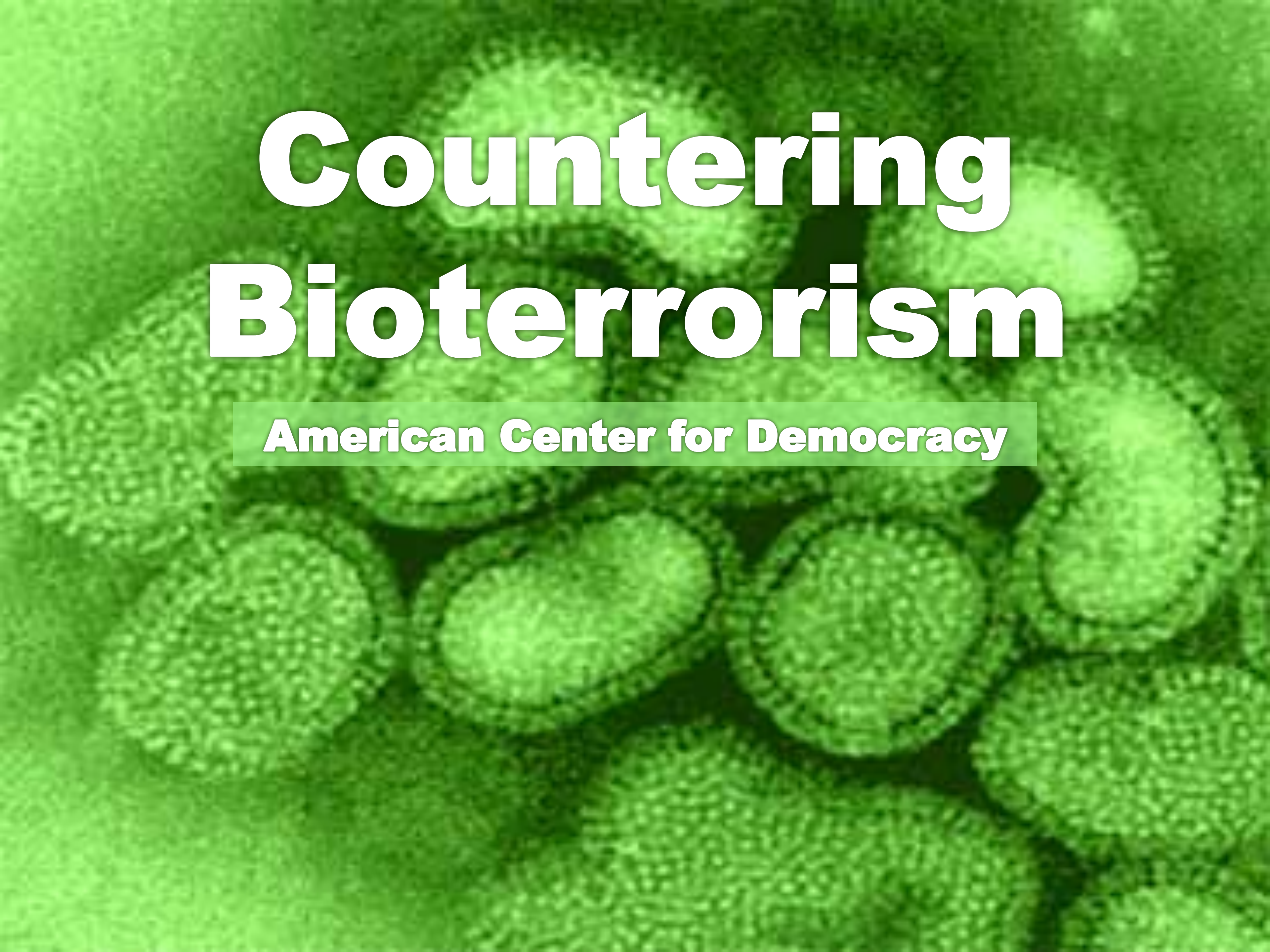
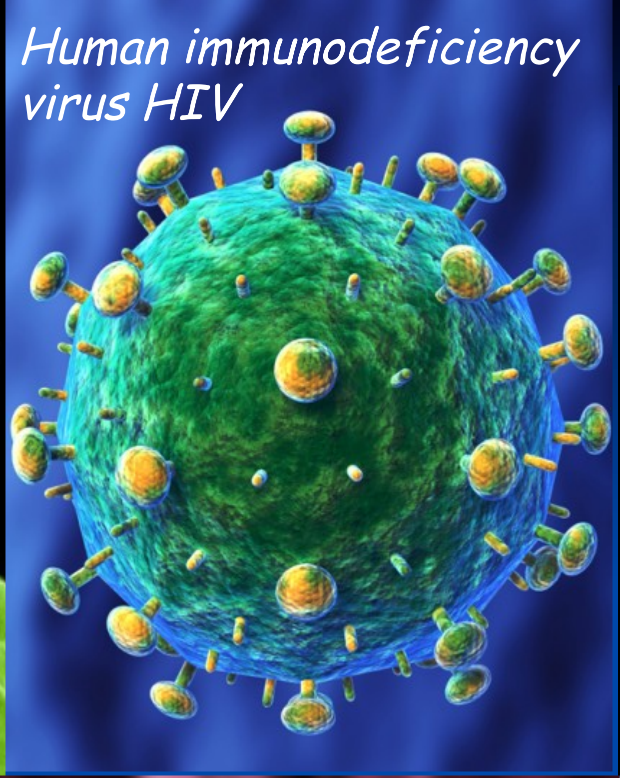
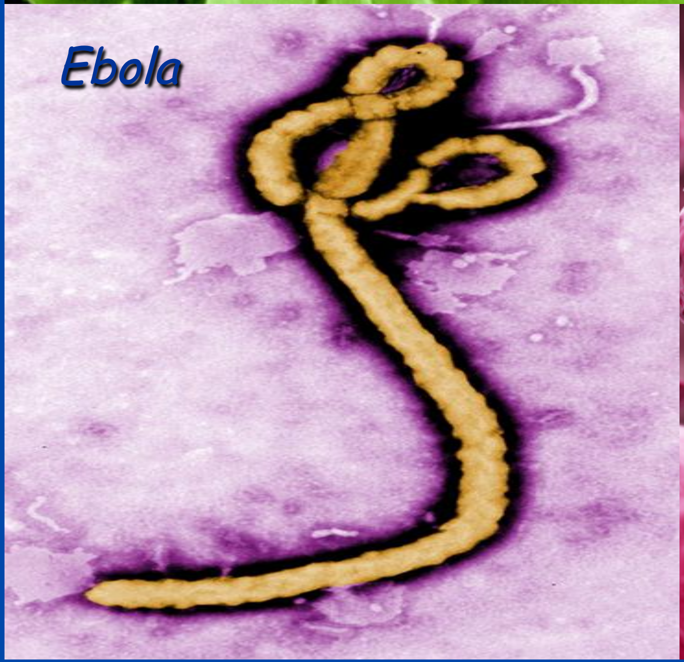
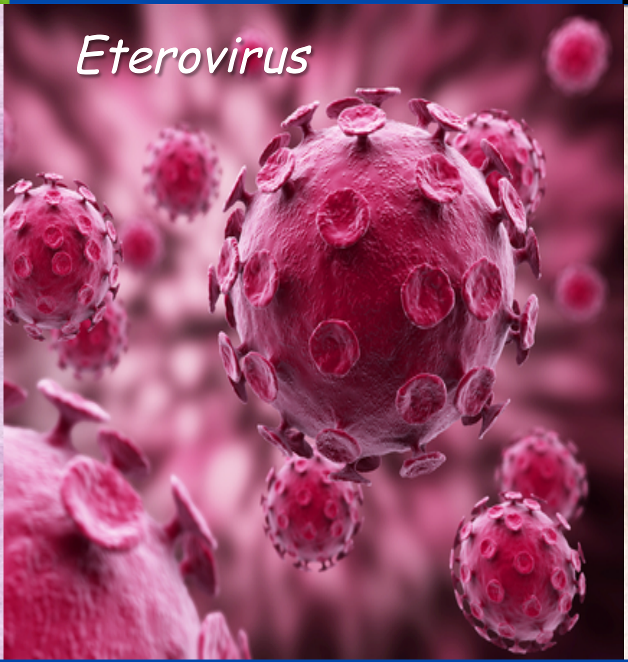

Countering Bioterrorism
American Center for Democracy

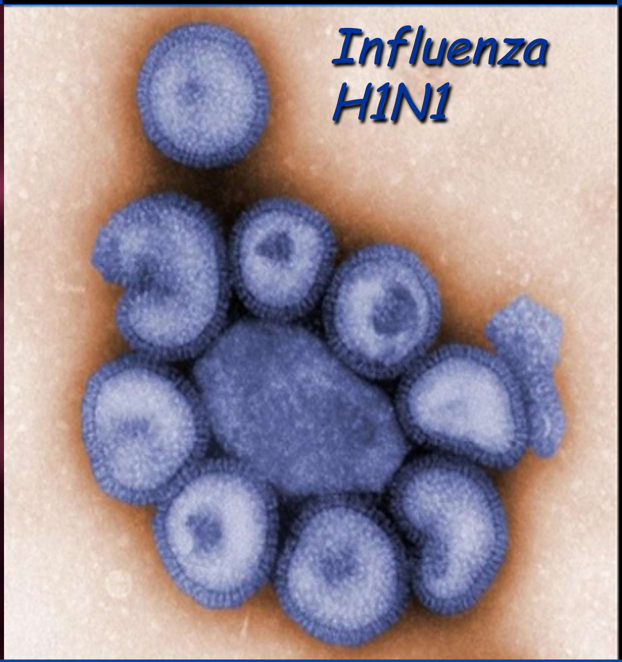

Streptococcus pyogenes


Human immunodeficiency virus HIV


Giardia lamblia


Ebola


Eterovirus


Influenza H1N1

Brief History

- Neolithic Period -- Aztecs tip darts with curare or amphibian toxins
- Roman Period -- Dead animals used to contaminate water supplies
- 1346 -- Tartars catapult plague corpses into the besieged city of Kaffa
- 1347-51 -- Black Death (*y. pestis*) killed 25 million Europeans
- 1763 -- British distribute smallpox contaminated blankets to Indian allies of the French during French-Indian War*
- 1881 -- Pasteur develops and employs bacterial vaccination
- 1925 -- Geneva Protocols prohibit use of biological warfare agents
- 1932 -- Japanese establish Unit 731 at Pin Fan, Manchuria; over 3,000 Chinese prisoners died from exposure experiments
- 1941 -- Japanese spread cholera in Changteh, China; 11,000 killed

*British and Colonial soldiers were immune having been inoculated with cow pox vaccine.

Revolutionary Advances in Biotechnology

In 1975, only a few biotech companies existed. Today, US industry employs around 100,000 biotech scientists in over 2,000 biotech companies. About 700 companies exist in Europe. Many developing countries, including states with ties to terrorist organizations, have their own biotech industries. Genetic engineering has become a mature, widely-exploited discipline.

Specific Developments of Interest to Department of Defense

- Rapid detection and characterization of pathogens
- Vaccines effective against all strains of specific pathogens
- Technologies for bio-agent defeat and remediation
- Identification of possible sources of pathogens
- High confidence indicators of possible bio-agent production and weaponization
- Indicators of covert production of pathogens
- Indicators of possible emerging pathogens
- Modeling of bio-agent release and infection paths/ patterns

Starting Biocultures Are Readily Available

Cultures of many deadly bio-agents can be obtained from hospitals or from one's own environment. For example, strains of *anthracis* can be found in some potting soils. *Lyssavira* (rabies) might be found in bat droppings in caves. A dead rodent might be a source of *pestis*. Iraq allegedly obtained its *anthracis* cultures, by overnight courier, from ATCC, a biotech company in the US (not possible today because…………)

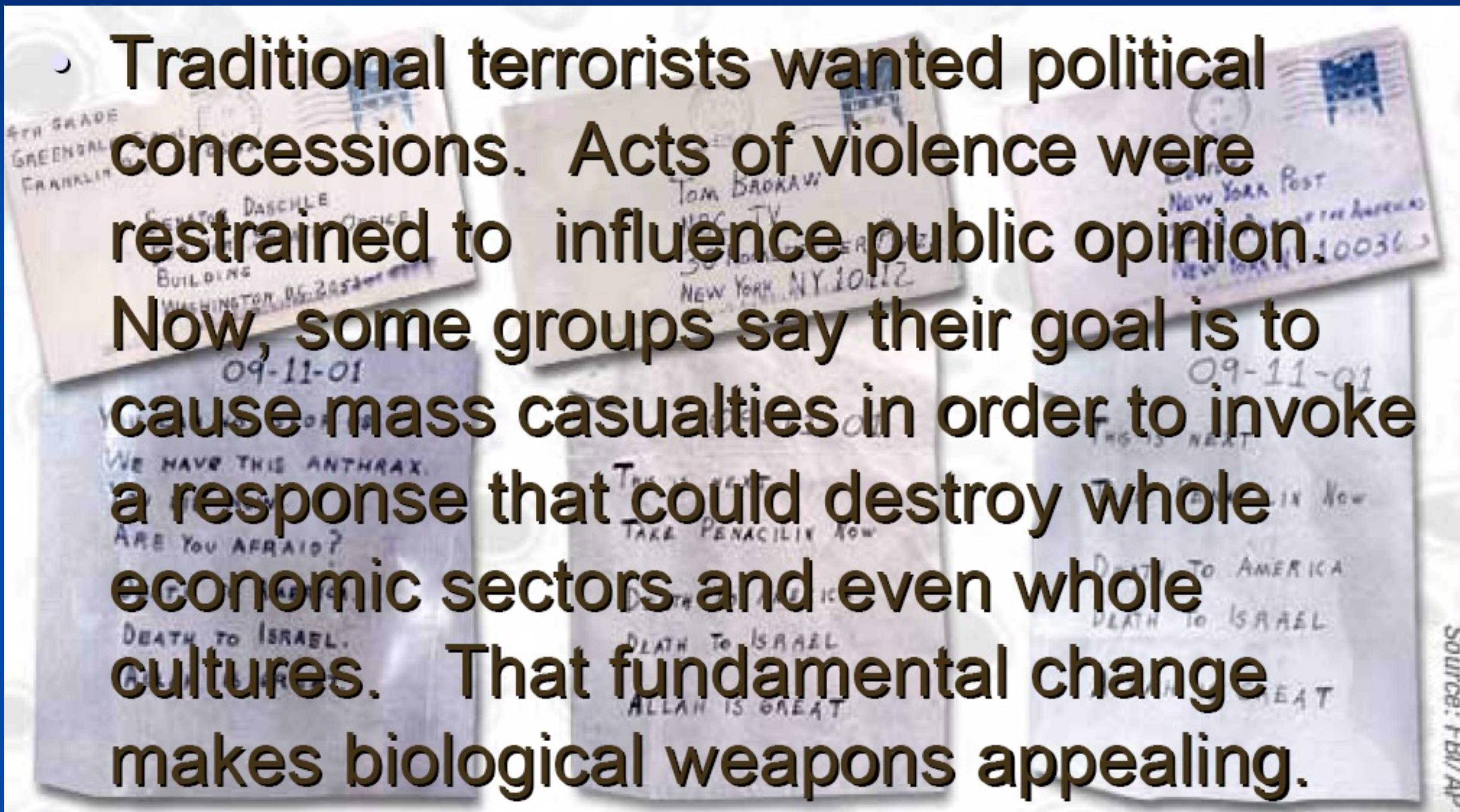
American Type Culture Collection

NEW WARNING NOTICE

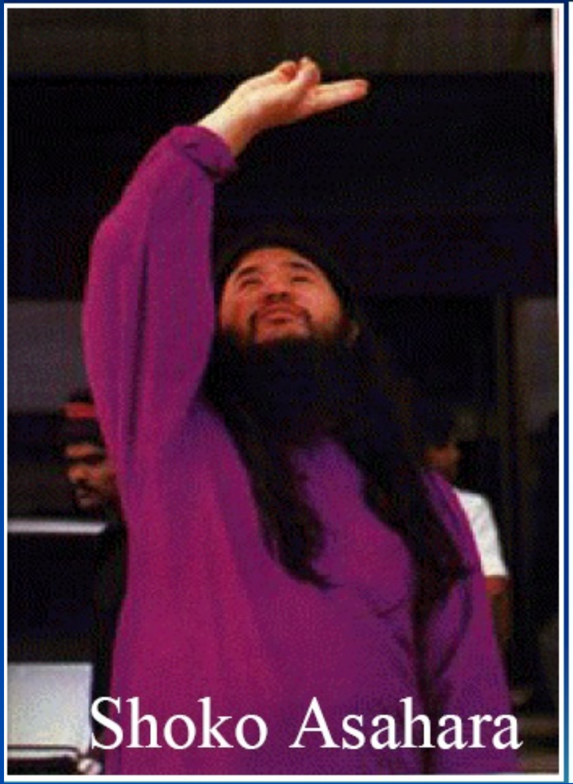
…..ATCC now has a strict policy to ensure that cultures are distributed only to qualified organizations and researchers with legitimate and justifiable scientific uses for these materials. The U.S. Department of Commerce (DOC) regulates exports of controlled commodities to foreign countries or their agents and also prohibits persons in certain countries from receiving controlled commodities from the ATCC.

