developed for clinical sampling**
  - Immunoassays for rapid estimate of exposure (not yet CDC authorized)
  - PCR techniques being deployed in some laboratories to provide strain specificity and drug resistance
  - Culture still used to provide “gold standard” for pathogen ID
- **New technology developments could greatly increase the speed, sensitivity, and multiplicity of environmental assays**
  - Protein microarrays could offer highly multiplexed, rapid ID capability on collected samples
  - DNA microarrays could offer hundreds to thousands of pathogen tests on single-chip format, provided sample preparation can be made compatible



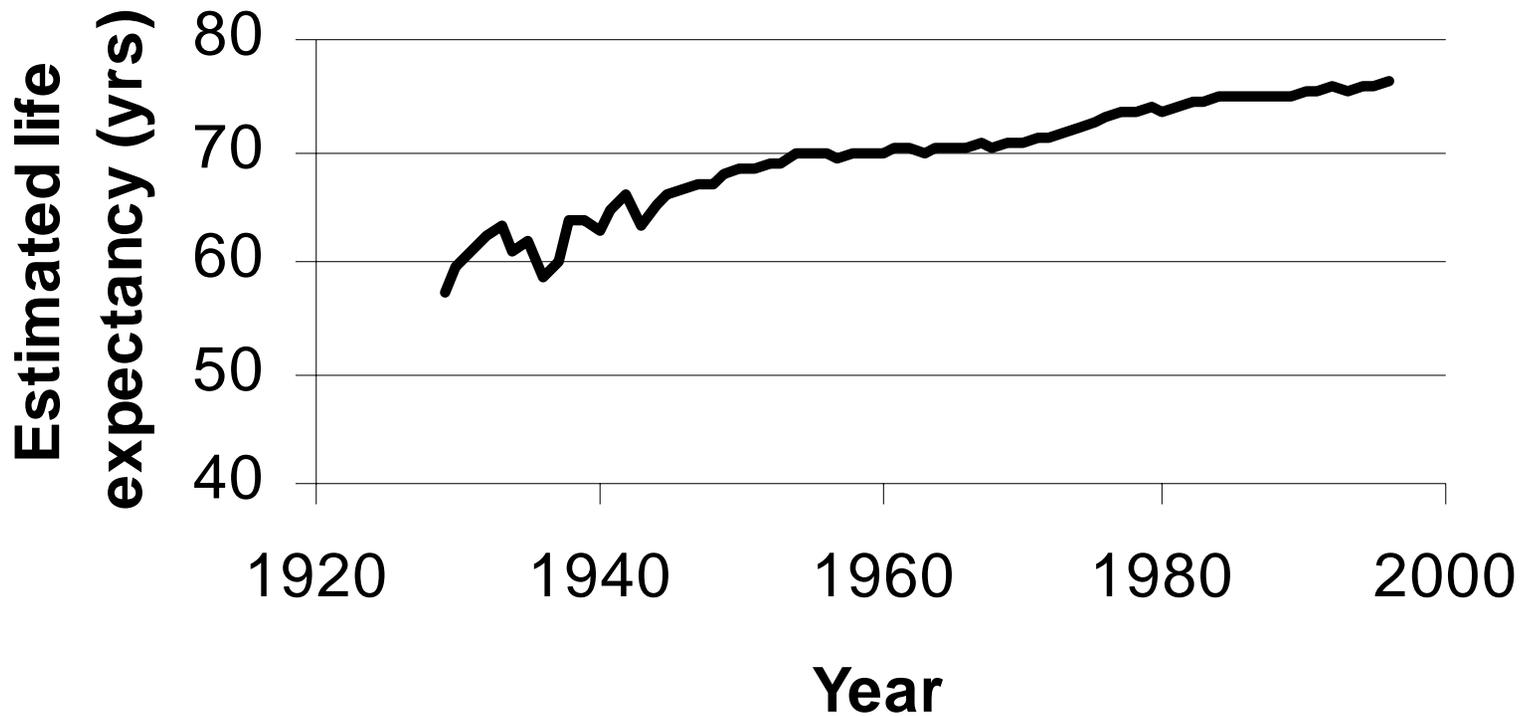
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# Where are we?

- The Age of Ignorance (no understanding of science, no control, all R no B)
  - Cotton Mather on colonial times: “A dead child was a sight no more surprising than a broken pitcher”
- The Age of Discovery (revolution in science, ability to understand and control disease, take R to get B)
- The Age of Miracles (idea of the magic pill or magic bullet, science can cure any problem, pursue B with abandon)
- The Age of Risk Management (science is critical, but we have to make good choices to avoid overkill, balancing R and B)

•  
•  
•

# What does this mean for public health? Longer lives...



# • • Great progress – a few examples

- Diagnosis of disease based on gross physical characteristics --> laboratory analyses of body fluids and genetic testing and interventions that save lives
- Sulfanilamide --> numerous antibiotics
- Focus on feeding and milk composition for infants --> pasteurization, refrigeration, infant formulas, dehydration treatments, and improvements in medical care

# • Hunnewell Building, Circa 1914



Photograph courtesy of Children's Hospital Archives, Boston, MA.

© 2002, Kimberly M. Thompson, Sc.D.

# Great progress

- The iron lung and deformities associated with polio --> immunizations for polio and many other diseases and eradication of small pox



Photograph courtesy of Children's Hospital Archives, Boston, MA.

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# Public health improvements

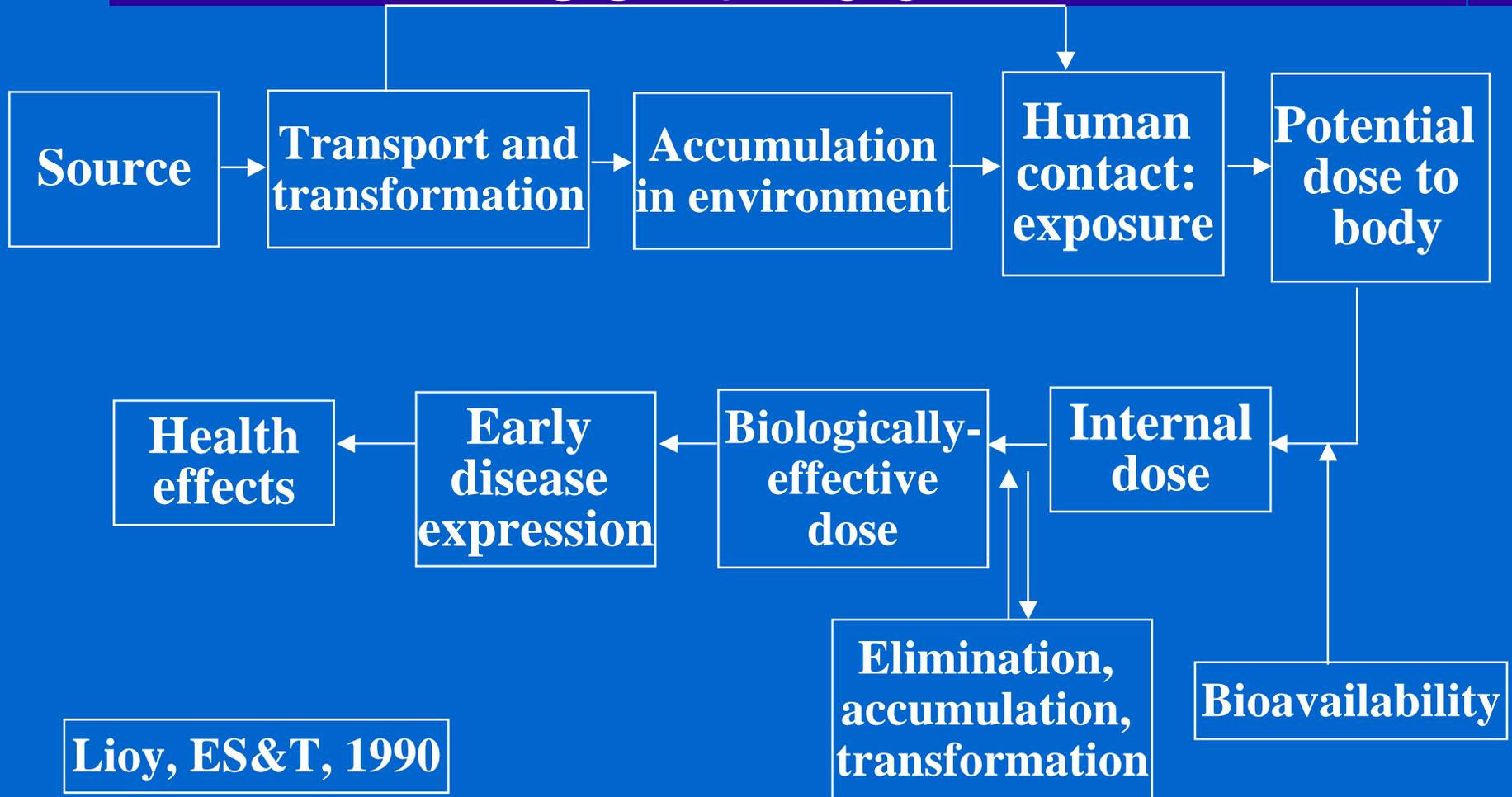
- Are we winning the war with germs?
  - Certainly doing better with respect to health outcomes (e.g., saving lives once lost to some infections, and reducing the severity and spread of infections)
  - Public perception now that infectious disease is not as much of a problem (immunization)
- Wait
  - BIG issues remain with antibiotic resistance/“Superbugs”/ new diseases
  - Prevailing assumption that releases of organisms would be unintentional (i.e., we’re fighting nature)
  - Infectious disease still a leading cause of death

- 
- 
- 

## Context

- Given the background of ID, what does BT preparedness look like and how does it fit in with basic public health?
- What tools can help us understand the risks and measure the impacts of interventions?
- How will we know that a BT preparedness program works?
- What decisions get made about characterizing the different agents?

# Human health risk continuum



Liroy, ES&T, 1990

# The need for risk and decision analyses

- Risk analysis and decision analysis are used to integrate information and sift it down into a usable form
- Used support many actions:
  - Initiating regulatory activity or treatments
  - Setting protective standards
  - Selecting products, technologies, or substances
  - Siting hazardous facilities, isolation choices
  - Cleaning up or control of contaminated areas
  - Initiating research and establishing priorities
  - Others....
- Key component of decision (but not only)



- 
- 
- 

# Decision tools

- Risk analysis
- Benefit-cost analysis
- Cost-effectiveness analysis
- Decision analysis
- Comparative risk analysis

All share common elements to some degree, but differences do matter

# Variability vs. uncertainty

- Variability - heterogeneity or diversity in a well-characterized population which is usually not reducible through further measurement or study
- Uncertainty - ignorance about a poorly characterized phenomenon that is sometimes reducible through further measurement or study
- Variability and Uncertainty = f(decision context)
  - NRC (1994): “Uncertainty forces decision makers to judge how probable it is that risks will be overestimated or underestimated for every member of the exposed population, whereas variability forces them to cope with the certainty that different individuals will be subjected to Techniques exist to maintain these separately
  - risks both above and below any reference point one chooses.”

# Risk estimates do matter

- Example 1 – uncertainty about the effectiveness of airbags in motor vehicles
- Example 2 – variability in the mortality risk to people on the ground from crashing airplanes

# Cost-effectiveness analysis

- One of many tools
- Growing role in medical decision making
- Panel on Cost-effectiveness in Health and Medicine
  - Total costs/Total effectiveness (Incremental ratio)
  - Recommended methods (QALYs, 3% discount rate, societal perspective)
- Typical CEA ignores uncertainty, variability, time, preferences and other attributes, troubles with zeros, criteria for “acceptability”

# Why care about dynamic nature?

- Optimal strategies change with time
- Dynamics may be very important to model to characterize the benefits of herd immunity
- Times of major shifts (e.g., perceptions of risk and benefits change going from wild type cases to vaccine-associated cases, with eradication risk shifts to polio in bio warfare)
- When we assess the CE ratio may matter in terms of policy

# Changing CE model components

- Most vaccine CEA's assume constant probabilities of getting infection (for both vaccinated and unvaccinated children) – may not capture big herd immunity effects (e.g. mass vaccination reduces risks for unvaccinated as well as vaccinated people)
- Other time-dependent factors:
  - Costs (For single vaccine and program, do these go up, down, or stay the same over time?)
  - Preferences and values
  - Societal dynamics (urbanization, more women working so staying home has greater opportunity costs)
  - Technology

- 
- 
- 

## Do these matter?

- Consider a case study on polio
  - Long history
  - ... but not too long
  - Numerous interventions
  - Near eradication
  - Good time to remind people
  - Story of many successes
  - Could make the transition from ID to possible BW agent if public health community successful

- 
- 
- 

## Project: Background

- Herd immunity effects following polio vaccination.
- E.g. mass vaccination of 95% of infants will reduce the probability for unvaccinated persons as well.
- Other time-dependent factors:
  - price of vaccine
  - with discounting of health and dollars: ->point of time of disease is important
  - demography, technology, etc.

# Retrospective Polio CEA Model(1)

- Ideally, we have for all vaccine programs:

- $\text{Cost}(t) = (V(t)tg(t)vc(t) + (D(t) - D^0(t))H(t))e^{rt}$

- $\text{Effectiveness}(t) = (D^0(t) - D(t))Q(t)$

$vc(t)$  = vaccine coverage (as function of time)

$V(t)$  = vaccine costs per completed vaccine schedule

$tg(t)$  = target group       $r$  = interest to year 2000 dollars

$D(t)$  = disease burden (incidence) under mass vaccination

$D^0(t)$  = incidence in absence of immunization program

$H(t), Q(t)$  = health costs resp. QALYs lost per disease case

•  
•  
•

# Retrospective Polio CEA Model(2)

- Cumulative cost-effectiveness ratio:

$$\text{CCE}(t) = \frac{\text{integrated discounted costs until } t}{\text{integrated discounted health gains until } t}$$

- Cost-effectiveness ratio:

$$\text{CE} = \text{CCE}(T_{\text{end}})$$

Suggested  $T_{\text{end}}$  : 2015

# Retrospective Polio CEA Model(3)

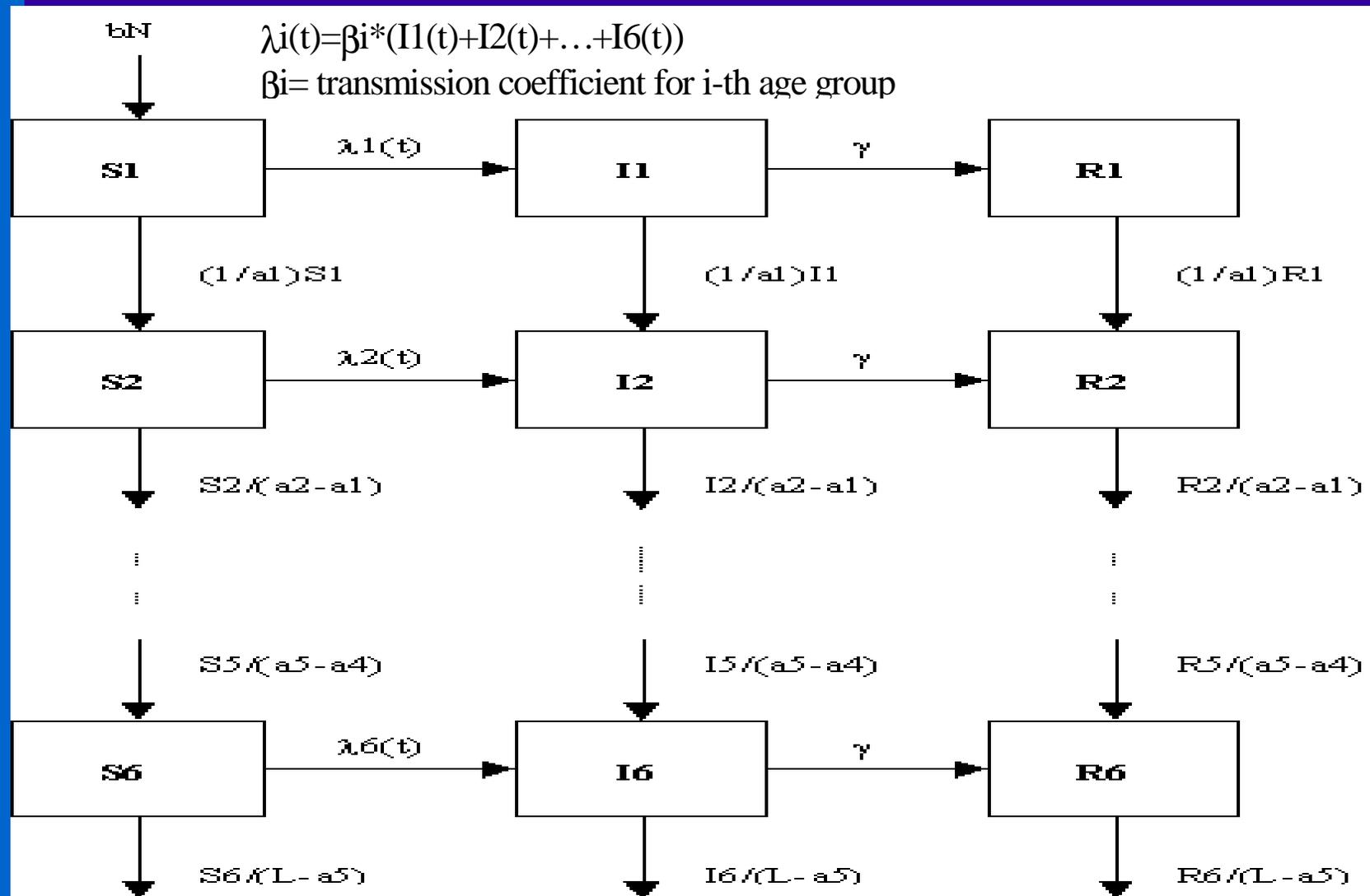
- The disease incidence with or without immunization program can be calculated with a transmission model -> requiring assumptions about transmission, and data
- For every variable except incidence, real historic data will be used.

# Concept of Transmission

## Models: SIR Models

- $S(t)$  = number of *susceptibles*: those individuals that could get infection
- $I(t)$  = number of *infecteds*: those that are infectious: they can contaminate susceptibles
- $R(t)$  = number of *removeds*: those that are immune to infection (*recovereds, resistants*)
- Transition rates between S, I, R  $\rightarrow$  differential eqns.
- $\lambda(t) = \beta * I(t)$  = force of infection = per susceptible rate of infection,  $\beta$  is the *transmission coefficient*

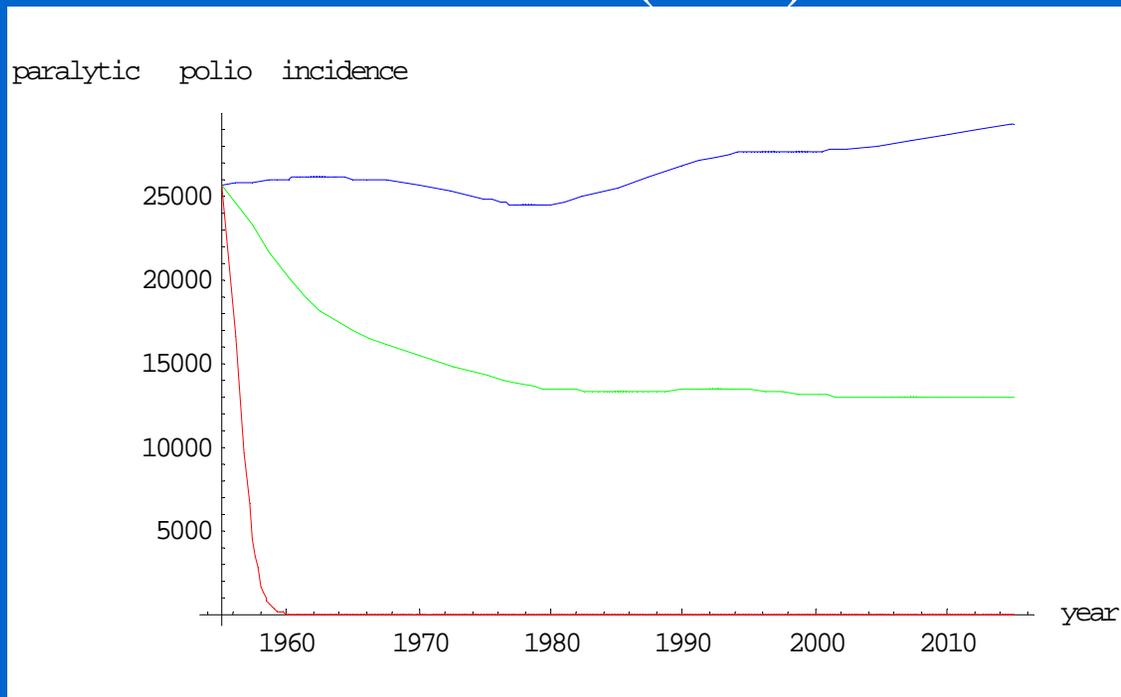
# Transmission model (1)



- 
- 
- 

# Example Results

Vaccine 1, paralytic polio incidence with static(green) and dynamic(red) transmission model and without vaccine(blue):



- 
- 
- 

# Insights

- Risk analysis and decision analysis tools have evolved to the point where they are helpful in characterizing and understanding the trade-offs associated with tough choices
- Must consider the dynamics of the disease to accurately quantify the health benefits
- Complex problem – analysis is needed
  - No zero risk
  - Real trade-offs

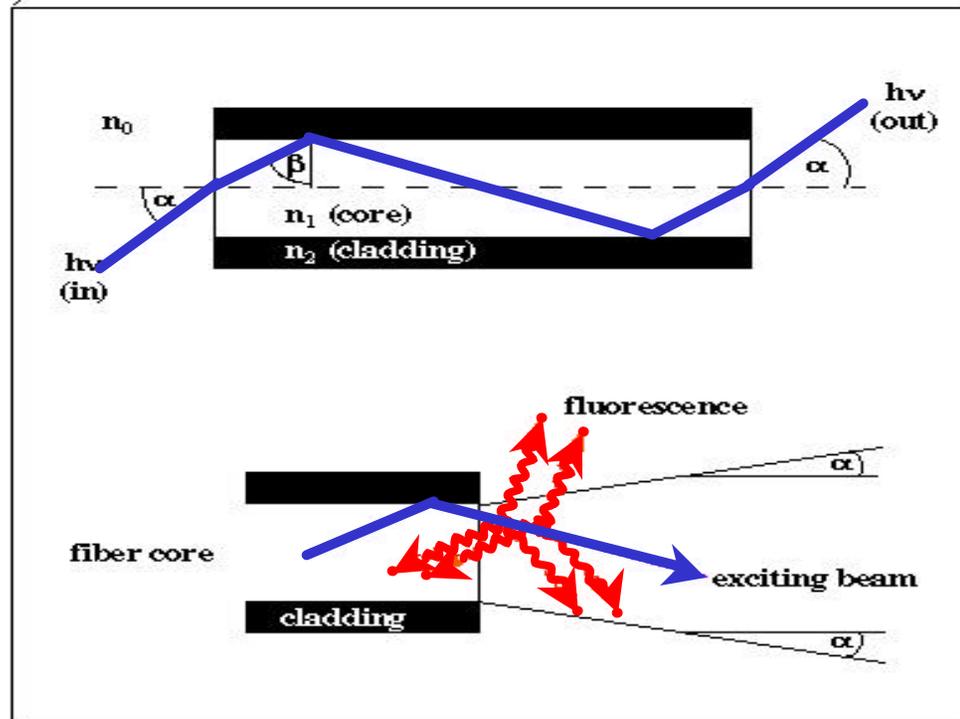
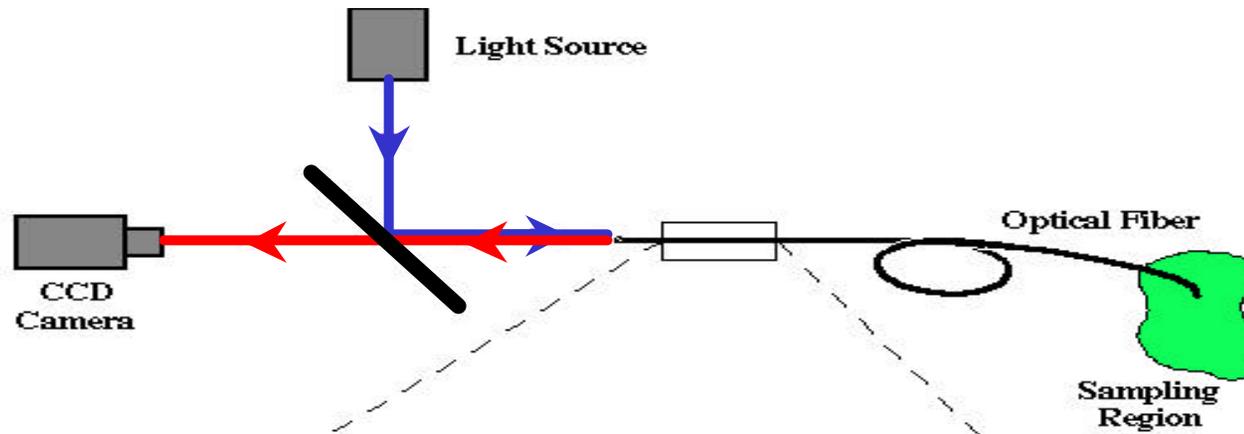
# **Microarrays**

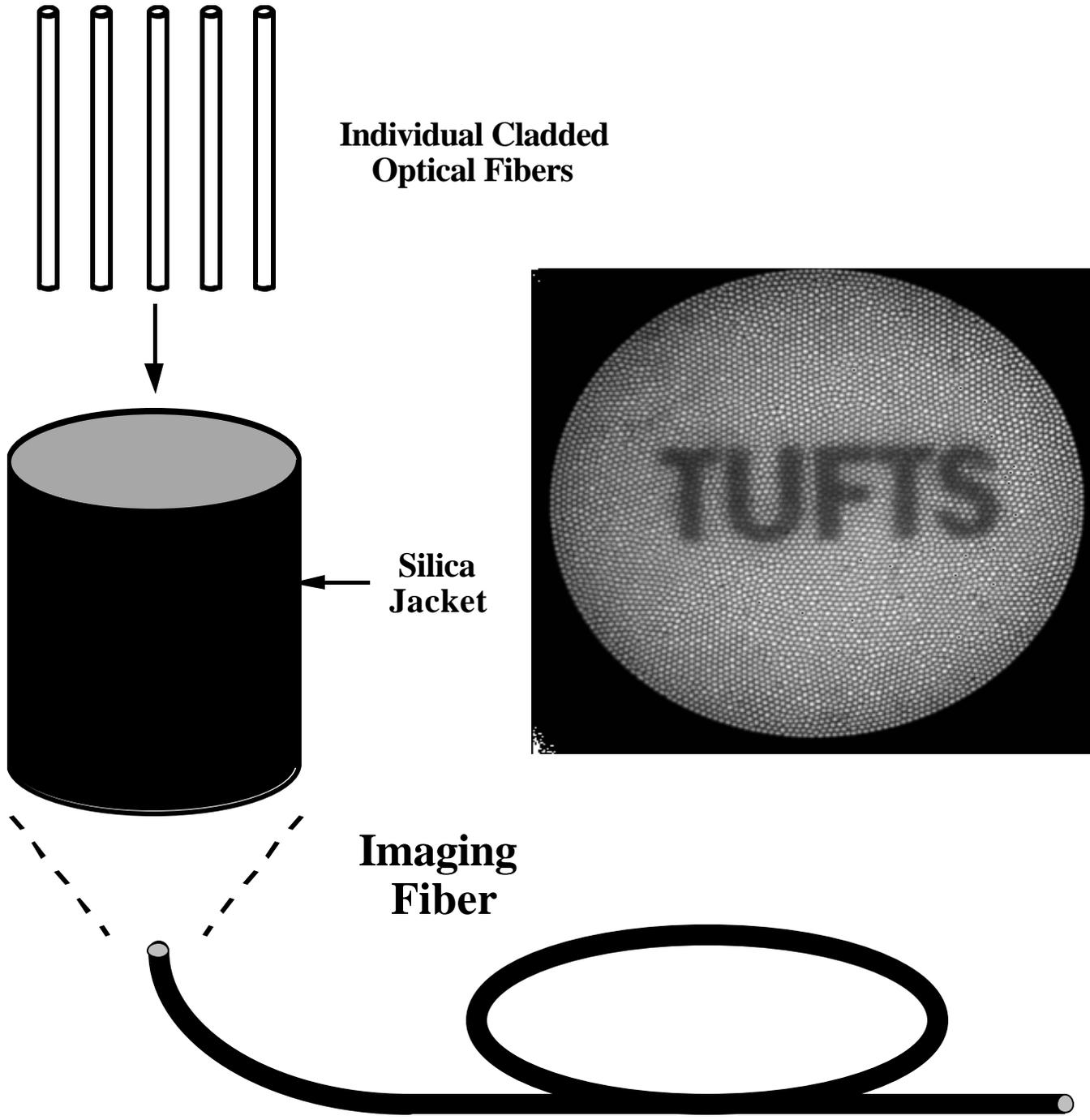
**New England Bioterrorism Preparedness Workshop**

**Dr. David Walt**

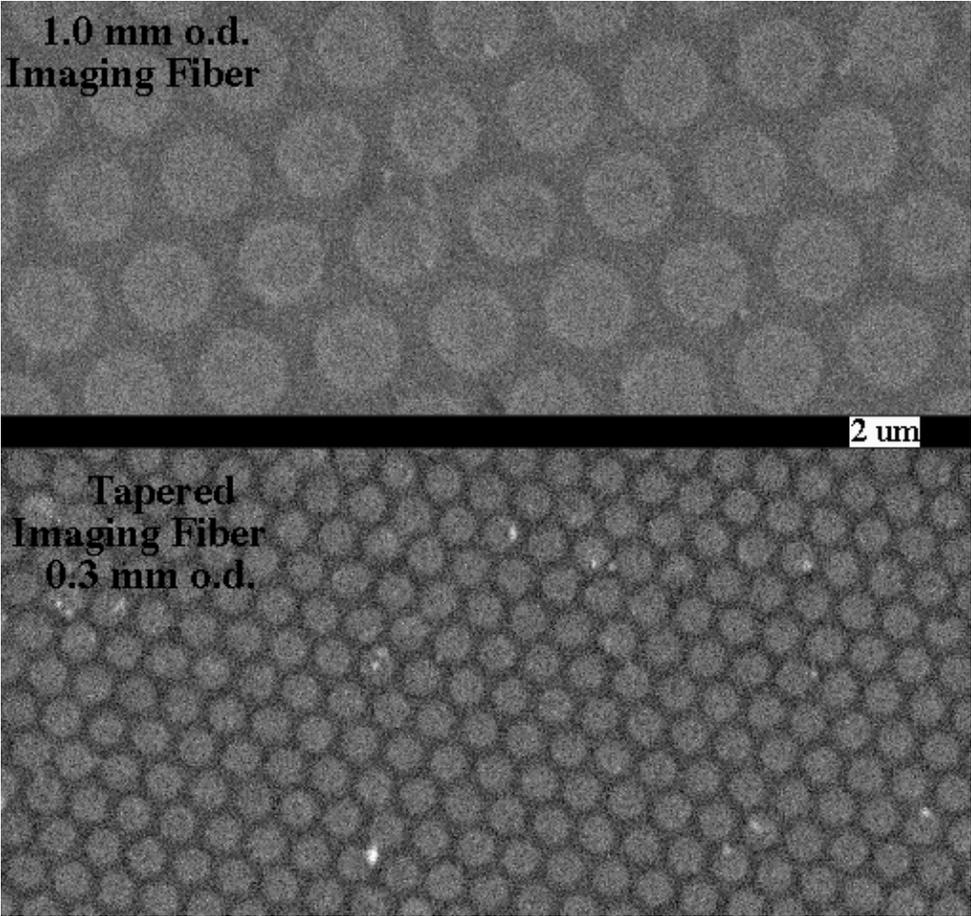
**Tufts University**

**4 April 2002**





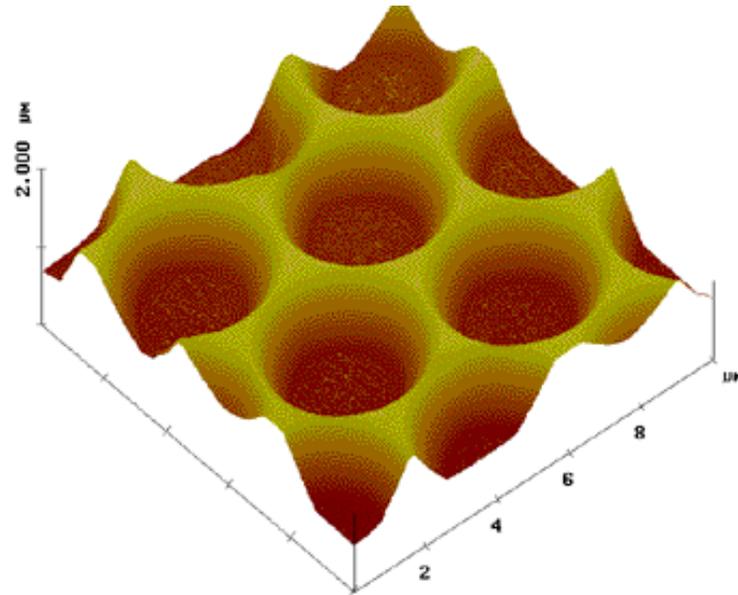
# Optical Imaging Fiber Before and After Tapering



