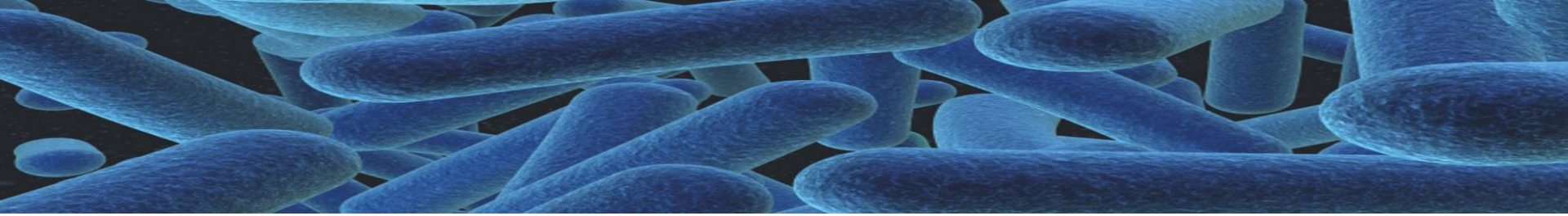
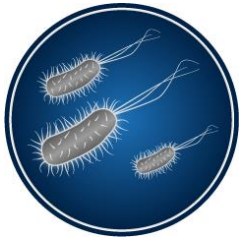


Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods

Micro-Electro-Mechanical Systems (MEMS)

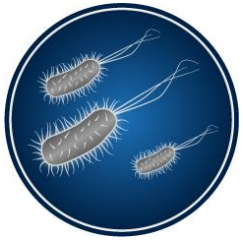
Michael J. Miller, Ph.D.

MICROBIOLOGY CONSULTANTS, LLC



Micro-Electro-Mechanical Systems

- Imagine a machine so small that it cannot be seen by the human eye
- Imagine working machines no bigger than a grain of pollen
- Imagine thousands of these machines batch fabricated on a single piece of silicon
- Imagine a world where gravity and inertia are no longer important, but atomic forces and surface science dominate

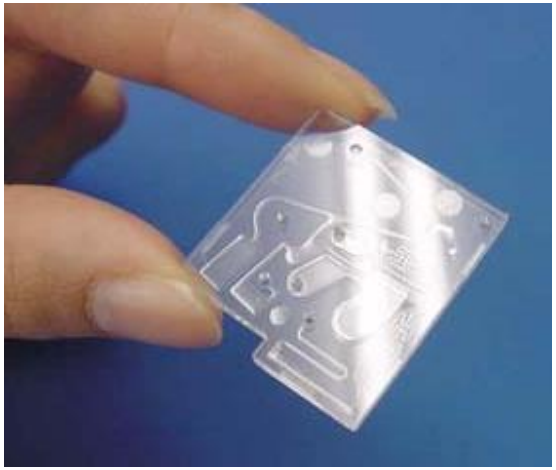


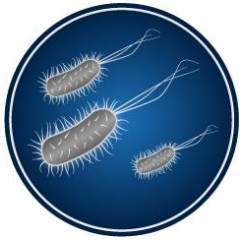
Micro-Electro-Mechanical Systems

- MEMS is the integration of mechanical, electrical, fluidic and optical elements, sensors, and actuators on a common silicon substrate through microfabrication technology
- MEMS for biomedical applications have the fastest growth rate within the MEMS market, particularly for drug discovery and delivery, Lab-On-A-Chip and microfluidics devices, microarrays and biochips, biosensors, nanoparticles, DNA test and diagnostics, biotelemetry, and genomics

Lab-On-A-Chip

- An automated, micro-scale (or nano-scale) laboratory that enables sample preparation, fluid handling, analysis and detection steps to be carried out within the confines of a single microchip