Greater Awareness of the Potential of Bioagents to Disrupt Society

- Traditional terrorists wanted political concessions. Acts of violence were restrained to influence public opinion. Now, some groups say their goal is to cause mass casualties in order to invoke a response that could destroy whole economic sectors and even whole cultures. That fundamental change makes biological weapons appealing.

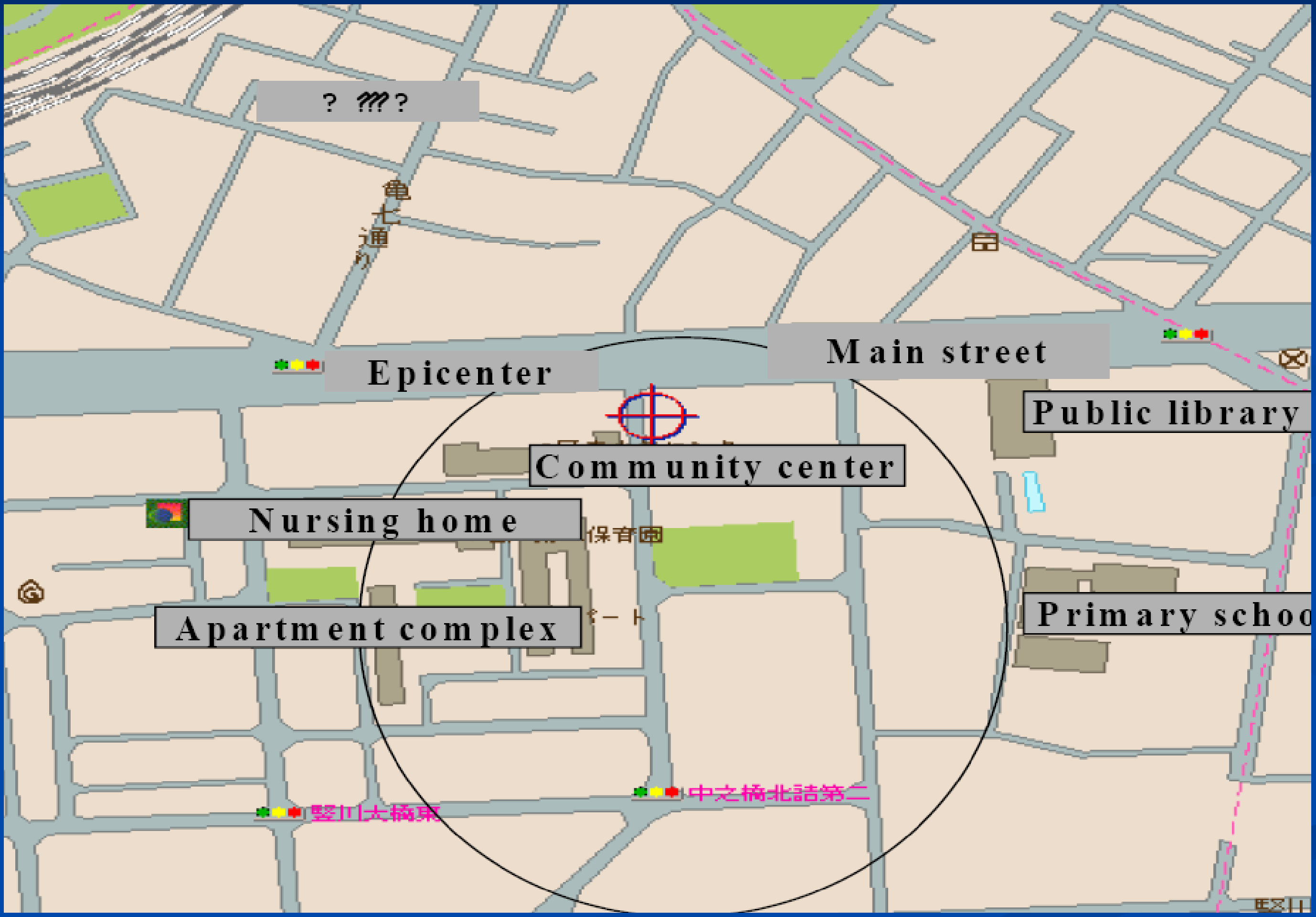


Example: Aum Shinrikyo Laboratory near Mt. Fuji

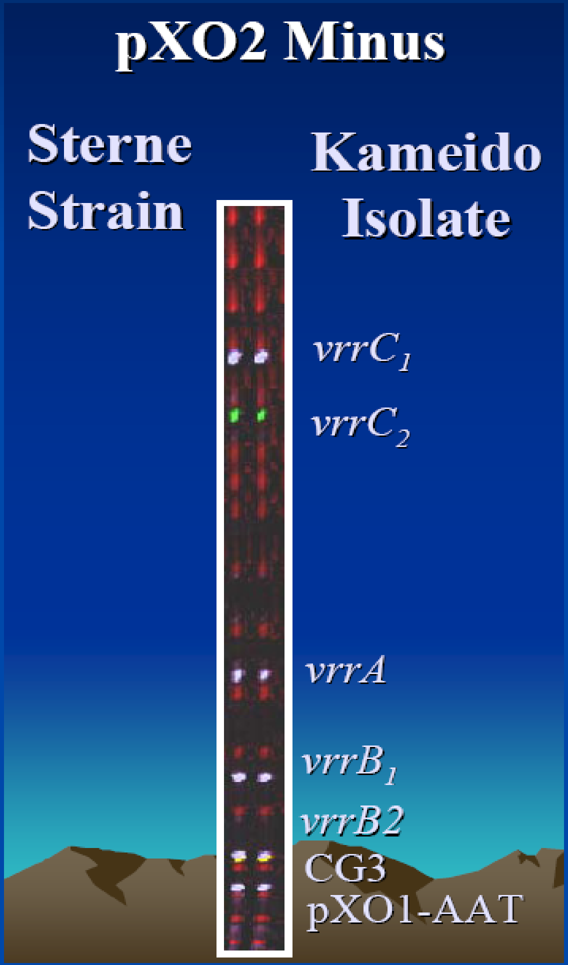


Aum Shinrikyo Kameido Facility

Exposure Area in Kameido



Kameido Isolate Consistant with Sterne Strain



Certain Biological Agents Are the Most Toxic Materials Known

- In theory, a cup of the biotoxin generated by *Chlamydophila psittaci* (Parrot Fever) could kill every human.
- One ounce of dart frog toxin is enough to kill more than 2,500,000 people.
- Saxitoxin derived from dinoflagellates is 1,000 times more toxic than Sarin nerve agent.
- A few hundred kilograms of a properly 'weaponized' bacterial preparation, carefully dried and milled to a precise particle size, has the potential to kill the inhabitants of an entire city in a single strike.
- Fortunately, these potentials are extremely difficult to achieve.

Toxicity: The Case of *Yersinia Pestis*

One particle of *yersinia pestis* potentially could be a lethal dose

Production of Bioagents is Not Difficult: Try Opening a Package of Raw Chicken*

Campylobacter

- **Methods for culturing large quantities of bacteria are commonly available in the public domain. Anyone who is determined, trained in basic microbiological techniques, and willing to take a few short-cuts on safety could conceivably produce significant amounts of lethal biological material.**

***Most poultry (~95%) contain one or more of the following pathogens:** *Campylobacter, Clostridium perfringens, E. coli O157:H7, Staphylococcus aureus, Listeria monocytogenes* **and/or** *Salmonella sp.*

A Useful Analogue: A Brewery

But For Some, Bigger is Better

One of ten 20,000-liter fermenters in Russia's Biopreparat's Scientific Experimental and Production Base (SNOPB) Stepnogorsk, Kazakhstan

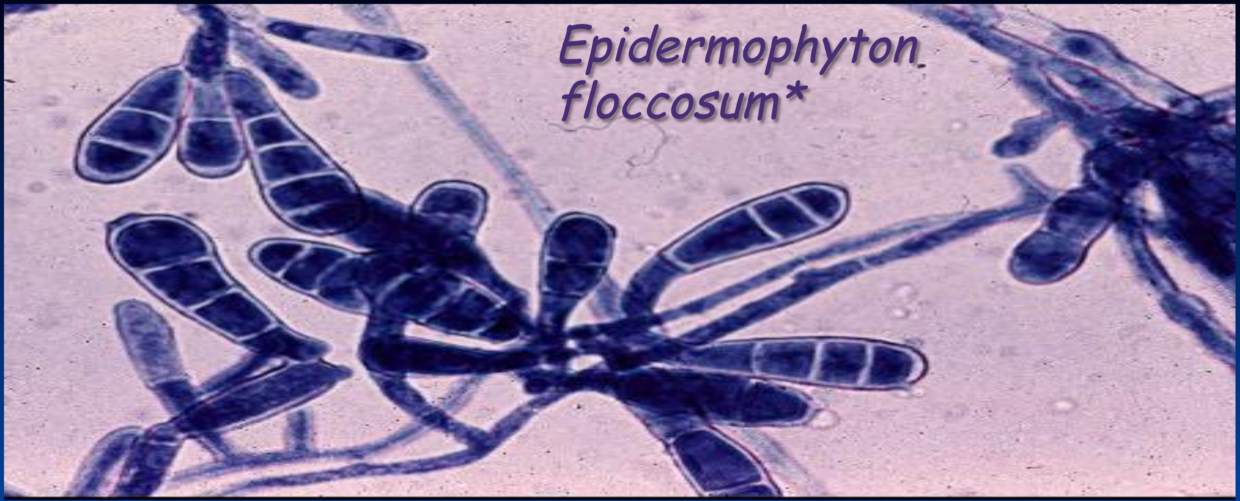
Imported “Know-How” for Terrorists and Rogue States

- Facts: The Former Soviet Union maintained an extensive biological weapons program called “Biopreparat” that produced tons of bioagents and conducted research on engineered pathogens.
- Question: Thousands of scientists became unemployed with the closure of Biopreparat. For whom are they working now?

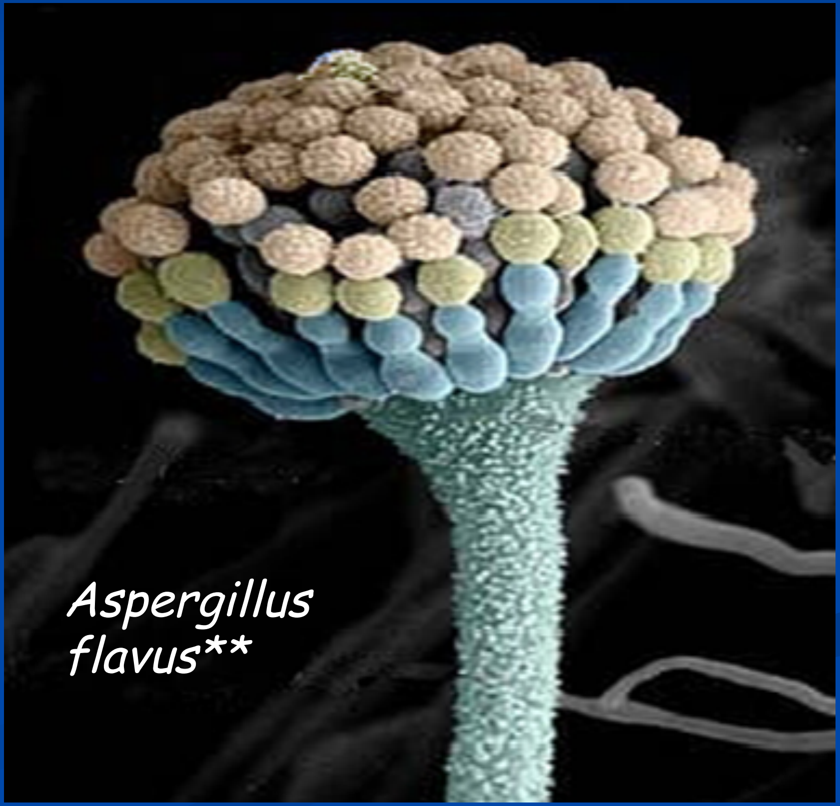
Specific Types

- Fungi: multi-cell parasites capable of causing severe disease in humans, and plants.
- Rickettsia: intracellular parasites that are about 100 times smaller than bacteria and usually carried by anthropoids such as lice or ticks.
- Bacteria: single-cell organisms that are the causative agents for numerous diseases.
- Viruses: intracellular parasites that can only reproduce inside host cells.
- Prions: proteinaceous infectious particles that lack nucleic acid.
- Bio-toxins: poisonous substances made by a living system or a synthetic analogue of a naturally occurring poison.

Fungi



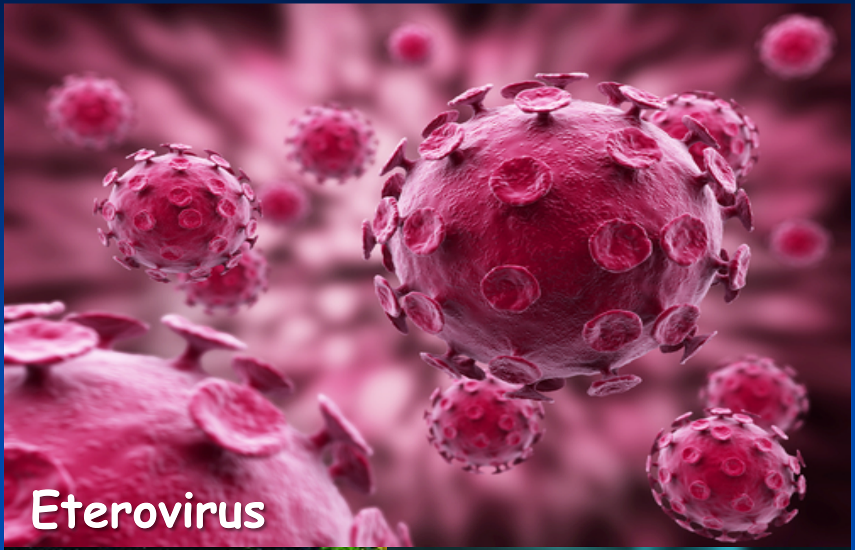
Fungi are parasitic, multicellular organisms composed of filaments called *hyphae*. The body of the organism is called *mycelium*. When reproductive *hyphae* are produced, they form a organized structure called a *sporocarp*, or mushroom.