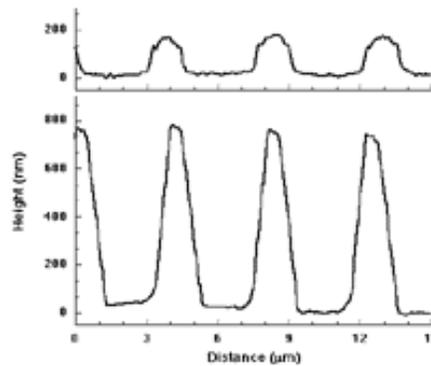
**Individual Core  
Diameter ~ 2.6 ? m**

**Individual Core  
Diameter ~ 0.85 ? m**

# AFM of a Chemically-Etched 1000- $\mu$ m Diameter Imaging Fiber



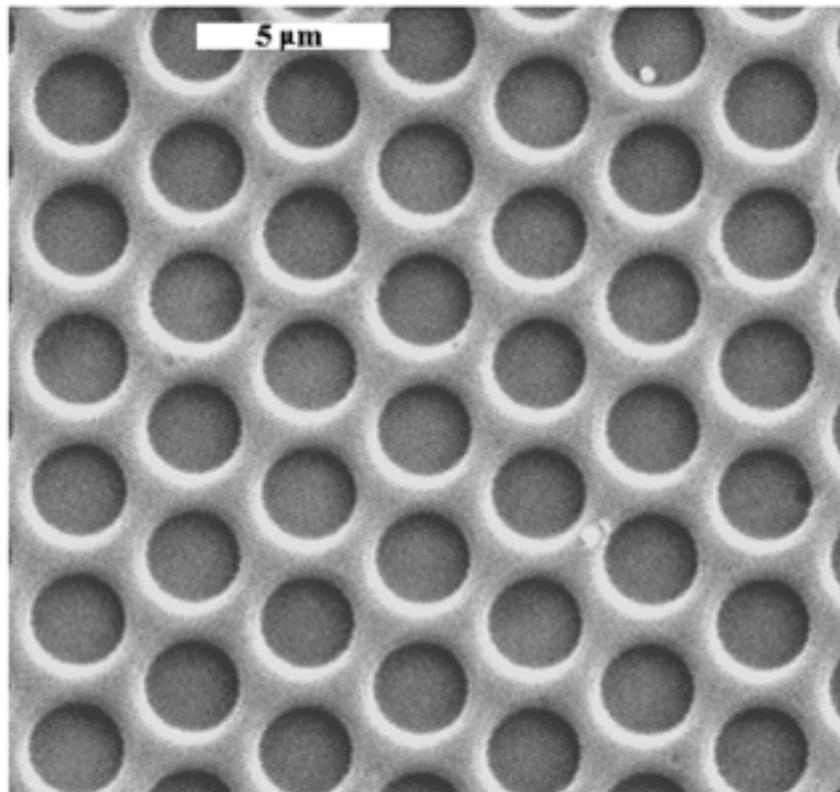
## Well Profiles



15 s etch

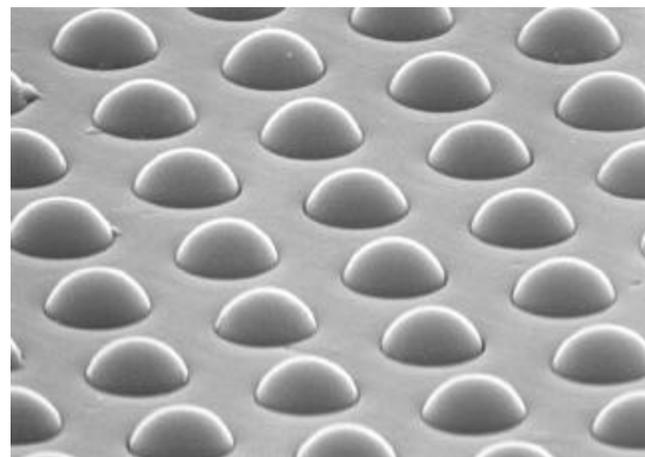
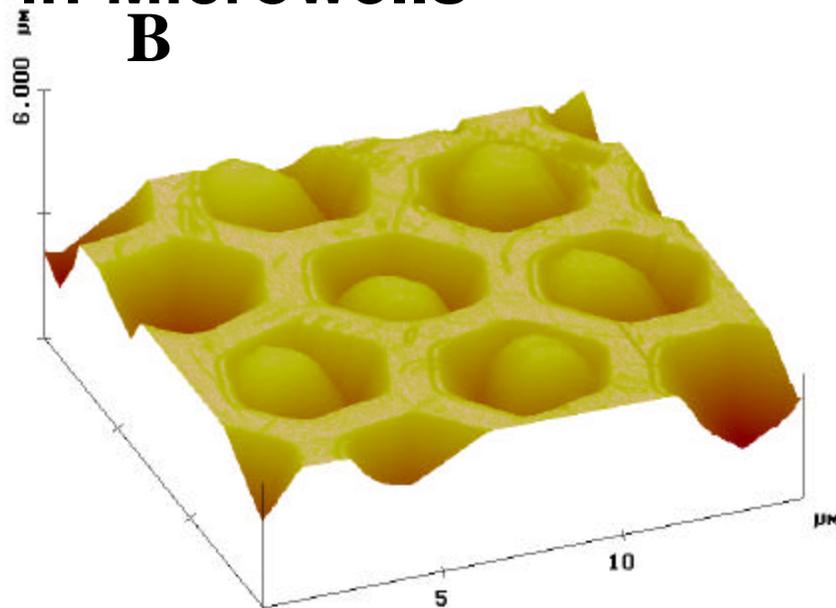
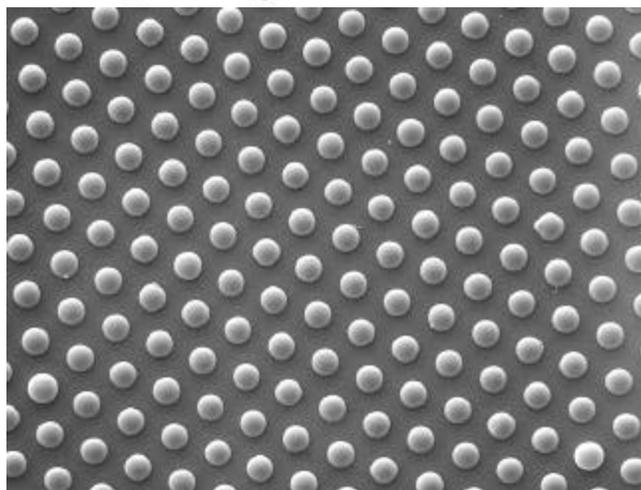
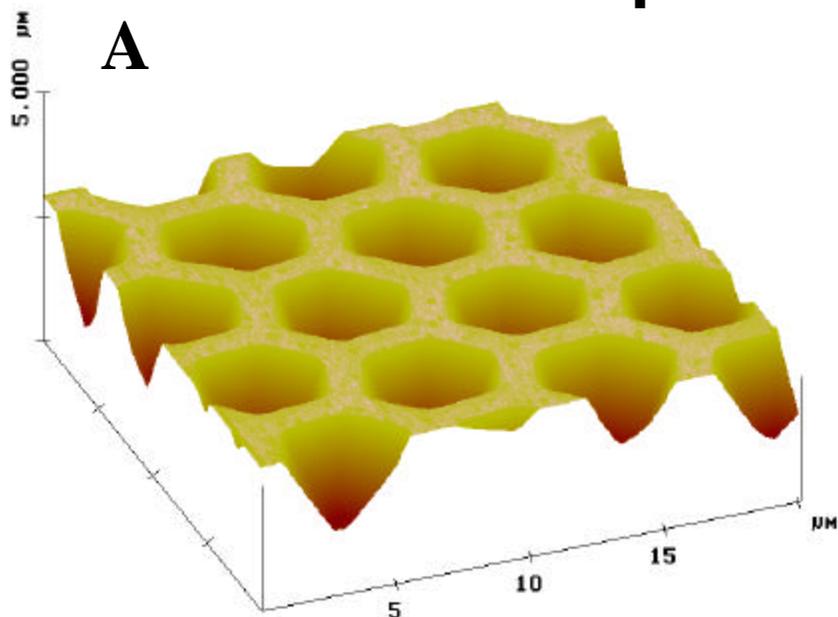
60 s etch

## SEM of a Chemically-Etched 1000- $\mu$ m Diameter Imaging Fiber

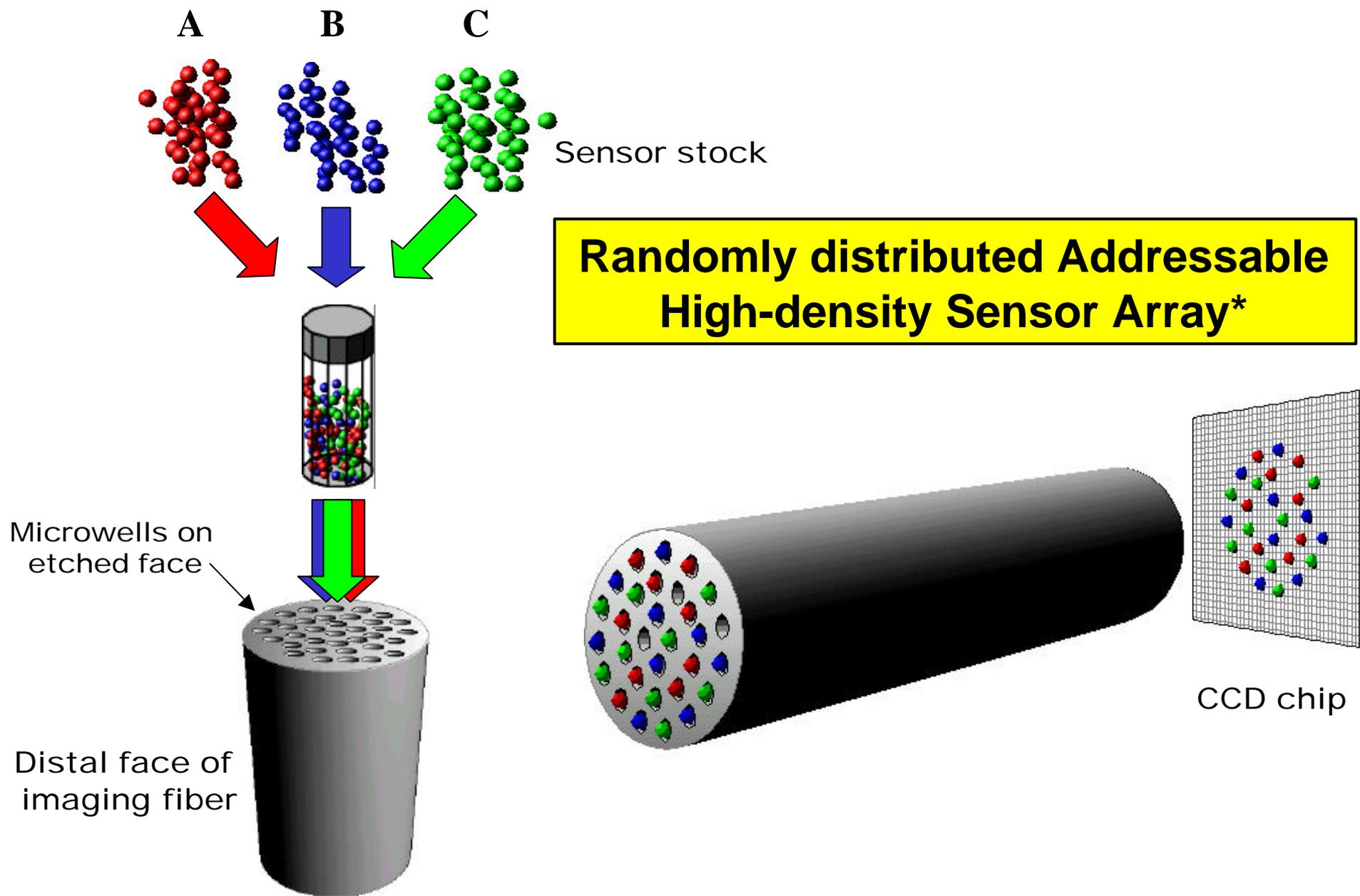


Pantano, P.; Walt, D.R. *Chem. Mater.* **1996**, 8, 2832-2835

# Microspheres in Microwells

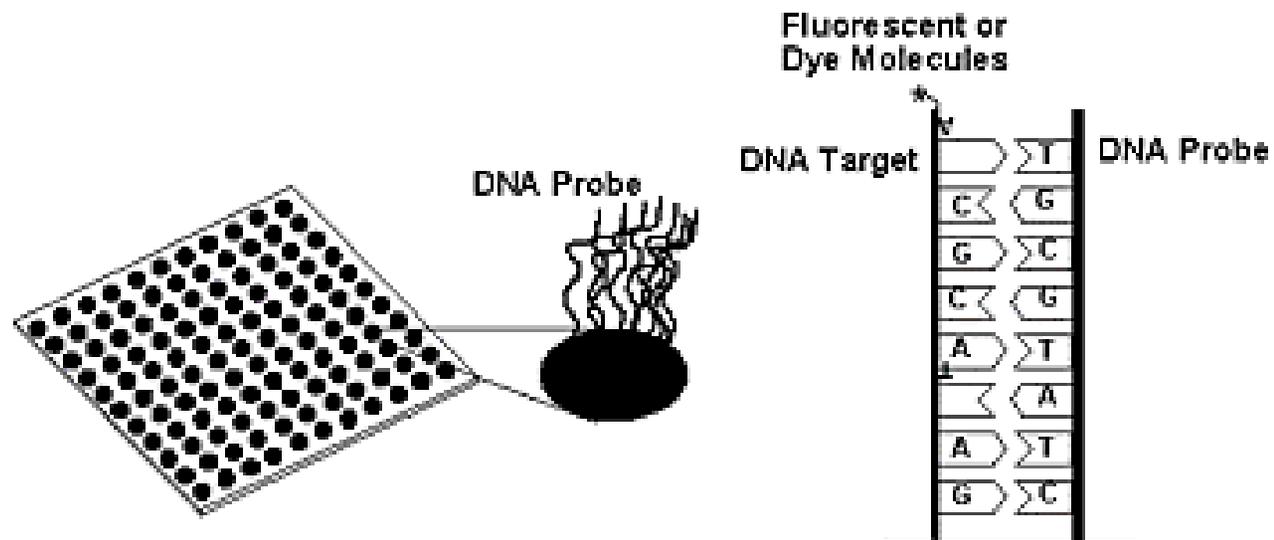


Michael, K.L. *et al. Anal. Chem.* 70 (7): 1242-1248 (1998).

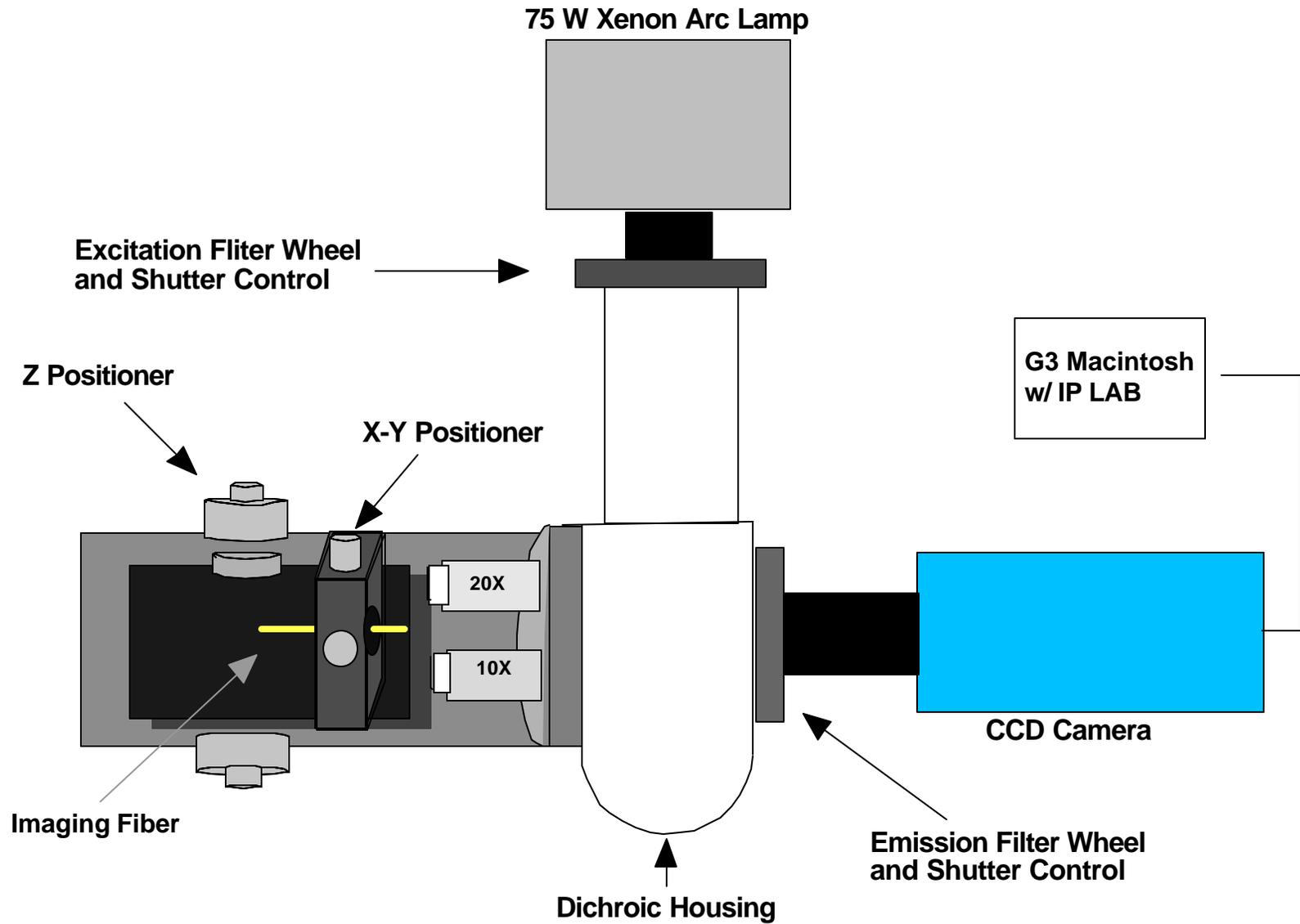


\*Michael et al. **1998** *Anal. Chem.* **70**: 1242-1248

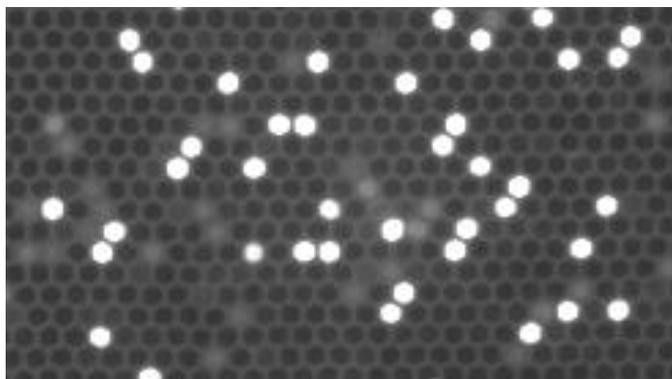
# DNA Array Principle



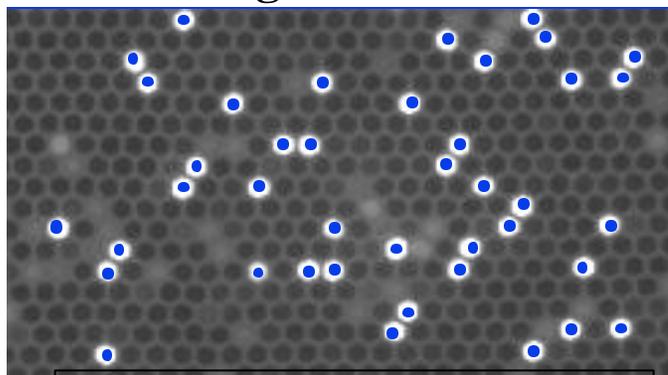
# Instrumentation: Modified Fluorescence Microscope



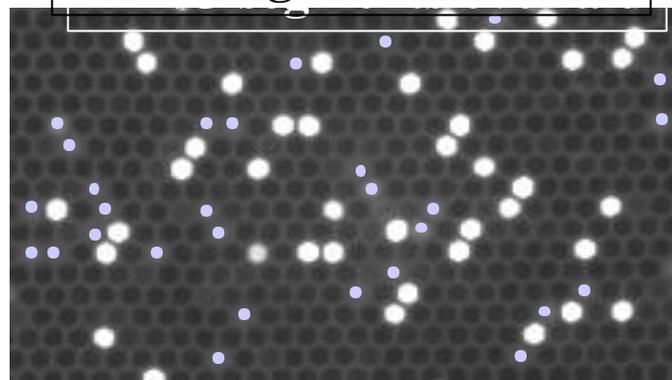
**Encoding Signal of Dye 1**



**Signal 530**

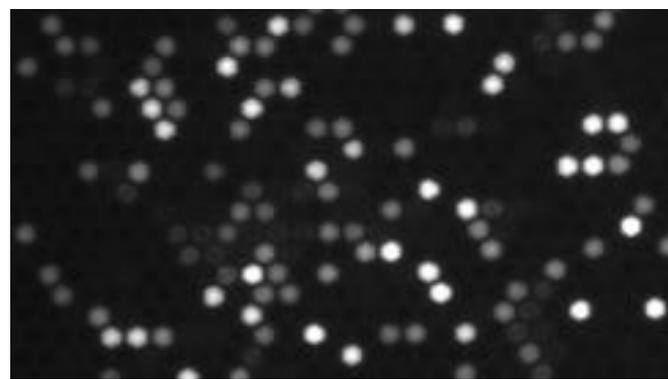


**IFNG segments overlaid**

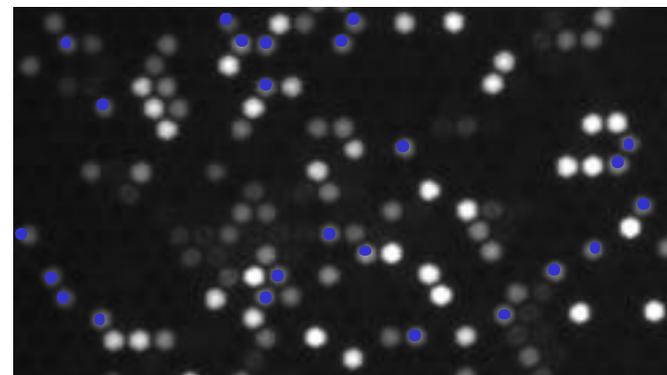


**IL2 segments overlaid**

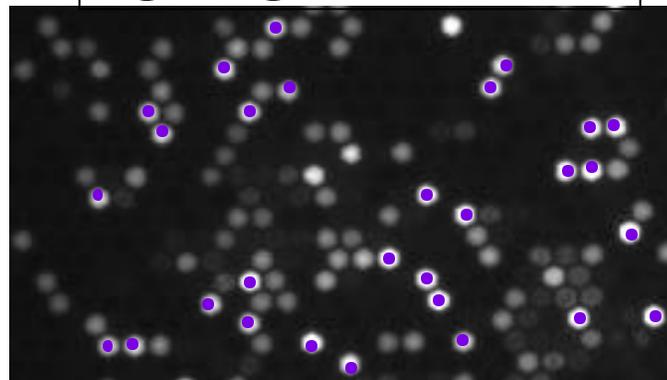
**Encoding Signal of Dye 2**



**Signal 620**



**Bglo segments overlaid**



**Hwt segments overlaid**

## Sequences of 25 Probes used together in a Microsphere Array

1) ?-glo (segment of human ?-globin)<sup>26</sup>  
TCA ACT TCA TCC ACG TTC ACC

2) IFNG (interferon gamma 1)<sup>26</sup>  
IFNG TGG GTT CTC TTG GCT GTT ACT

3) IL2 (interleukin-2)<sup>26</sup>  
TA CAA GAA TCC CAA ACT CAC CAG

4) IL4 (interleukin-4)<sup>26</sup>  
CC AAC TGC TTC CCC CTC TGT

5) IL6 (interleukin-6)<sup>26</sup>  
GT TGG GTC AGG GGT GGT TAT T

6) K-ras WT<sup>27</sup>  
GGA GCT GGT GGC GTA

7) H-ras WT<sup>27</sup>  
CCG GCG GTG T

8) CFTR (cystic fibrosis exon 11)<sup>13</sup>  
CAT TAT ACT TGT AGA G

9) R553X (cystic fibrosis exon 10)<sup>13</sup>  
TGT AGA ATT ATC TTC

10) PAN132<sup>16</sup> (human peripheral lymphocyte)  
CCT CTA TAC TTT AAC GTC AAG

11) Schena-2<sup>16</sup>  
AAG TTT AAC CTA TAC CCT GTC

12) Hakala-1<sup>20</sup>  
CCT ATG ATG AAT ATA G

13) Hakala-2<sup>20</sup>  
AAT ATG ATA ATG GCC T

14) complement to probe 1  
TG AAC GTG GAT GAA GTT G

15) complement to probe 2  
AG TAA CAG CCA AGA GAA CCC AAA

16) complement to probe 3  
CT GGT GAG TTT GGG ATT CTT GTA

17) complement to probe 4  
AC AGA GGG GGA AGC AGT TGG

18) complement to probe 5  
AA TAA CCA CCC CTG ACC CAA C

19) complement to probe 6  
TAC GCC ACC AGC TCC

20) complement to probe 7  
ACA CCG CCG G

21) complement to probe 8  
CTC TAC AAG TAT AAT G

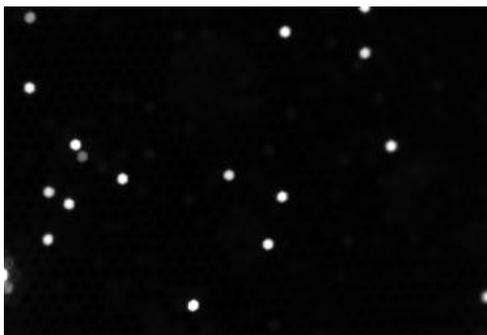
22) complement to probe 9  
GAA GAT GTT AAA GTA TAG AGG

23) complement to probe 10  
CTA GAC GTT AAA GTA TAG AGG

24) complement to probe 12  
CTA TAT TCA TCA TAG G

25) complement to probe 13  
AGG CCA TTA TCA TAT T

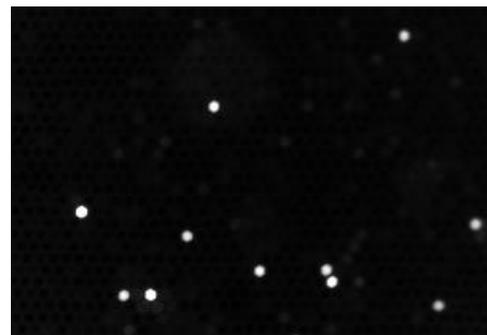
2



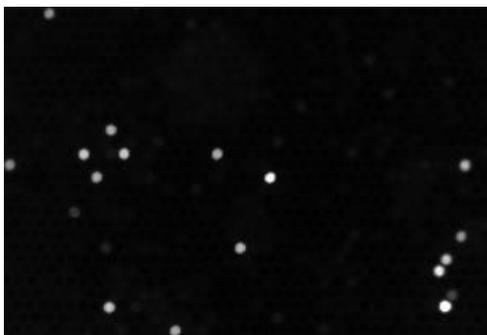
15



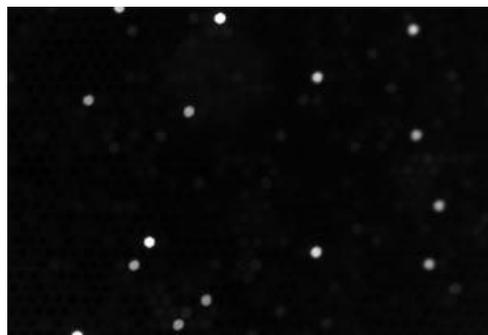
14



3



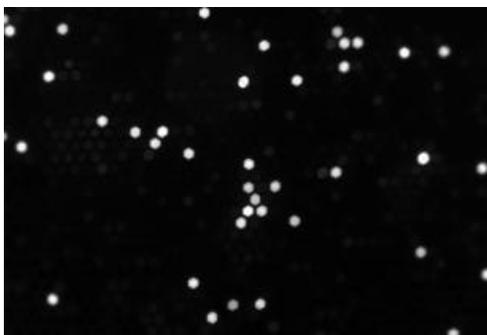
18



11



6



23



9



8



24



5

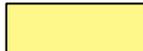


# *E. coli* Allelic Discrimination

## ycgW locus\*

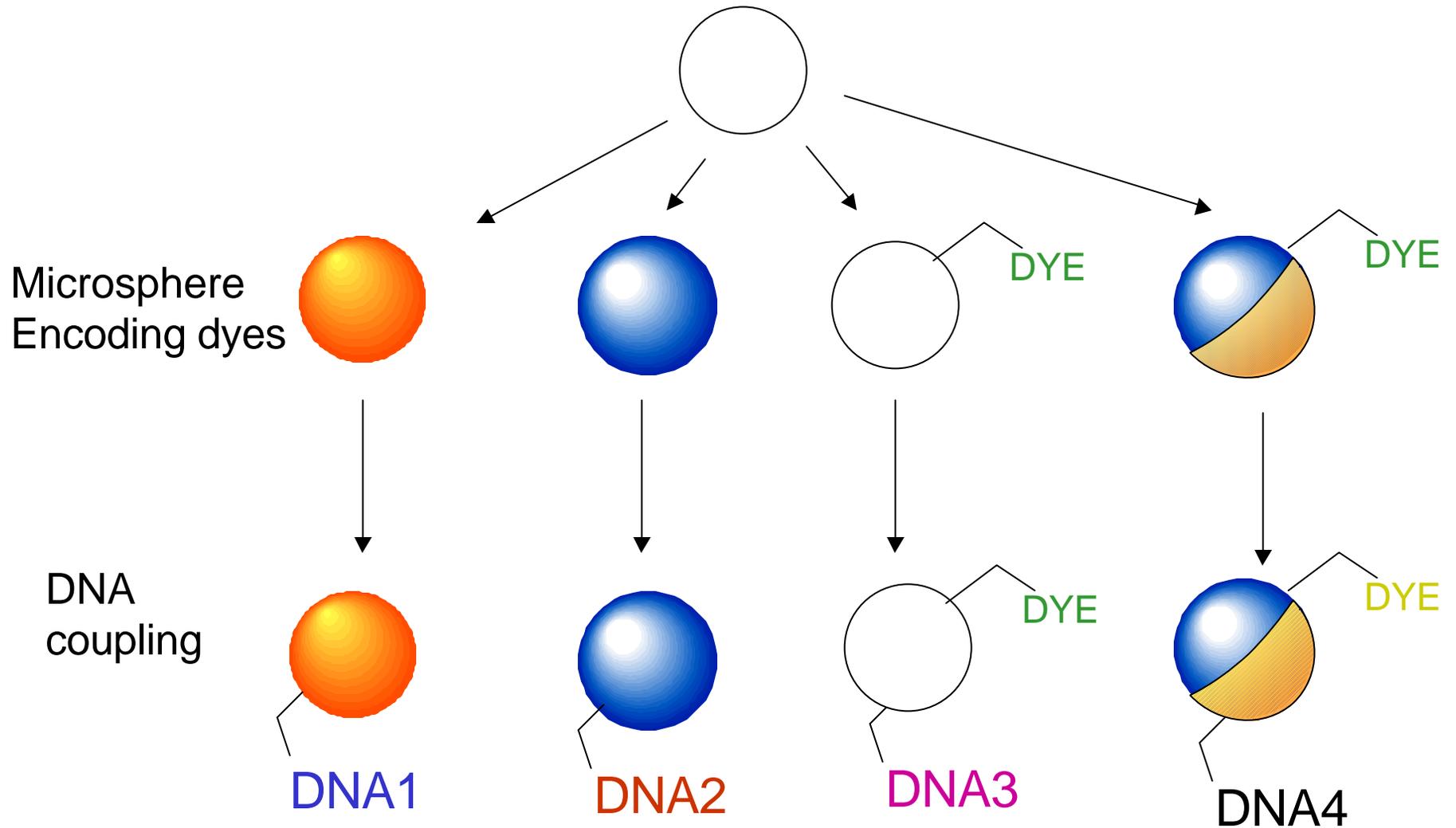
No.	strain	strain sequence									No.	strain	strain sequence									
1	BSR9b	T T T T T G A	A	G	G	G	G				19	HER1265	no PCR product									
2	BSR9c	T T T T T G A	A	G	G	G	G				20	HER1266	no PCR product									
3	"ETEC"	T T T T T T G	A	G	G	G	G				21	EC68	no PCR product									
4	O111NM	T T T T T G A	A	G	G	G	G				22	EC69	T T T T T T G	A	G	G	G	G				
5	O113:H2	T T T T T G A	A	G	G	G	G				23	EC63	no PCR product									
6	O157NM	T T T T T T G	A	G	G	G	G				24	EC54	no PCR product									
7	HER1058	no PCR product									27	O86:H10	T T T T T T G A	A	G	G	G	G				
8	K12DH5a	T T T T T T G	A	G	G	G	G				37	O86:H18	T T T G T T T T T T G									
9	K12W4100	T T T T T T G	A	G	G	G	G				30	O8:H9	T T T T T G A	A	G	G	G	G				
10	O55:H7	T T T T C G A	A	G	G	G	G				34	O9:H33	T T T T T T G	A	G	G	A	G				
11	"EPEC"	T T T T T T G	A	G	G	G	G				38	O153:H-	T T T T T T T G	A	G	G	G	G				
12	K12W3110	T T T T T T G	A	G	G	G	G				43	O26:H11	T T T T T T G A	A	G	G	G	G				
13	O22:H8	T T T T T T G A	A	G	G	G	G				48	O127:H21	T T T T T T G A	A	G	G	G	G				
14	O26:H-	T T T T T T T G	A	G	G	G	G				52	EC1	T T T T T T T G	A	G	G	G	G				
15	O42:H2	T T T T T T G A	A	G	G	G	G				53	EC7	T T T T T T T G	A	G	G	G	G				
16	O157:H7	no PCR product									54	EC18	T T T T T T T G	A	G	G	A	G				
17	HER1057	no PCR product									55	EC47	T T T G T T T T T T G G									
18	HER1261	no PCR product									56	EC52	no PCR product									
	Cons.	T T T * * * * *	*	*	*	*	G				Cons.	T T T * * * * *	*	*	*	*	G					