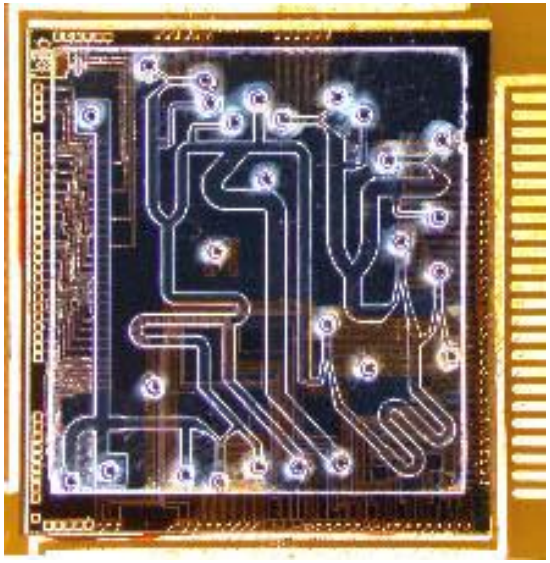


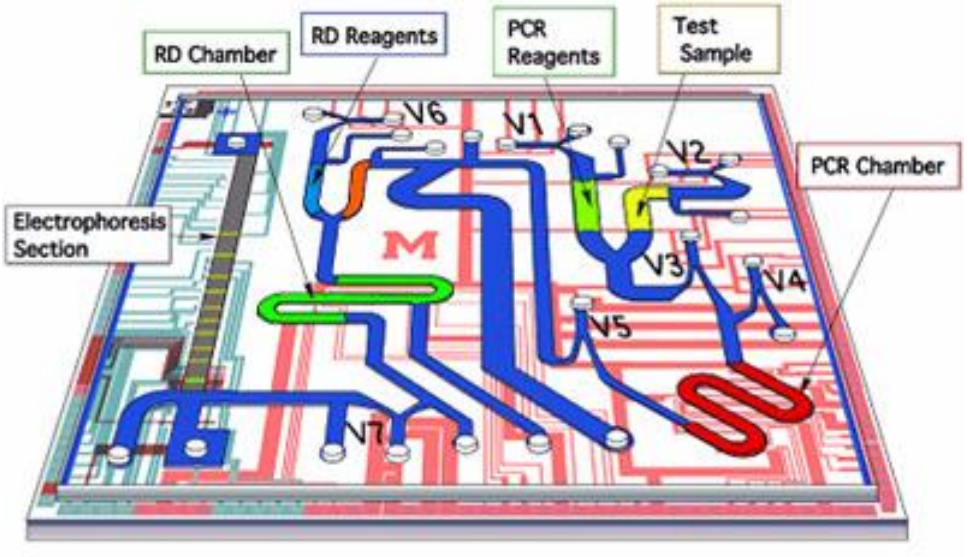


Lab-On-A-Chip

- Microfluidics
  - Most familiar application is ink-jet printing
  - Manipulation of minute amounts of liquid in miniaturized systems
  - Network of channels and wells that are etched onto glass or polymer chips
  - Pressure or voltage gradients move pico or nano liter volumes in a finely controlled manner through the channels
  - Enables sample handling, mixing, dilution, electrophoresis and chromatographic separation, staining and detection
- Lab chips are available to analyze protein, DNA, RNA and whole cells in fluid samples

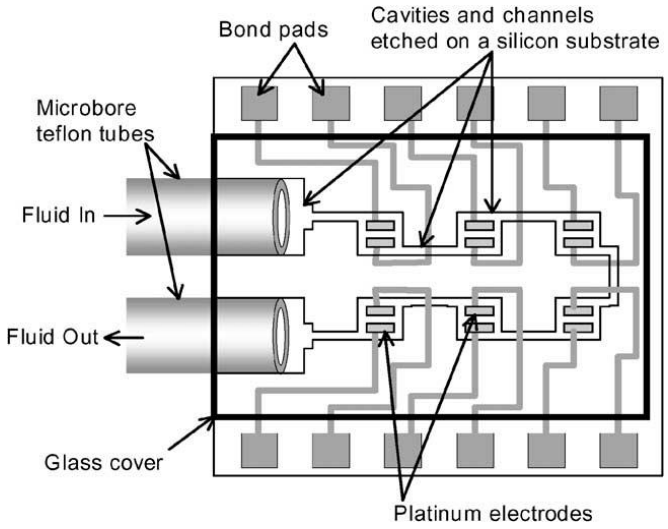
Lab-On-A-Chip



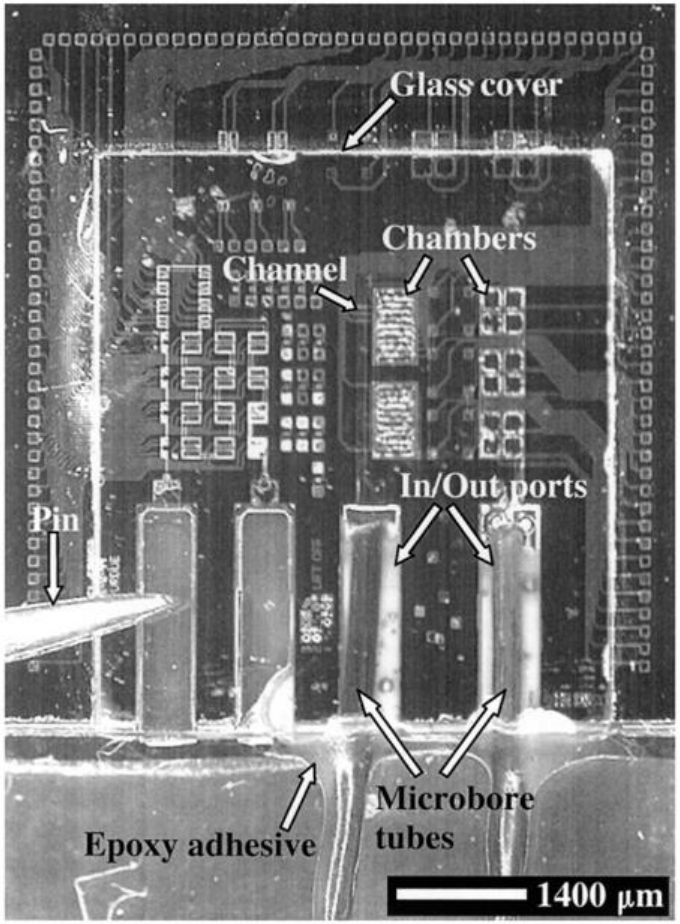

RD Chamber
RD Reagents
PCR Reagents
Test Sample
PCR Chamber
Electrophoresis Section
V1
V2
V3
V4
V5
V6
V7
M