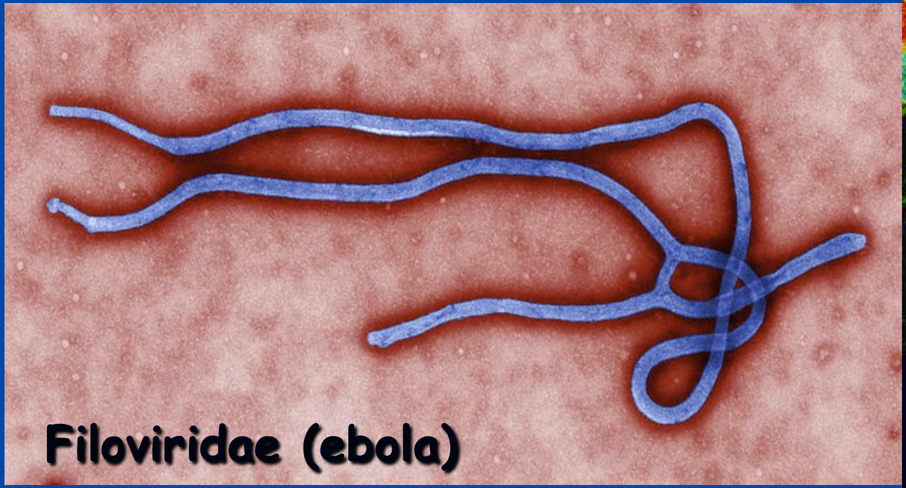


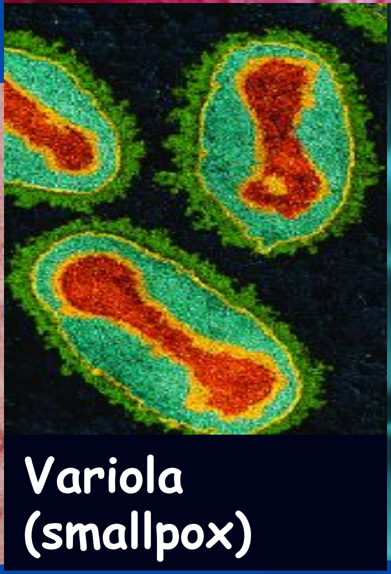
*Athlete's foot disease **Source of alfatoxin

Viruses

Submicroscopic pathogens consisting essentially of a core of single nucleic acid surrounded by a protein coat, having the ability to replicate only inside a living cell.

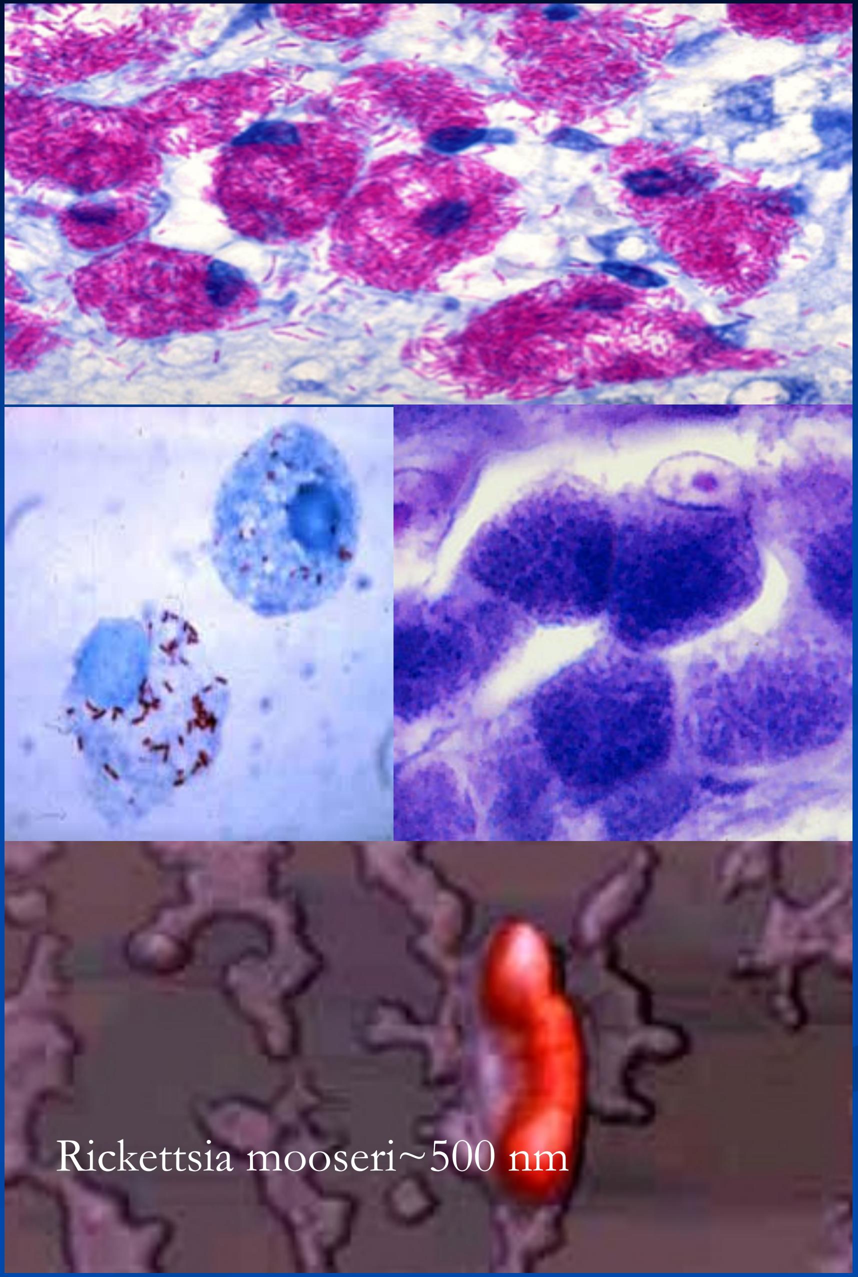






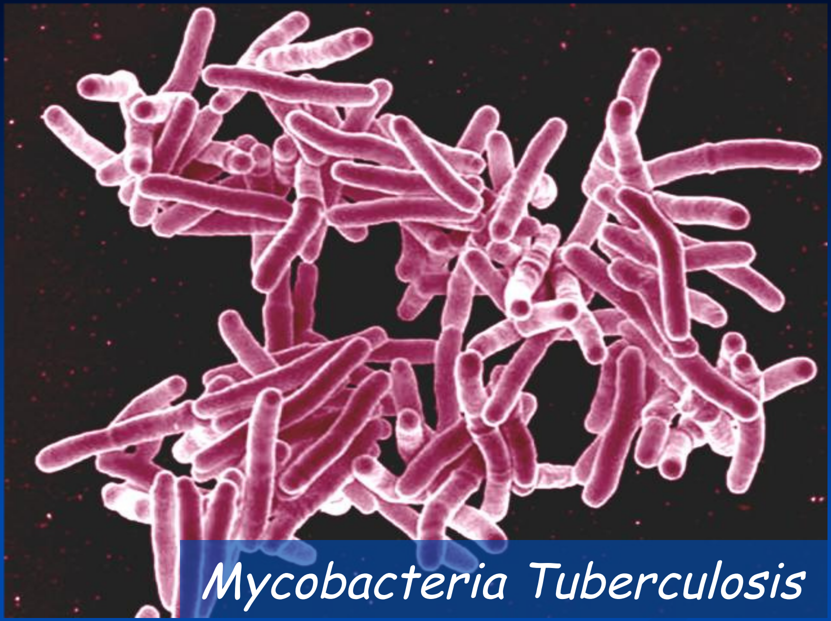
Rickettsia

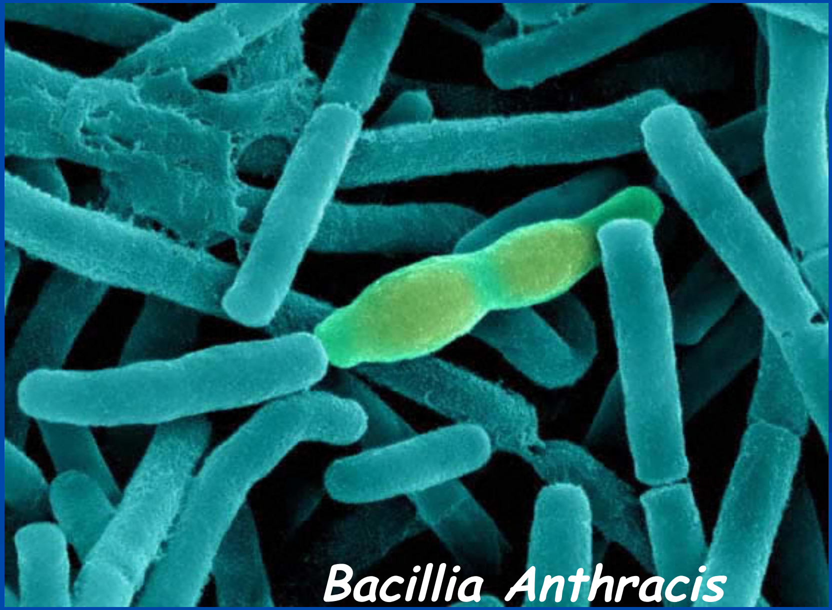
Intracellular parasites that are intermediate in size between viruses and most bacteria possessing characteristics of both, e.g., they only reproduce within host cells but they contain metabolic enzymes and have developed cell membranes.



Bacteria

Unicellular micro-organisms of the class *schizomycetes* with varying pathological expressions in plants and animal and existing in wide variety of forms as free-living organisms or parasites.



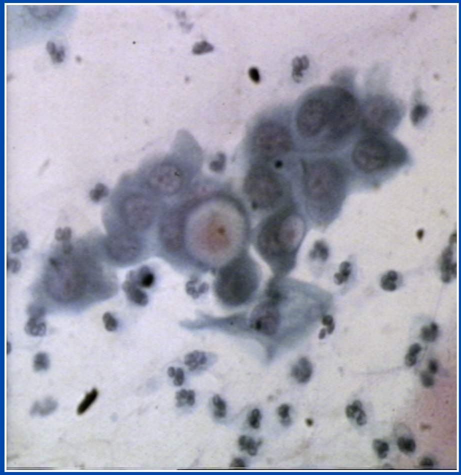


Bio-toxins

Bio-toxins are complex poisonous substances derived from a living organism or a synthetic analogue of a naturally occurring poison. These materials can be among the most toxic substances known. For example, one ounce of psittaci toxin in theory could be fatal to more than two-and-a-half million people.

Assassin Beetle

Dart Frogs



Chlamydophila Psittaci

Castor Beans (Ricin)

Coral Snake

Black Widow

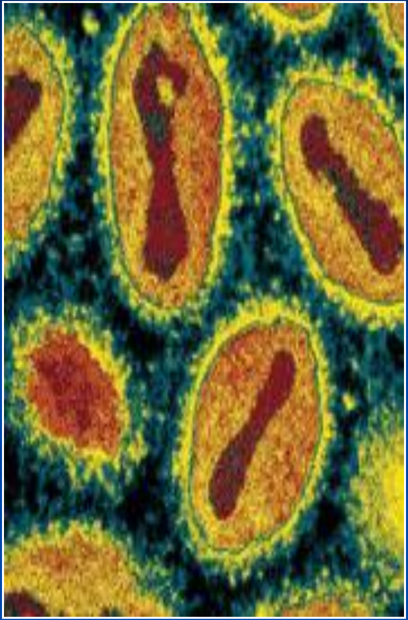
Bio-toxins

Brazilian wandering spider (*phoneutria nigriventer*) 0.006 mg bite lethal dose to mice

Specific Biological Agents

(Percent of morbidity)

- **AFLATOXIN (from *Aspergillus Favus* in grains or legumes)**
- **ANTHRACIS BACILLUS (*Anthrax*, 80%)**
- **CLOSTRIDIUM BOTULINUM (*Botulinum*, 10%)**
- **CLOSTRIDIUM TETANI (*Tetanus*, 40%)**
- **COXIELLA BURNETII (*Q-fever*, <1%)**
- **DENGUE FEVER VIRUS (four serotypes of *Flavivirus*)**
- **FILOVIRIDAE VIRUS (aka *Ebola, Hemorrhagic Fever*, >90%)**
- **FRANCISELLA TULARENSIS (*Rabbit Fever*, 10%)**
- **MARBURG VIRUS (*Marburg Hemorrhagic Fever*, >90%)**
- **ORTHOPOX-VIRUS VARIOLA (*Smallpox*, high mortality)**
- **CHLAMYDOPHILA PSITTACI (aka *Parrot Fever*, ~100%)**
- **PUCCINIA CORONATA (a fungus that attacks oats)**
- **PUCCINIA SORGHI (a fungus that causes cornrust)**
- **RICINUS COMMUNISMS (a toxic protein from castor beans, aka *Ricine*)**
- **T-2 TRICHOTHECENE MYCOTOXIN (aka "Yellow Rain" from fungi in the feces of wild bees and as *Fusarium* in whole grains)**
- **VARIOLA MAJOR (*Smallpox*, 30%)**
- **YERSINIA PESTIS (*Plague*, 90%)**



Smallpox

Others

- **Bacteria**: *burkholderia mallei, chlamydia psittaci, salmonella typhi, shigella dysteriae, vibrio cholerae, brucella militensis*
- **Viruses**: *lyssavira, chikungunya, equine encephalitis, hunta sin nombre, lassa fever, encephalitis, rift valley fever, eterovirus*
- **Rickettesia:** *rickettsia felis, rickettsia prowazekii, rickettsia rickettsii, rickettsia typhi, rickettsia conorii, rickettsia africae*
- **Fungi (plant pathogens):** *colletorichum coffeanum virulans, cochliobolus miyabeanus, microcyclus ulei, puccinia graminis, puccinia striiformis, pryicularia grisea*

Possible Bioterrorism Symptoms

- Single case caused by an uncommon agent
- Large number of ill persons with similar disease
- Unusual illness in one population without precursor infections
- Higher morbidity than expectations with a common disease
- Multiple disease entities coexisting in same patients
- Disease with an unusual geographic or seasonal distribution.
- Multiple atypical presentations of disease

- Similar genotype agents from distinct sources
- Atypical, genetically engineered, or antiquated strains
- Endemic disease with unexpected increased incidence.
- Simultaneous clustering of similar symptoms in non-contiguous areas
- Atypical aerosol, food, or water transmission.
- Many ill persons presenting at same time
- Concurrent increase in animal presentation of similar symptoms

Required Competencies

- **Biomedical technology**: detectors, sensors, stable isotopes, lasers, biomechanics, modeling, and informatics
- **Cellular analysis**: flow cytometry, digital fluorescence, cell growth, cycle control, and transformation
- **Biomolecular structure, dynamics, and functional analysis**: scanning tunneling and transmission electron microscopy, x-ray and neutron scattering
- **Genome analysis**: chromosome sorting, clone libraries, genome mapping and sequencing, pathogen data bases positional cloning
- **Fate and forensics**: early-stage epidemic identification, symptomology, modeling dispersal, consequence analysis, strain identification, decontamination methods, epidemiological modeling, risk assessments

Competencies Applied to Address Particular Difficult Challenges

Detection & Characterization

Goal: To provide early warning, identify people to treat, and identify contaminated areas with high sensitivity and low false alarms.