 = probe 1 signal

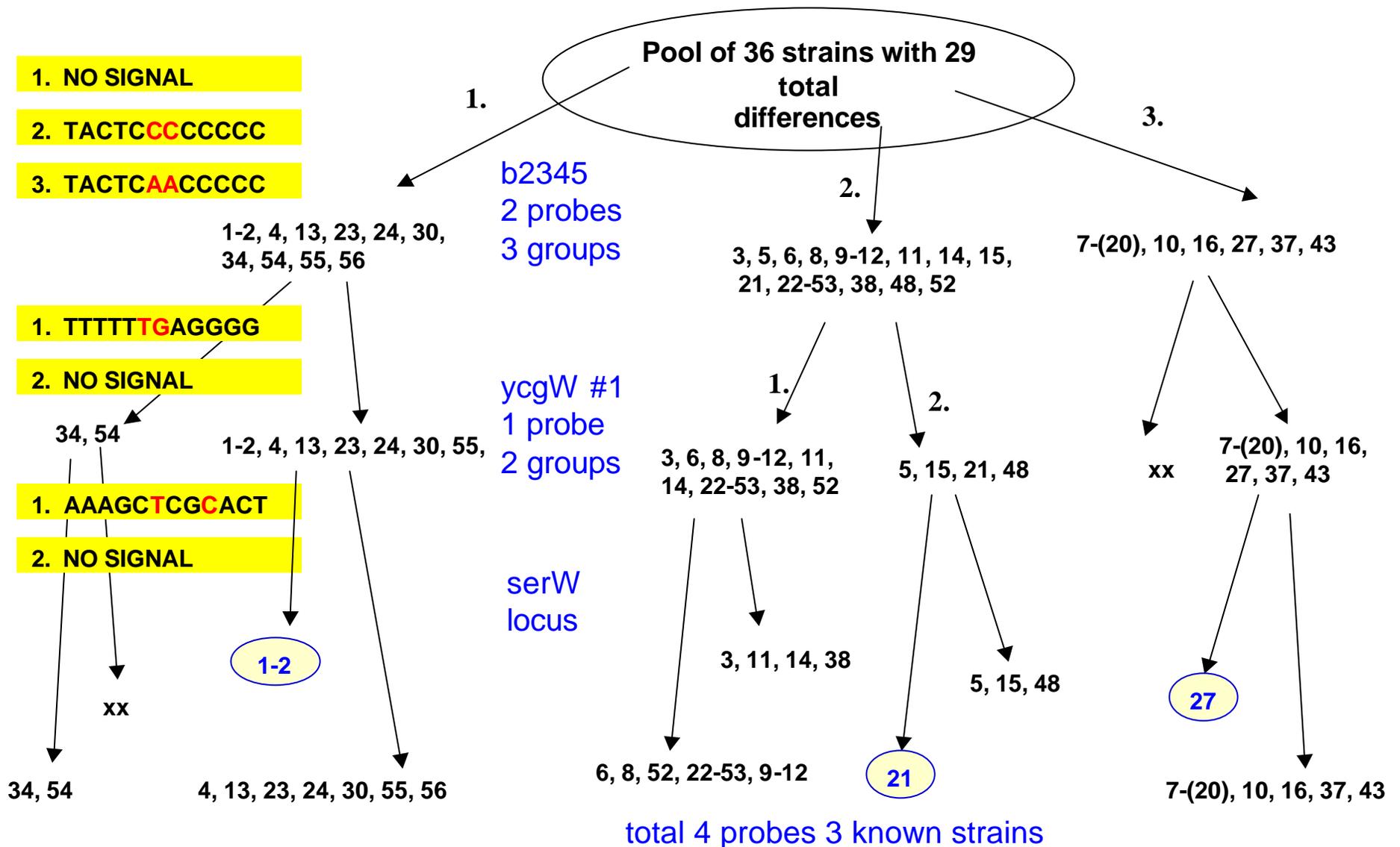
 = no signal

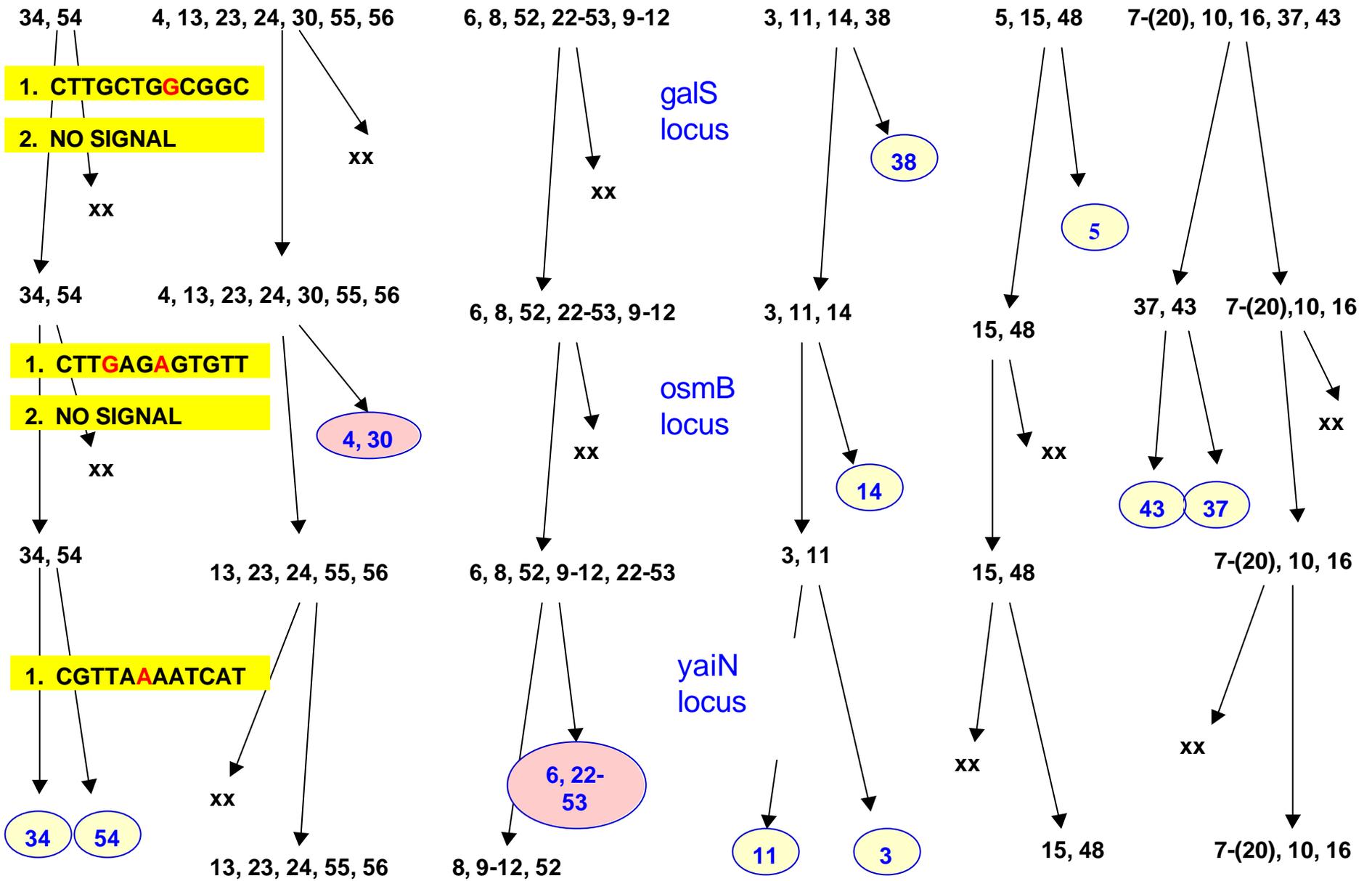
\*ycgW locus is 77 nucleotides long

# Microsphere Functionalization

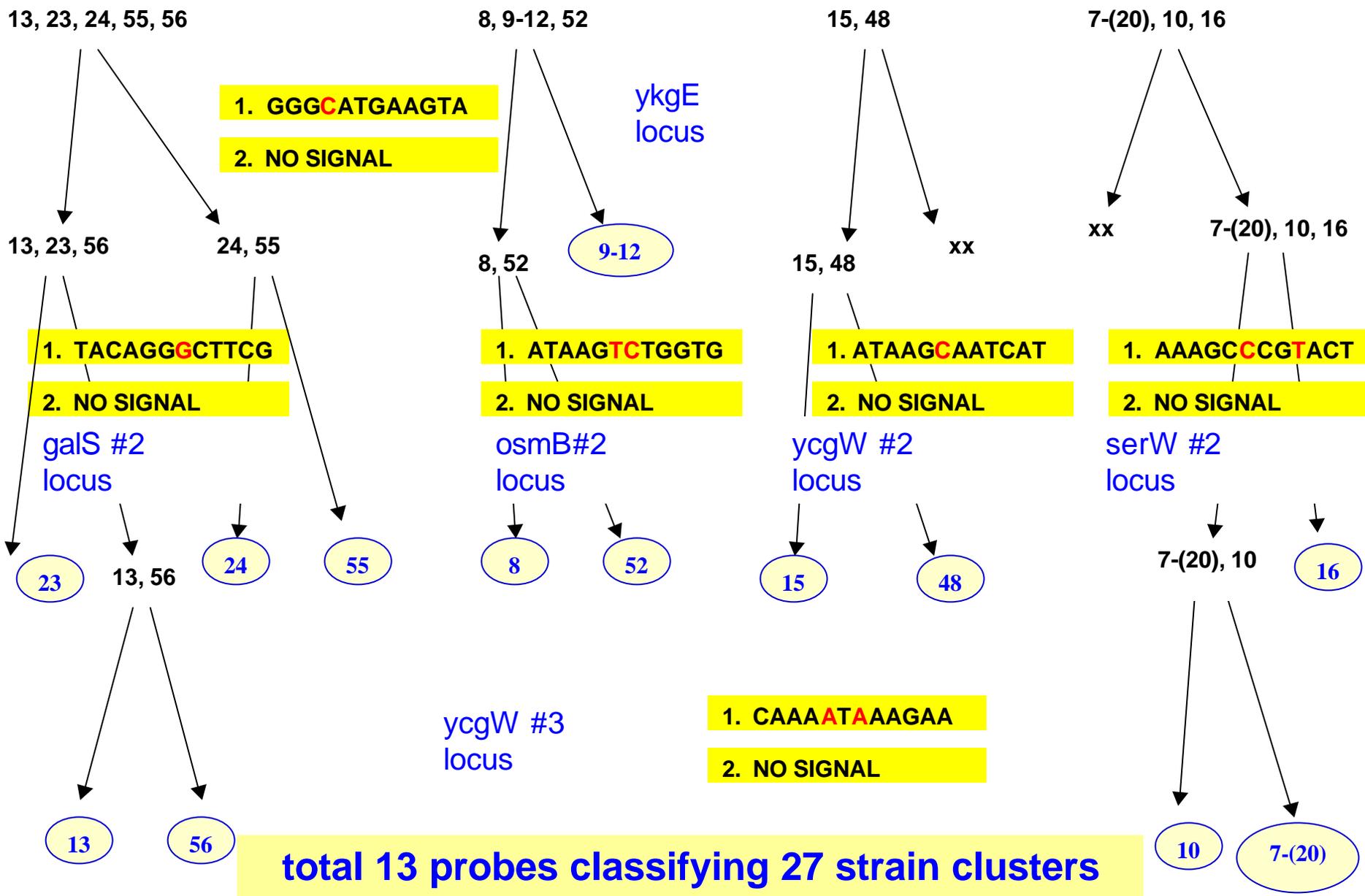


# *E. coli* Genomic Discrimination Flowchart





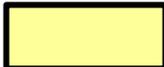
total 7 probes 12+ known strains



# *E. coli* Genomic Pattern Response

No.	strain	ycgW	serW	osmB	yaiN	ykgE
14	O26:H-	signal response	no signal	no signal	signal response	signal response
11	"EPEC"	signal response	no signal	signal response	signal response	signal response
3	"ETEC"	signal response	no signal	signal response	no signal	signal response
6	O157NM	signal response	signal response	signal response	no signal	signal response
22	EC69	signal response	signal response	signal response	no signal	signal response
9	K12W4100	signal response	signal response	signal response	signal response	no signal
12	K12W3110	signal response	signal response	signal response	signal response	no signal
8	K12DH5a	signal response				

 = signal response

 = no signal

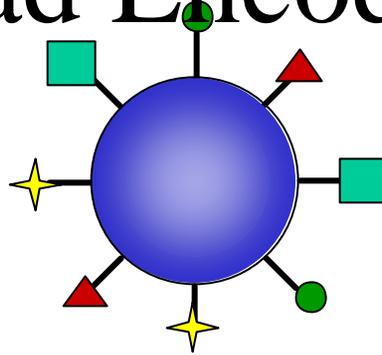
# *E. coli* Genomic Pattern Response

No.	strain	ycgW	b2345	serW	galS	Osmb	yKgE	ycgW#3	serW#2
21	EC68	no signal	signal response	signal response					
1	BSR9b	no signal	no signal	signal response					
2	BSR9c	no signal	no signal	signal response					
15	O42:H2	no signal	signal response	no signal	signal response				
5	O113:H2	no signal	signal response	no signal	no signal				
4	O111NM	no signal	no signal	no signal	signal response	no signal			
24	EC54	no signal	no signal	no signal	signal response	signal response	no signal		
13	O22:H8	no signal	no signal	no signal	signal response	signal response	signal response	signal response	
23	EC63	no signal	no signal	no signal	signal response	signal response	signal response	no signal	
10	O55:H7	no signal	no signal	no signal	no signal	signal response	no signal	signal response	
16	O157:H7	no signal	no signal	no signal	no signal	signal response	no signal	no signal	no signal
7	HER1058	no signal	no signal	no signal	no signal	signal response	no signal	no signal	signal response
17	HER1057	no signal	no signal	no signal	no signal	signal response	no signal	no signal	signal response
18	HER1261	no signal	no signal	no signal	no signal	signal response	no signal	no signal	signal response
19	HER1265	no signal	no signal	no signal	no signal	signal response	no signal	no signal	signal response
20	HER1266	no signal	no signal	no signal	no signal	signal response	no signal	no signal	signal response

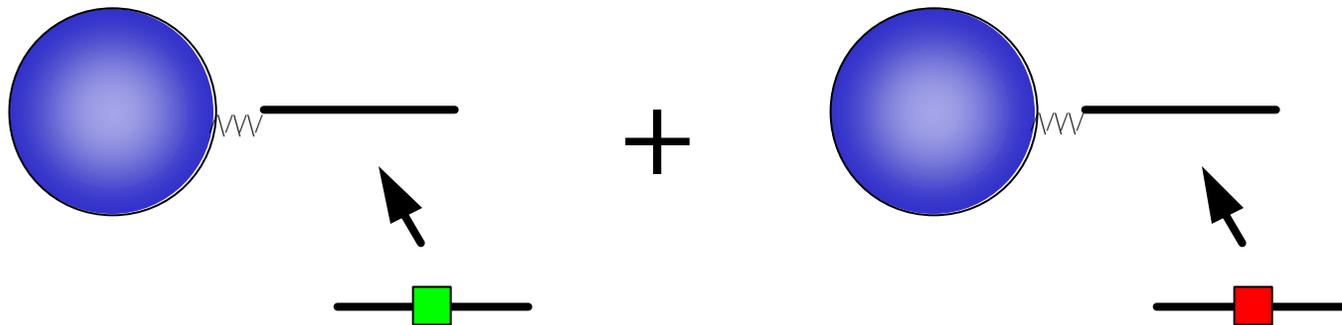
 = signal response

 = no signal

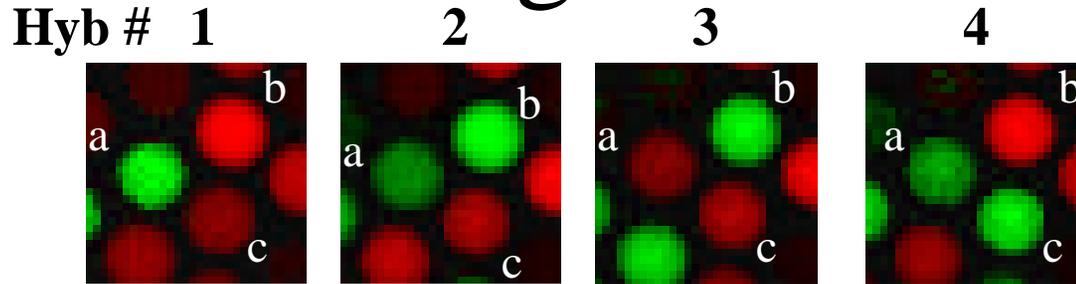
# Bead Encoding



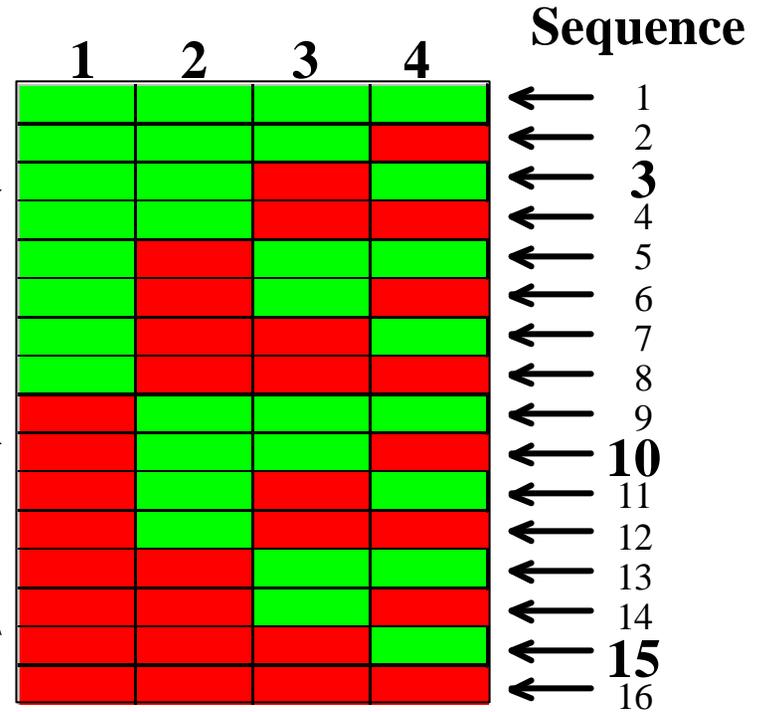
# Sequential Decoding



# Decoding 16 Probes



	Hyb.#1	Hyb.#2	Hyb.#3	Hyb.#4
<b>a</b>	green	green	red	green
<b>b</b>	red	green	green	red
<b>c</b>	red	red	red	green

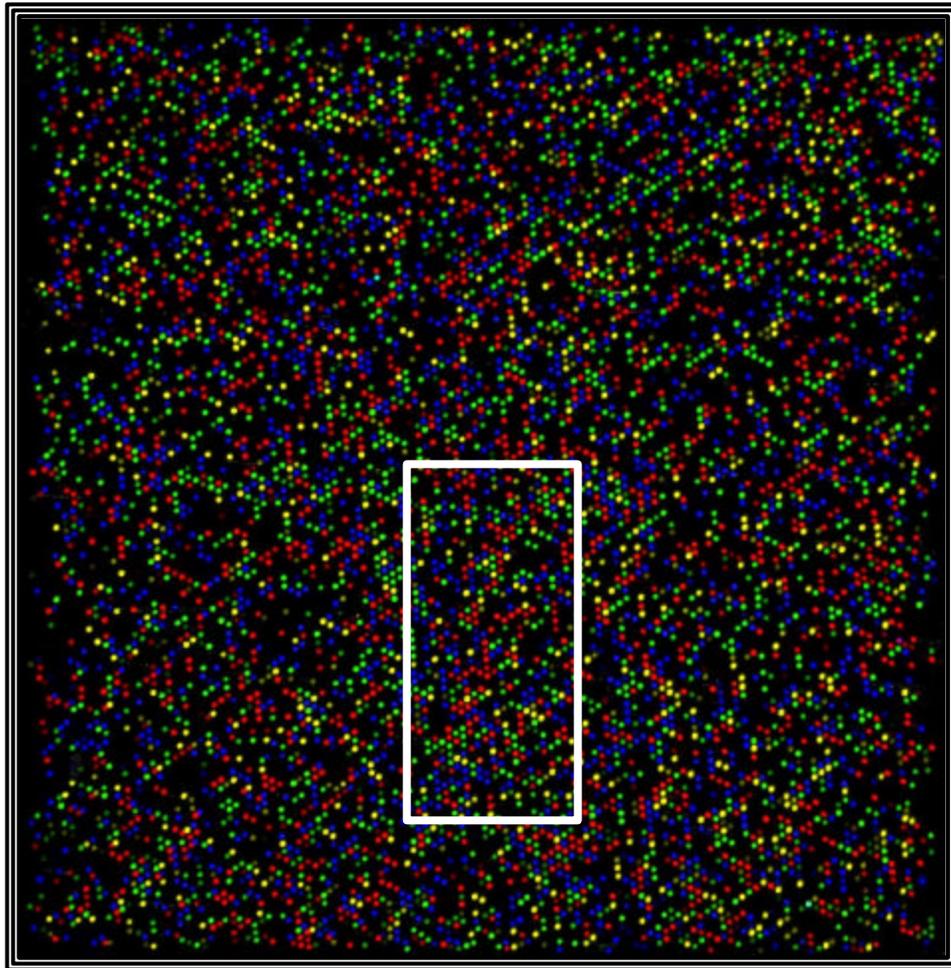


# Decoding is Exponential

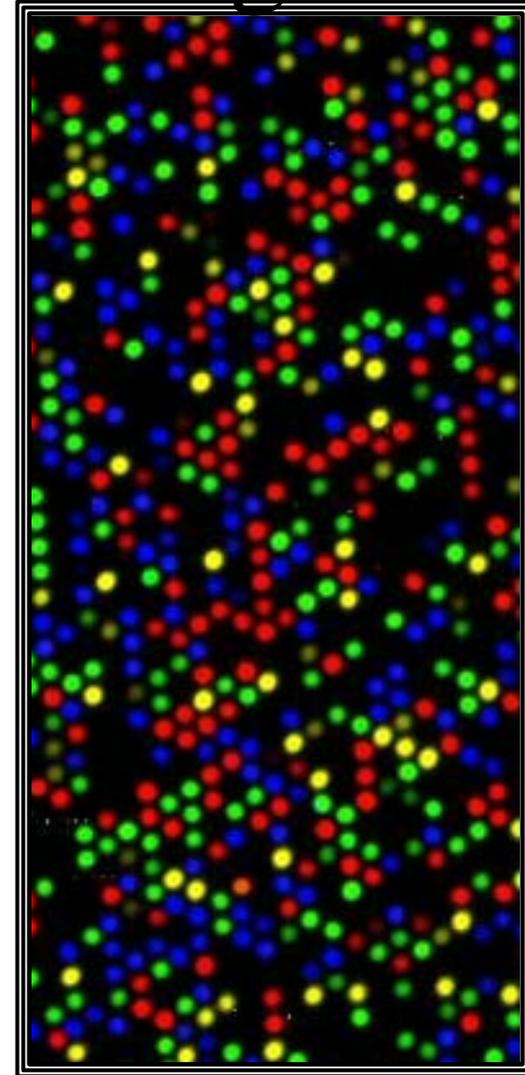
**$x$  labels ,  $z$  steps =  $x^z$  codes**

- **2** Dyes <sup>**4**</sup> Steps = **16** Codes
- **4** Dyes <sup>**6**</sup> Steps = **4,096** Codes

# Four-Color Decoding

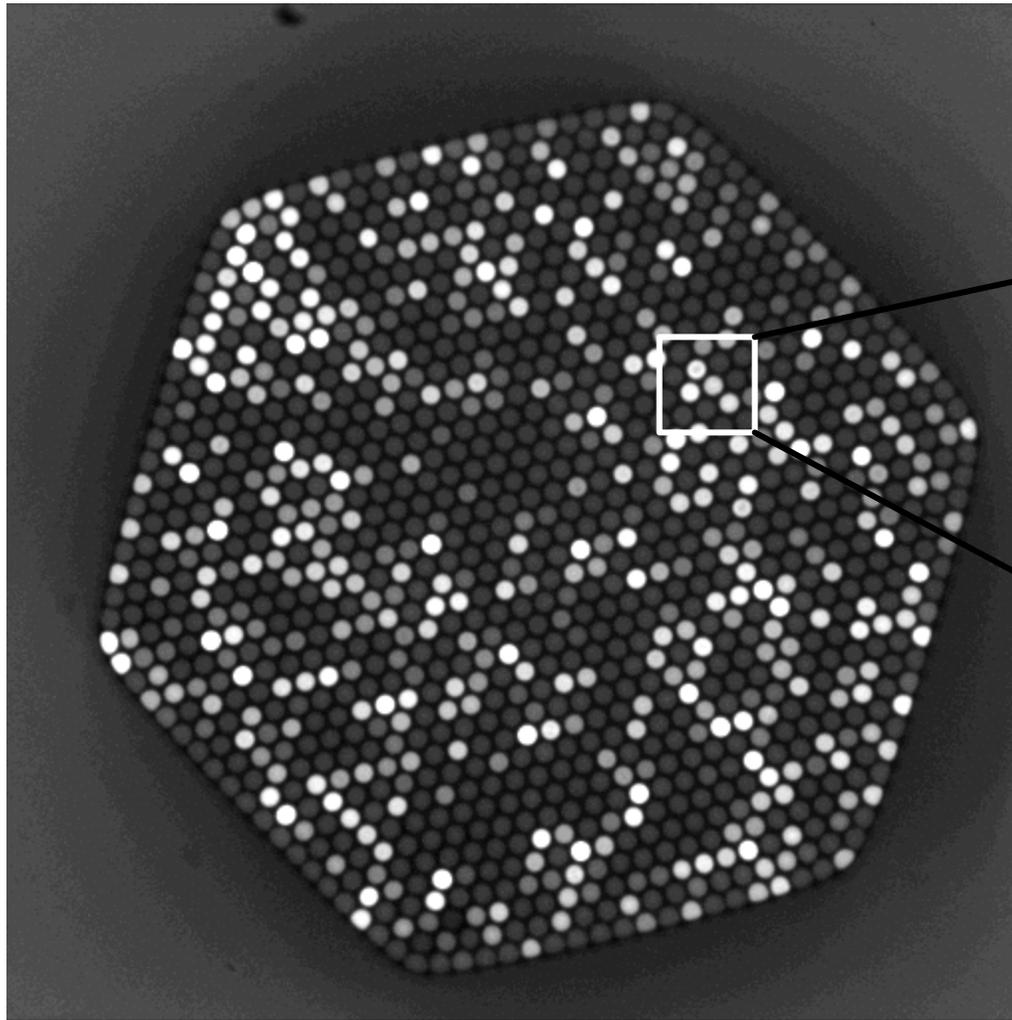


*~13,000 Wells, 16 Probe Sequences*

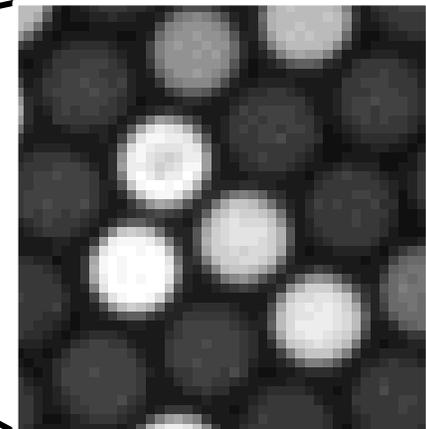


**D.R. Walt, *Science*, 2000**

# 1K Fiber Bundle

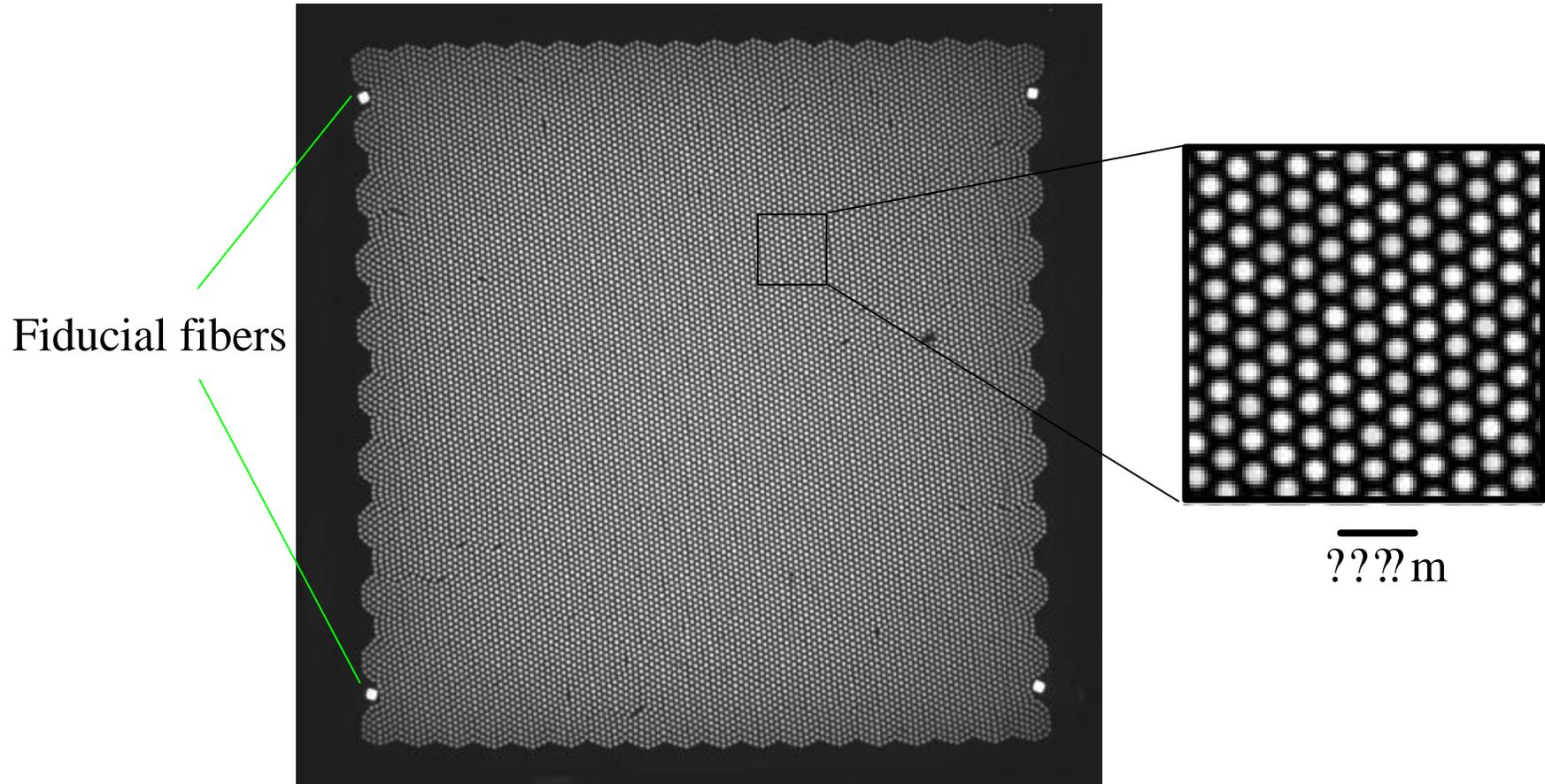


100? m

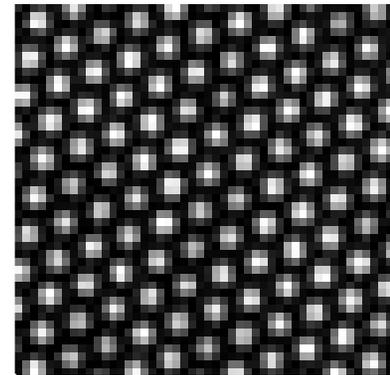
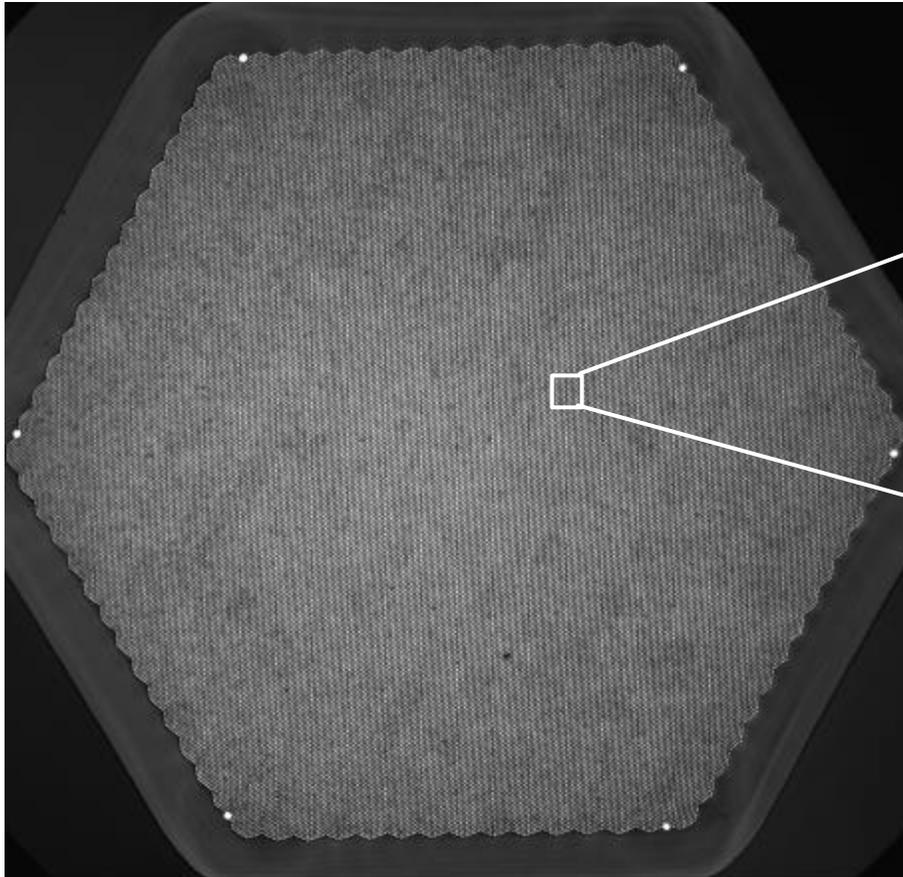