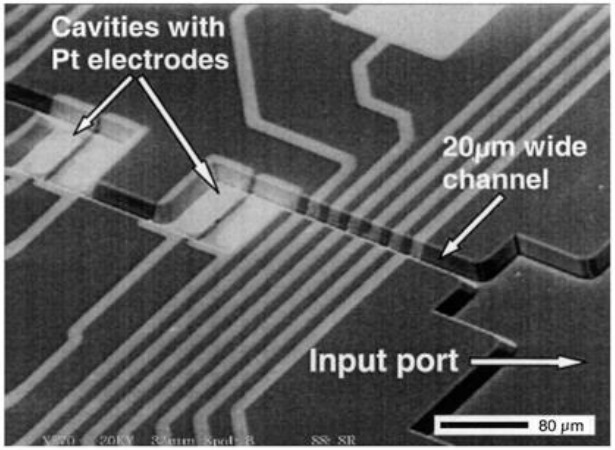
Microscale Impedance

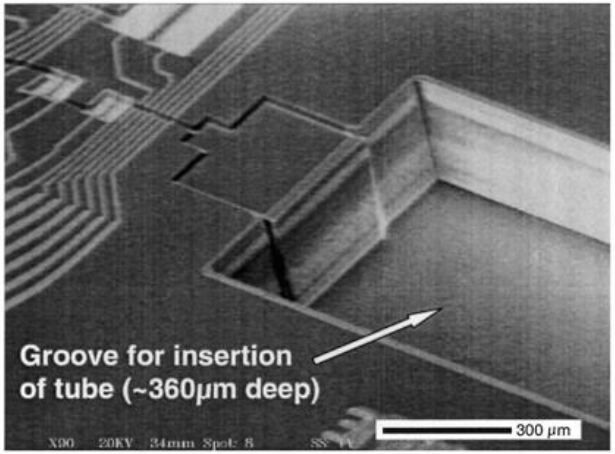
- Purdue University developed an impedance system with channels and chambers etched onto a crystalline silicon substrate
- Platinum electrodes measure the impedance changes
- Incubation/measurement chamber volume less than 1 nL



Microscale Impedance

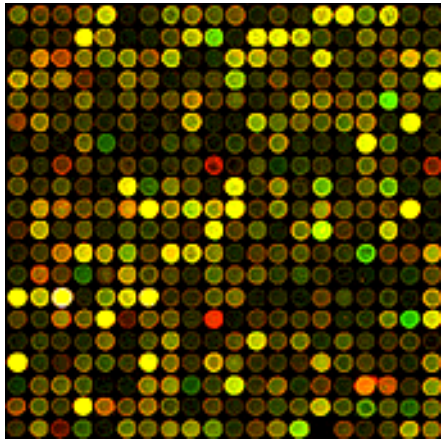






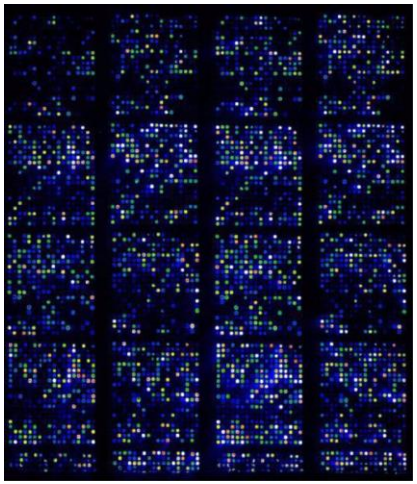
Microarrays

- A collection of miniaturized test sites arranged on a solid substrate that permits many tests to be performed simultaneously
- They are composed of an orderly arrangement (e.g., spots) of protein or thousands of DNA or RNA fragments on glass, silicon or nylon substrates



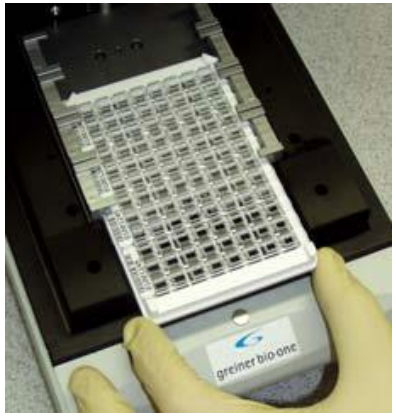
Microarrays

- Applications include nucleic acid sequence identification and measuring expression levels of genes
  - Influenza A and Avian H5N1
- The GeneChip (Affymetrix) contains the entire human genome (~50,000 known genes and gene variants) on a single chip