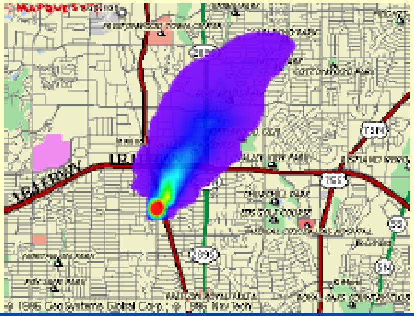
Biological Foundations

Goal: To provide essential biological information for detection, and medical countermeasures.

Sterilization & Decontamination

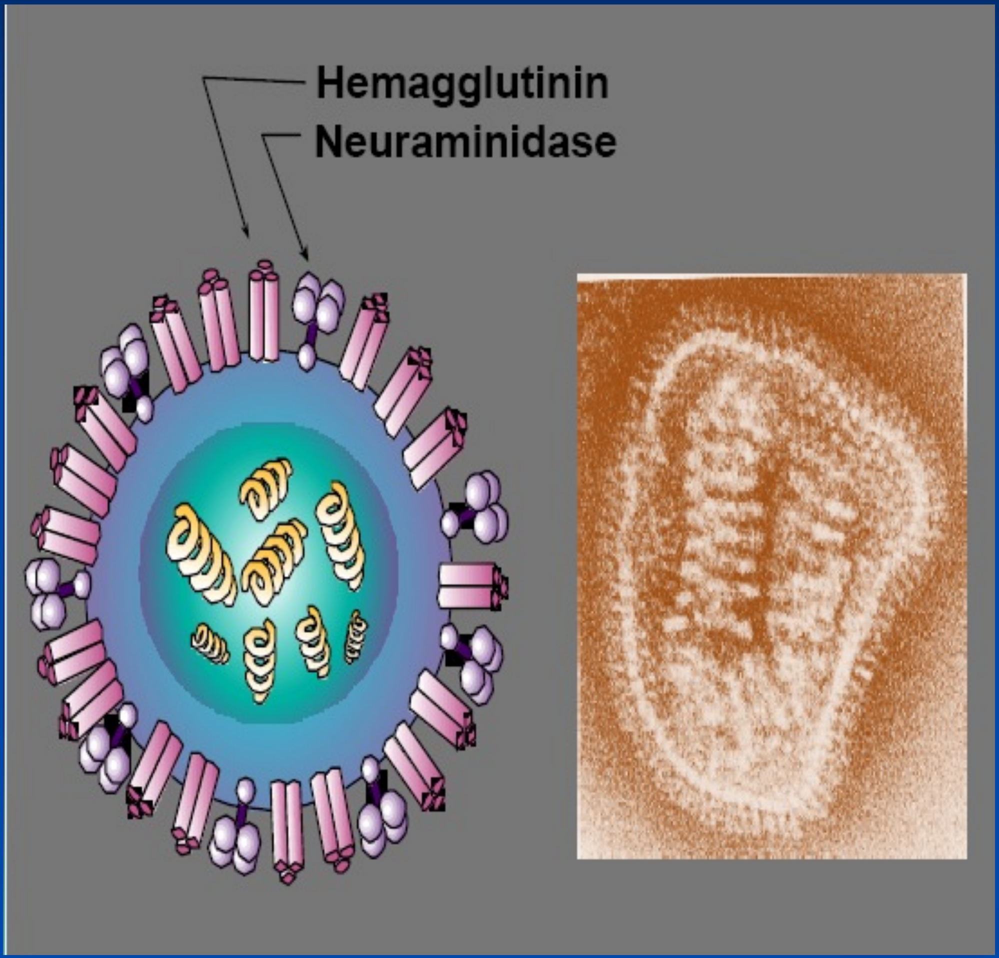
Goal: To destroy pathogens in situ or quickly restore defense and civilian facilities.

Modeling & Prediction



Goal: To develop predictive modeling tools for urban environments (inside & outside of facilities).

The Influenza Detector

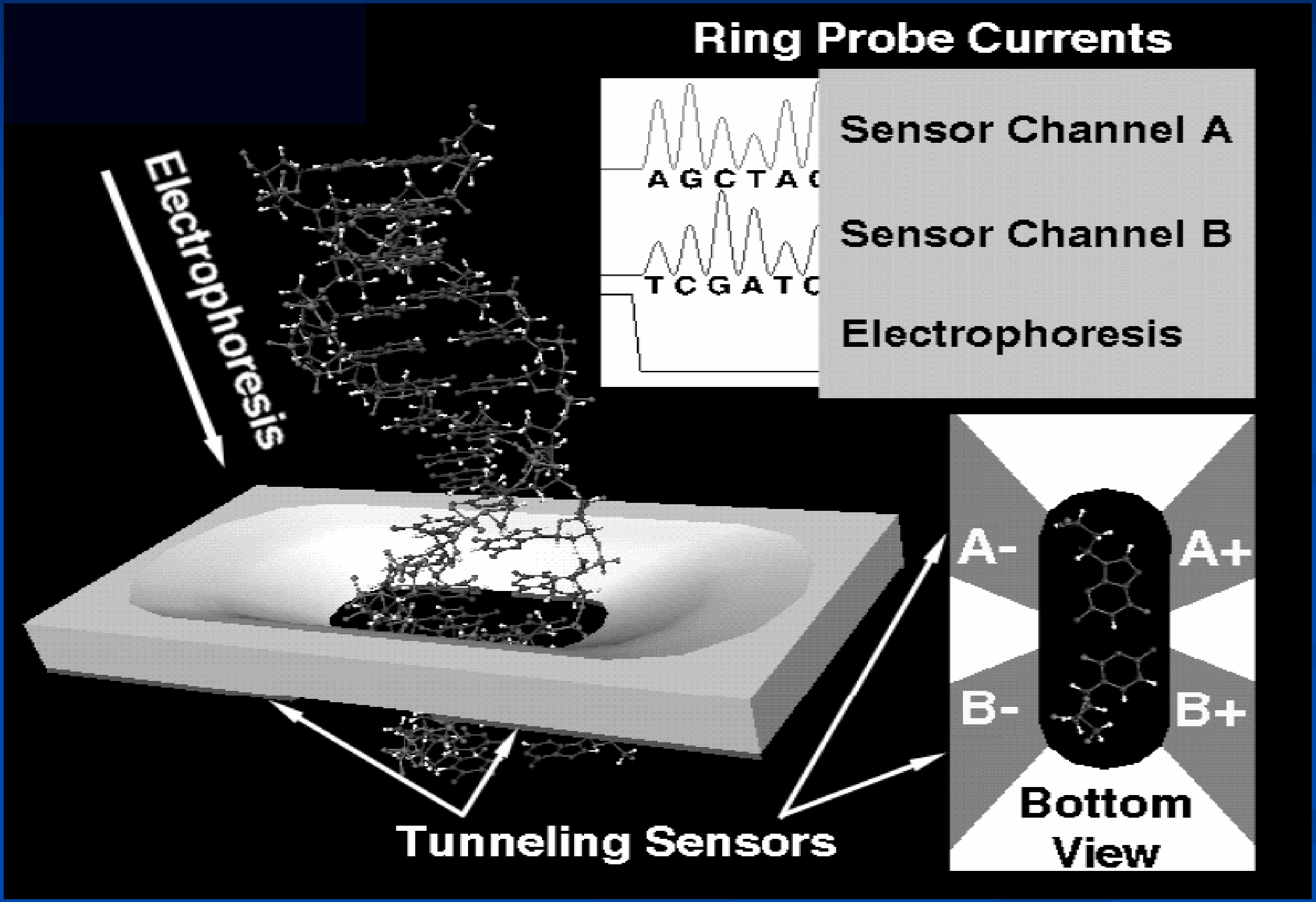


- Optical waveguide biosensor membrane loaded with labeled hemagglutinin and neuraminidase receptors.
- Biosensor detects flu viruses in body fluids with high sensitivity.
- Use sensor to rule out bioagent infection when patients present with flu-like symptoms.

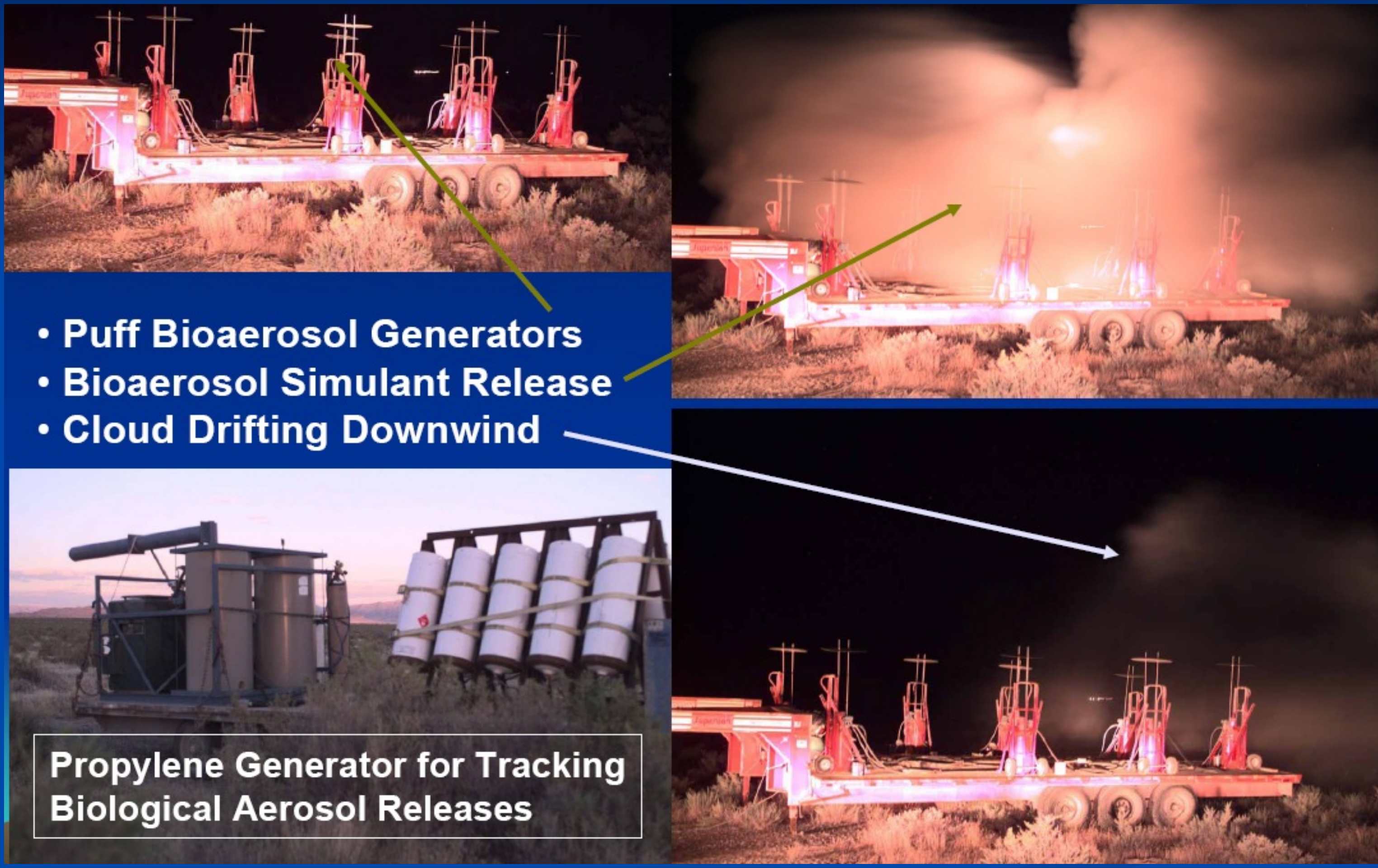
Long Range Biological Stand-off Detection System