6? m

# 13K Fiber Bundle

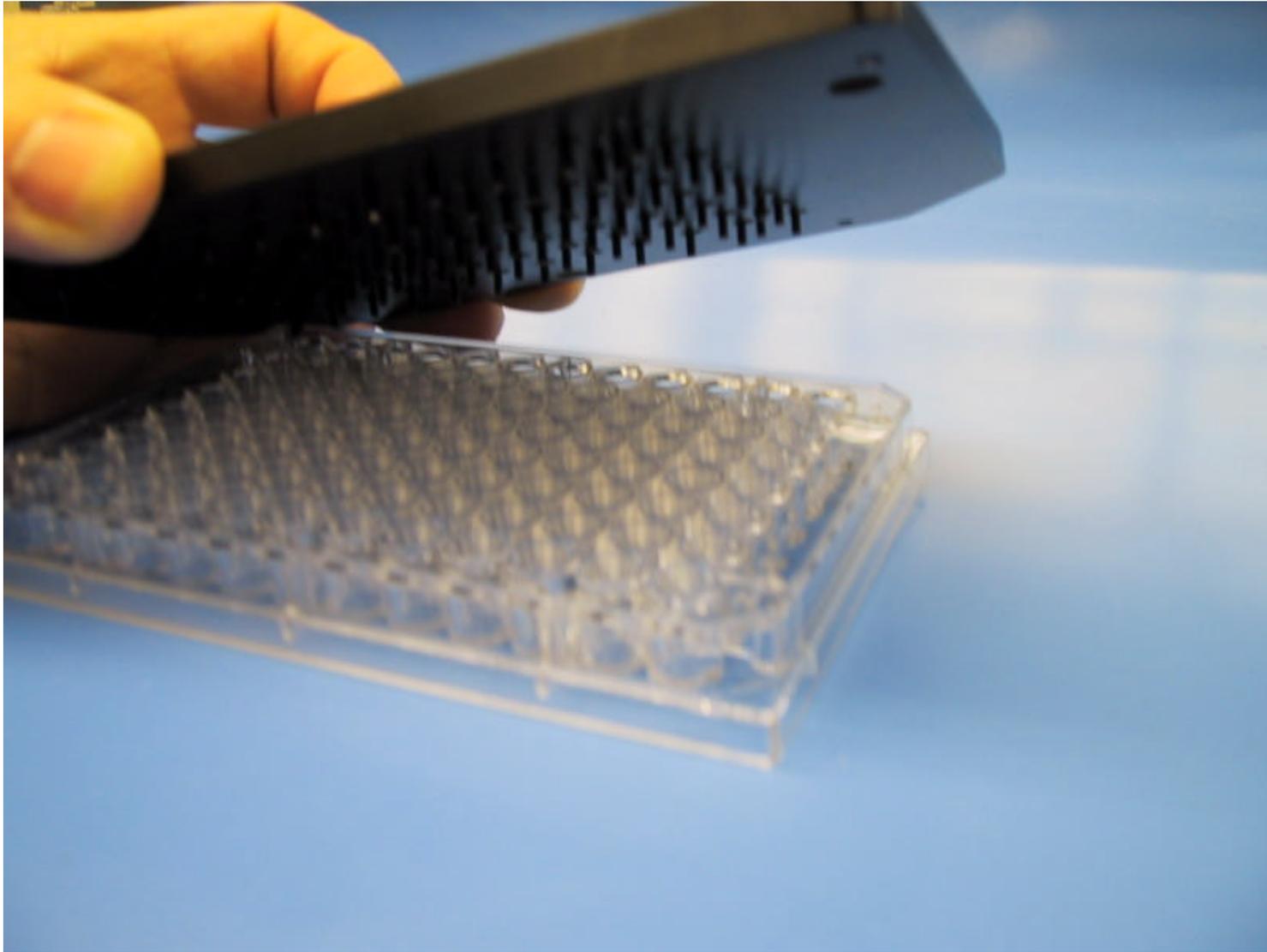


# 57K Fiber Bundle



??m

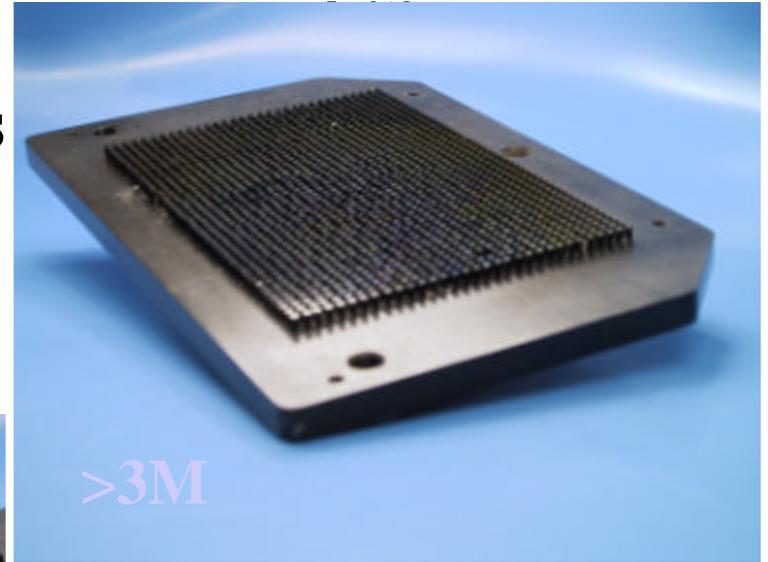
# Array of Arrays™



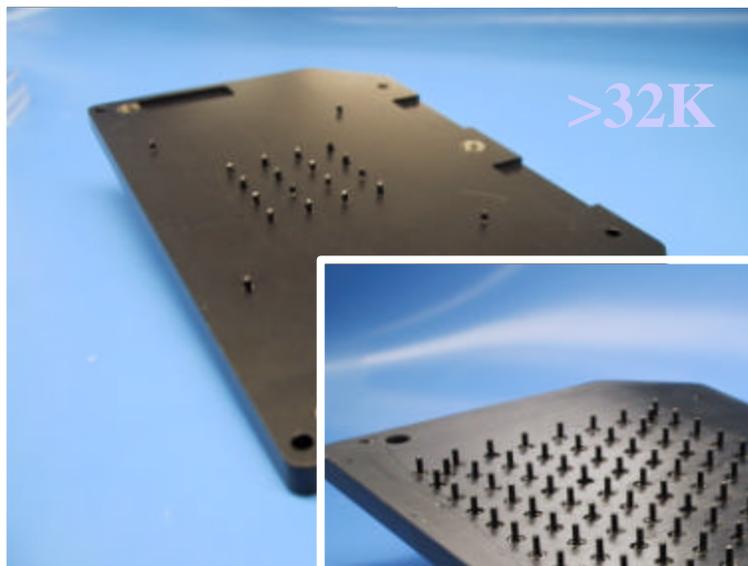
# Scalability of Technology

>2K

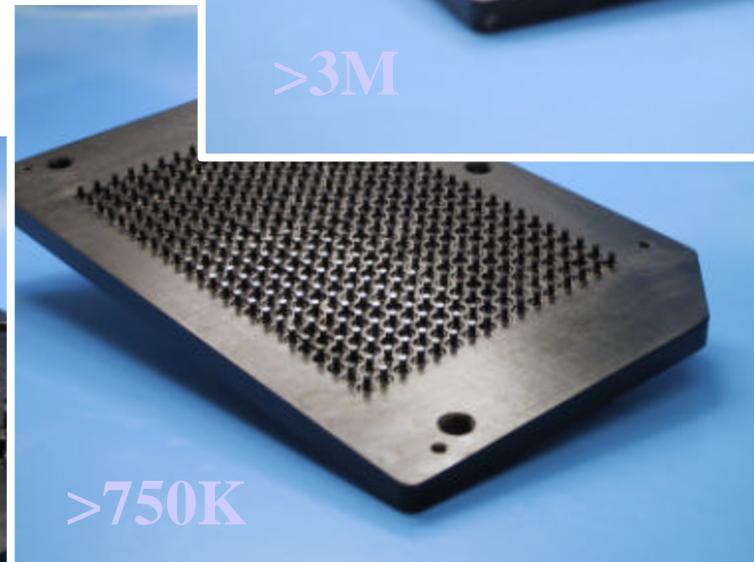
**Unique Experiments**  
(with ~20-fold redundancy)



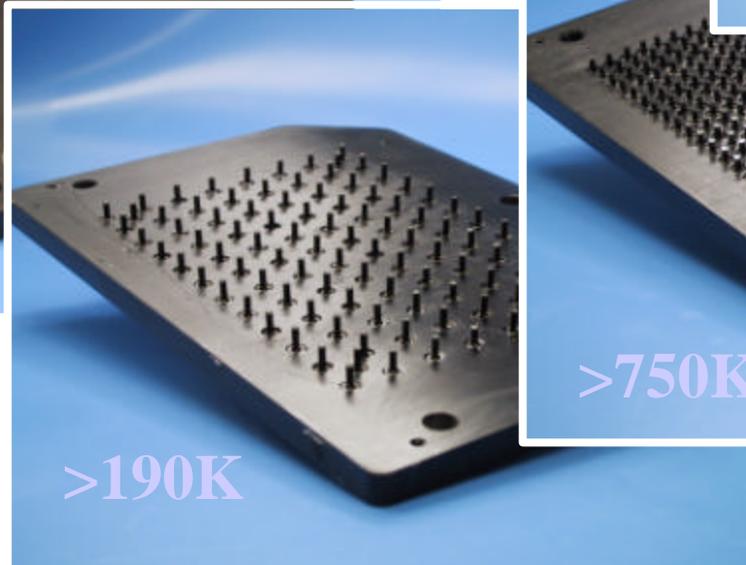
>3M



>32K



>750K



>190K

# Bead Summing

Concentration	Hybridization Time: No Summing	Hybridization Time: 100 Bead Summed
100 pM	10 minutes	4 minutes
10 pM	30 minutes	7 minutes
100 fM	4 hours	20 minutes
10 fM	17 hours	30 minutes

# Size and Concentration

Volume			1 M	1 nM	1 pM
$(1 \text{ m m})^3$	1 L	$10^{-6} \text{ L}$	$6 \times 10^{11}$	$6 \times 10^8$	$6 \times 10^5$
$(100 \text{ } \mu\text{m})^3$	1 nL	$10^{-9} \text{ L}$	$6 \times 10^8$	$6 \times 10^5$	$6 \times 10^2$
$(10 \text{ } \mu\text{m})^3$	1 pL	$10^{-12} \text{ L}$	$6 \times 10^5$	$6 \times 10^2$	$6 \times 10^{-1}$
$(1 \text{ } \mu\text{m})^3$	1 fL	$10^{-15} \text{ L}$	$6 \times 10^2$	$6 \times 10^{-1}$	
$(0.1 \text{ } \mu\text{m})^3$	1 aL	$10^{-18} \text{ L}$	$6 \times 10^{-1}$		

# Probe and Target Sequences for DNA Microarray Detection Limits

## Probe

IL2 (interleuken-2) 5'-TA-CAA-GAA-TCC-CAA-ACT-CAC-CAG-3'

IL6 (interleuken-6) 5'-GT-TGG-GTC-AGG-GGT-GGT-TAT-T-3'

F508C 5'-TAG-GAA-ACA-CCA-CAG-ATG-ATA-3'

## Target

IL2 (interleuken-2) 5'-CT-GGT-GAG-TTT-GGG-ATT-CTT-GTA-3'

IL6 (interleuken-6) 5'-AA-TAA-CCA-CCC-CTG-ACC-CAA-C-3'

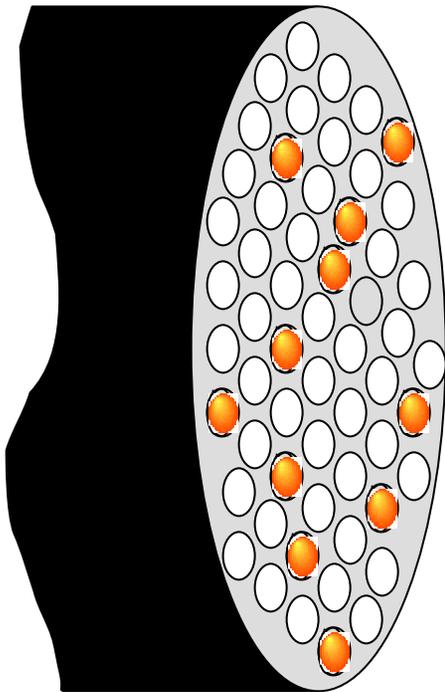
F508C 5'-TA-TCA-TCT-GTG-GTG-TTT-CCT-A-3'

## DNA Minimum Hybridization Time with ICCD Camera

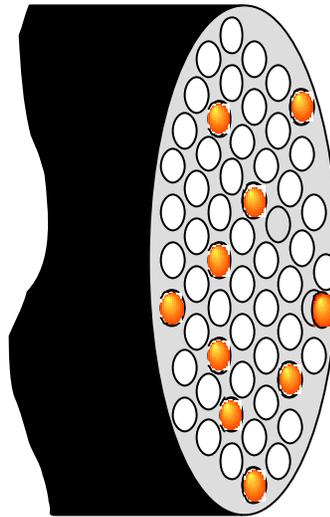
DNA Concentration	Hybridization Time (min)
1 pM	10
100 fM	20
10 fM	30
1 fM	60

# Detection Limit Problem

Multiple beads provides a signal averaging benefit.



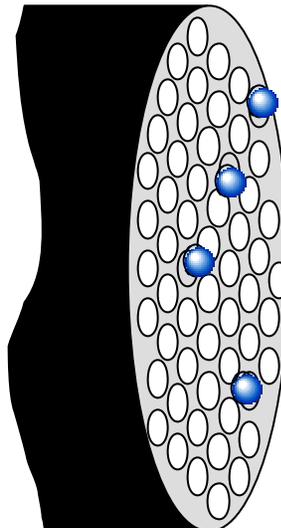
$S/N$  increases by  $\sqrt{n}$



$\frac{1000 \text{ target molecules}}{10 \text{ beads}}$

=100 target molecules/bead

Fewer beads provide more target molecule numbers per bead.



$\frac{1000 \text{ target molecules}}{4 \text{ beads}}$

=250 target molecules/bead

# Multiplexed Array Sensitivity and Selectivity with 1 fM IL2 Target Solutions

IL2 Target - 1 fM concentration - 12 hour hybridization time

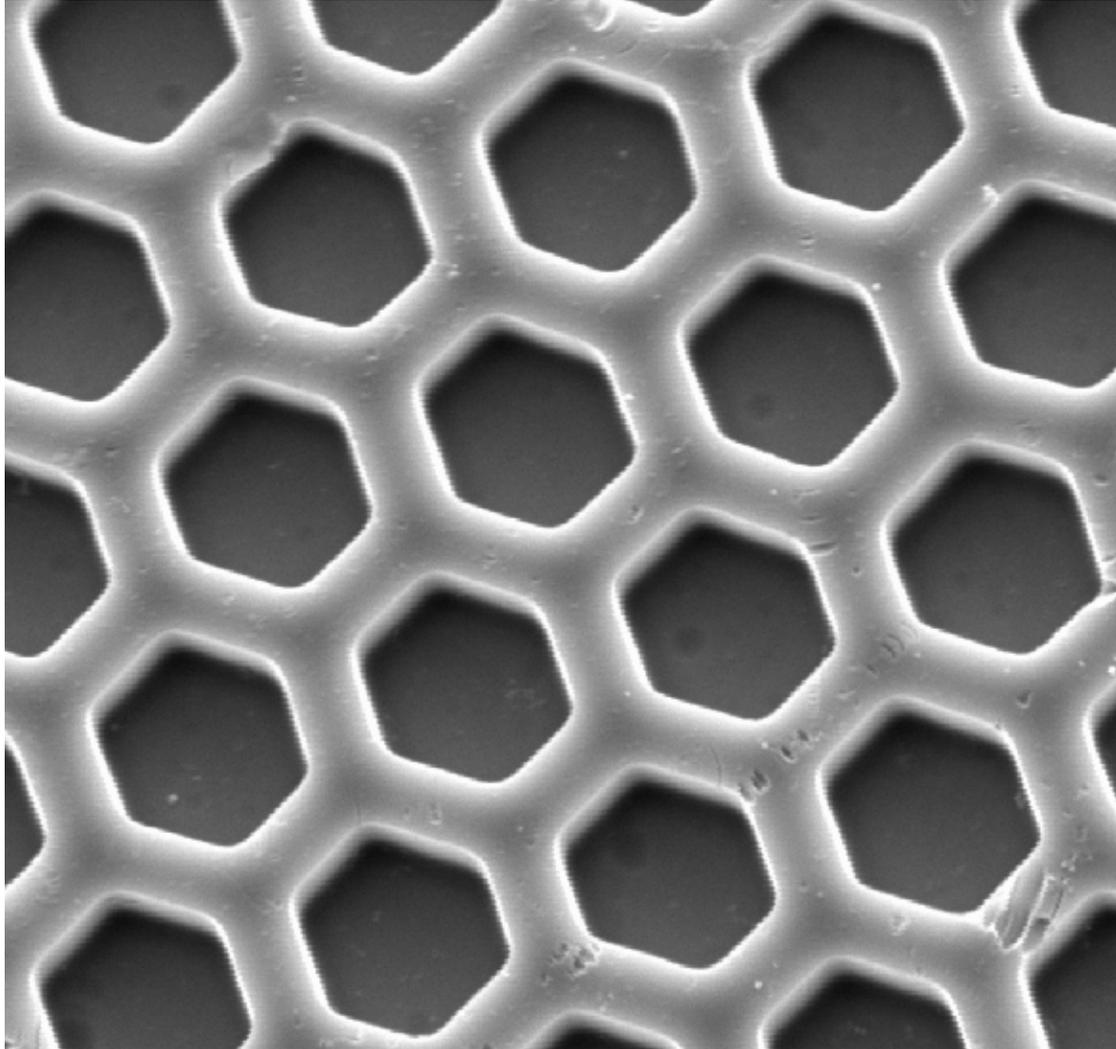
Target/Probe		Mean background $\pm$ s.d	Hybridization $\pm$ s.d.	Signal $\pm$ s.d
F508	F508C	530.43 $\pm$ 1.8	550.17 $\pm$ 7.5	{19.74} $\pm$ 7.7
	IL2	563.99 $\pm$ 7.7	677.08 $\pm$ 8.1	113.09 $\pm$ 11
	IL6	445.99 $\pm$ 3.9	449.16 $\pm$ 1.4	{3.17} $\pm$ 4.1
IL2	F508C	439.64 $\pm$ 3.5	443.34 $\pm$ 5.6	{3.70} $\pm$ 6.6
	IL2	432.52 $\pm$ 5.6	503.31 $\pm$ 6.6	70.79 $\pm$ 8.7
	IL6	431.11 $\pm$ 2.1	432.13 $\pm$ 2.8	{1.02} $\pm$ 3.5
IL6	F508C	454.84 $\pm$ 3.6	465.82 $\pm$ 1.4	{10.98} $\pm$ 3.8
	IL2	429.42 $\pm$ 0.92	517.38 $\pm$ 2.6	87.96 $\pm$ 2.8
	IL6	459.81 $\pm$ 3.0	467.82 $\pm$ 5.3	{8.01} $\pm$ 6.1

# Microsphere Array Sensitivity and Selectivity with 100 aM IL2 Target Solutions

IL2 Target - 100 aM concentration - 12 hour hybridization time

Probe/Target	Mean background $\pm$ s.d	Hybridization $\pm$ s.d.	Signal $\pm$ s.d
IL2 F508C	$386.97 \pm 3.2$	$387.98 \pm 1.4$	$\{1.01\} \pm 3.5$
IL2 IL2	$378.55 \pm 2.3$	$394.00 \pm 3.7$	$15.32 \pm 4.4$
IL2 IL6	$382.80 \pm 6.3$	$393.81 \pm 6.1$	$\{11.01\} \pm 7.1$
IL2 F508C	$268.66 \pm 2.3$	$274.22 \pm 8.5$	$\{5.56\} \pm 8.8$
IL2 IL2	$297.73 \pm 2.3$	$310.02 \pm 2.3$	$12.29 \pm 3.2$
IL2 IL6	$247.59 \pm 2.7$	$248.70 \pm 6.9$	$\{1.11\} \pm 7.4$
IL2 F508C	$410.73 \pm 2.6$	$413.63 \pm 2.6$	$\{2.90\} \pm 2.9$
IL2 IL2	$410.69 \pm 2.7$	$455.26 \pm 6.5$	$44.57 \pm 7.0$
IL2 IL6	$390.24 \pm 7.4$	$392.88 \pm 2.8$	$\{2.64\} \pm 7.9$

# SEM of a Microwell Array

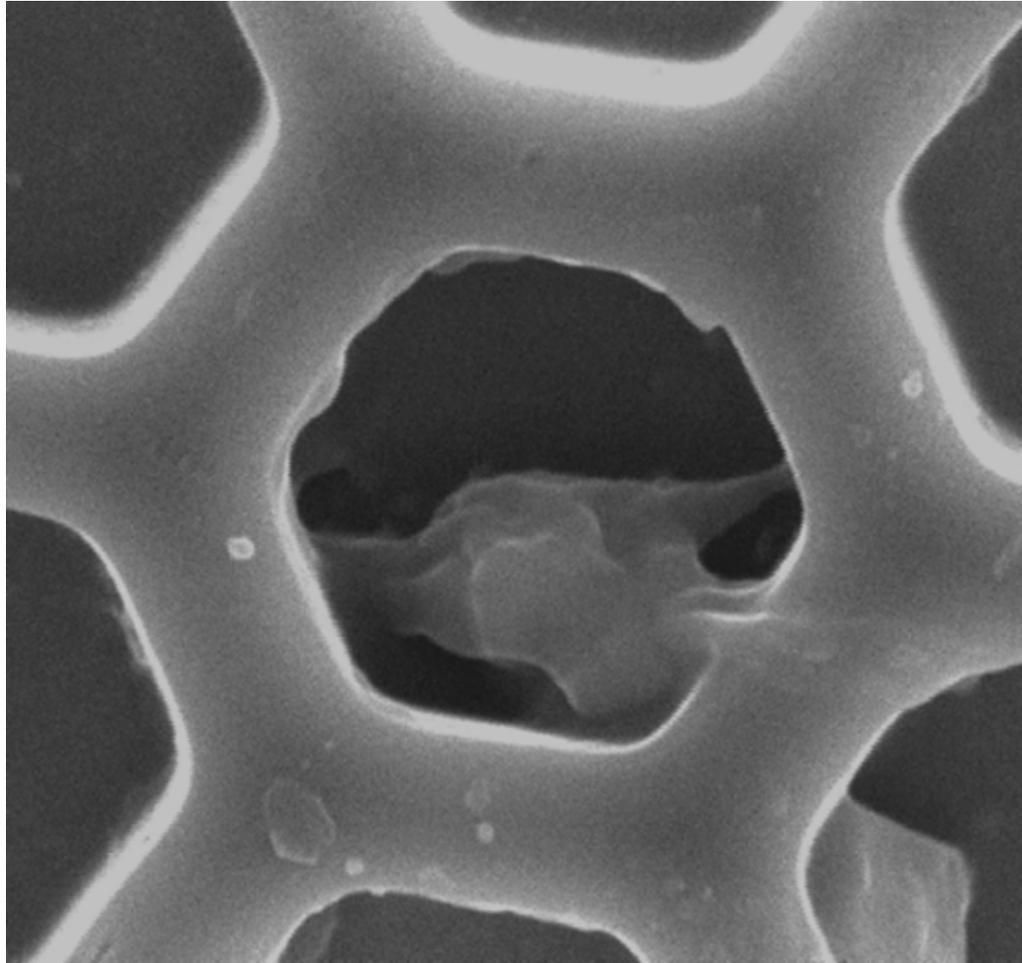


**7 ? m well diameter**

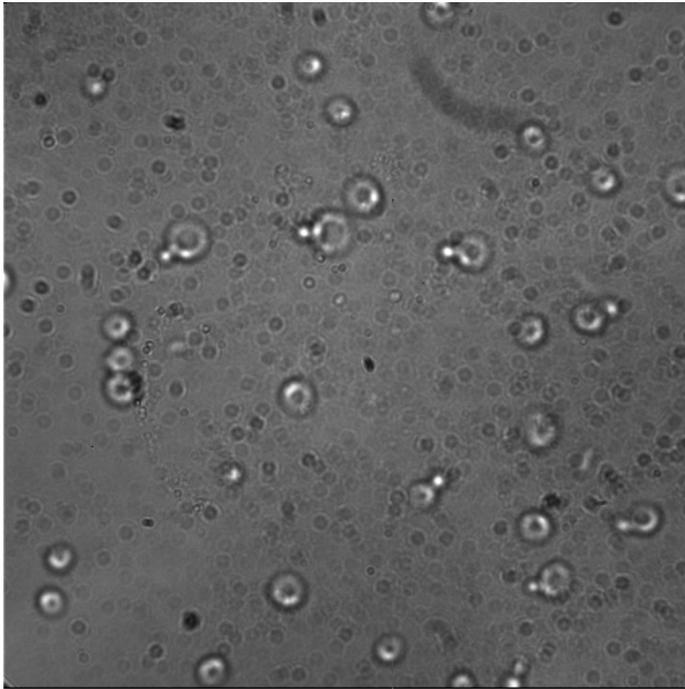
**~3 ? m well depth**

**~90 fL well volume**

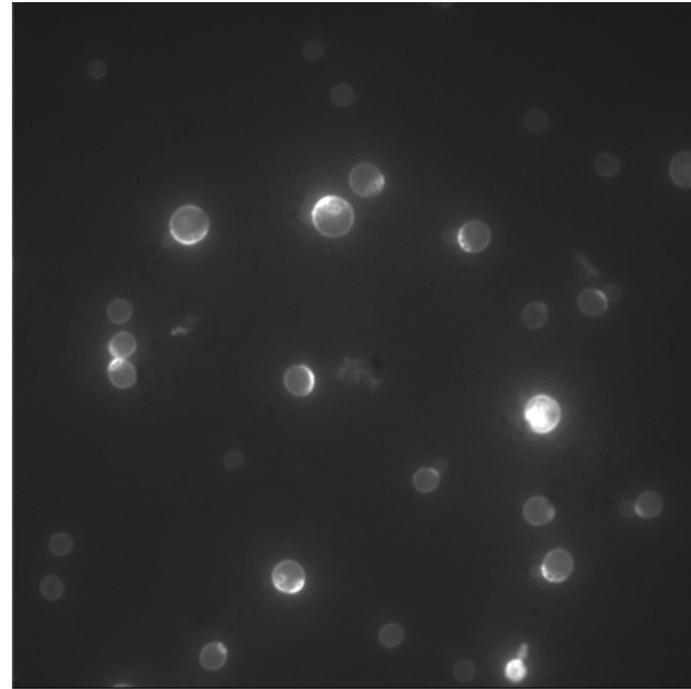
# Single NIH 3T3 Mouse Fibroblast Cell in a Fiber-optic Microwell