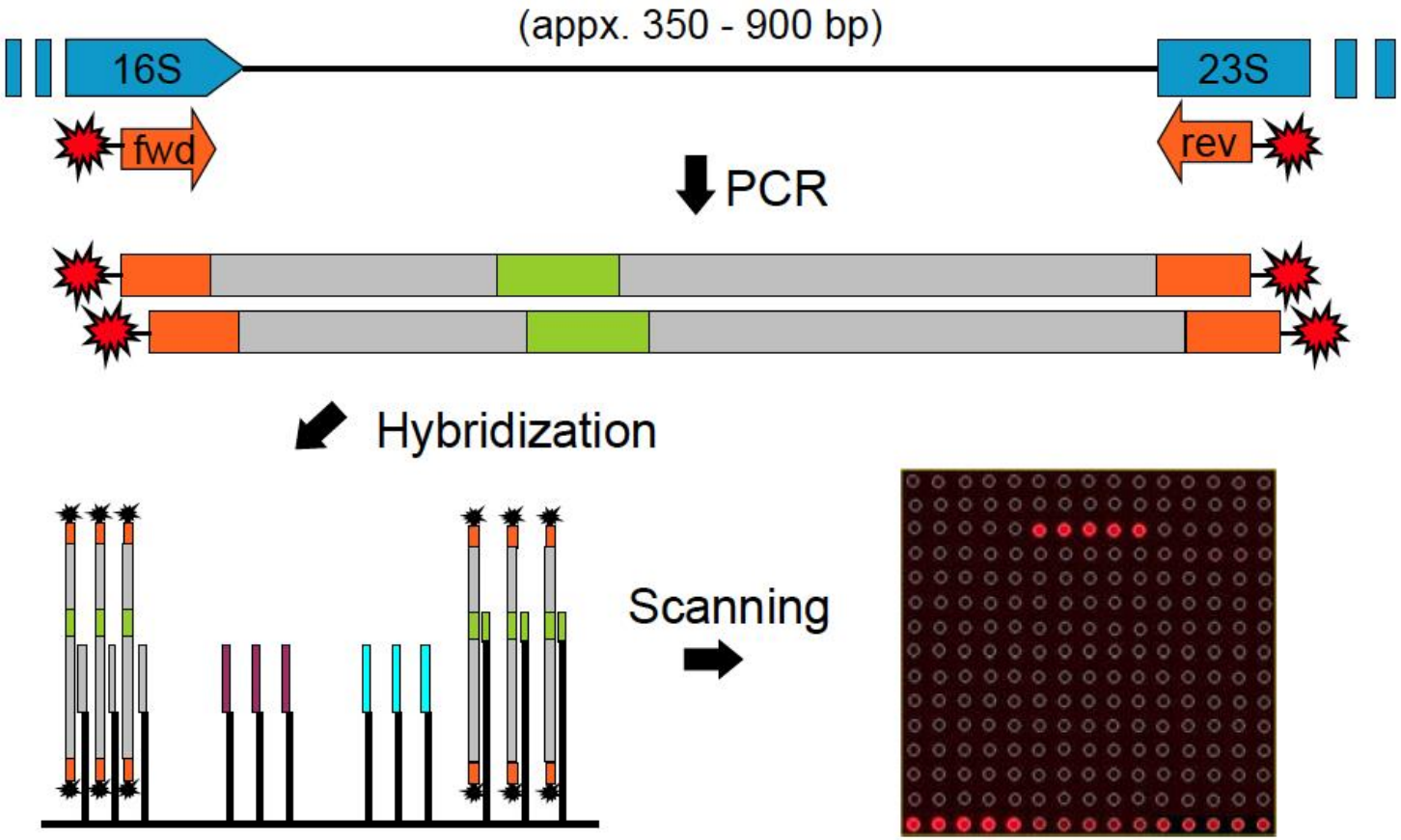


greiner bio-one CytoCheck

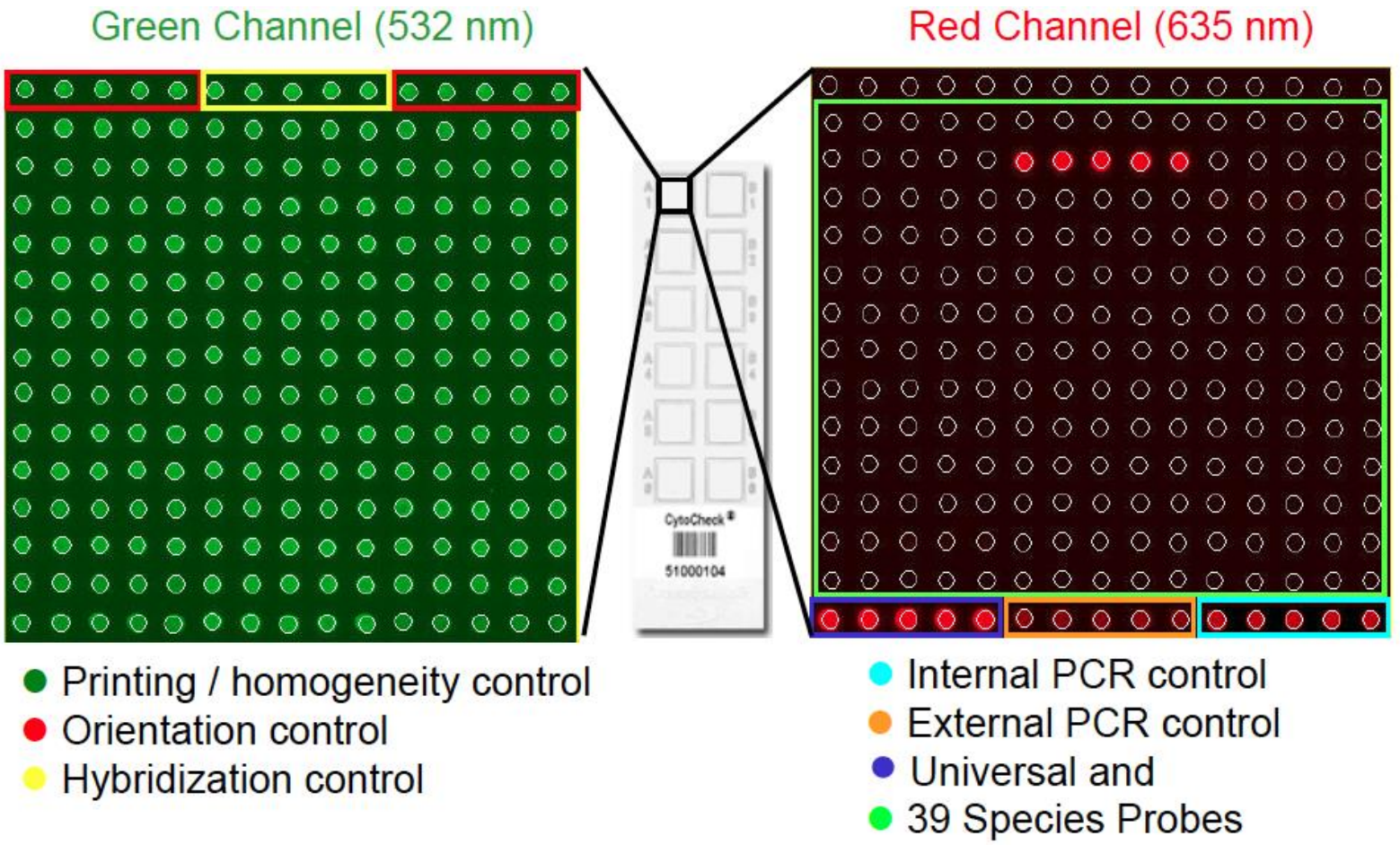
- Detection and identification of 40 Mycoplasma species in a microarray
- DNA is extracted and PCR performed using primers specific for conserved and species-specific regions of the 16S-23S rRNA intergenic transcribed spacer (ITS) of Mycoplasma DNA
- The fluorescently labeled fragments are then hybridized to the microarray chip
- The chip contains probes for both species-specific targets and a universal probe for all Mycoplasma