Biological Integrated Detection System (BIOS) Miniature Flow Cytometer

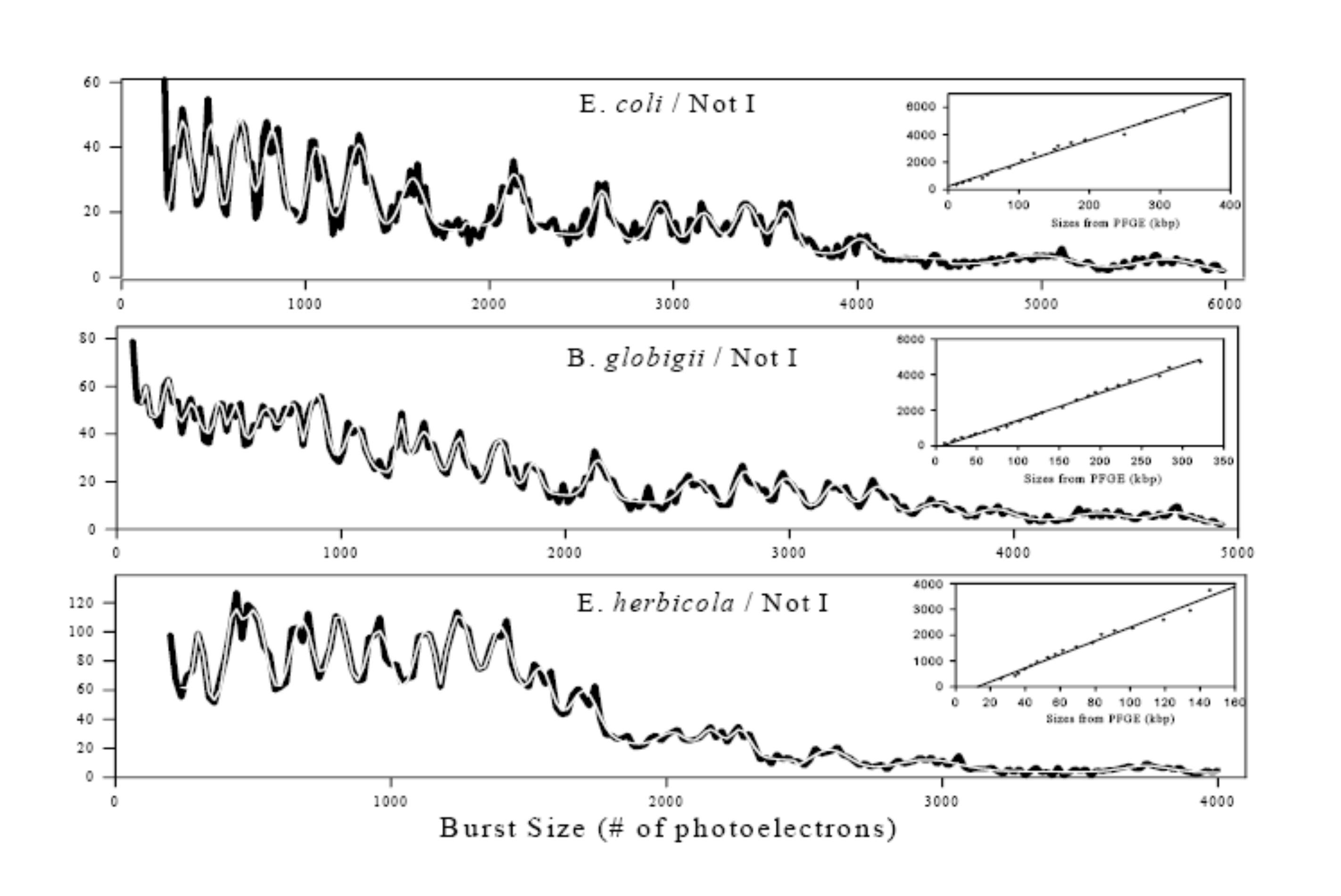
Radial Probe Sequencer



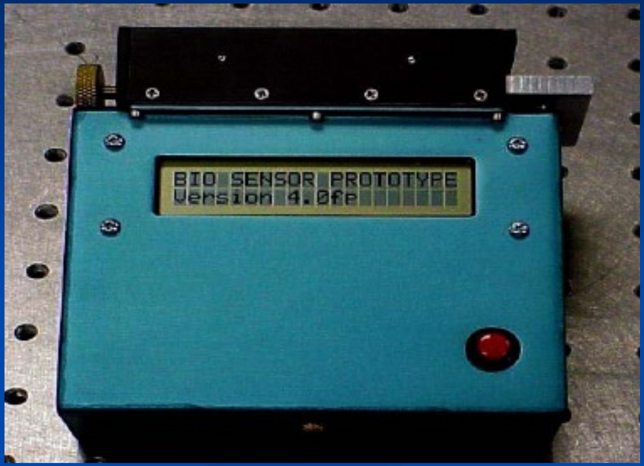
Puff Bioaerosol Stimulant Generator



DNA Fragment Sizing Flow Cytometry

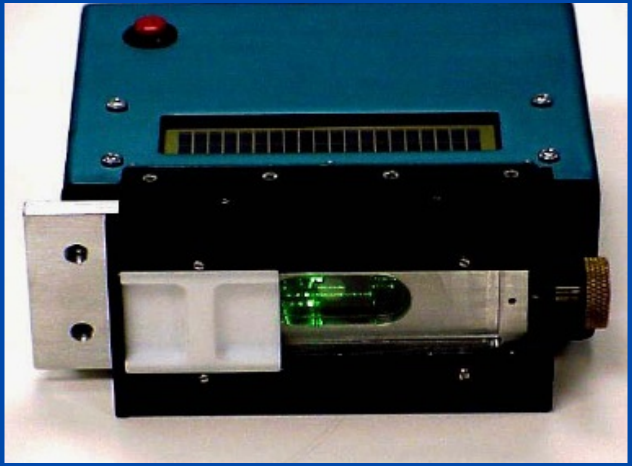


Field Deployable Biosensors



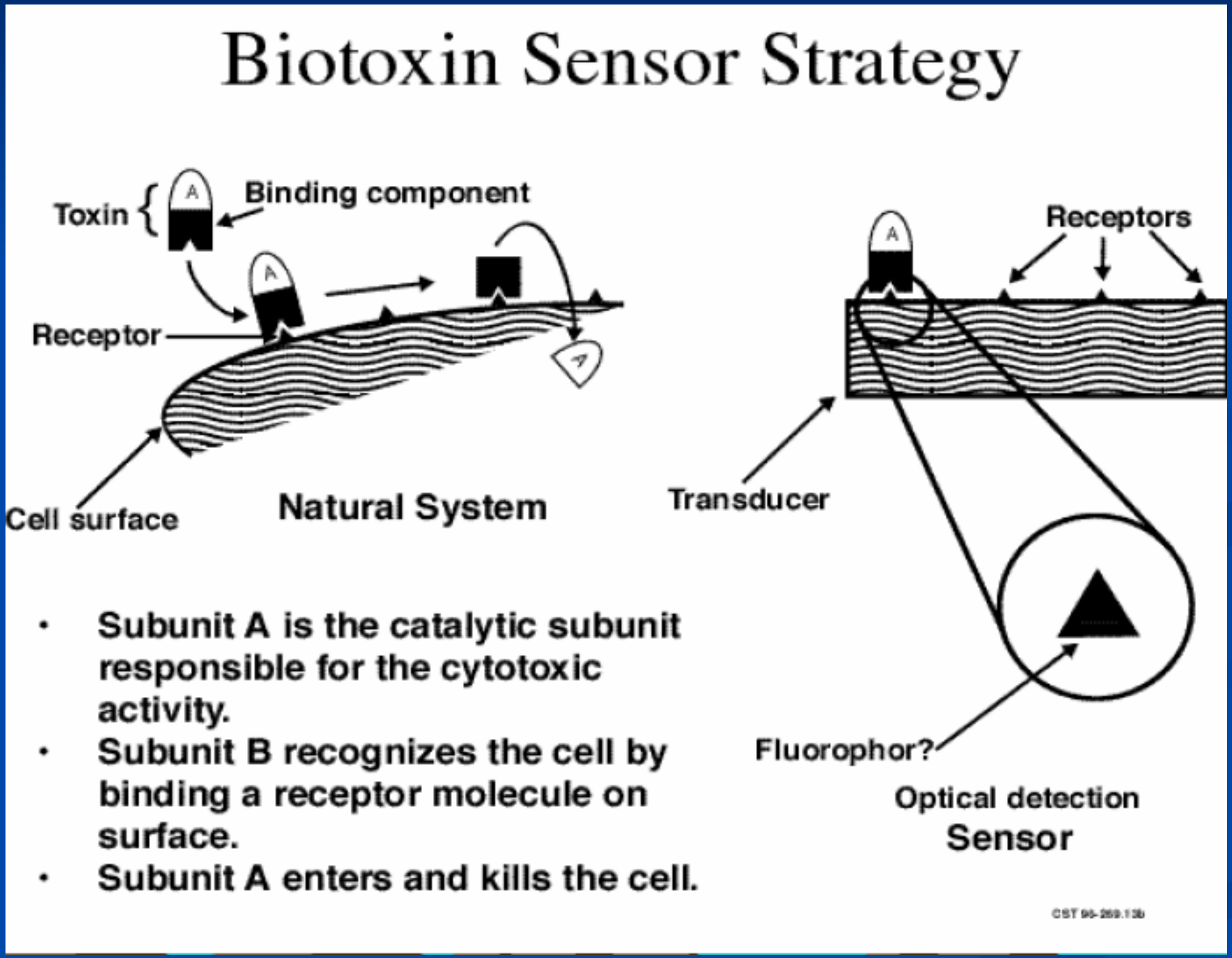
Sensor Attributes

- Hand-Held and Low Power Requirements
- Single-Step Reagentless Assay
- Exchangeable Sensor Elements for Reuse
- Ultrahigh Sensitivity and Specificity

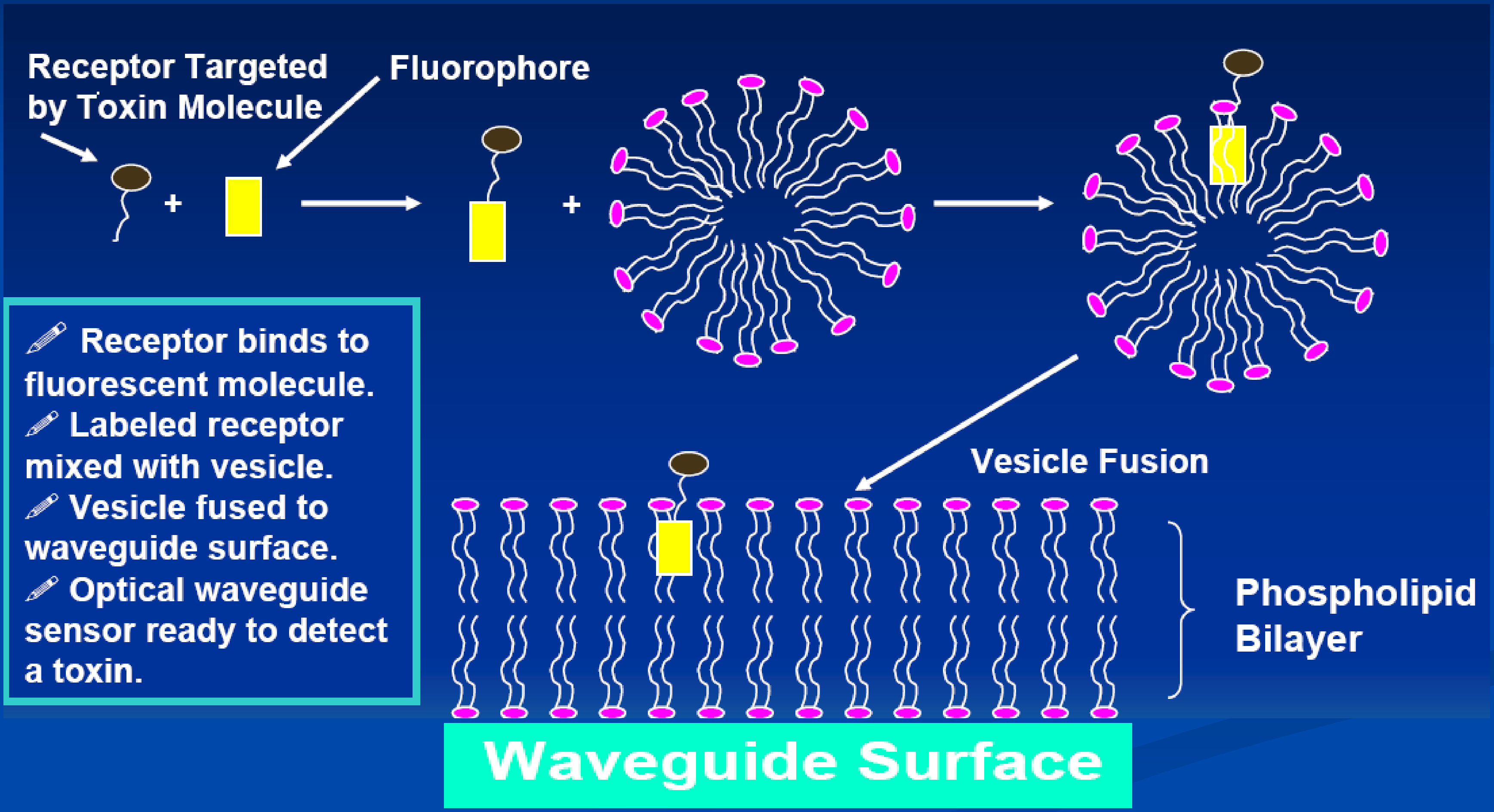


- Rapid Detection of Toxins and Pathogens
- Pre-symptomatic Diagnosis of Infection
- Environmental Detection for Alarm
- Adaptable to Multiple Agents
- Suitable for Use by First Responders

Biometrics: Mimicking Cell Surface Chemistry

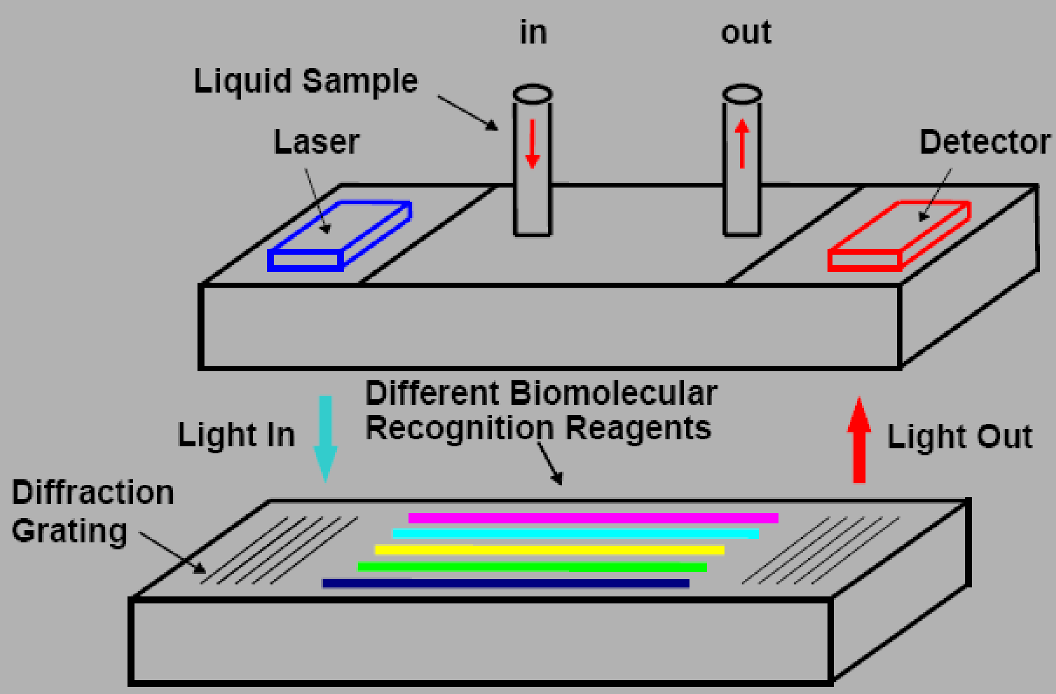


Creating the Waveguide Sensor



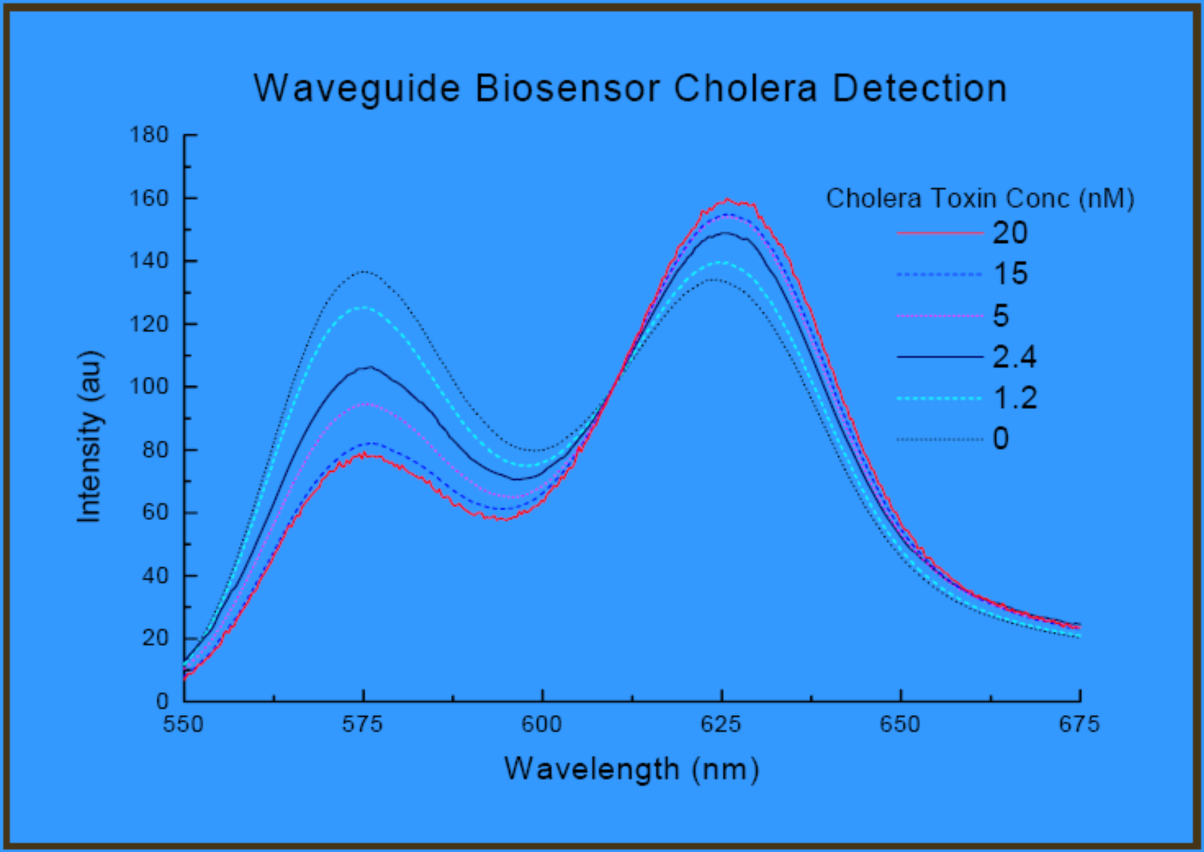
- Receptor binds to fluorescent molecule.
- Labeled receptor mixed with vesicle.
- Vesicle fused to waveguide surface.
- Optical waveguide sensor ready to detect a toxin.