# Single Yeast (*Saccharomyces cerevisiae*) Cells Array

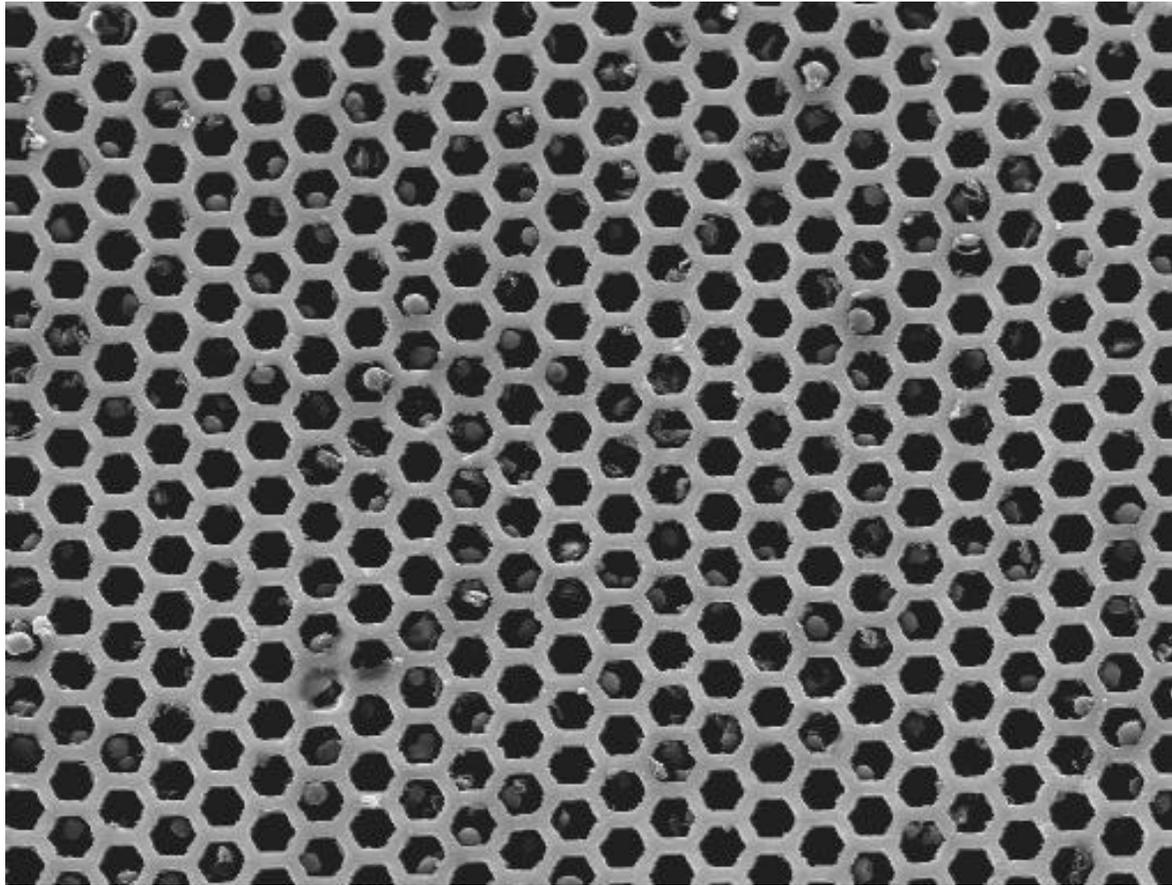


White light

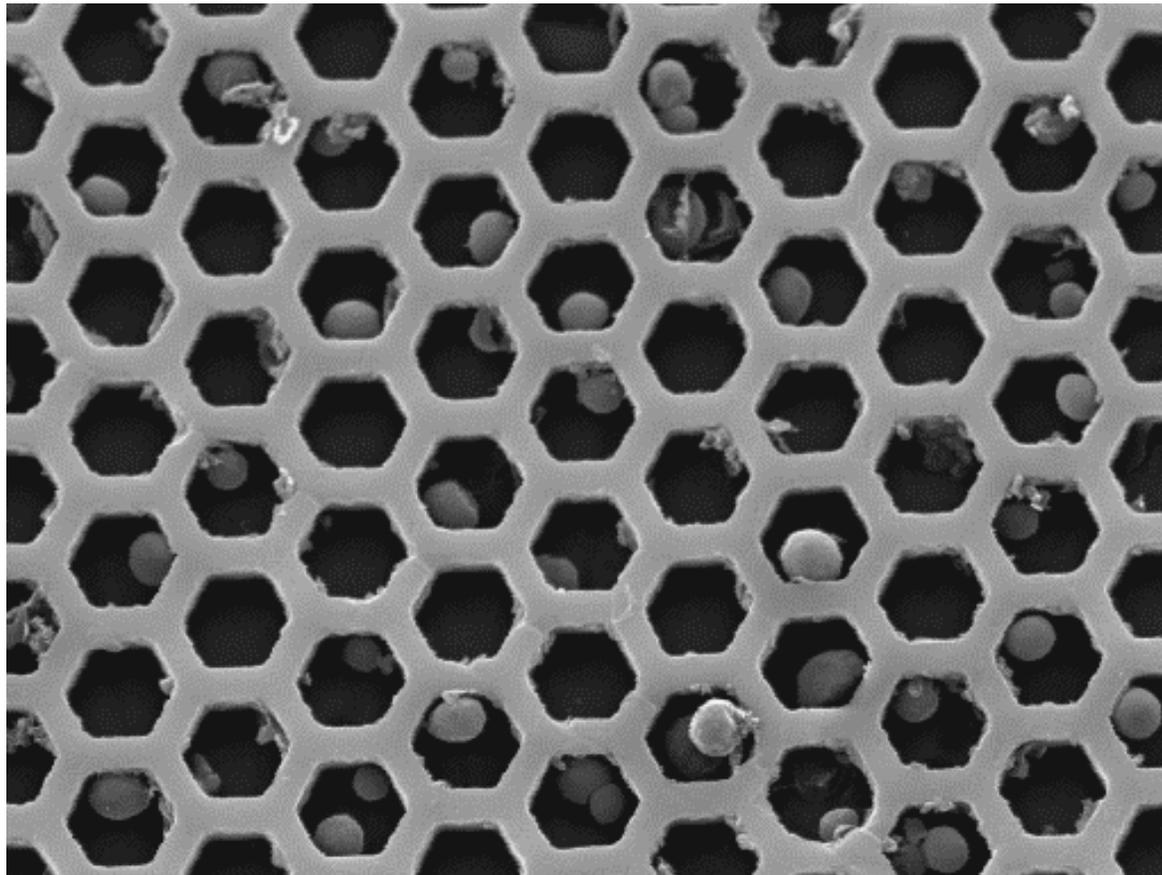


Calcofluor White 360/440

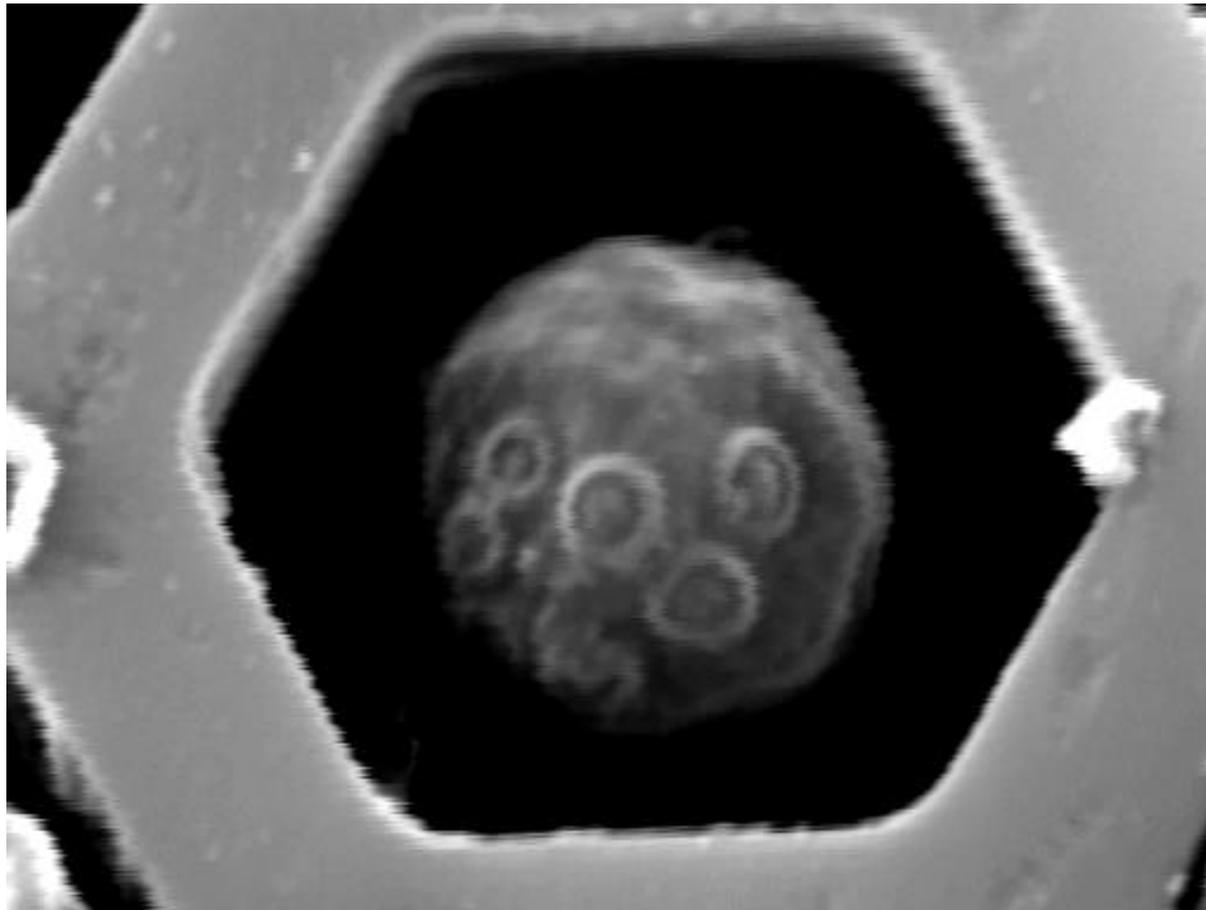
# SEM images of Single Yeast Cells on the Microwells array



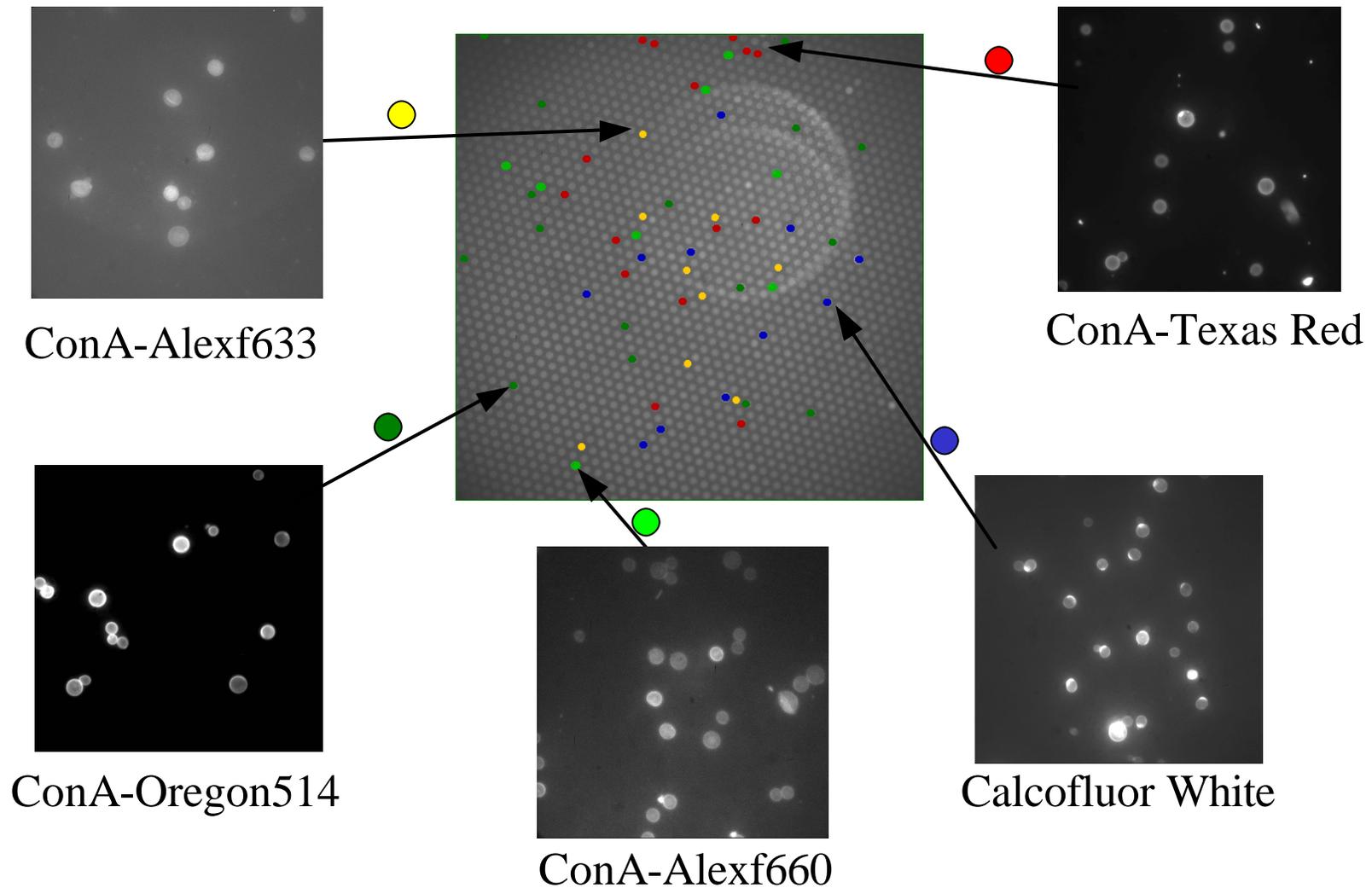
# SEM images of Single Yeast Cells on the Microwells array



## SEM images of Single Yeast Cells on the Microwells array

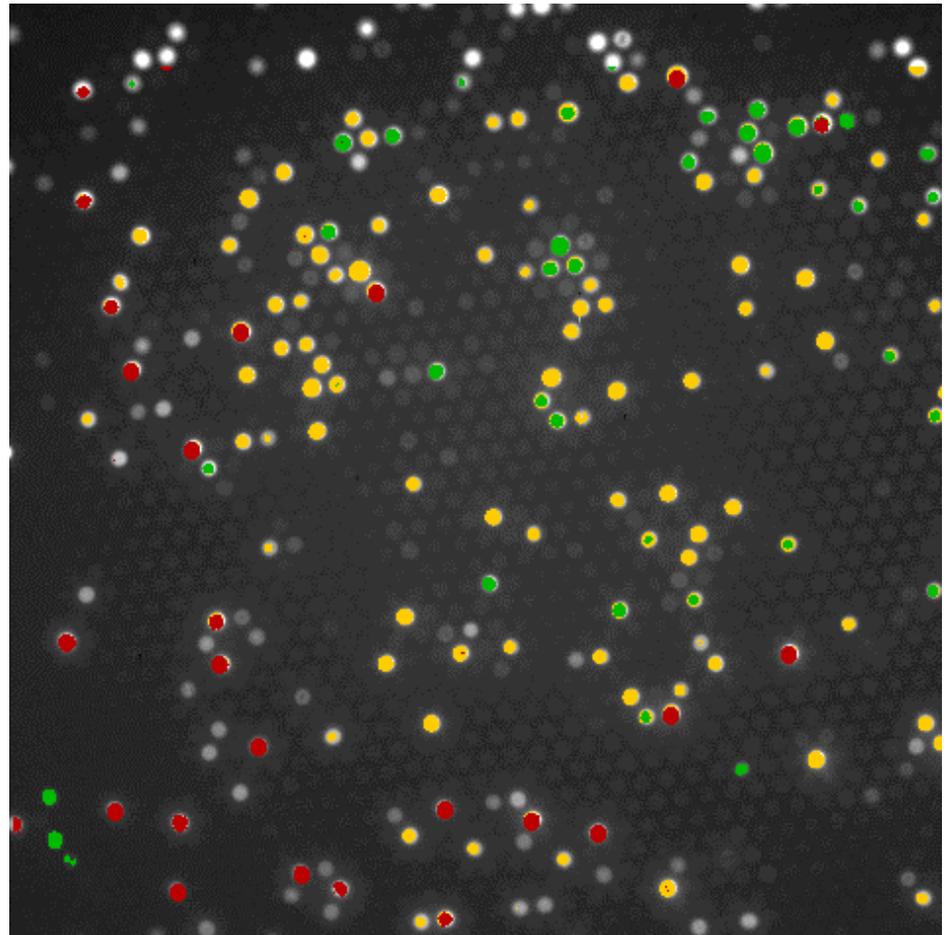


# Encoded Yeast Cells on the Fiber Array



# pH Measurement of Single Yeast Cells Microenvironment in the Array

- Concanavalin A-FITC
- Concanavalin A-FITC  
+  
Concanavalin A-Alexa fluor 660
- Concanavalin A-FITC  
+  
Concanavalin A-Texas Red



# Smarter Sensors- Anticipatory

Is it bad?

What does it resemble?

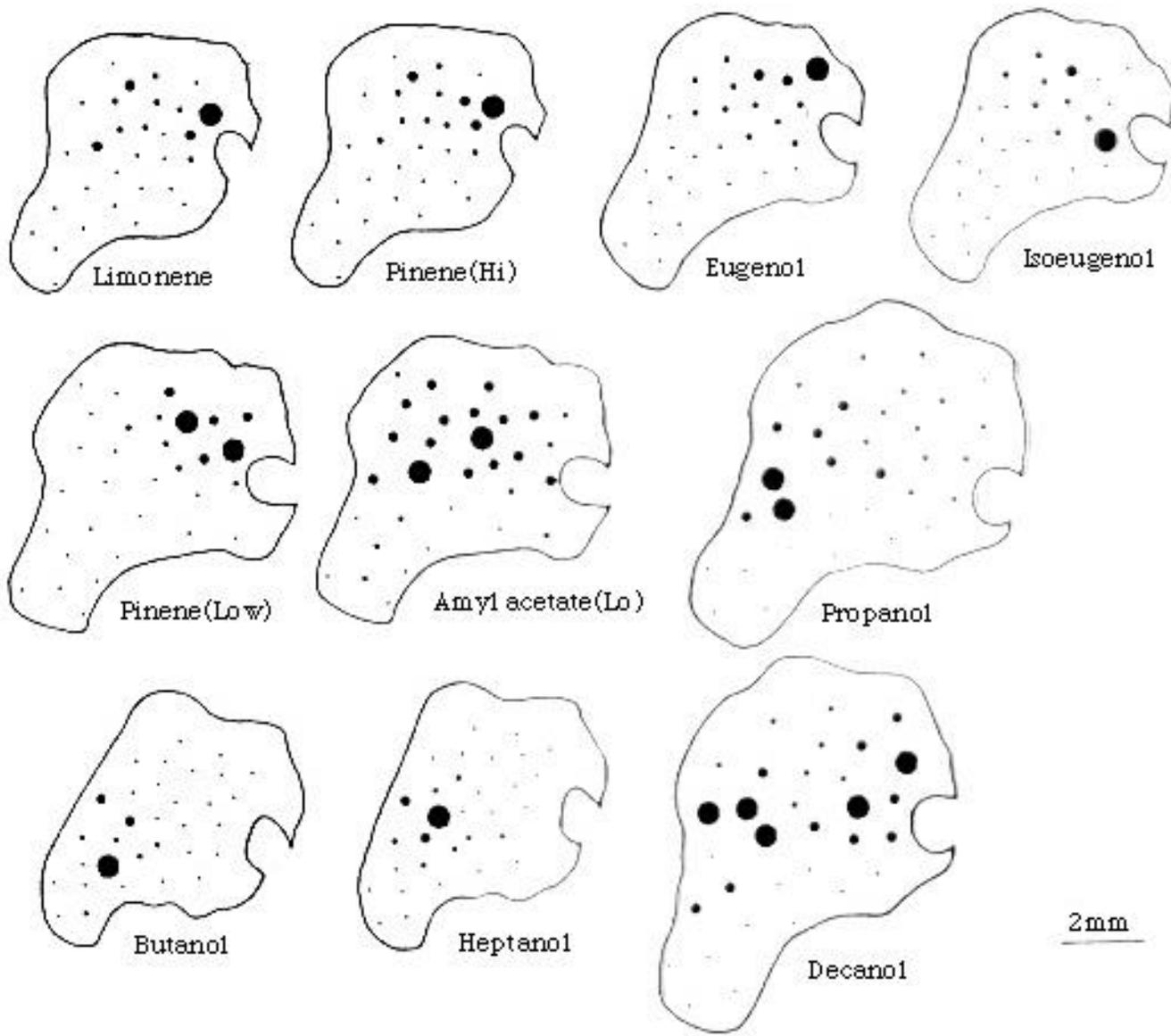
What will it do?

e.g. GI, neurotoxic, etc.

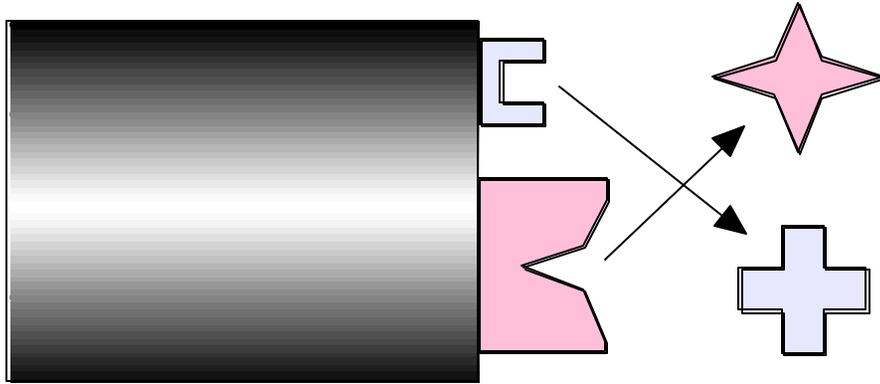
“common virulence mechanisms”



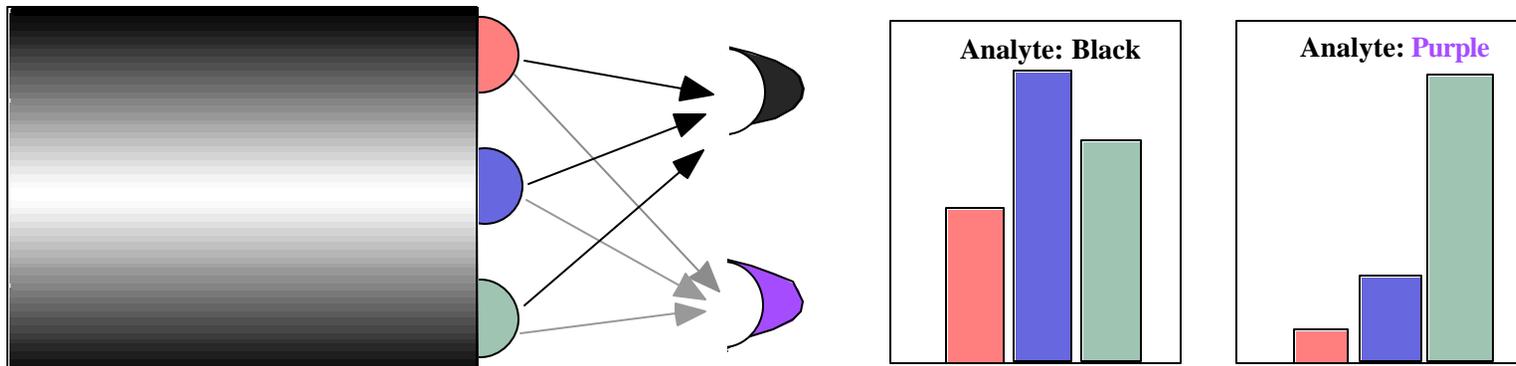
surrogates



# Sensor Design

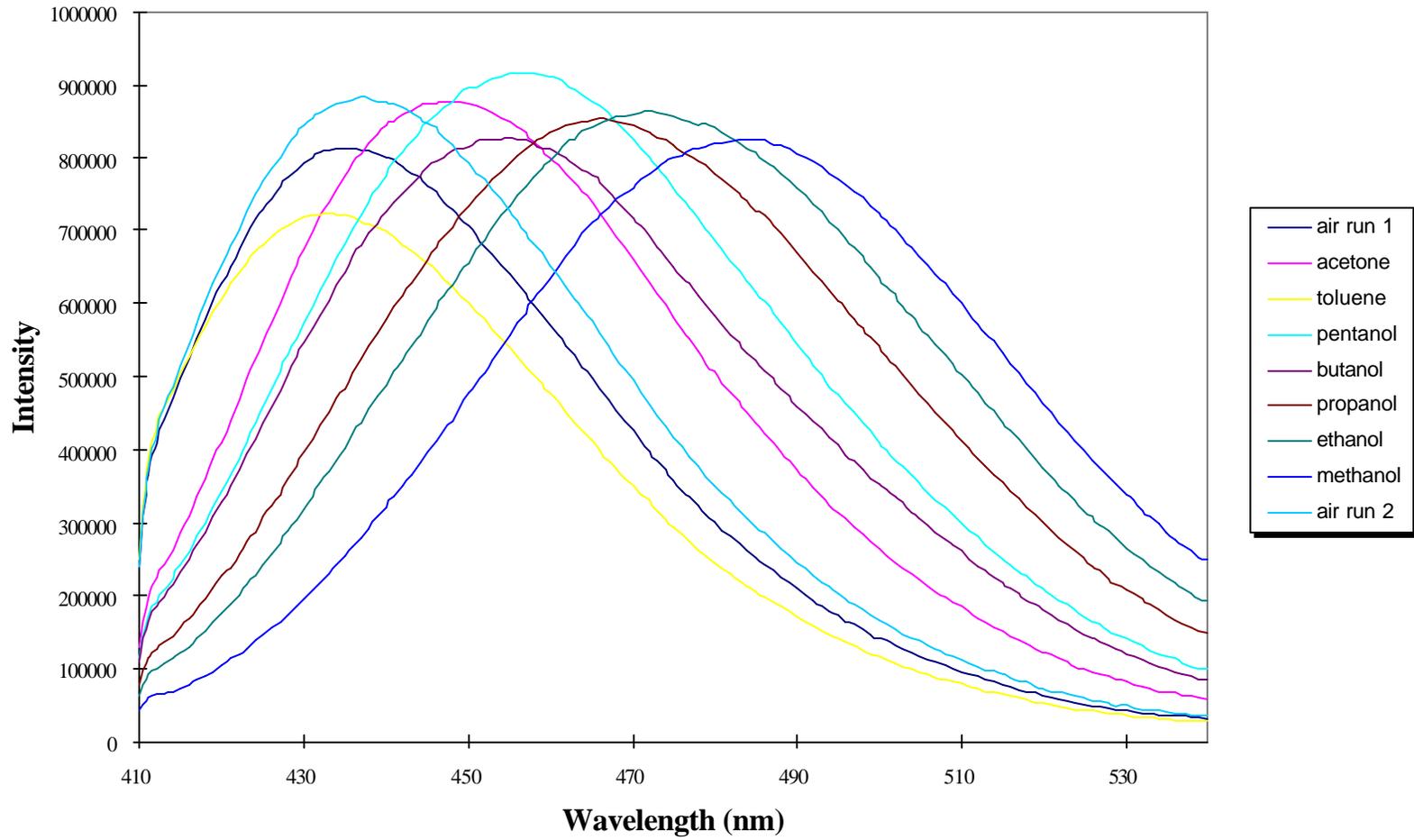


A) Lock-and-key Sensor

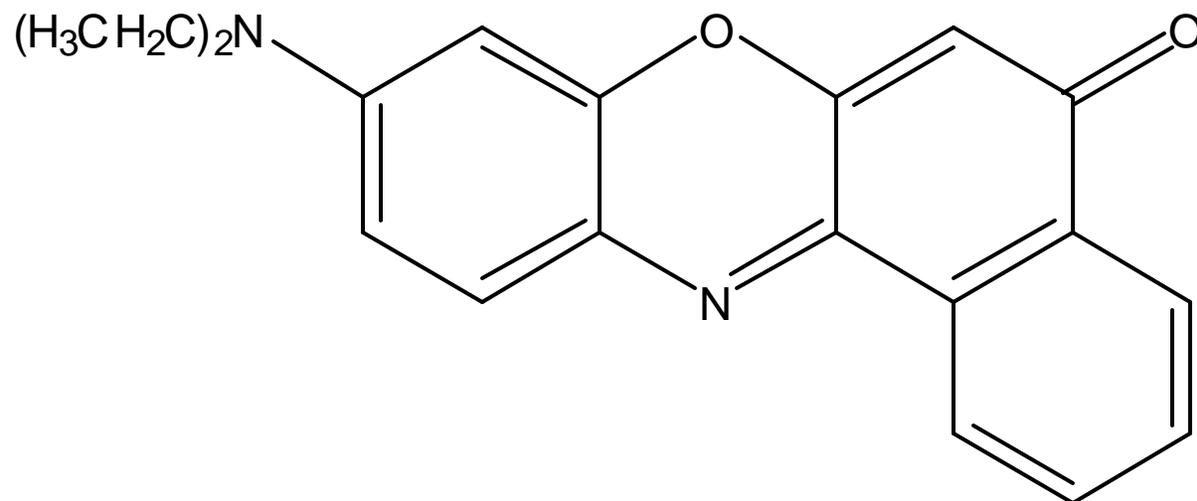


B) Cross-reactive Sensor

# Solvatochromic Effect

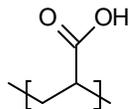


# Nile Red

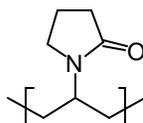


# Role of Polymer Polarity

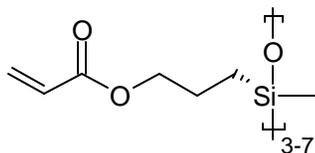
poly(acrylic acid), **PAA**



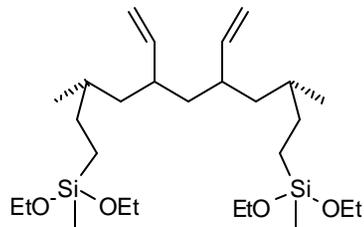
poly(N-vinyl pyrrolidone), **PVP**



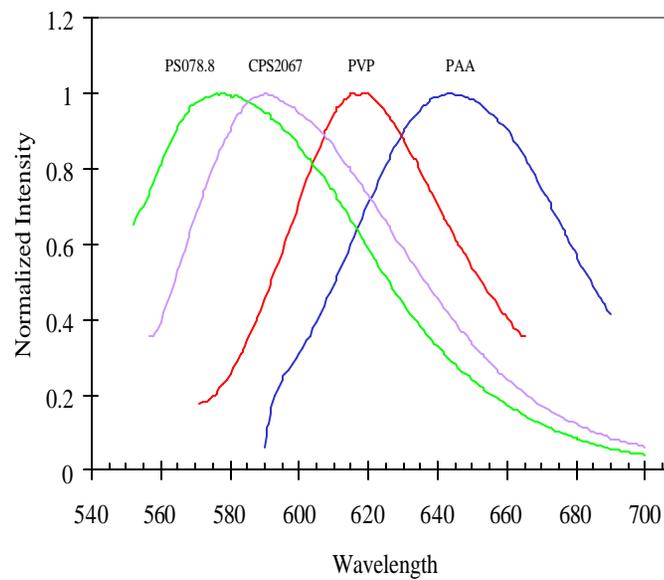
acryloxypropylmethyl-  
cyclosiloxane, **CPS2067**



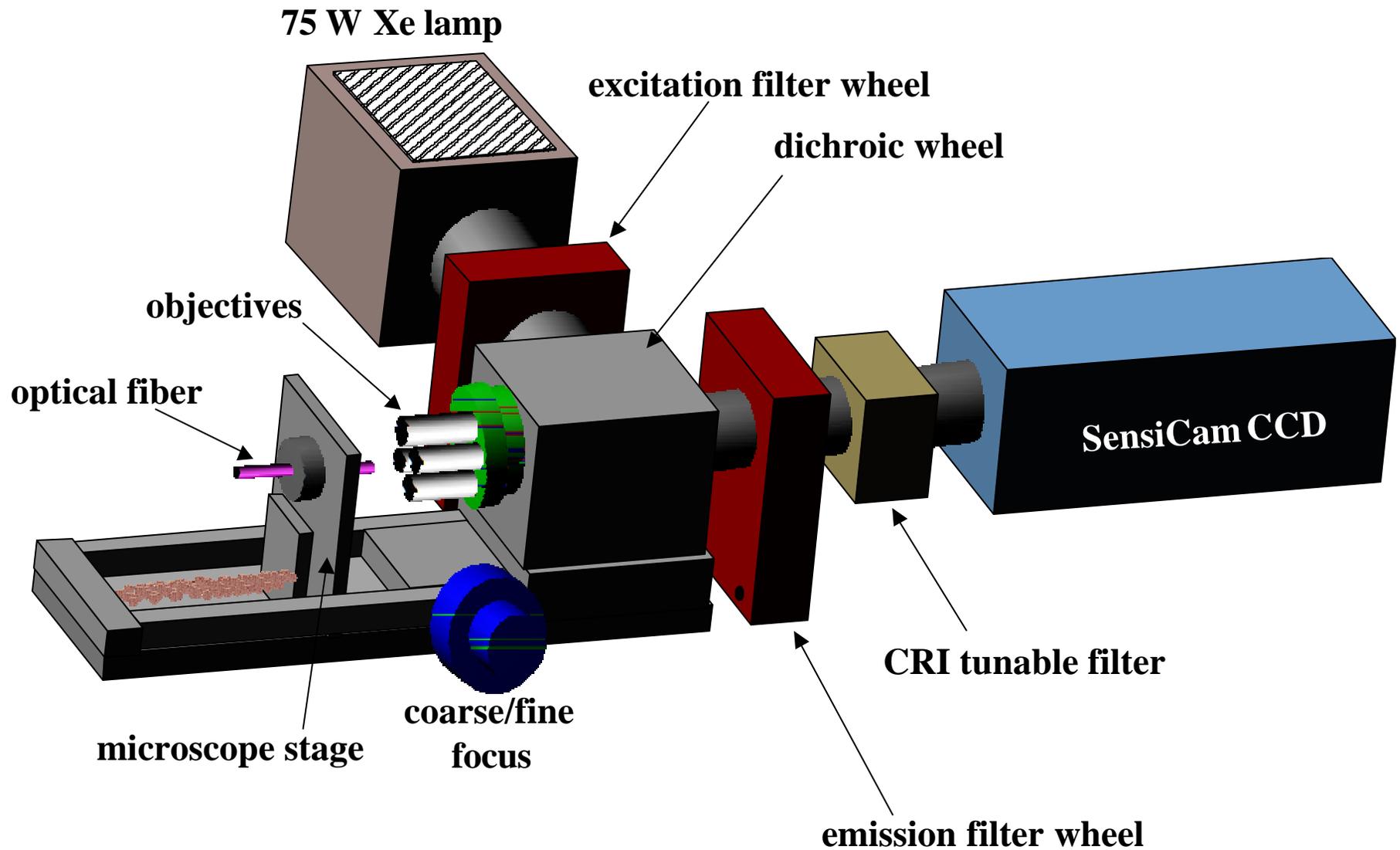
diethoxymethylsilyl-modified  
polybutadiene, **PS078.8**