greiner bio-one CytoCheck



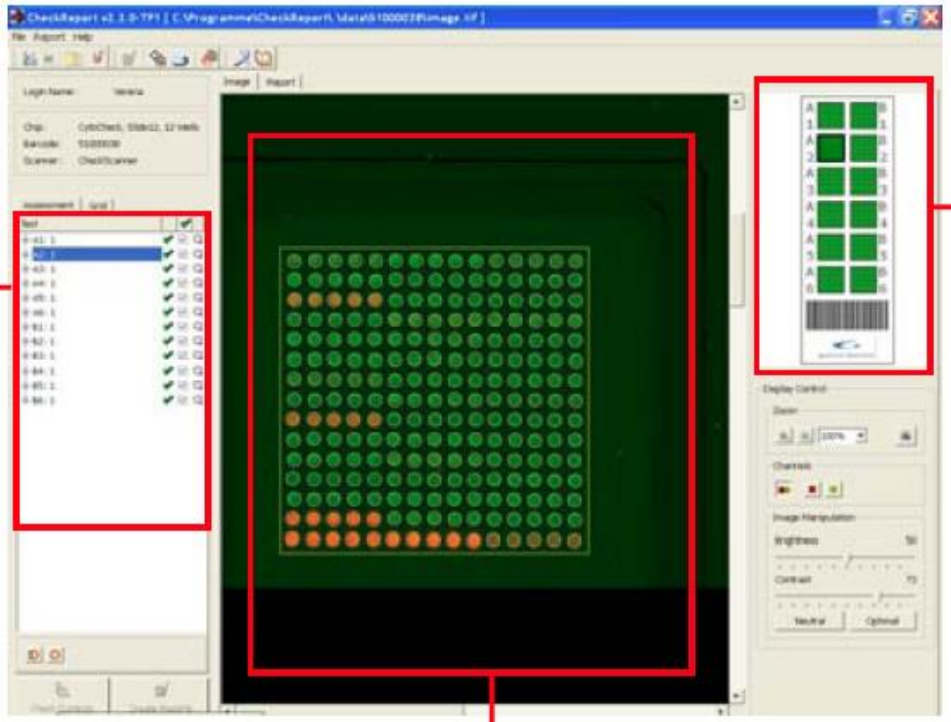
greiner bio-one CytoCheck



greiner bio-one CytoCheck

Automatic quality assessment of

- Extraction
- PCR
- Hybridisation
- Contamination status

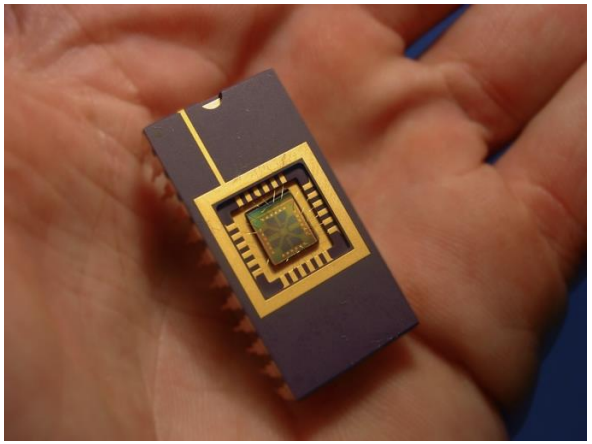


HTA™Slide12 overview

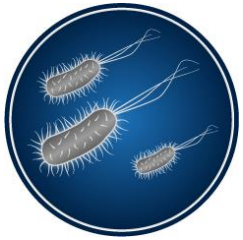
Visual image inspection of the DNA array

Biosensors

- A device for the detection of an analyte that is comprised of a biological component combined with a physicochemical detector component

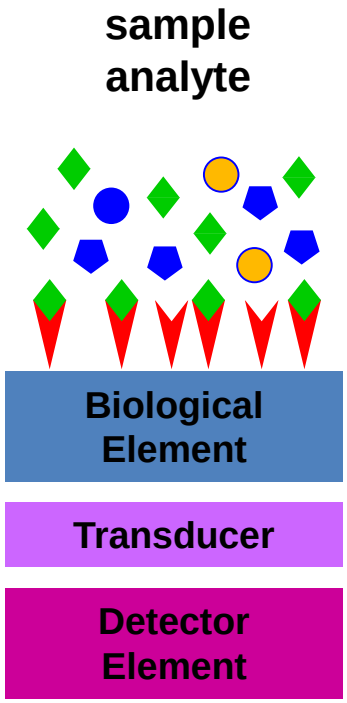


- A canary in a cage, as used by miners to warn of gas, may be considered as an early type of biosensor



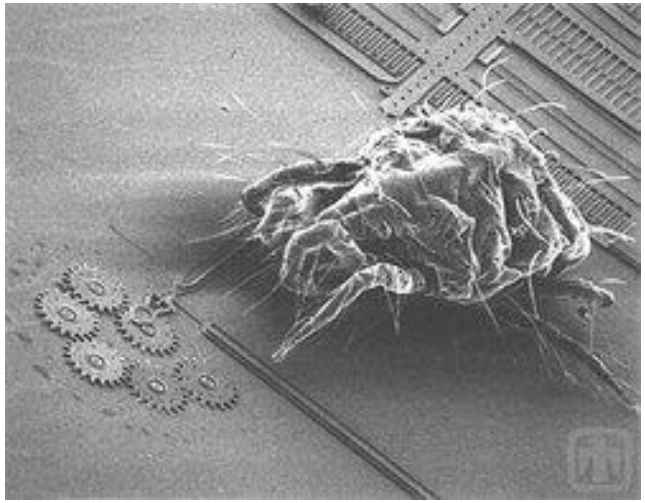
Biosensors

- *Sensitive biological element*
  - Tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, whole cells
- *Transducer* in between
  - Associates both components
- *Detector element*
  - Works in a physicochemical way
  - Optical, electrochemical, thermometric, piezoelectric or magnetic
- Future uses will include remote sensing of airborne bacteria and detection of pathogens