Integrated Optical Biometric Sensor (IOBS)



- 532-nm solid state laser illumates waveguide via diffraction grating.
- Green light excites yellow fluorescence in membrane-bound receptors.
- Toxin pulls receptors together.
- Fluorescence energy transfer enables yellow fluorophores to excite nearby red fluorophores.
- Red fluorescence emission is detected with high S/N ratio.

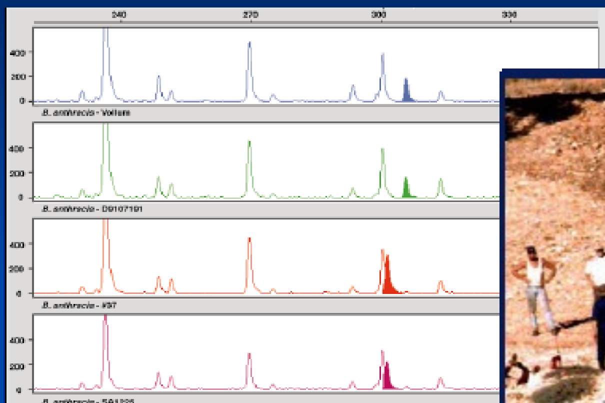
IOBS Results: Cholera Toxin Detection



- Fluorescence intensity vs wavelength for waveguide biosensor.
- Donor fluorescence is left hand peak.
- Acceptor fluorescence is right hand peak.
- Low background fluorescence.

Portable DNA Extractor for Environmental Samples

Forensics: Polymorphism in B. Anthracis

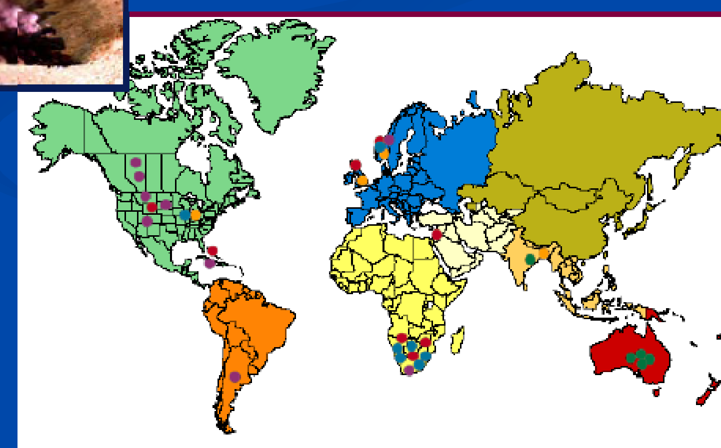


Amplified Fragment Length Polymorphism

Note variances at 301 and 306

Australia, 1997:

Destroying livestock killed by anthrax

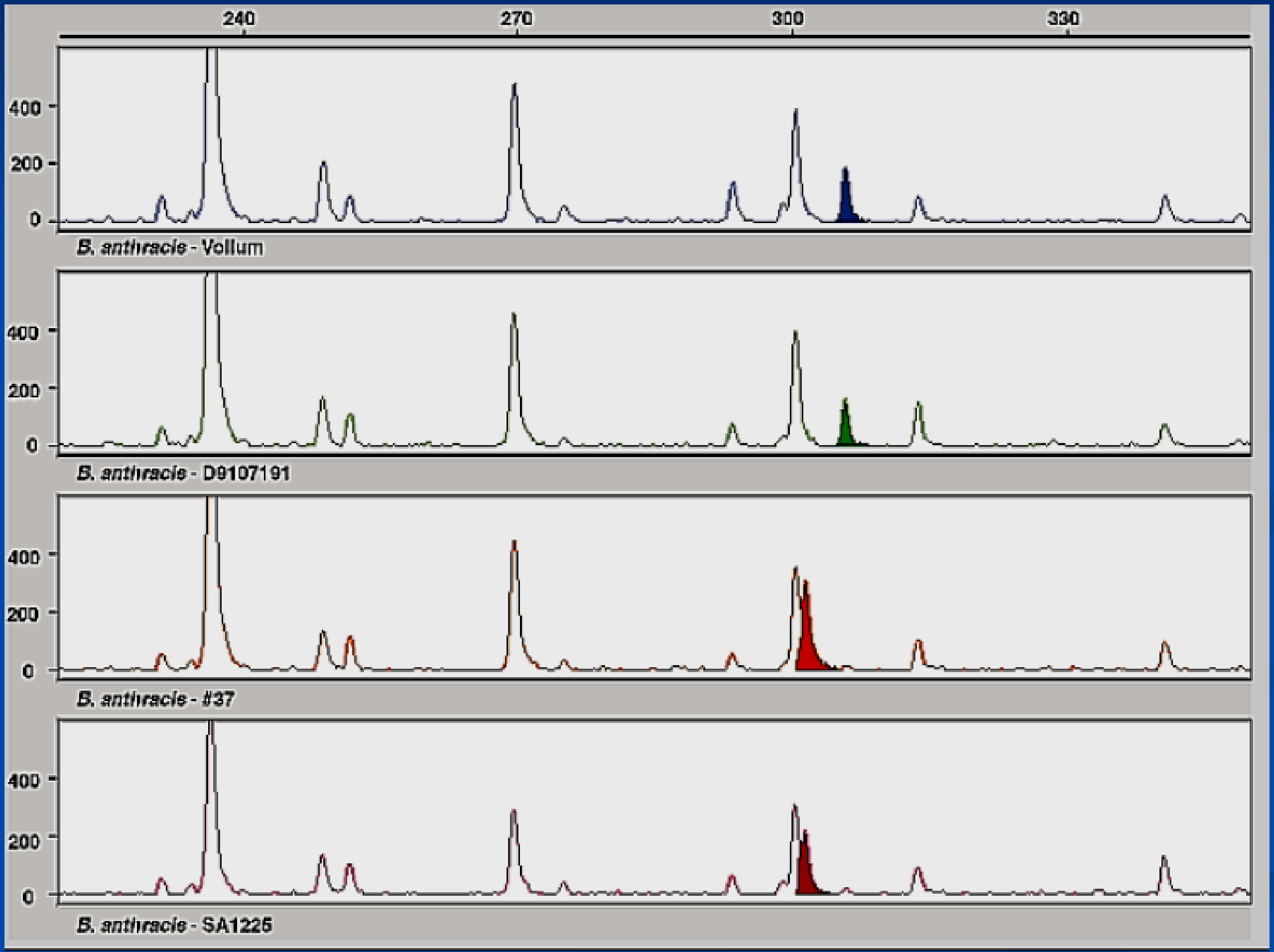


Anthrax Outbreaks

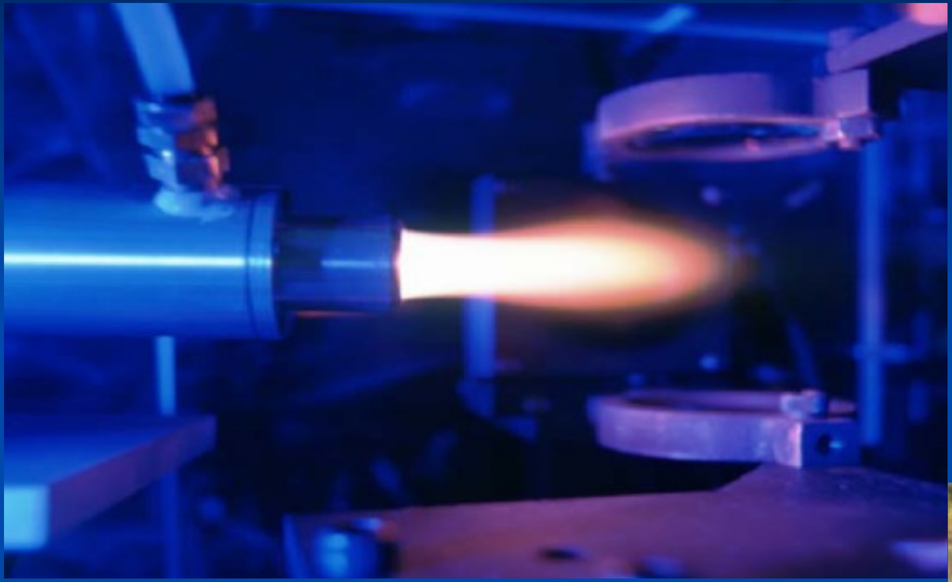
Colors on world map indicate strain relation

Australian outbreak in 1997 related to 1850's India epidemic

B. Anthracis Polymorphic Variances at 301 and 306

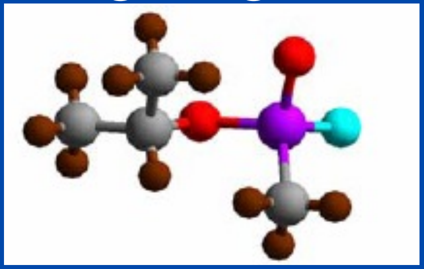


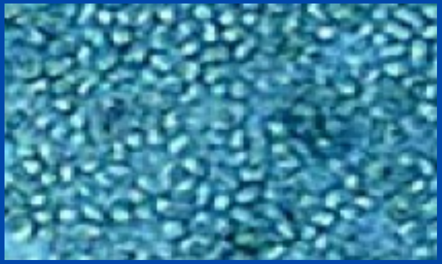
Low Temperature Plasma for Destroying CBW Agents



- Reactive metastable & atomic oxygen (~100ppm) generated
- Dry, fast, non-destructive, safe, portable, and "green" decontamination
- Safe for enclosed areas such as subways

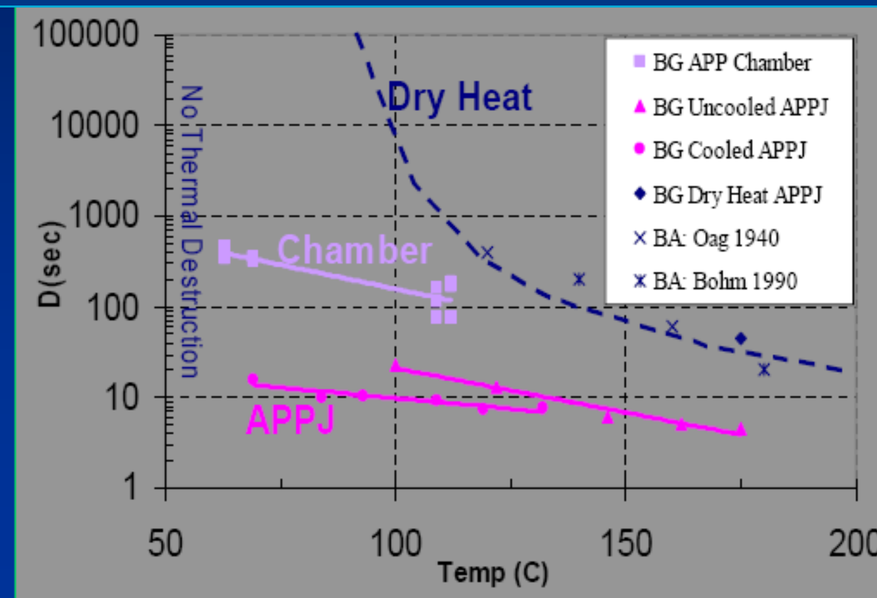
Effective against both chemical and biological agents





Sarin Nerve Agent   Spores

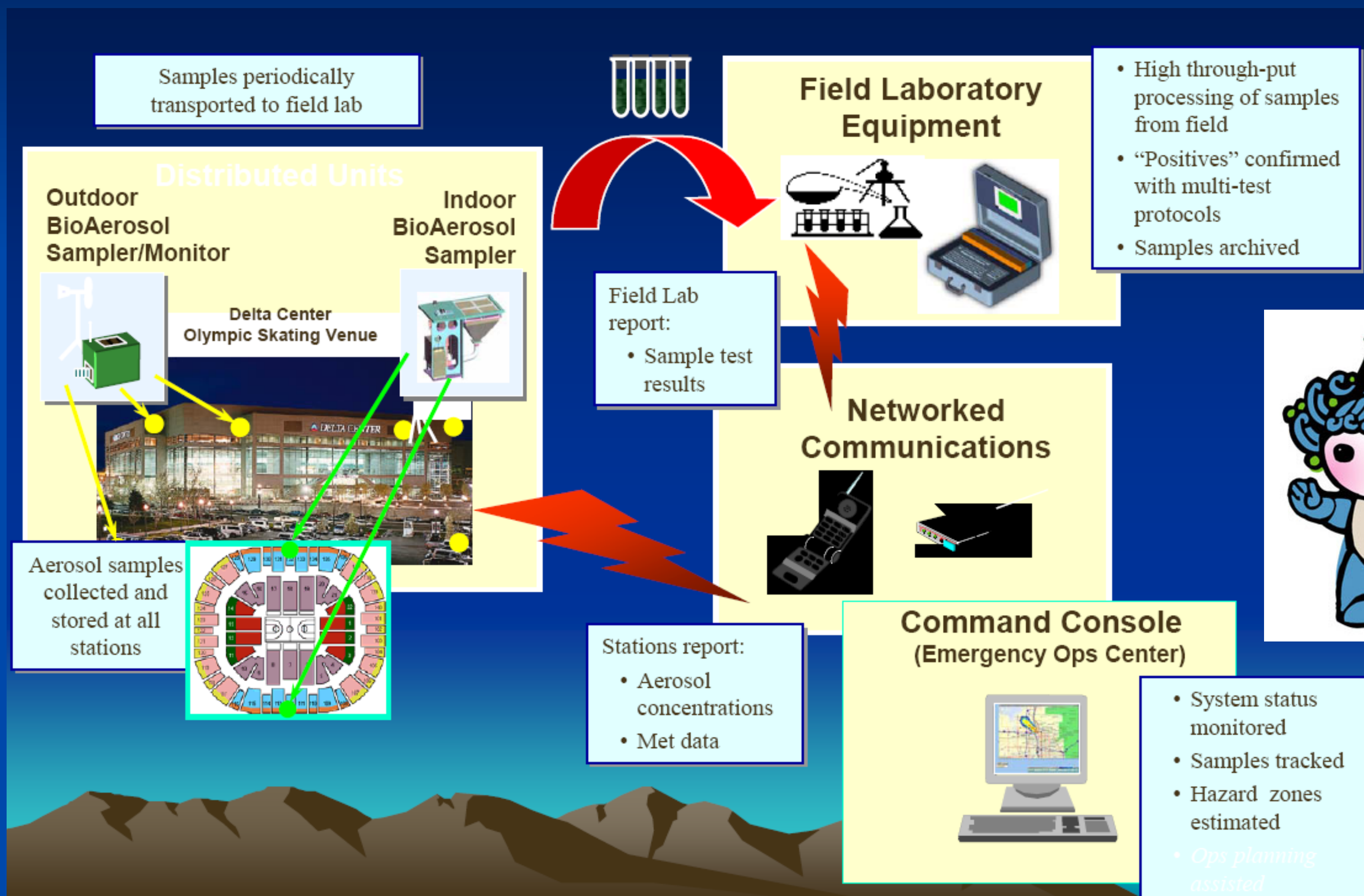
Plasma Chamber Decontamination Results



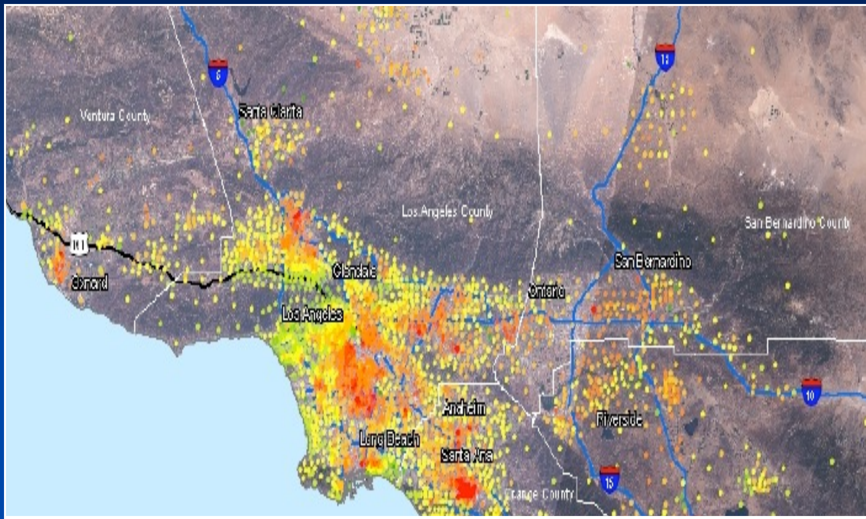
Decontamination chamber without recirculation capability tested (not optimized)