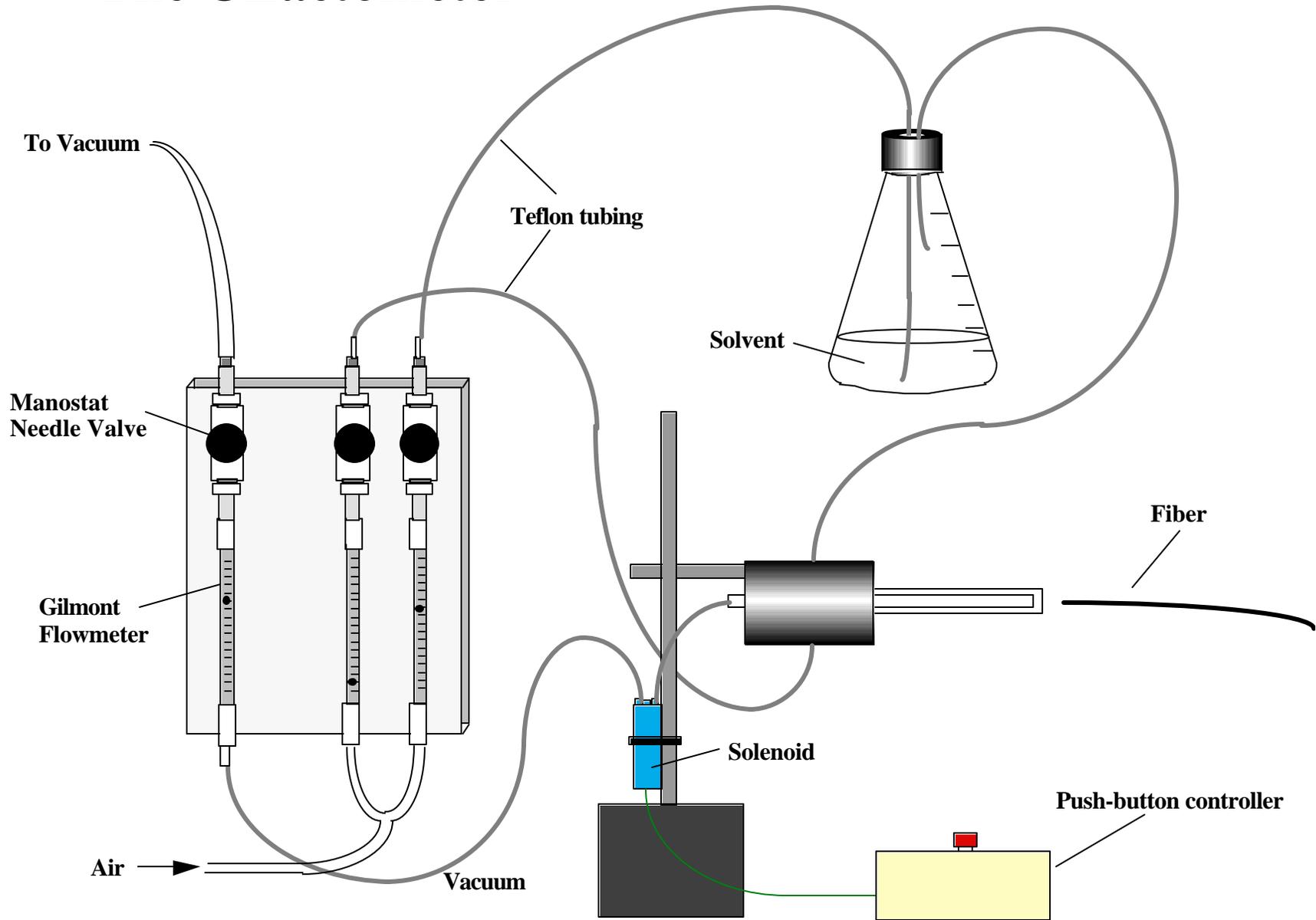
Decreasing  
polarity



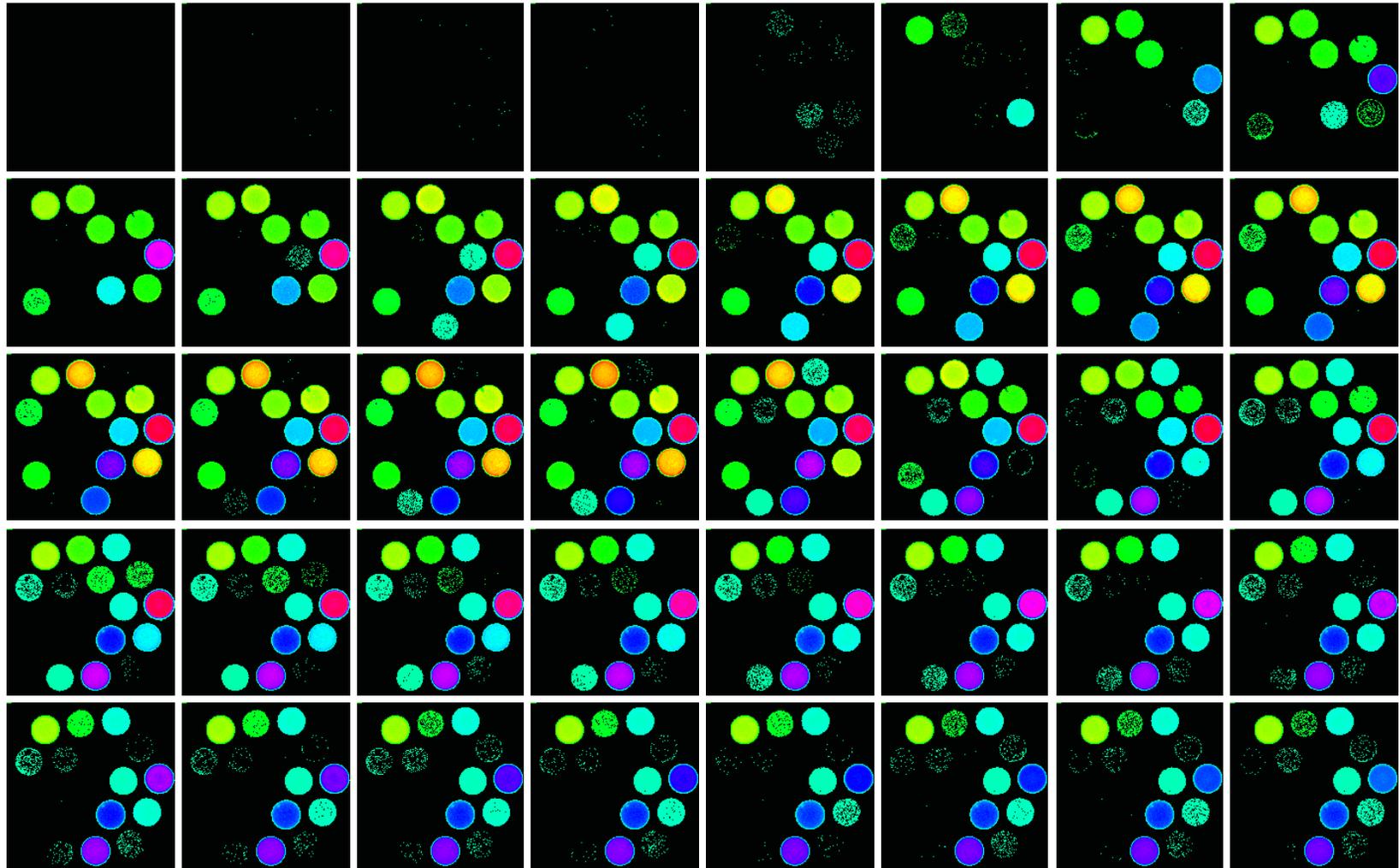
# CCD-based imaging system



# The Olfactometer — J. Kauer



# Sensor Array Response to Benzene Vapor Pulse



Dickinson, T. A., *et al.* (1996) *Nature*, 382: 697-700.



# Classification Results

## Learning Vector Quantization Approach

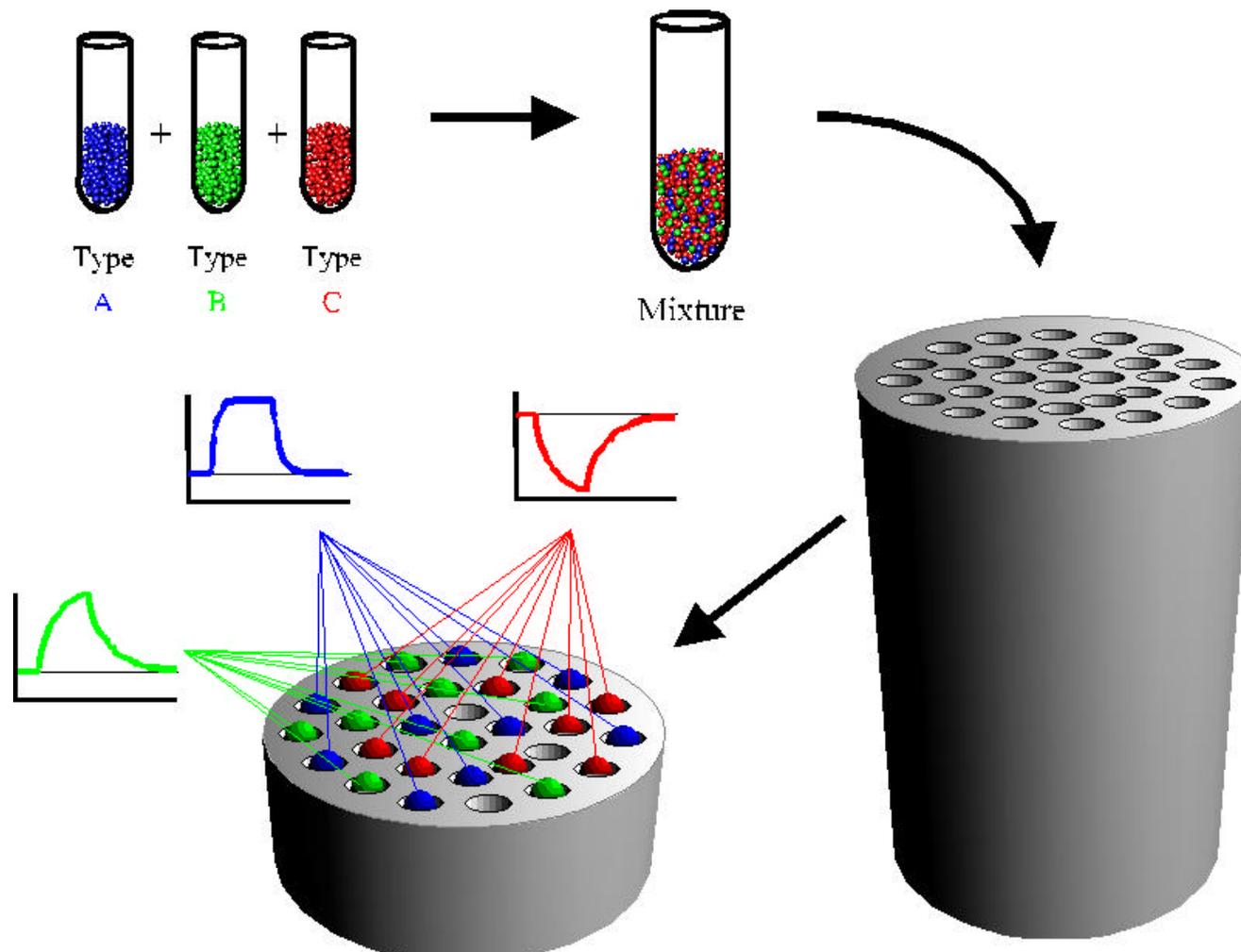
True  
Identity

Network Output

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	24																				
2		23										1									
3			20							3			1								
4			1	19					1					1					2		
5			1		17	4			2												
6				1	2	19					1				1						
7							18	4		1			1								
8							2	21					1								
9				1					23												
10			3							19			1			1					
11											21					1			2		
12												23							1		
13										1			23								
14									1					20			2		1		
15		1														23					
16																	24				
17																		24			
18																			24		
19											4									20	
20		1																			23

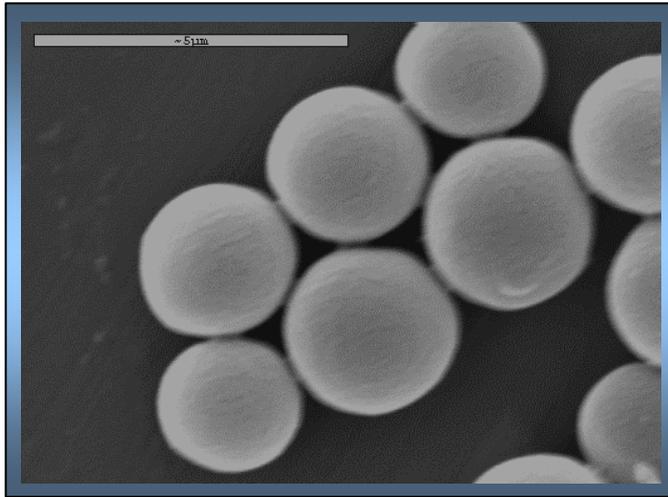
- 1) Acetone
- 2) Butyl Acetate
- 3) Beauty
- 4) Camphor
- 5) Carvone -
- 6) Carvone +
- 7) Chloroform
- 8) Dichloroethane
- 9) DMSO
- 10) Drakkar Noir
- 11) Water
- 12) Heptane
- 13) Isopropanol
- 14) Indole
- 15) Mercaptoethanol
- 16) Methanol
- 17) Propanol
- 18) Propionic Acid
- 19) Pseudoexplosive
- 20) Toluene

# SENSOR ARRAYS are Assembled 'Randomly' in ONE Fabrication Step

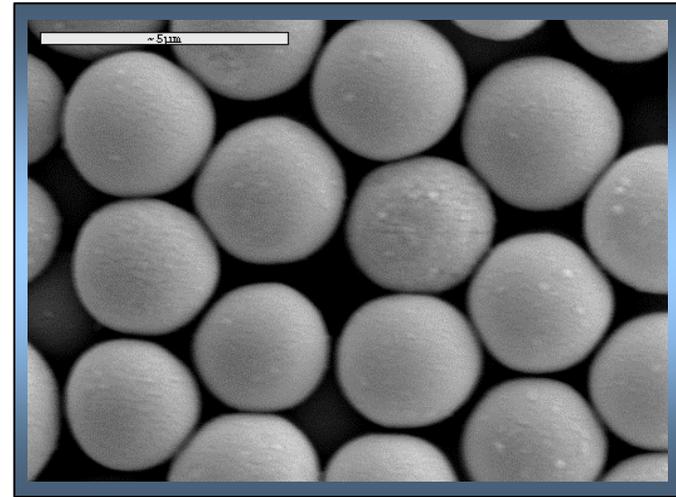


**NOTE: the Sensor Array is a 'Self-Encoding' Bead Array (SEBA). Billions of Sensors are Fabricated at Once.**

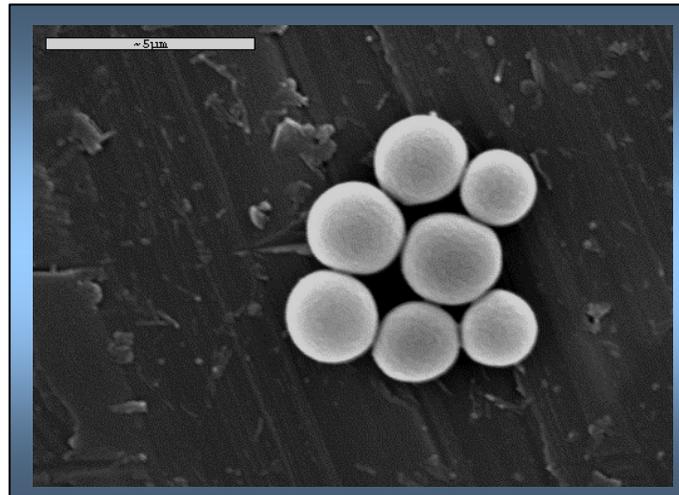
# Hollow Poly(benzyl methacrylate) Spheres



3.5 h polymerization



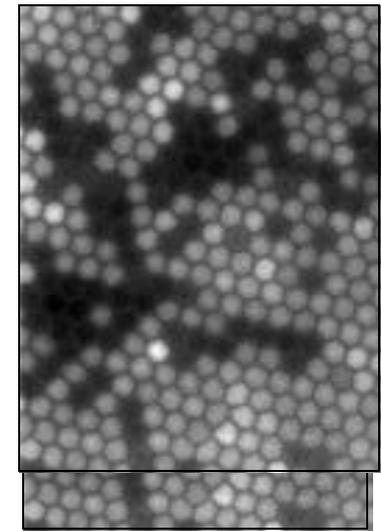
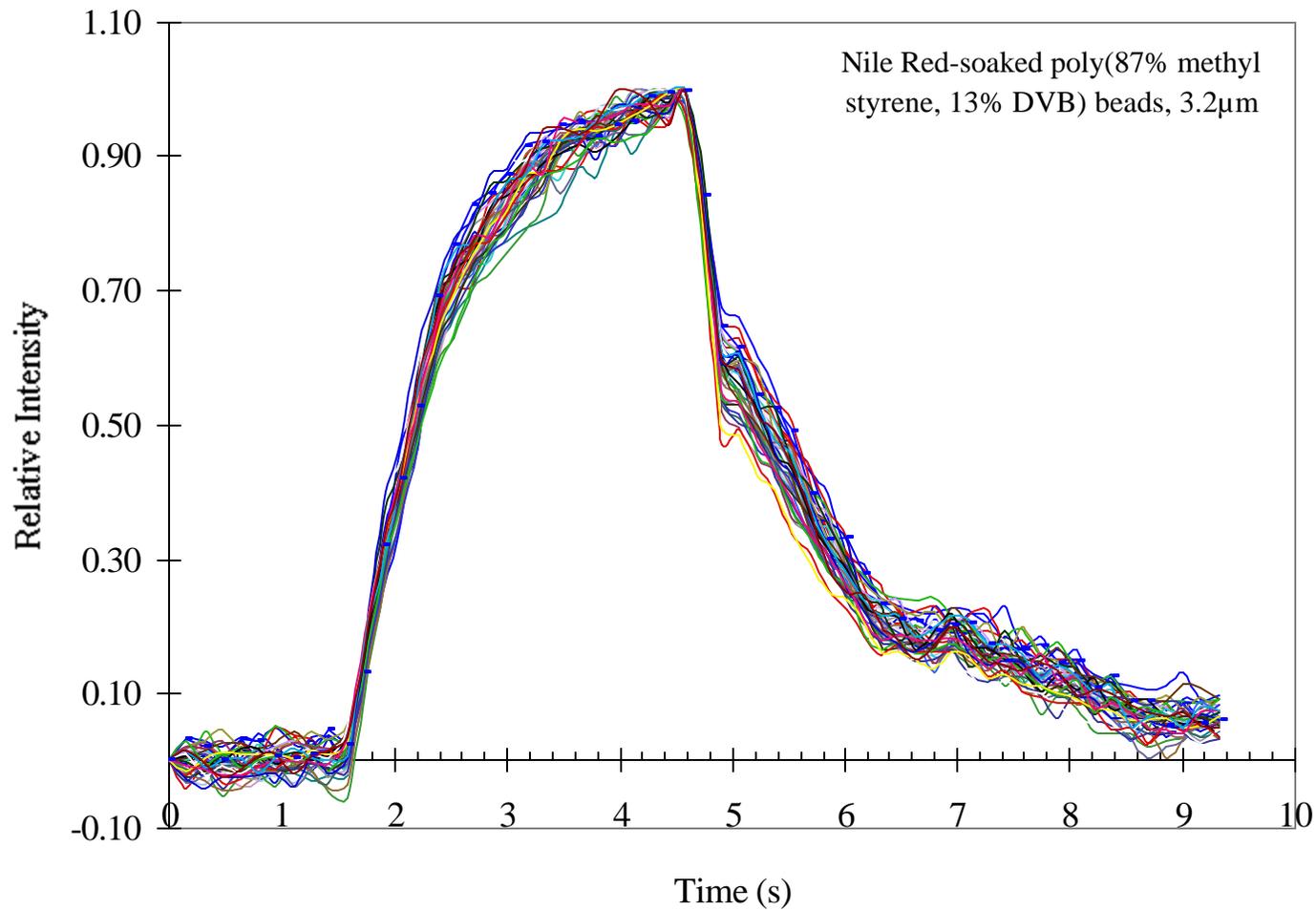
6.5 h polymerization



14h polymerization

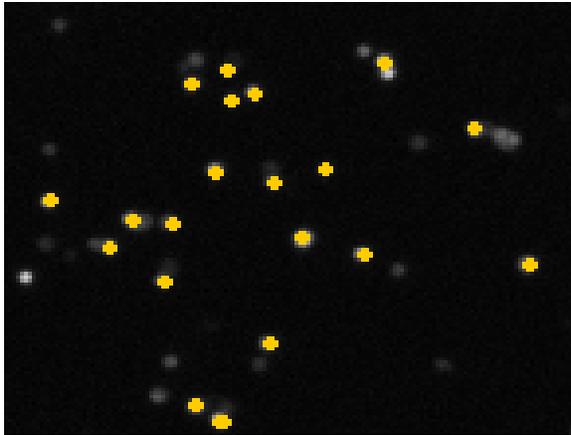
Chem. Mater. 2000

# Nile Red/PolyMethylStyrene Beads in Wells: Response of 40 beads to methanol pulse

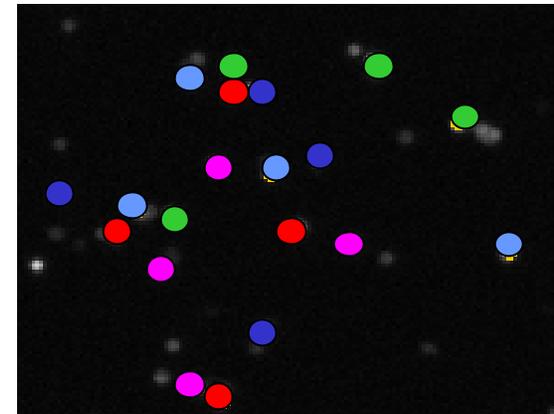


bright = wells with beads  
dark = empty wells

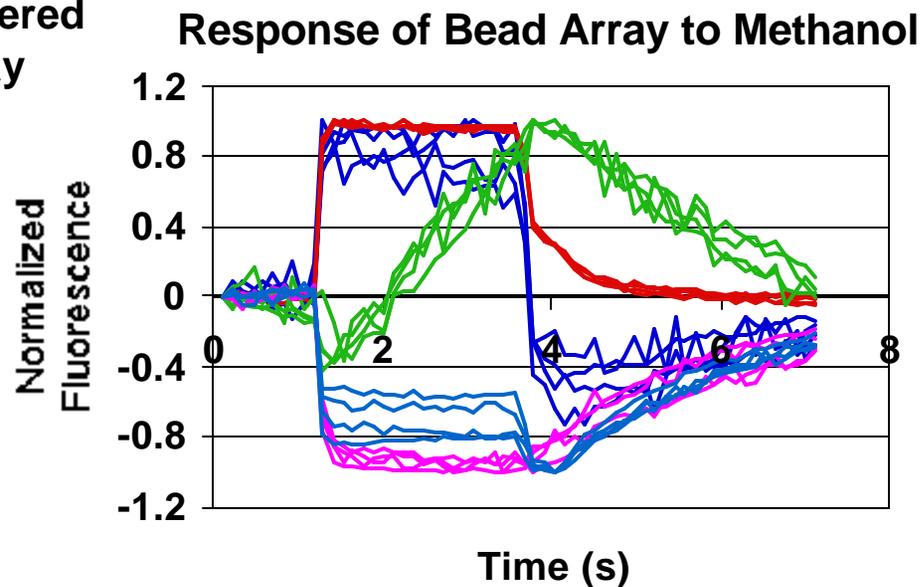
# Sensor Registration Problem



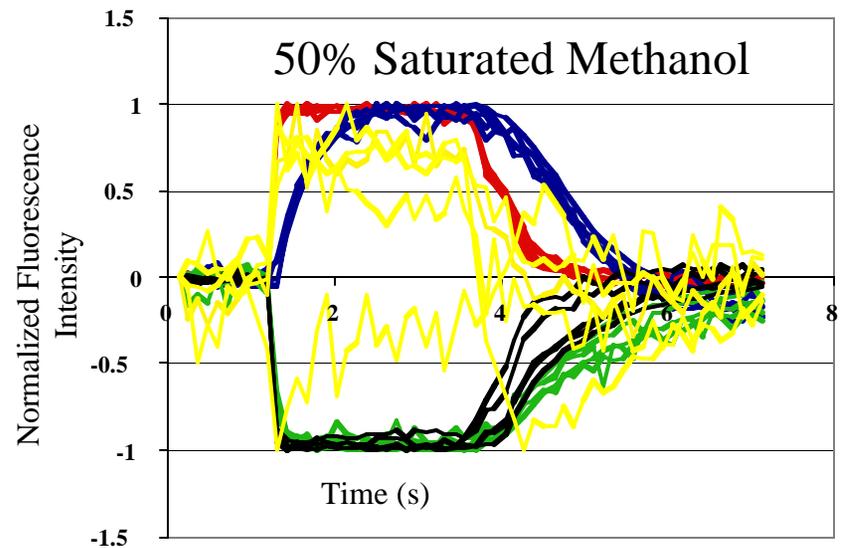
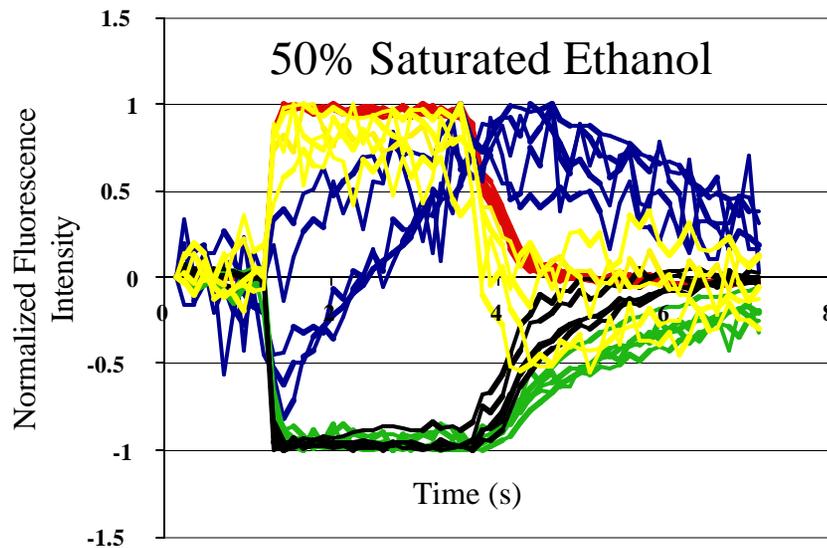
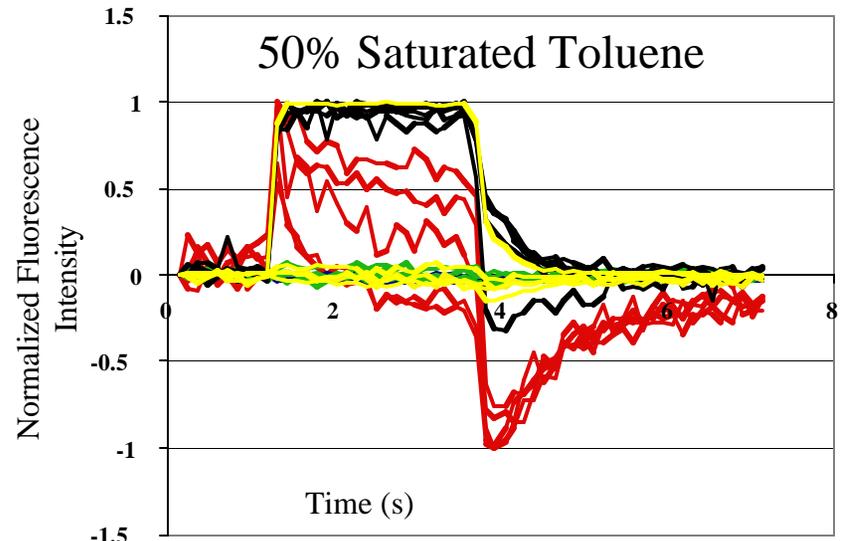
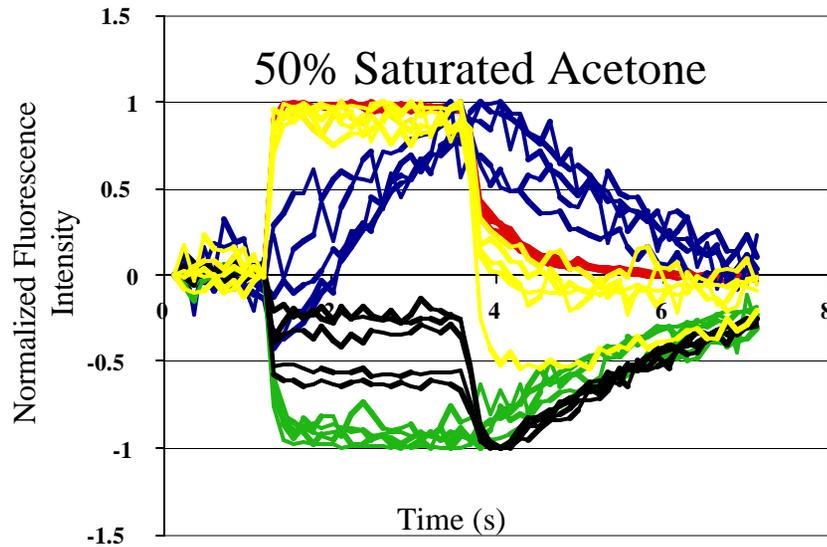
Randomly Ordered  
5 Bead Array



Decoded 5  
Bead Array



# 5 Sensor Types with 4 Analytes

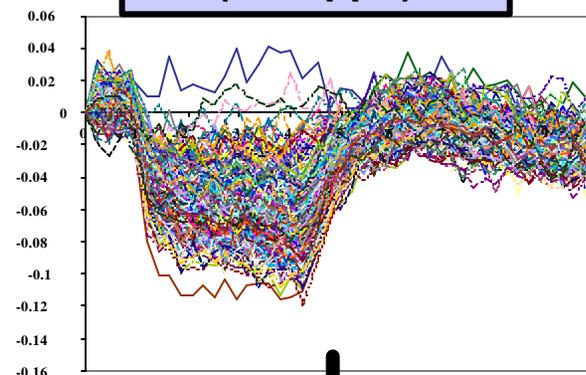
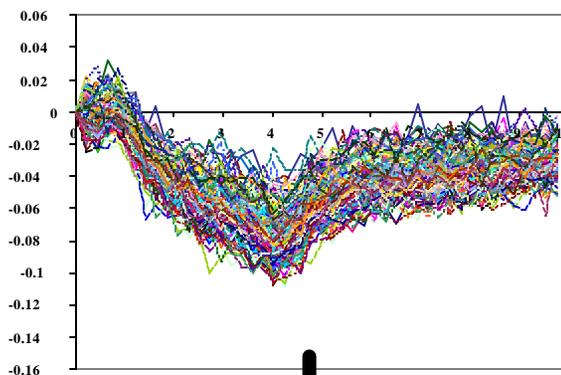
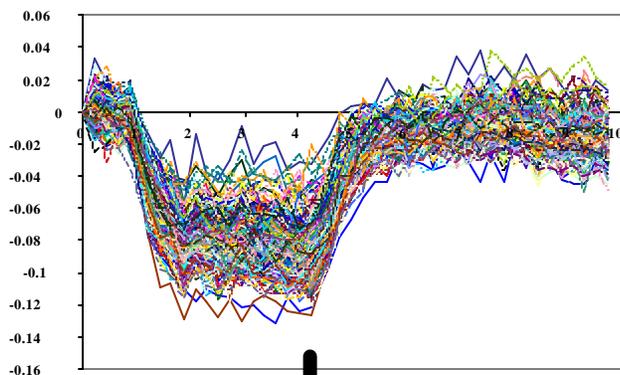


# TIME (s) vs. FLUORESCENCE RESPONSE: 250 INDIVIDUAL Bead Sensors

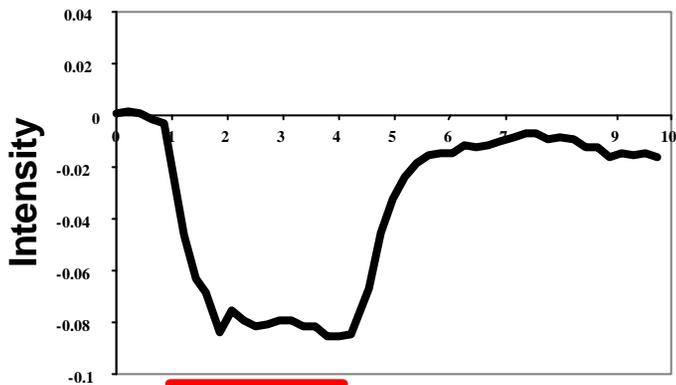
~23 ppb 2,4-DNT

~80 ppb 1,3-DNB

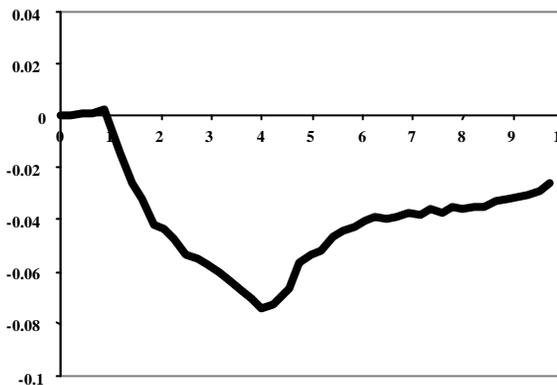
8% saturated  
TNT vapor Strips  
(~0.4 ppb)



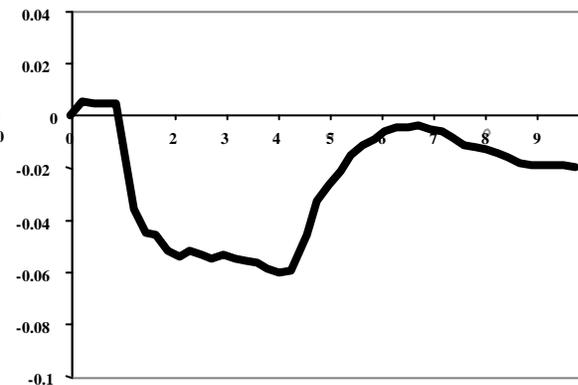
Signal/Noise Improvement:  
Average of 1000 Sensors



3.25 s vapor exposure



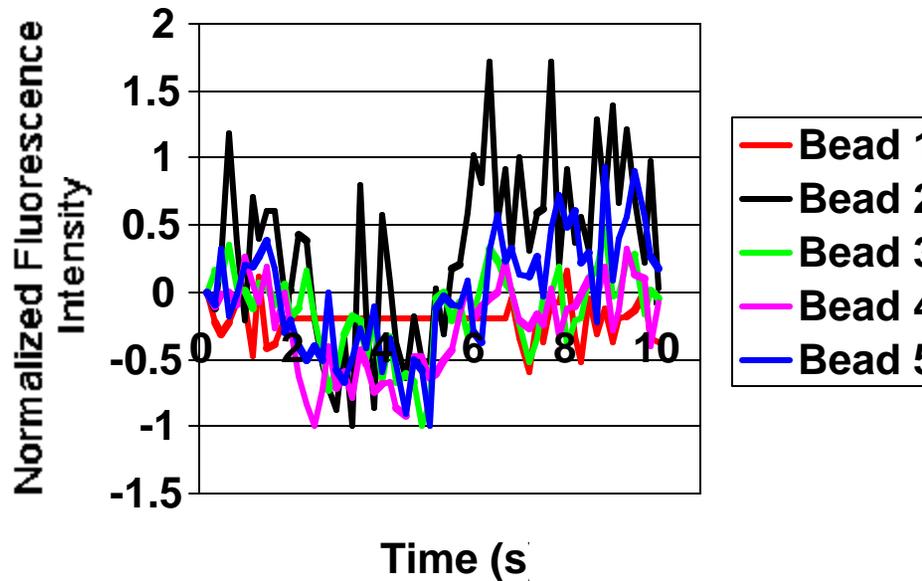
Time (s)



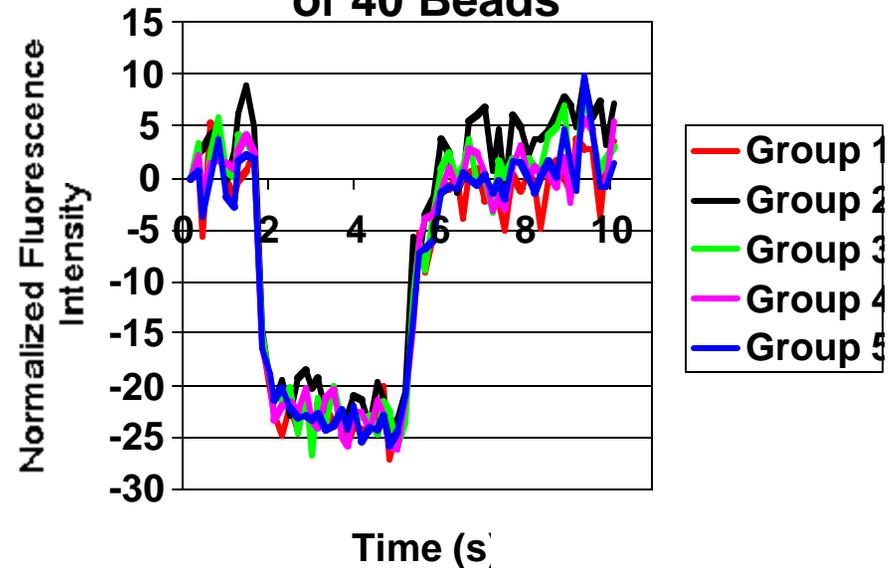
Albert, K. J. and D. R. Walt (2000) *Anal. Chem.* in press.