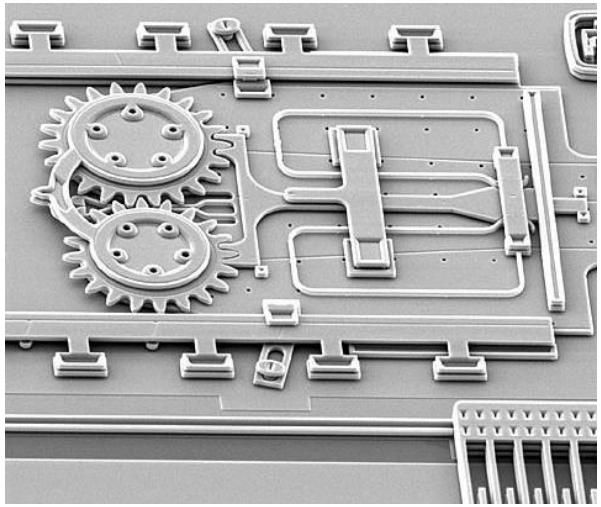


Nanotechnology

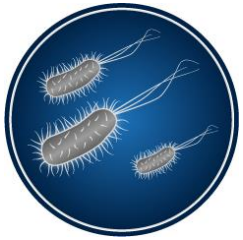
- Technology development at the atomic, molecular, or macromolecular range of approximately 1-100 nanometers to create and use structures, devices, and systems that have novel properties



Dust mite next to nanogears



Clutch mechanism;
gears are 50 microns across

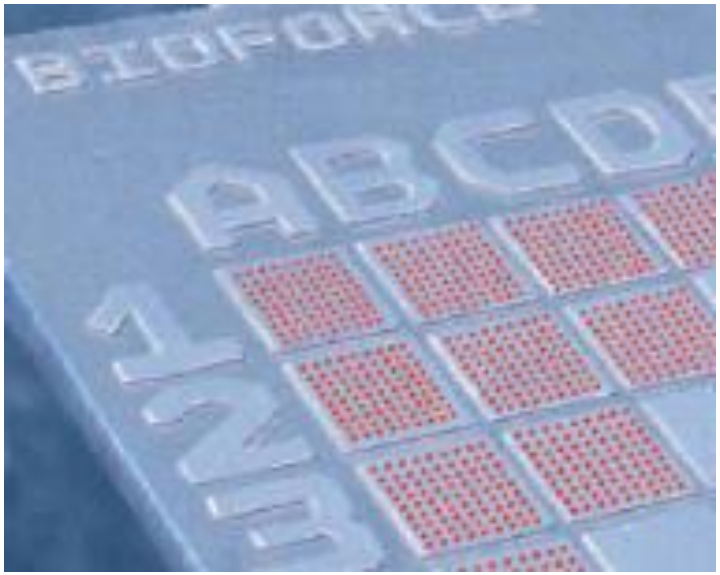


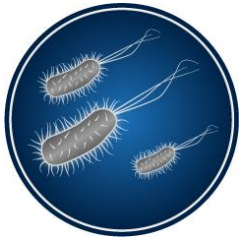
Nanoarrays

- Arrays with molecules placed at defined locations on a surface with nanometer spatial resolution
- Spots can include biological samples such as proteins, DNA, RNA and whole viruses, as well as non-biological samples
- Nanoarrays utilize approximately 1/10,000th of the surface area occupied by a conventional microarray
- Over 1,500 nanoarray spots can be placed in the area occupied by a single microarray spot

Nanoarrays

- The BioForce NanoArray System prints biological and non-biological materials onto 4x4 mm silicon chips and other surfaces with ultra-micro spot sizes ranging from 1-20 microns, and in the nanometer range to 250 nm