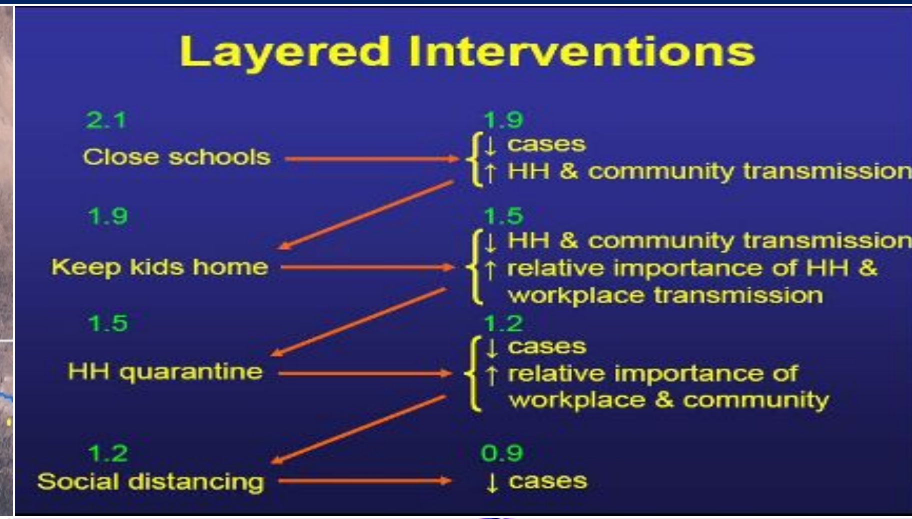
Dimensions → 6” ID x 12” (BG spores placed in center)

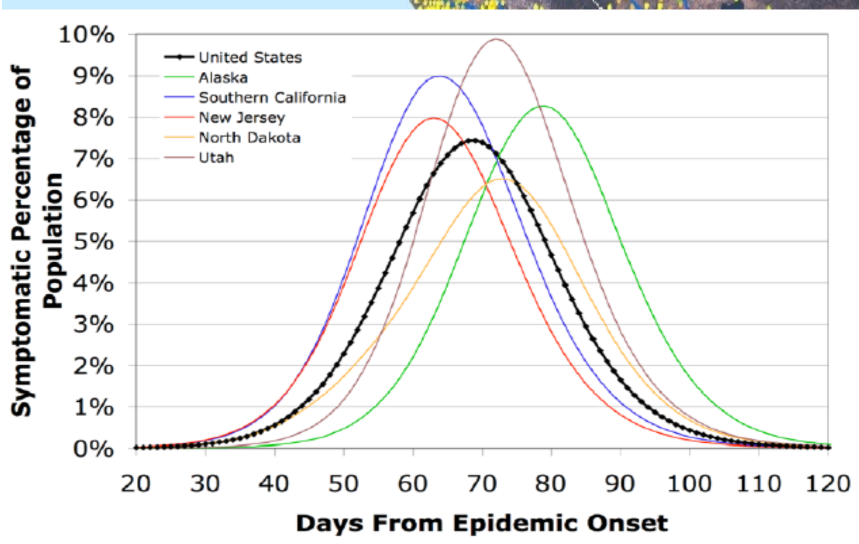
SCMIS Architecture for the 2008 Beijing Olympics

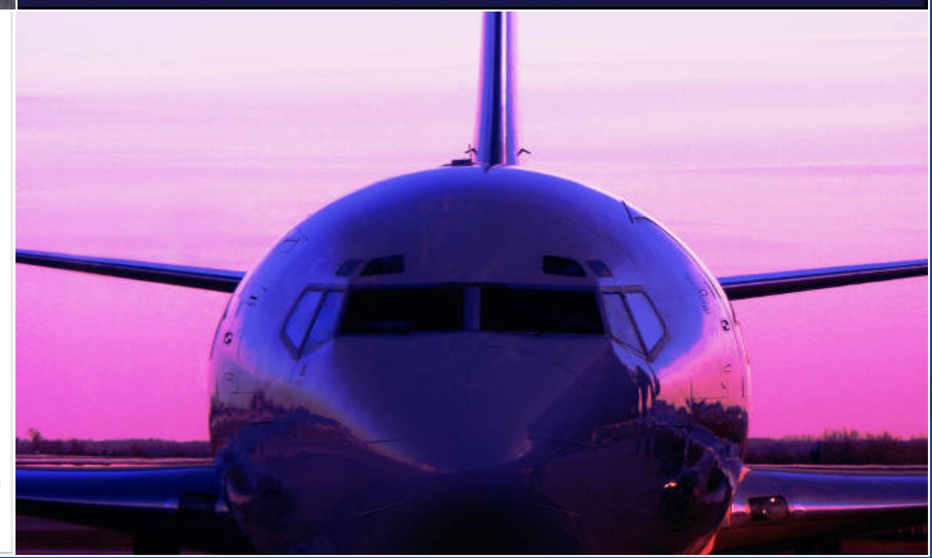


EpiCast: Agent Based Epidemiology Model





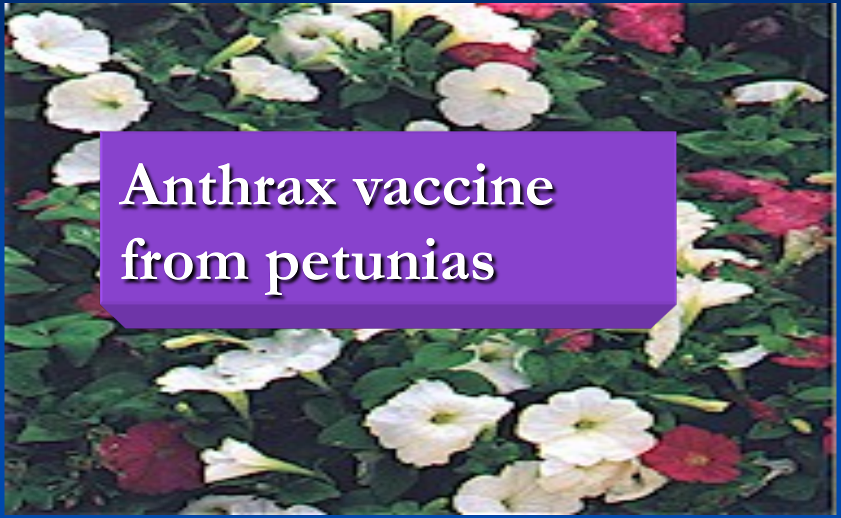


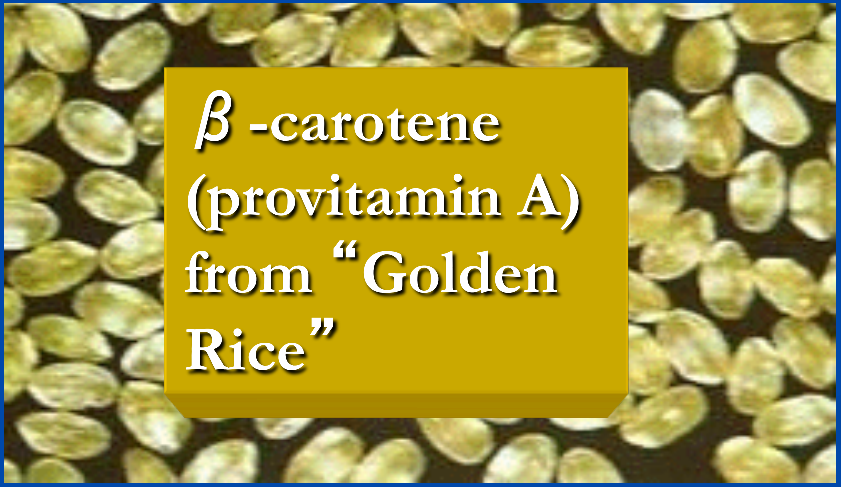


http://public.lanl.gov/stroud/PI_report_LAUR067966.pdf

Moving into the Future: Using Bio-science as a Benevolent Ally

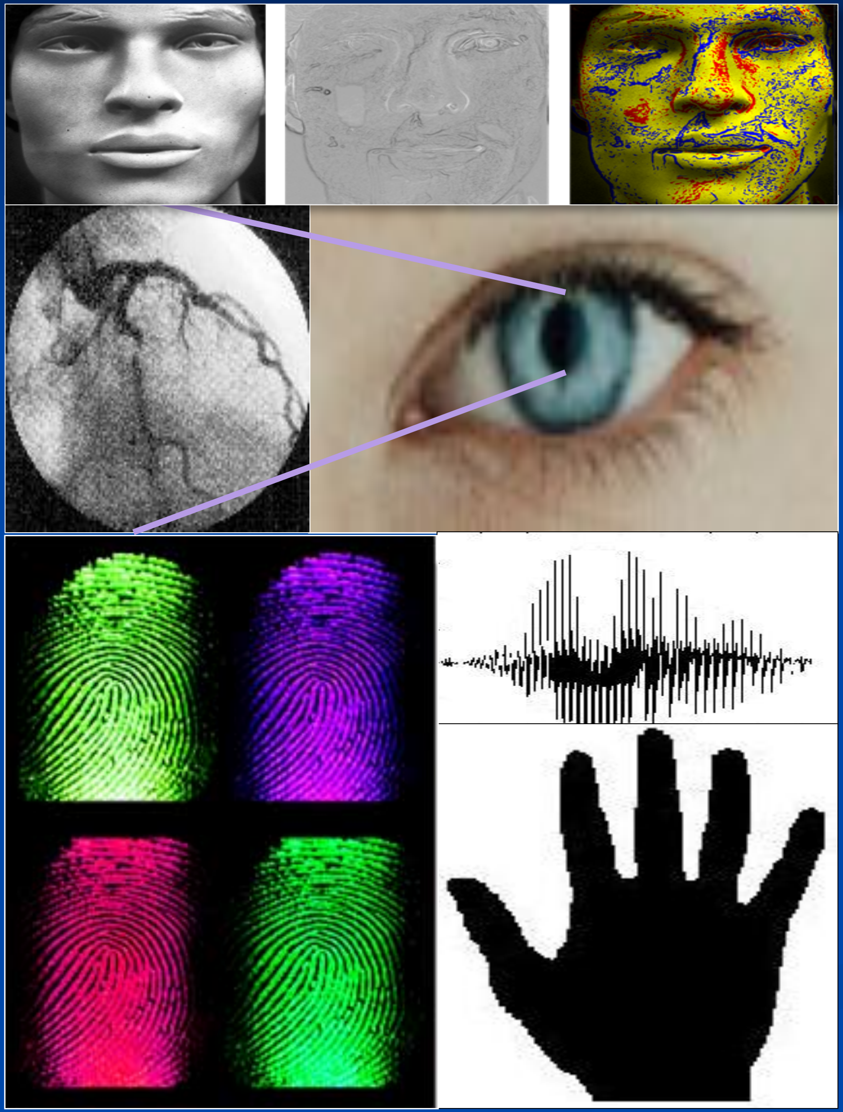
Transient Gene Expression





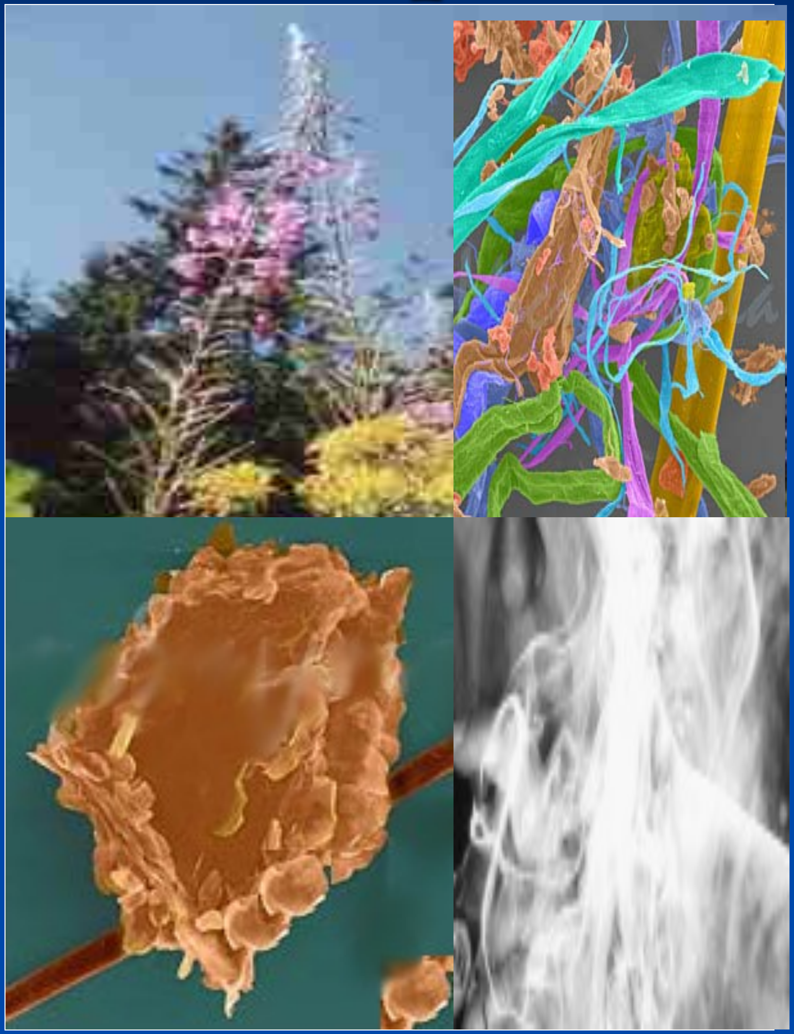
- Transient gene expression and similar techniques will enable the production of vaccines, proteins, vitamins and pharmaceuticals in various plants and animals including humans.