# Signal Summing

Individual Response of  
5 Bead Sensors



Summed Responses of  
5 Random Groups  
of 40 Beads



**Summing improves signal-to-noise ratio.**

# Analytes for Two Class Problem

- Pure Analytes
  - Acetone
  - Benzene
  - Chloroform
  - Ethanol
  - Ethyl Acetate
  - Heptane
  - Methanol
  - Toluene
  - 1,3-Dinitrobenzene
  - 4-Nitrotoluene
- Binary Mixtures
  - Ethyl Acetate/Heptane
  - Methanol/Benzene
  - 4-NT/Benzene
  - 4-NT/Heptane
  - 4-NT/Methanol
  - 1,3-DNB/Ethyl Acetate
  - 1,3-DNB/Heptane

# Concentrations of Analytes

Table 1: The concentration of the pure analytes  $\pm 15\%$ .

The concentrations were calculated based on literature values for analyte vapor pressures.

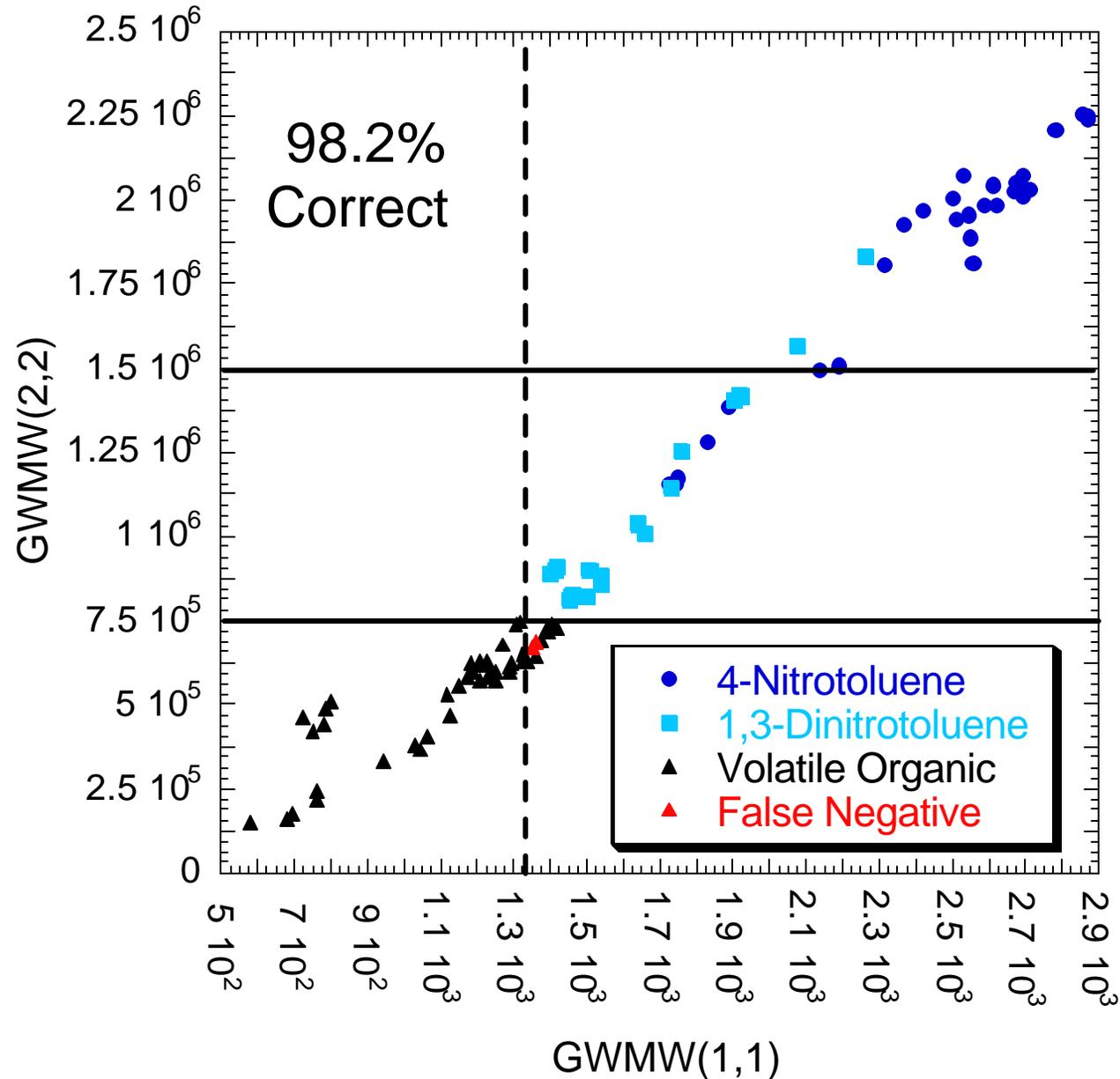
Analyte	Vapor Pressure @25 °C (mmHg)	Concentration (ppm)
Acetone	2.31E+02	7.6E+04
Benzene	9.53E+01	3.1E+04
Chloroform	1.97E+02	6.5E+04
Ethanol	5.90E+01	1.9E+04
Ethyl Acetate	9.45E+01	3.1E+04
Heptane	4.57E+01	1.5E+04
Methanol	1.27E+02	4.2E+04
Toluene	2.84E+01	9.4E+03
1,3-Dinitrobenzene	9.00E-04	6.0E-01
4-Nitrotoluene	1.64E-01	1.1E+02

Table 2: The concentration of the binary mixtures  $\pm 15\%$ .

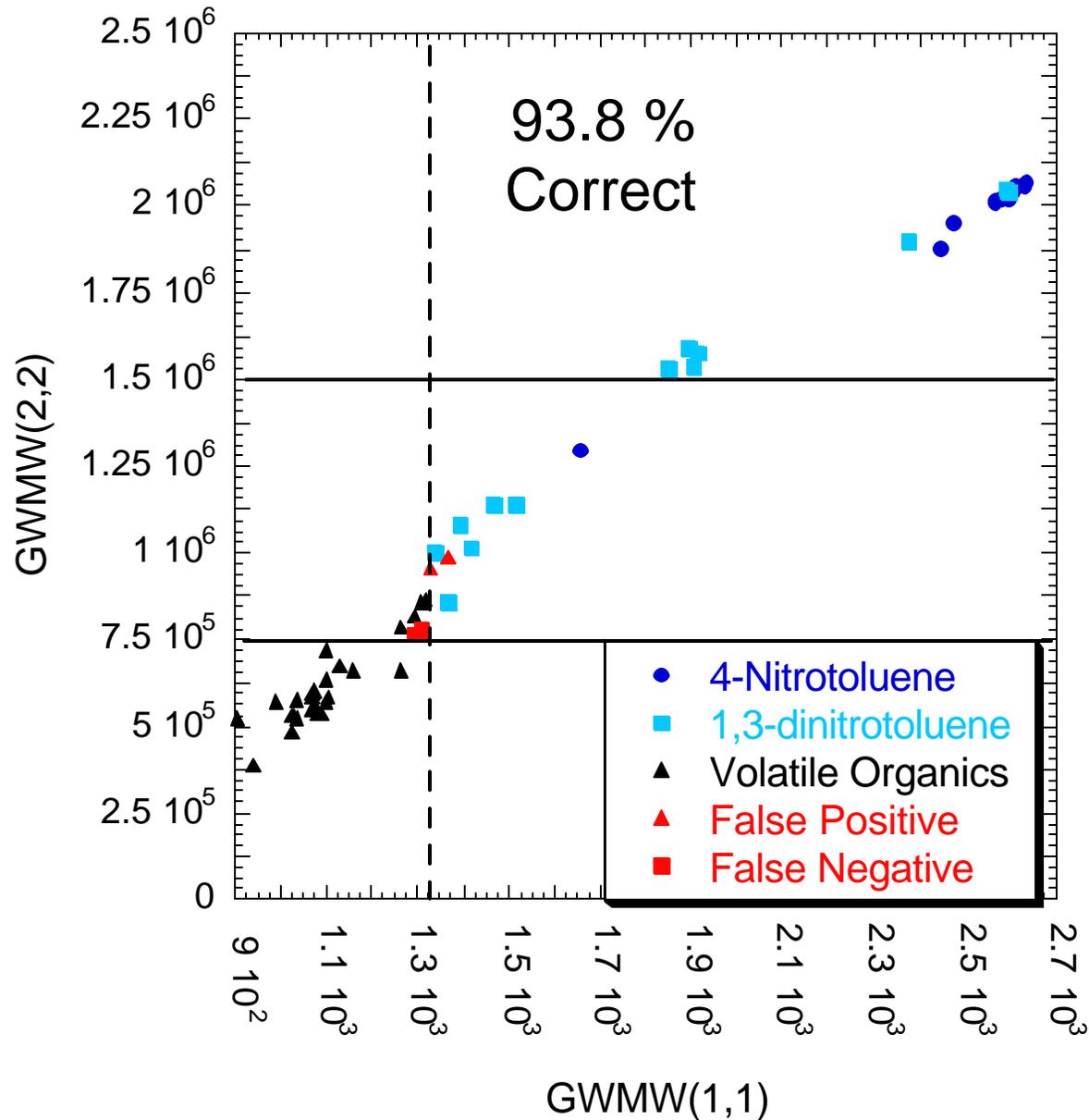
Analyte 1	Analyte 2	Concentration analyte1 (ppm)	Concentration analyte2 (ppm)
Benzene	Methanol	3.1E+04	4.2E+04
Benzene	4-Nitrotoluene	3.1E+04	5.5E+01
Benzene	4-Nitrotoluene	3.1E+04	1.1E+02
Ethyl Acetate	Heptane	3.1E+04	1.5E+04
Ethyl Acetate	1,3-Dinitrotoluene	3.1E+04	3.0E-01
Ethyl Acetate	1,3-Dinitrotoluene	3.1E+04	6.0E-01
Heptane	1,3-Dinitrotoluene	1.5E+04	6.0E-01
Heptane	4-Nitrotoluene	1.5E+04	1.1E+02
Methanol	4-Nitrotoluene	4.2E+04	5.5E+01
Methanol	4-Nitrotoluene	4.2E+04	1.1E+02



# First Testing Array (1 Month)



# Second Test Array (7 months)



# Live/Dead Bacteria Discrimination

## Calculated Identity

Actual Identity	Calculated Identity										
	Live B10	Live B4	Live B5	Live B8	Live B9	Dead B10	Dead B4	Dead B5	Dead B8	Dead B9	Medium
Live B10	4	1	0	0	0	0	0	0	0	0	0
Live B4	2	3	0	0	0	0	0	0	0	0	0
Live B5	0	0	5	0	0	0	0	0	0	0	0
Live B8	0	0	0	4	0	0	1	0	0	0	0
Live B9	0	0	0	0	5	0	0	0	0	0	0
Dead B10	0	0	0	0	0	5	0	0	0	0	0
Dead B4	0	0	0	0	0	0	5	0	0	0	0
Dead B5	0	0	0	0	0	0	0	5	0	0	0
Dead B8	0	0	0	0	0	0	0	0	5	0	0
Dead B9	0	0	1	0	2	0	0	2	0	0	0
Medium	0	0	0	0	0	0	0	0	0	0	10

85% Correct, 87% Variance (7PCs)

B10: *Acintobacterium*

B4: *M. luteus*

B5: *E. coli*

B8: *Salmonella*

B9: *Klebsiella pneumoniae*

# Acknowledgements

ONR



DARPA



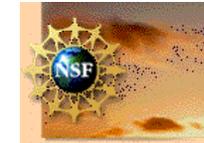
DOE



NIH



NSF



## Walt Group

Myoyong Lee  
Karri Michael  
Paul Pantano  
Caroline Schauer  
Jenny Tam

Keith Albert  
Todd Dickinson  
Jane Ferguson  
Michael Fleming  
Jason Epstein

Ferenc Szurdoki  
Frank Steemers  
Shannon Stitzel  
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## Johns Hopkins University

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## Illumina

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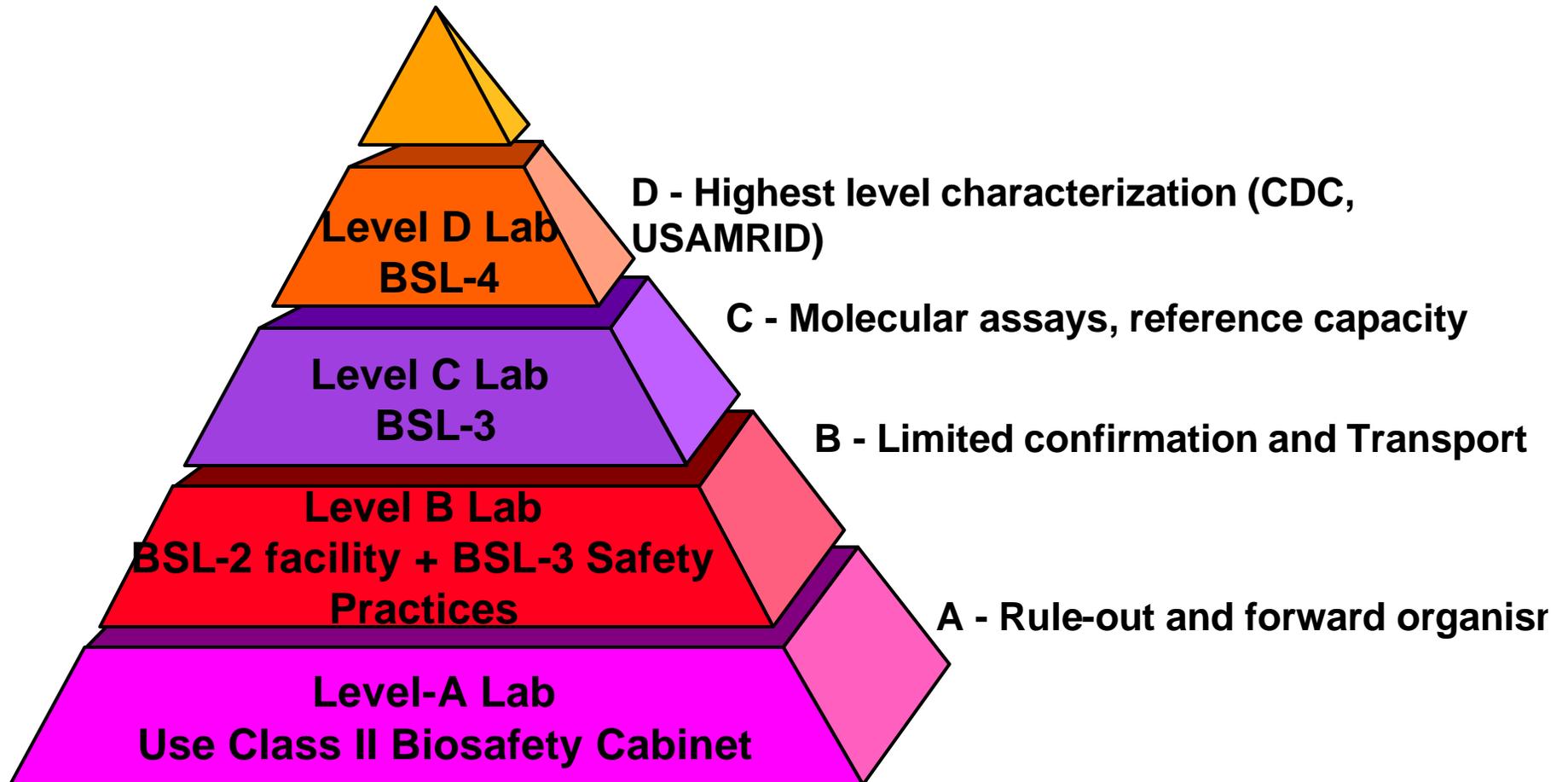
# Laboratory Response Network

Ralph Timperi

Massachusetts Department of  
Public Health, and

Association of Public Health  
Laboratories ([www.aphl.org](http://www.aphl.org))

# Laboratory Response Network For Bioterrorism





# CDC BT Rapid Response and Advanced Technology Lab

- **BSL -3**
- **Agent Identification and Specimen Triage**
- **Refer to and Assist Specialty Lab Confirmation**
- **Evaluate Rapid Detection Technology**
- **Rapid Response Team**

# LRN Capacity

## Specimen Collection and Transport

- **Appropriate specimens**
- **Forensic issues and chain of custody**
- **Timely transport & testing safety**

## Capacity to Diagnose

- **Surveillance**
- **Rapid screens - People/environment**
- **Definitive and trusted testing**
- **Secure, reliable means of electronic communication**
- **The right answer, to the right persons at the right time**

# LRN: Work-in-progress

- State and large city / county public health laboratories- secure internet website (reagents, protocols, capacity locator)
- Training and proficiency on 'highest priority agents'
- Conventional and rapid methods
- Validation of methods
- 'Surge capacity'

# LRN: Growing capacity

- Clinical microbiology laboratories collaboration- standard protocols, rule-out testing for clinical specimens, (future) definitive identification of agents
- Building a secure system for electronic laboratory reporting of test results- the technology is not the problem
- Surge capacity- build, protect, access
- Technology and reagents to more laboratories- capacity to validate and accept

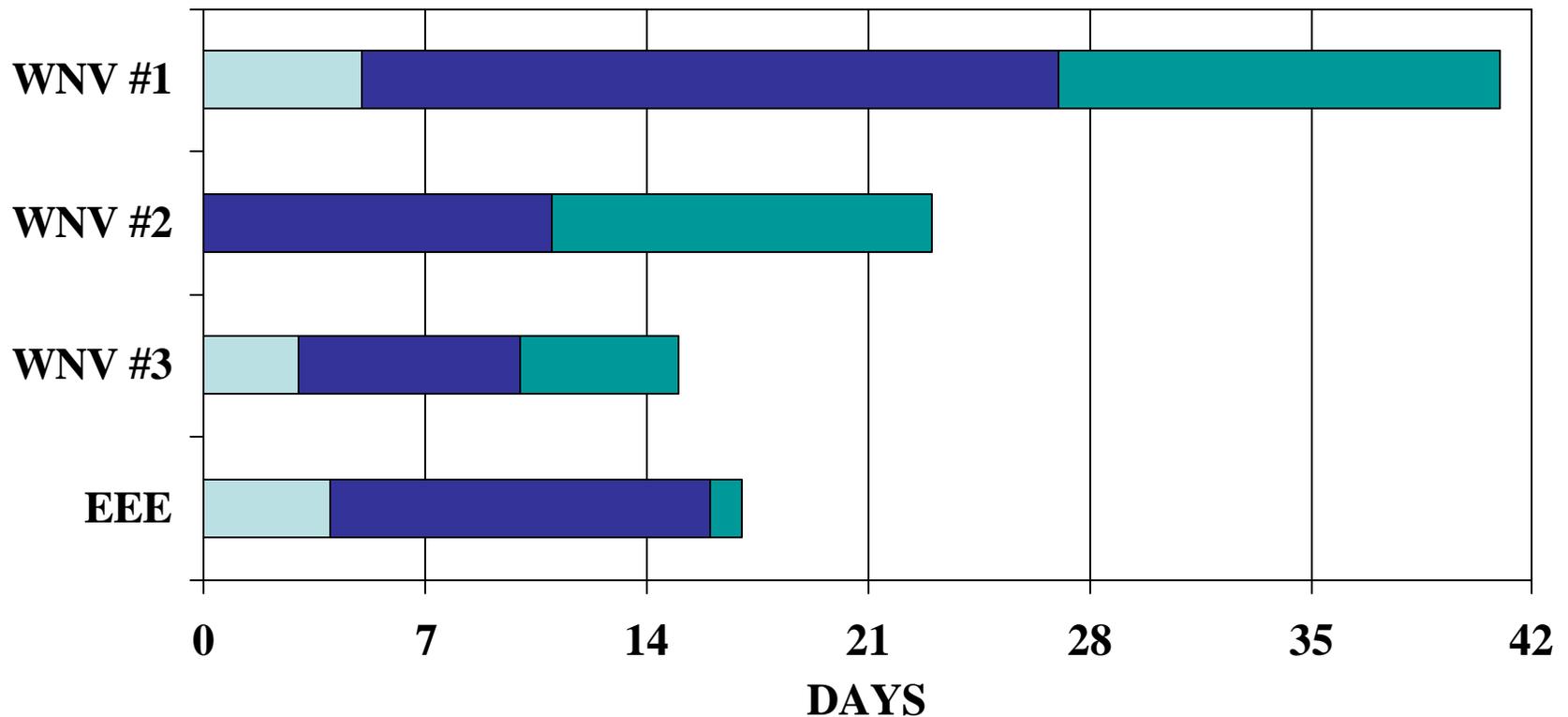
# Laboratory and Testing Issues

- Surveillance- Numbers of ill persons, general syndromes, laboratory-based species and DNA characteristics
- Field testing- First responders, environmental, risk characterization
- Laboratory diagnosis of human and animal illnesses- coordination and communication
- 24/7 available and accessible capacity

# Human Arbovirus Cases, MA

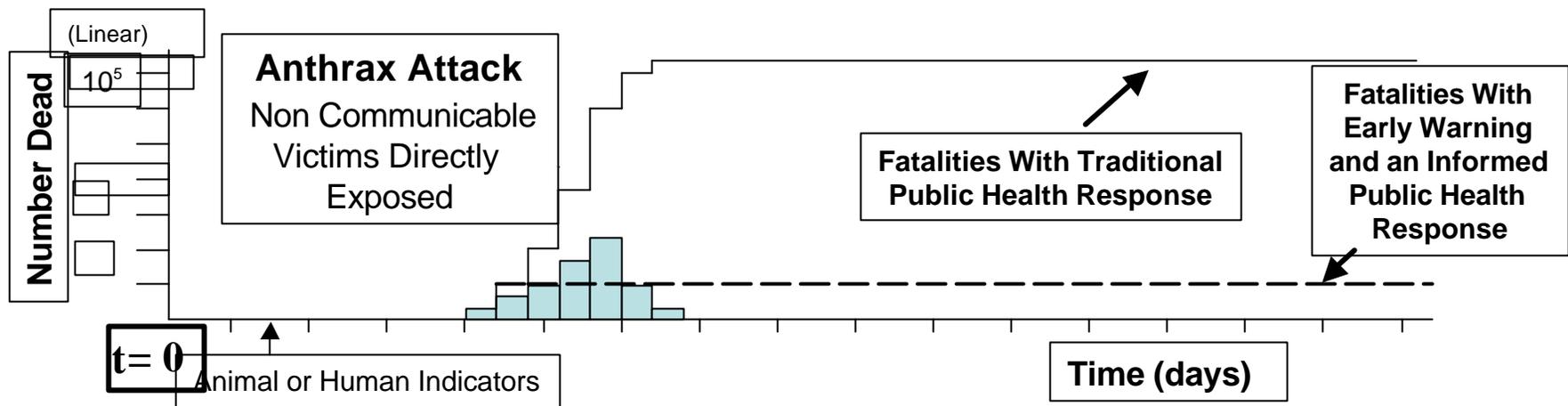
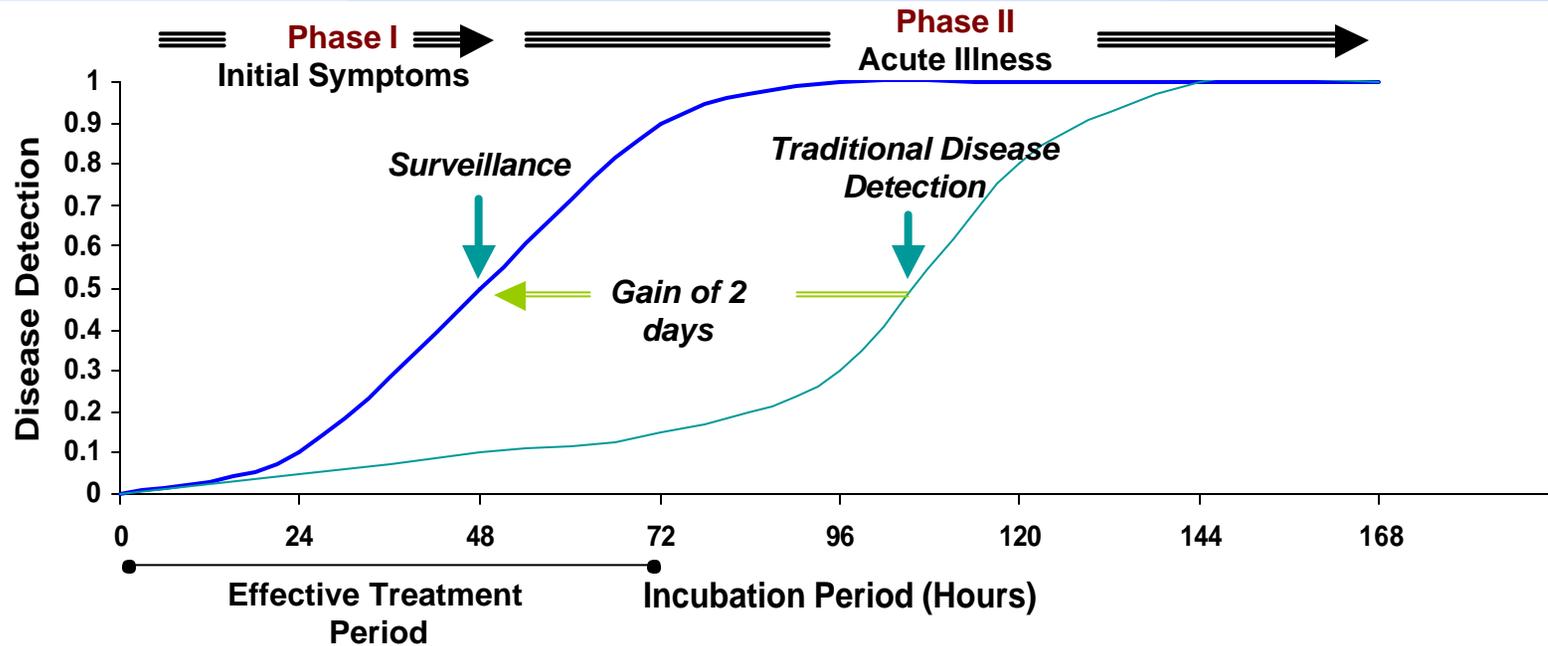
## Timelines: Onset to Diagnosis

■ Onset to Health Care   ■ Health Care to Notification   ■ Notification to Diagnosis