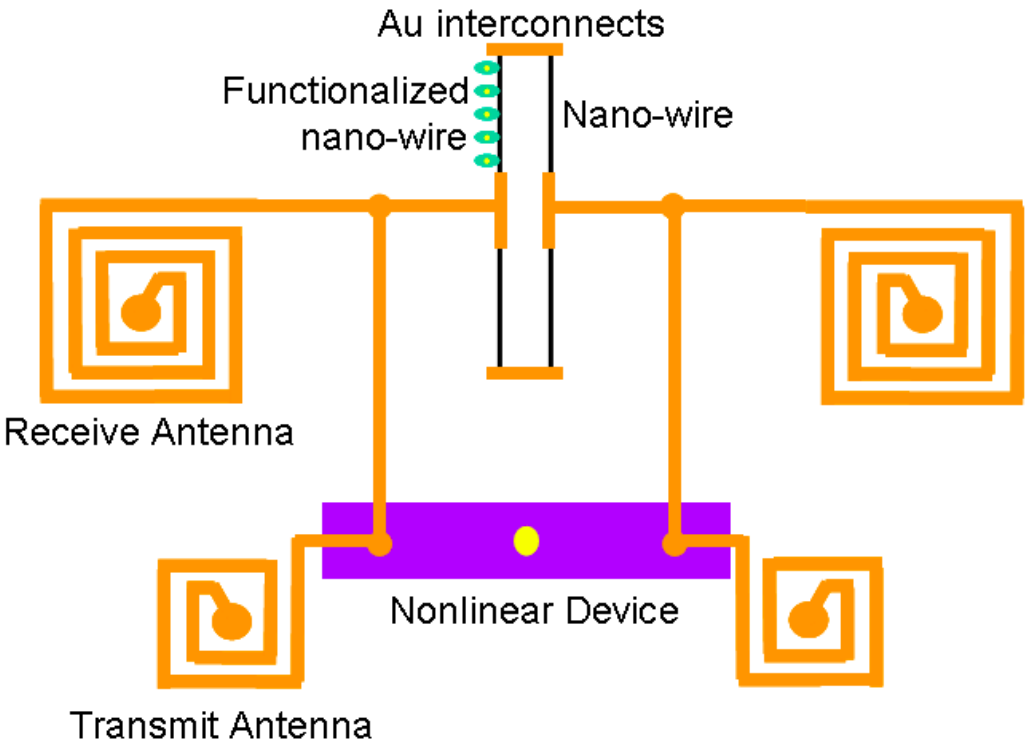


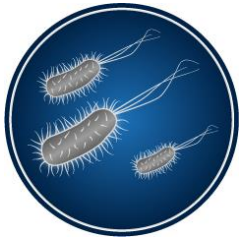


Battelle Nano Biosensors

- Development of a passive or remotely powered sensor that can be wirelessly monitored or probed with a non-contact device to detect a chem-bio exposure.
- Sensing mechanism based on the electrical property changes in a functionalized nano-wire
- Detection of airborne microorganisms
- Detection of contamination in packaging (raw materials, food)

Battelle Nano Biosensors



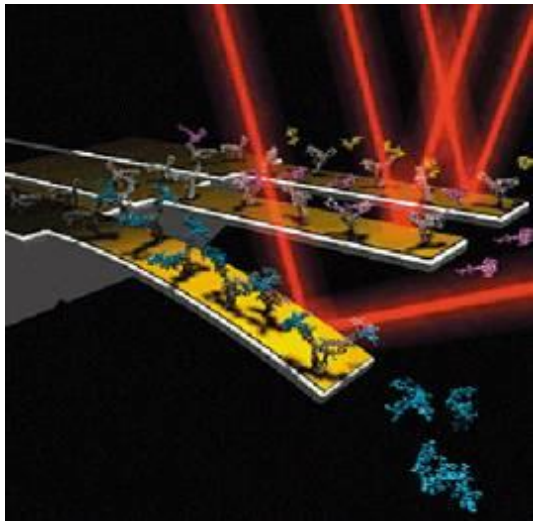


Microcantilevers

- A microcantilever is a device that can act as a physical, chemical or biological sensor by detecting changes in cantilever bending or vibrational frequency
- It is the miniaturized counterpart of a diving board that moves up and down at a regular interval based on mass
- The mass changes when contaminants land on the devices, causing them to vibrate at a different "resonant frequency, " which can be quickly detected
- Dimensions in microns and different shapes

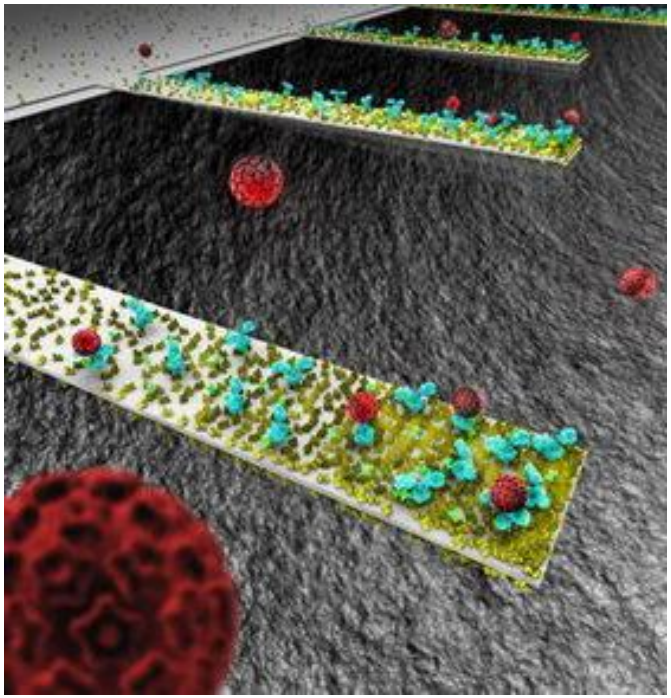
Microcantilevers

- BioCOM chip developed at the UC, Berkeley
- This chip-level microcantilever array is expected to provide a quantitative, label-free, and low-cost platform for detection of various biomolecules, such as DNA and proteins.



Nanocantilevers

- Purdue University is developing advanced sensors capable of detecting minute quantities of viruses, bacteria and other contaminants in air and fluids by coating the cantilevers with proteins and antibodies that attract the contaminants
- Applications in hospital environmental monitoring and homeland security
- Thousands of the cantilevers can be fabricated on a 1 cm^2 chip
- Cantilever length is a few microns and thickness is 20 nm



Technology Review Summary

- There are numerous platforms for the detection, enumeration and identification of microorganisms that are currently available or are in development
- The future of RMM technology is now!