Incontrovertible Identification



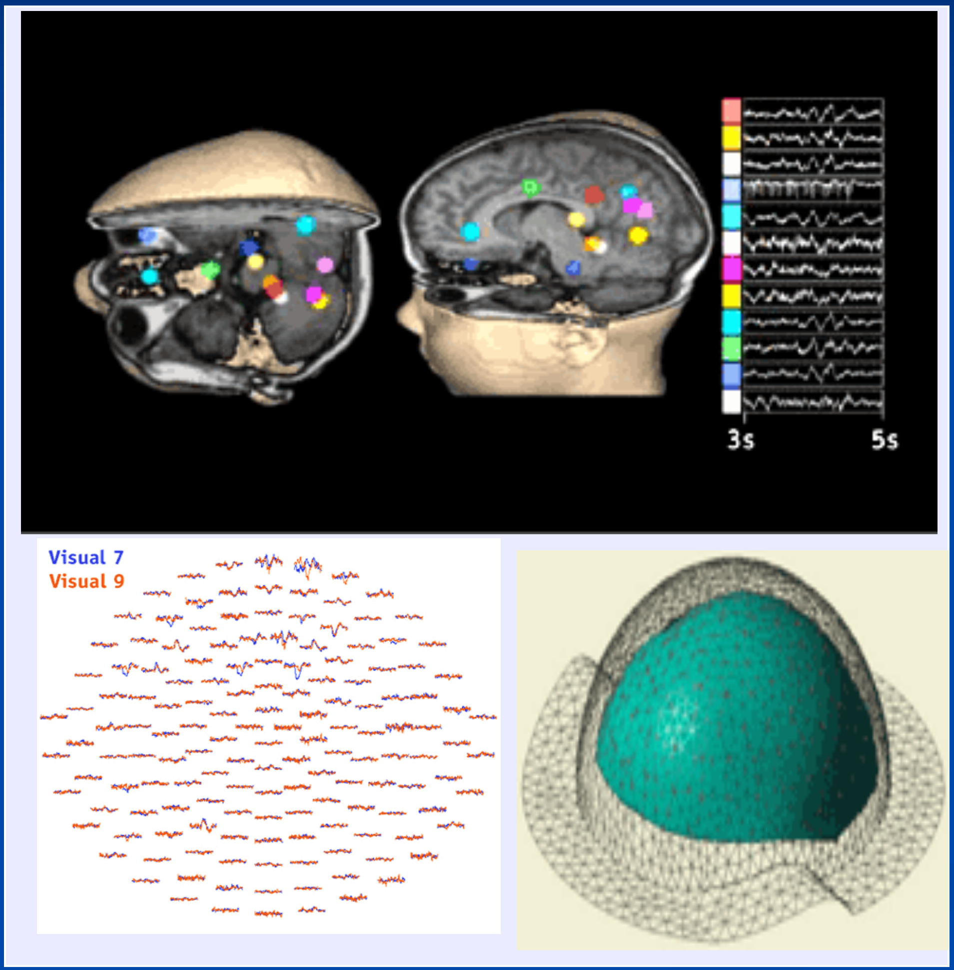
- Biometrics involve the precise measurement of unique human features such as epidermal features,iris and retina patterns, odors, fingerprints, voice prints, hand geometries and ultimately DNA.

Immunological Typing to Define Past Exposures



The immunological response of mammals to their environments leave semi-permanent records of the exposures to pollens, dust, dander, mold, mildews, chemicals, and minerals. The ability to rapidly determine a person's immunological profile will provide a thorough history of where a person has been, what they have eaten, what diseases have encountered, what specifically they were exposed to in the environments they visited, and ultimately even the identities of their associates.

Imaging Cognative and Precognative Thought



Measurements of brain neural currents can be used to diagnose disease, as well as to study brain functions. These tiny electrical currents can be observed by the magnetic fields they produce outside the skull, a technique "magnetoencephalography," or MEG.

Interactive surveillance "This mission is too important for me to allow you to jeopardize it."

Space Odyssey 2001

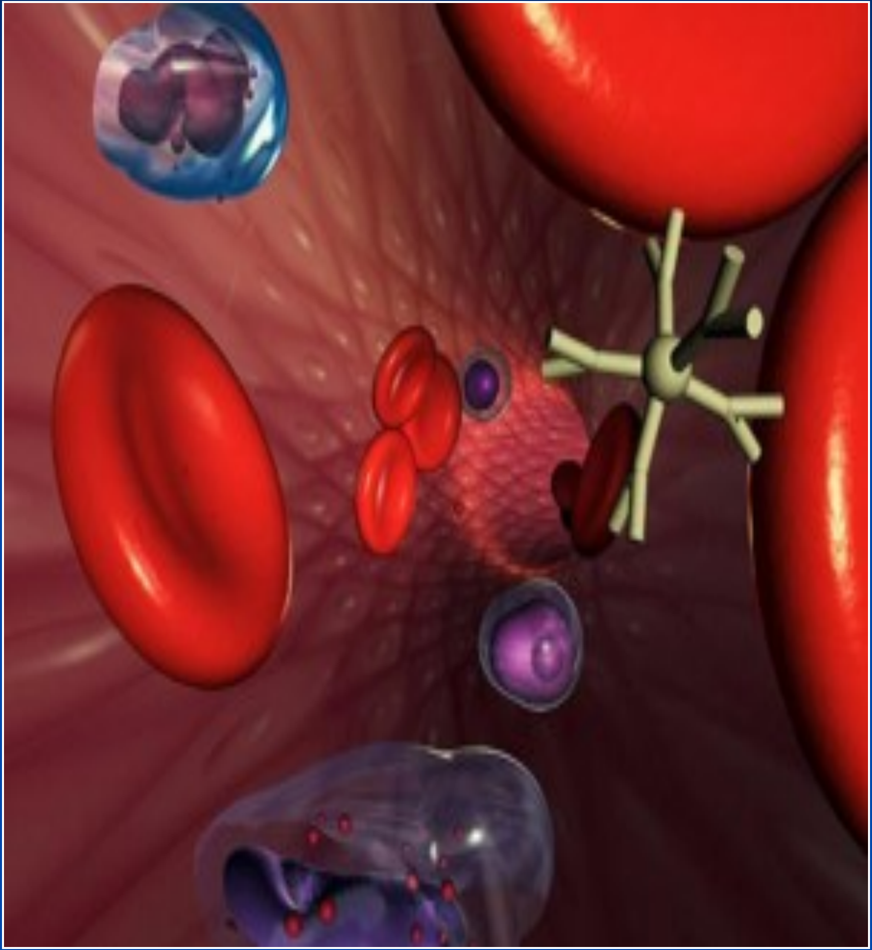
Within a work environment, surveillance systems could monitor human activity and, through "suggestions," ensure that important procedures have been accomplished. Example: Dr. Jones did you know you left a sponge in the patient? Captain Smith did you know you forgot to spin the dial on safe number four?

Cyber Security by Emulating Biological Encryption and Defense…………

Bacteriophage T4 contains ~168,800 base pairs of double stranded DNA containing the genetic blueprint necessary to recreate itself with a specificity of 10ex78,000. The phage binds to the host's surface, punctures the cell with its injection tube, and injects its own genetic blueprint. This genetic information subverts the host cell's biosynthetic machinery into creating replicas of the phage.

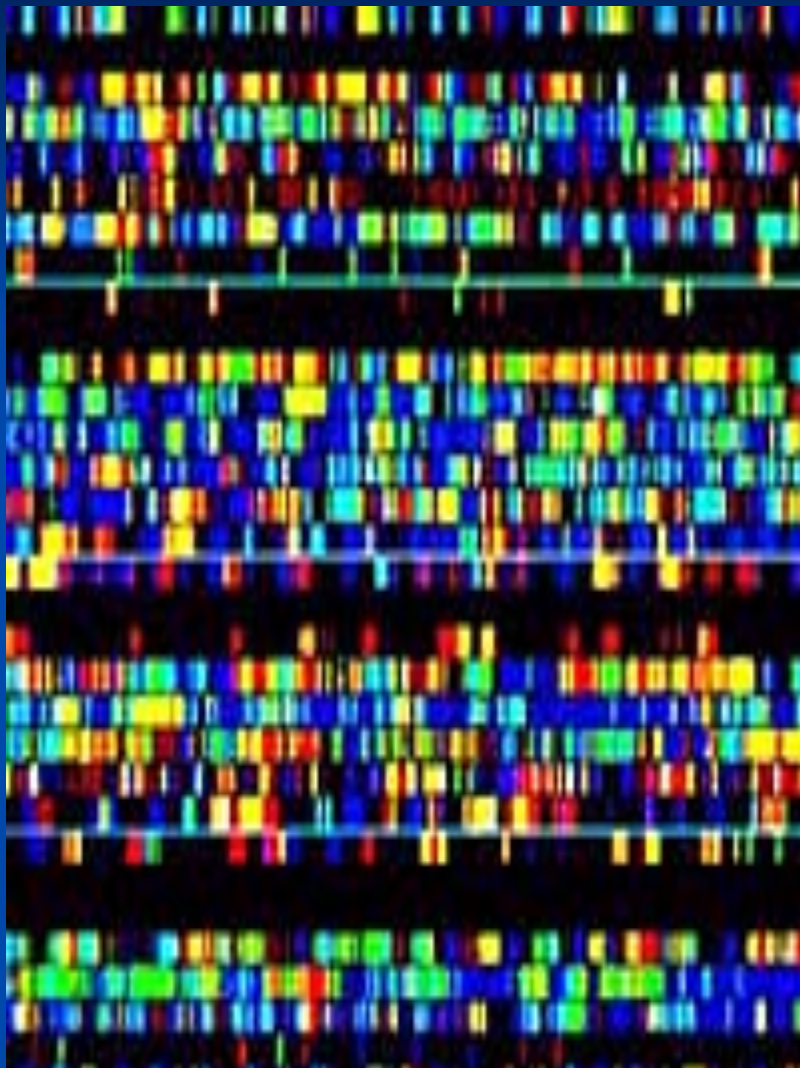
http://www.dform.com/projects/t4/virus.html

Nanoscale Bio-robitics



Biological nanoparticles or nanocapsules hold great potential for many fields of medicine, particularly cancer treatment. The capability is on the horizon to point deliver viral or tumor-killing poisons directly to compromised cells, thus averting the ravaging side-effects of potent medicines.

Laboratories on Chips



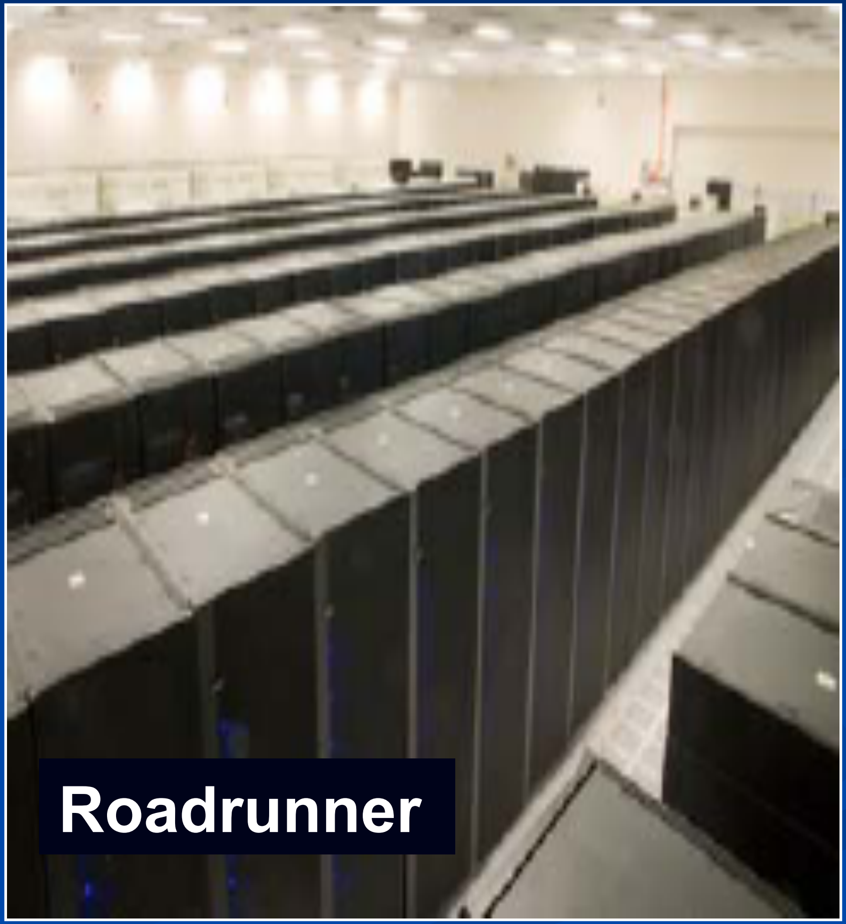
All the elements of a genetics lab capable of extracting DNA from cells automatically can be emplaced on microprocessors the size of a dime.

Some species of bacteria can detect viruses or single atoms of selected materials.

Photon emitting sequences can be inserted that ‘turn on” when the viruses or atoms of interest are detected.

Biotic Computers: if processors equate to neurons, these two computers have approximately the same computational power…

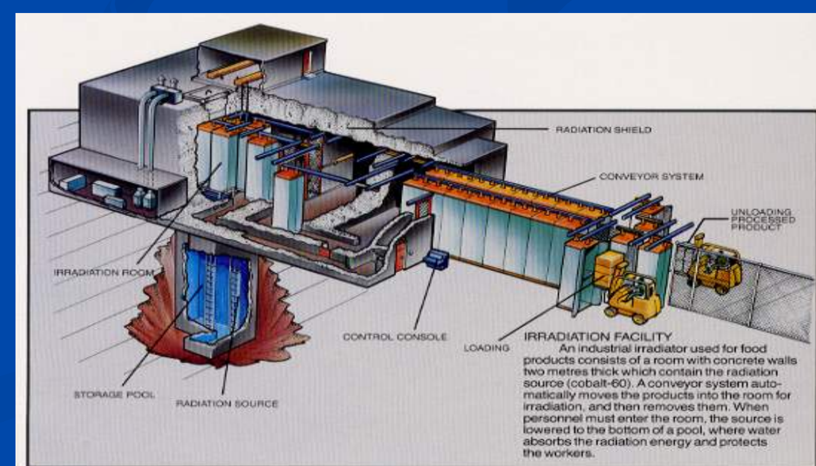
Size of a pecan, 0.001W, <1 cup of water per day



44,000 sq. ft., 25 MW, 200,000 gallons of water per day

Pathogen-free Food

- Smart packaging
- Active packaging
- Bioengineered sources
- Irradiation of food



Levels of Food Irradiation*

LOW < 1kGy

- Lessens vegetable sprouting and fruit ripening
- Eliminates some insects

MEDIUM 1-10 kGy

- Eliminates most pathogens, insects and parasites
- Elimination of most spoilage

HIGH >10k Gy

- Absolute sterilization of all fungi, bacteria and viruses

One Gray (Gy) = 100 rads

*foodsafety.psu.edu/…/sld001.htm

Possible Future

- Terrorism, fed by fatalism and hatred, has become a more significant challenge to our society and its values.
- Terrorists have developed or have access to means to produce biological agents and the systems to deliver same.
- Terrorism, involving biological agents, has been demonstrated and attacks are increasing in lethality and have caused major disruption in our food supplies.
- WMD terrorism has fundamentally changed our way of life and the rationale for sustaining our freedoms and liberties is being questioned.

A More Desirable Future

- Science and technology have enhanced public health services thereby lessening the possibility and impact of natural pandemics, bio-terrorism, and food-borne pathogens.
- Science and technology have developed methods for rapid detection and isolation of pandemics.
- Science and technology have made all acts of terrorism less probable and more costly to the terrorist.
- Science and technology have helped eliminate the underlying factors that made terrorism an option for achieving social change.
- Science and technology have combined to ameliorate the impact of counter responses on our freedoms.
- Science and technology, combined with global legal initiatives, have eliminated terrorist sanctuaries.