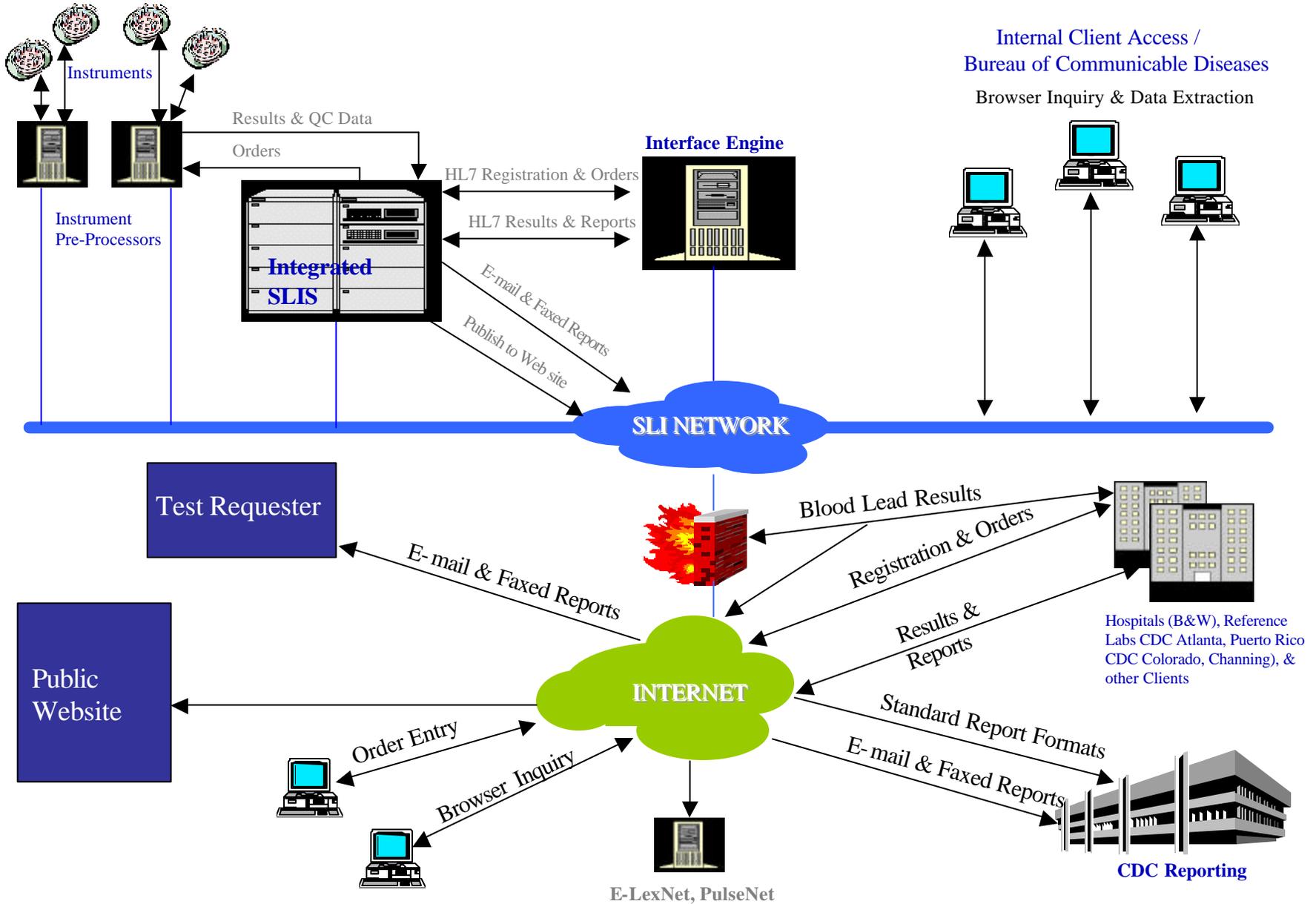




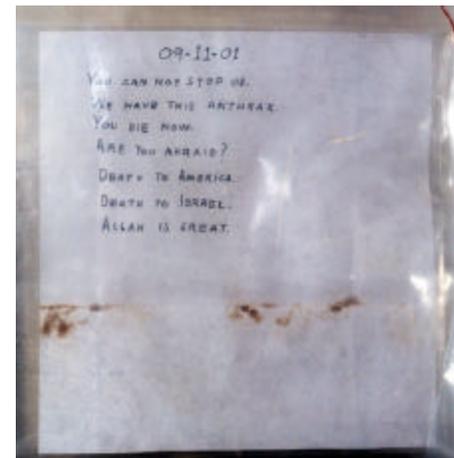
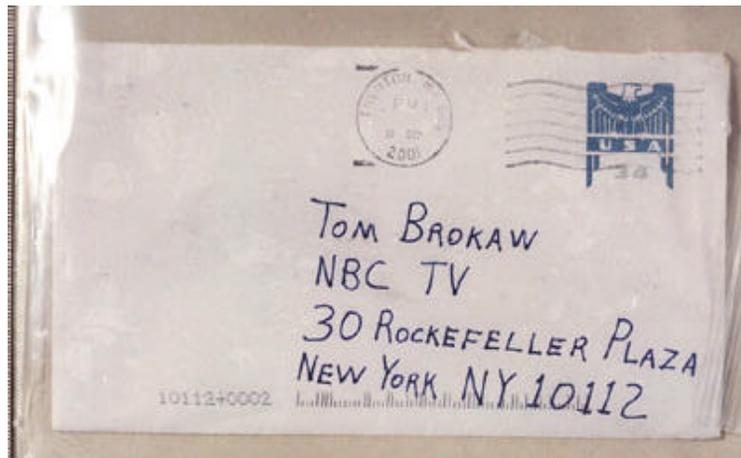
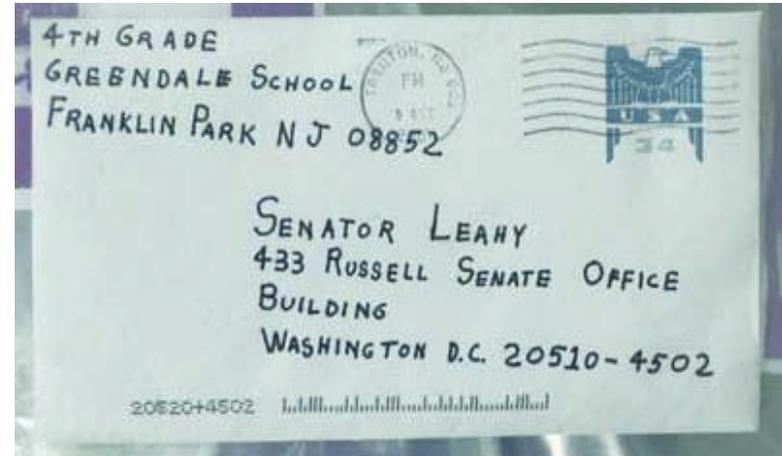
# Impact of Surveillance on Survivability (Anthrax)



# FRAMEWORK FOR AN INTEGRATED SLIS



# Anthrax Sent By Mail September / October 2001



## ENVIRONMENTAL SAMPLES

<b>Description</b>	<b>Number Submitted</b>	<b>Risk / Testing Priority</b>
<b>U.S. Mail contaminated with anthrax</b>	<b>0</b>	<b>Low to high / High</b>
<b>U.S. Mail with a suspicious powder (not anthrax or other pathogen) with or without a threat letter</b>	<b>52</b>	<b>None / High</b>
<b>Powders, particulate matter and various liquid or solid material on surfaces of floors, walls, furniture, clothing, appliances or food</b>	<b>~800</b>	<b>None / Low</b>
<b>Clothing, household items, business products, etc. without evidence of powder, particulate matter, etc.</b>	<b>~1800</b>	<b>None / None</b>

# LRN validated methods and reagents available

- ***Bacillus anthracis*: C, PCR, TRF**  
***Brucella* sp.: C**
- ***Francisella tularensis*: C, TRF**
- ***Yersinia pestis*: C, PCR, TRF**
- ***Clostridium botulinum*: C**

**Conventional, polymerase chain reaction,  
time resolved fluorescence**

# Methods in development

- **Ricin: TRF**
- ***Brucella* sp.: PCR, TRF**
- ***Francisella tularensis*: PCR**
- ***Staph.* enterotoxin B: TRF**
- ***Burkholderia mallei*: PCR**
- ***Burkholderia pseudomallei*: PCR**
- ***Coxiella burnetii*: TRF**
- ***Clostridium botulinum*: EIA, TRF**
- **Validation in progress**
- **Validation by summer**
- **Validation by late summer**
- **EDA not estimated**
- **Fall/Winter 2002**
- **Fall/Winter 2002**
- **EDA not estimated**
- **2004**

# Testing Methods - Environmental

- 1- Gross examination- (environmental samples only)
- 2- Microscopic examination for bacteria and spores
- 3- DNA test methods
- 4- Culture (growth of bacteria on artificial media)
- Most samples tested by methods 1, 4
- U.S. Mail and similar items tested by methods 1, 2, 4 and possibly 3
- Some items with no apparent contamination, no risk indicators tested by method 1 only

# **Targeting Immunity to Biothreats**

**David Scadden**

**Massachusetts General Hospital**

**Harvard Medical School**

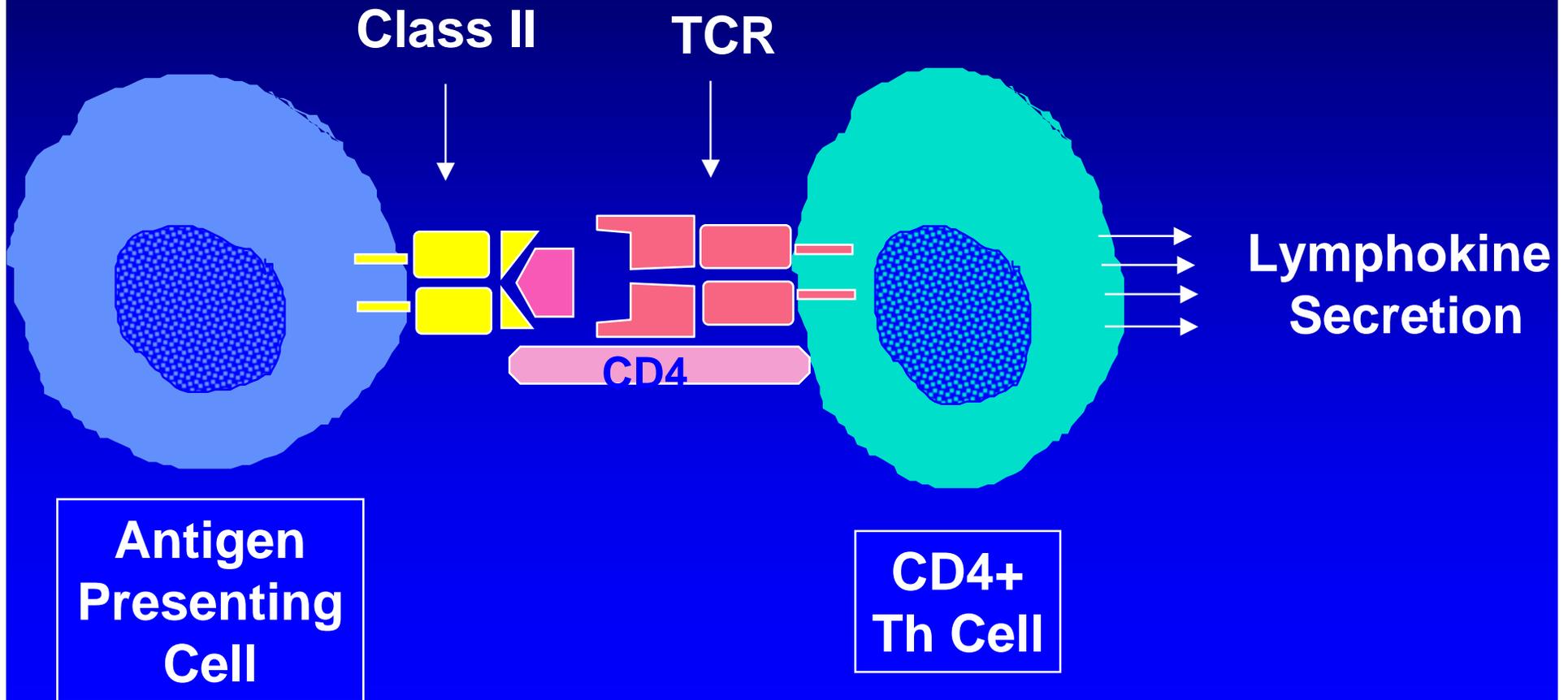
# Cellular immunity and HIV disease

Immune control of HIV infection is possible without anti-retroviral therapy

# Evidence for CTL control of HIV

- Negative correlation between CTL and viral load by more sensitive assays (Ogg et al)
- Increase in SIV viremia with CD8 cell depletion (Schmitz et al; Jin et al)
- Association between appearance of CTL and decline in viremia in acute infection (Koup et al; Borrow et al)

# Optimal CTL function depends on virus-specific T helper cells



**HIV-specific CD4+ T cell  
responses are associated with  
control of HIV**

**Rosenberg et al. Science 1997; 278, 1447**

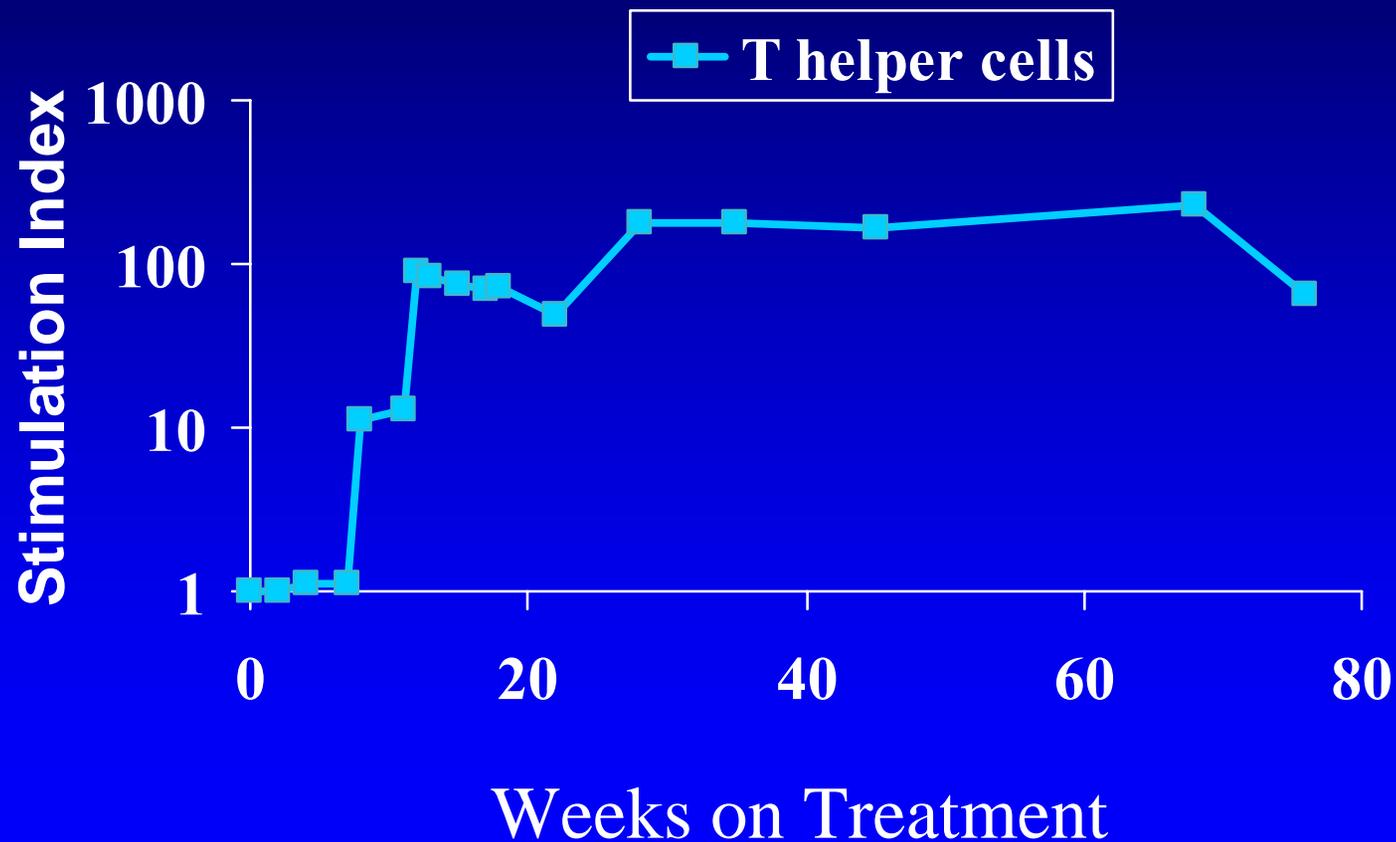




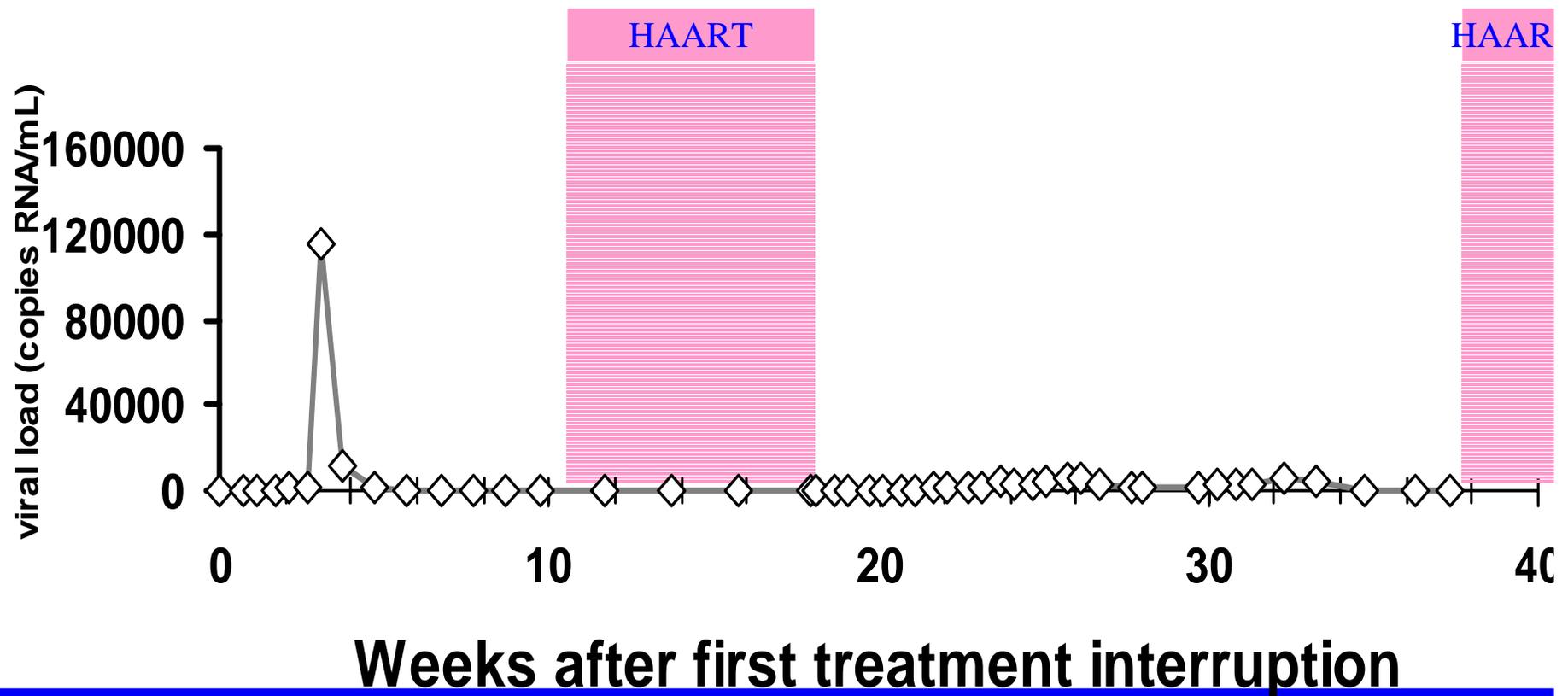




# Treatment of acute HIV-1 infection results in augmentation of T helper cell responses



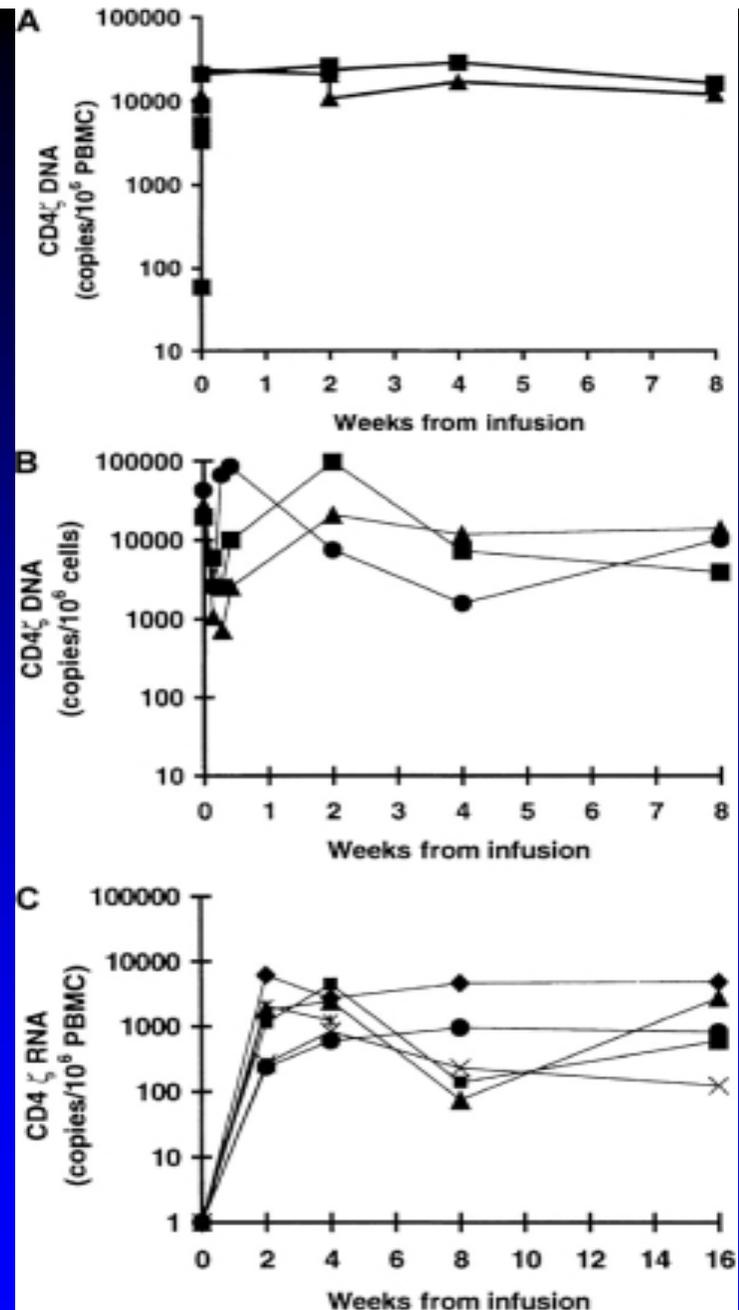
# Preserved HIV specific T cell helper function is associated with control of HIV without HAART



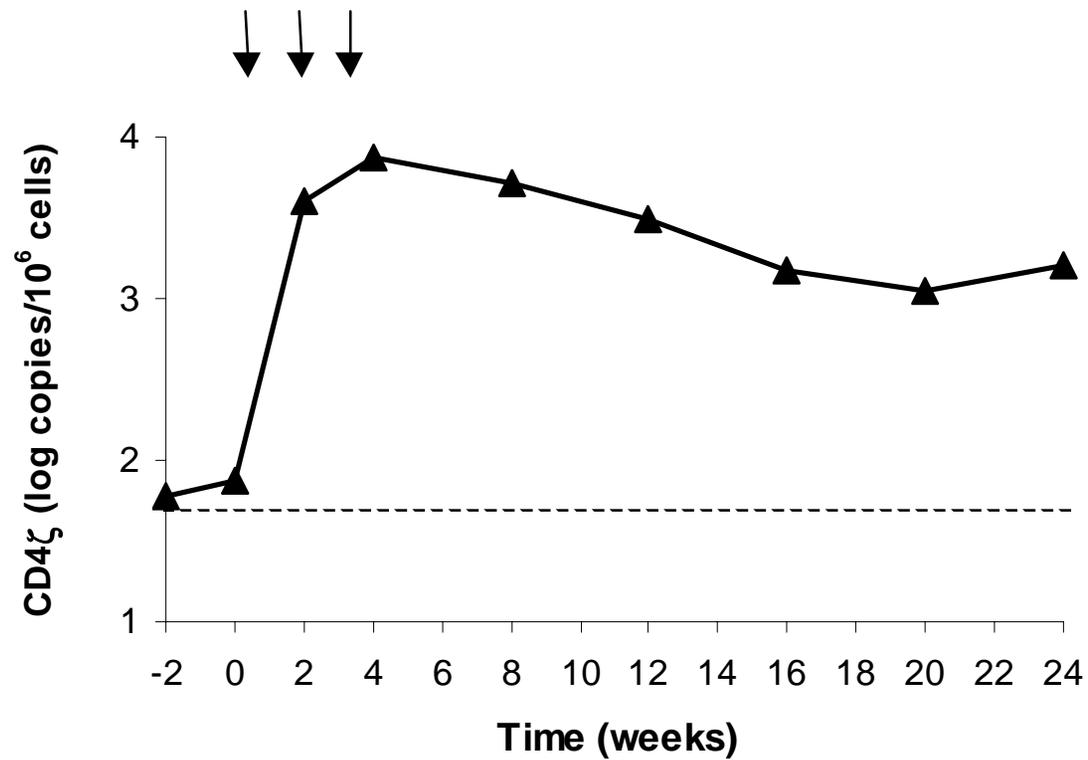
**Structured treatment  
interruptions (STI) in acute  
HIV infection may result in  
immunologic control of viremia**

# CD4 $\zeta$ -modified T-cell survival and gene expression in peripheral blood mononuclear cells (PBMCs)

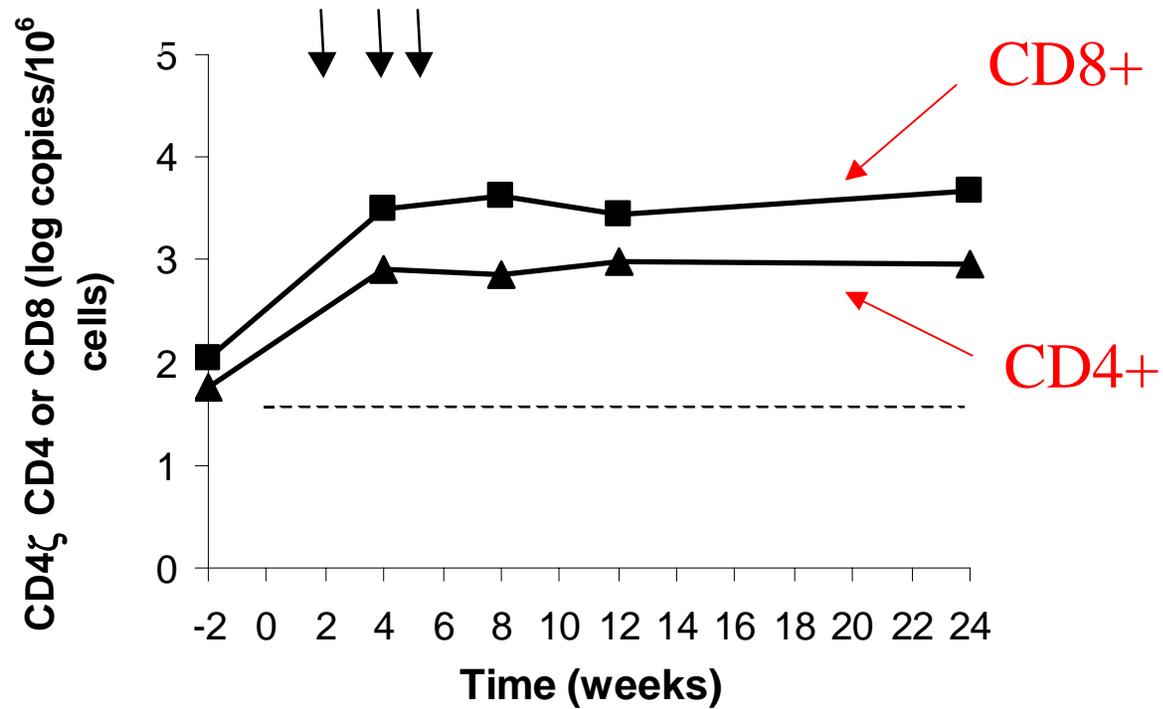
Mitsuyasu et al, *Blood* 2000; 96:785



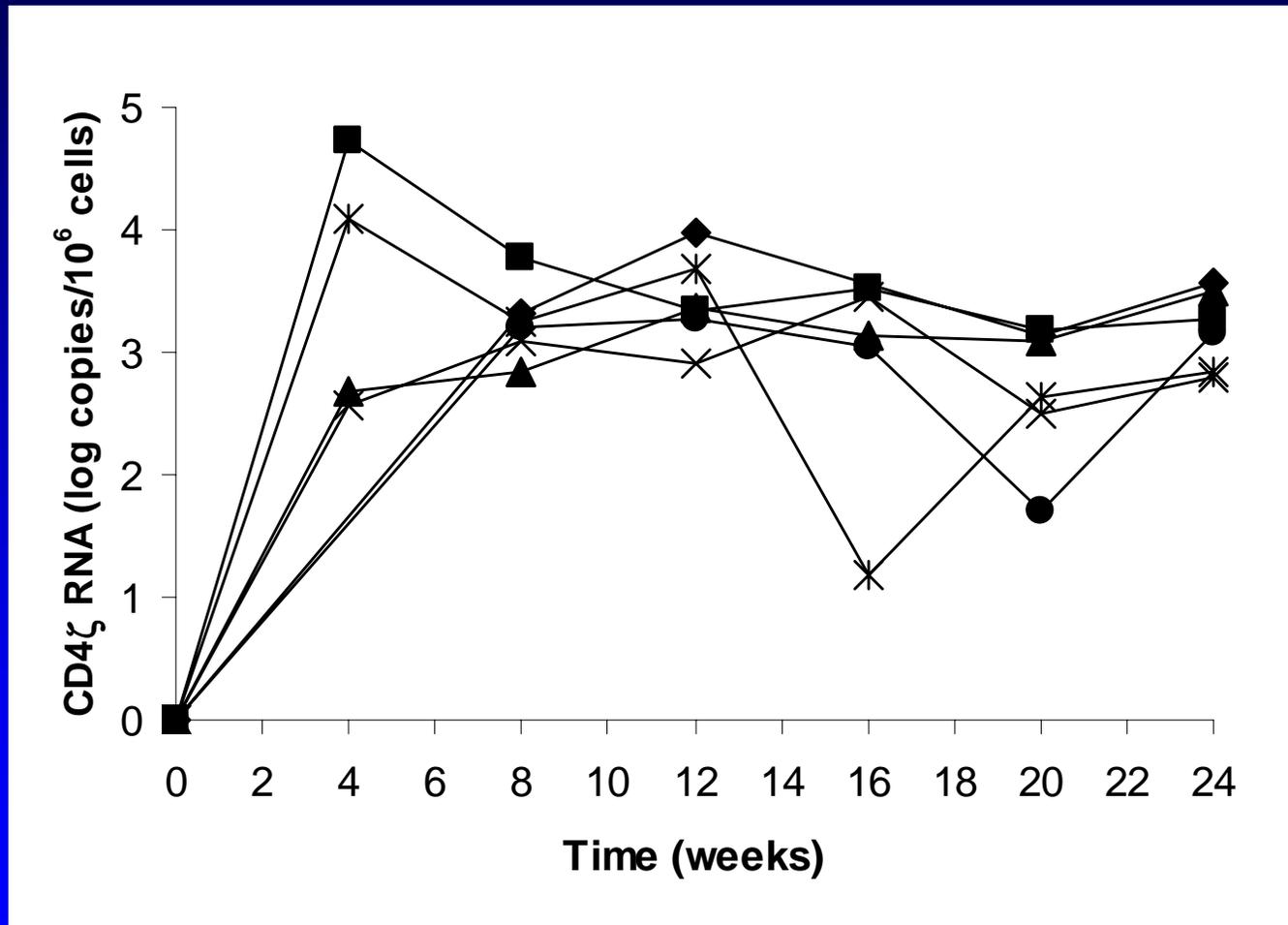
# Persistence of cells with chimeric TCR DNA



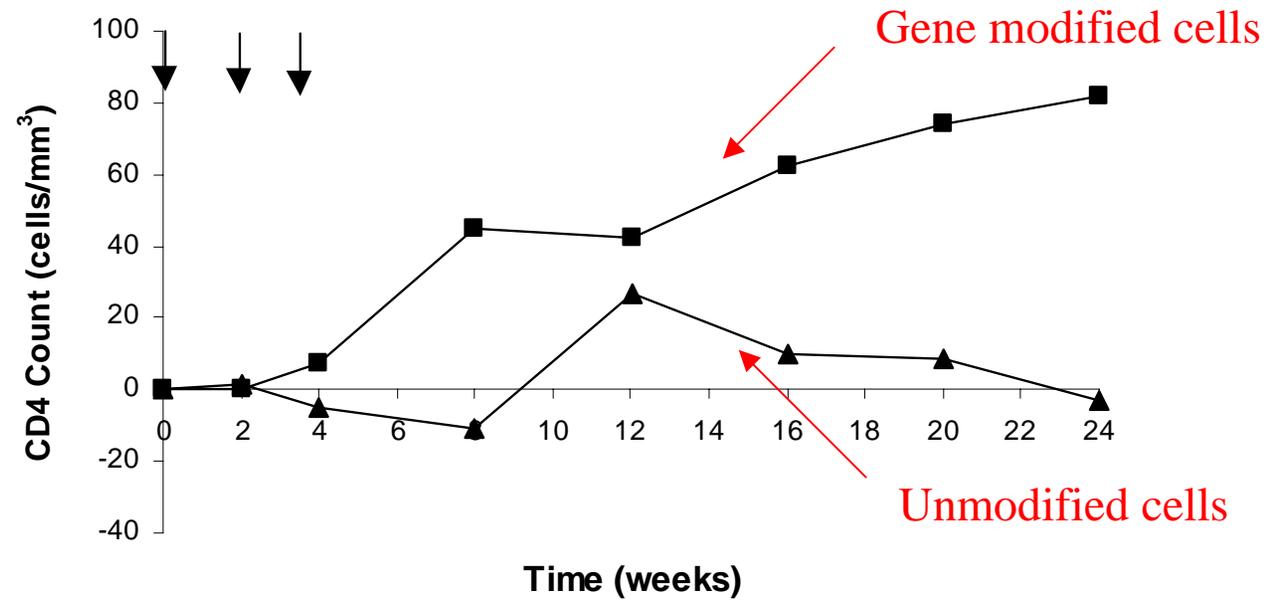
# Persistence of cells with chimeric TCR DNA



# Persistence of cells expressing chimeric TCR RNA



# CD4+ T cell counts after cell infusions



# Plasma viral load over time

