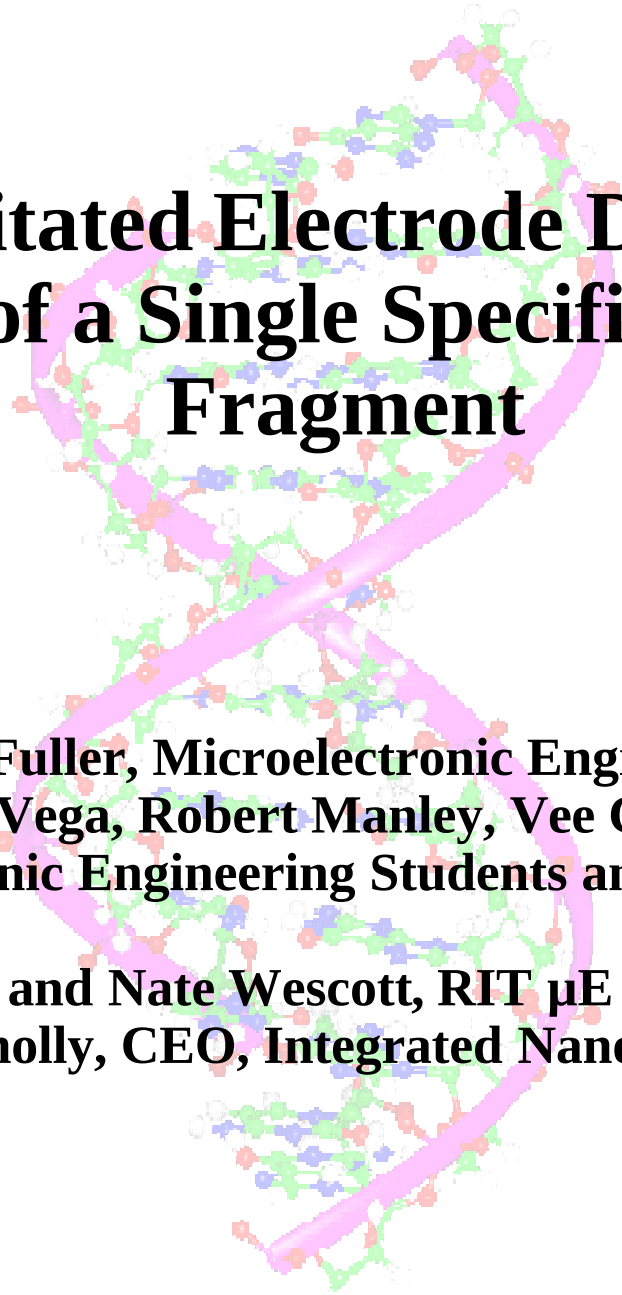
An Inter-digitated Electrode Detector for the Identification of a Single Specific DNA Molecule Fragment



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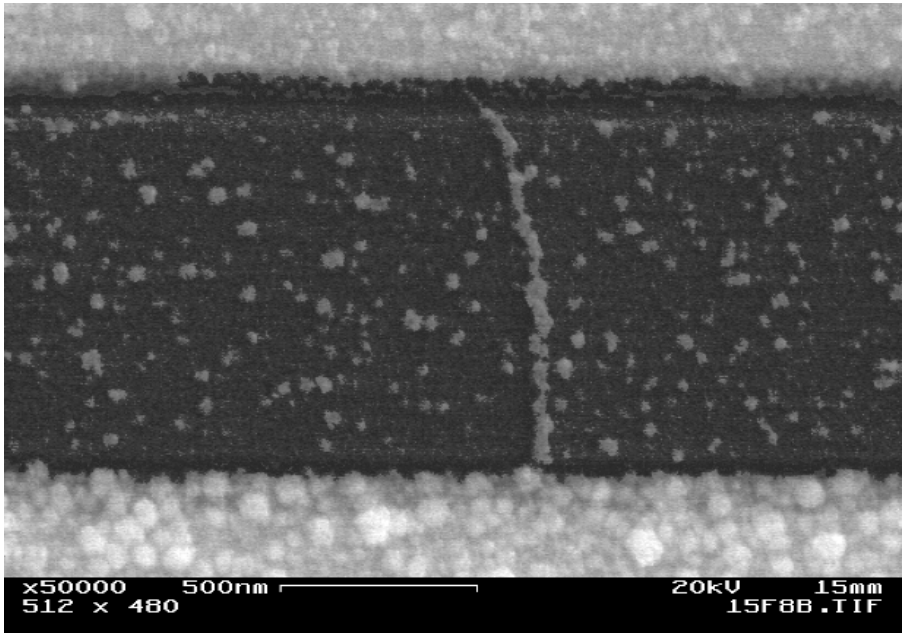
Abstract

The detection of a single specific DNA molecule fragment will allow for the identification of bacteria and viruses that could be harmful if not detected quickly. DNA probes attached to the sensor electrodes have a specific molecular sequence that results in a billion to one or better probability that any DNA that hybridizes with the probe is the DNA to be detected. The DNA is coated with a metal, resulting in a large decrease in the measured electrical resistance between the sensor electrodes. Thus the electrical detection of a specific single DNA molecule fragment is very easy. The design and fabrication of the sensor will be described.

The picture shows two metal lines 1 micrometer apart with a few DNA bridges coated with silver. The measured electrical resistance between the metal conductors dropped from infinity to 2 thousand ohms, making electrical readout of the detection of a single DNA very simple.



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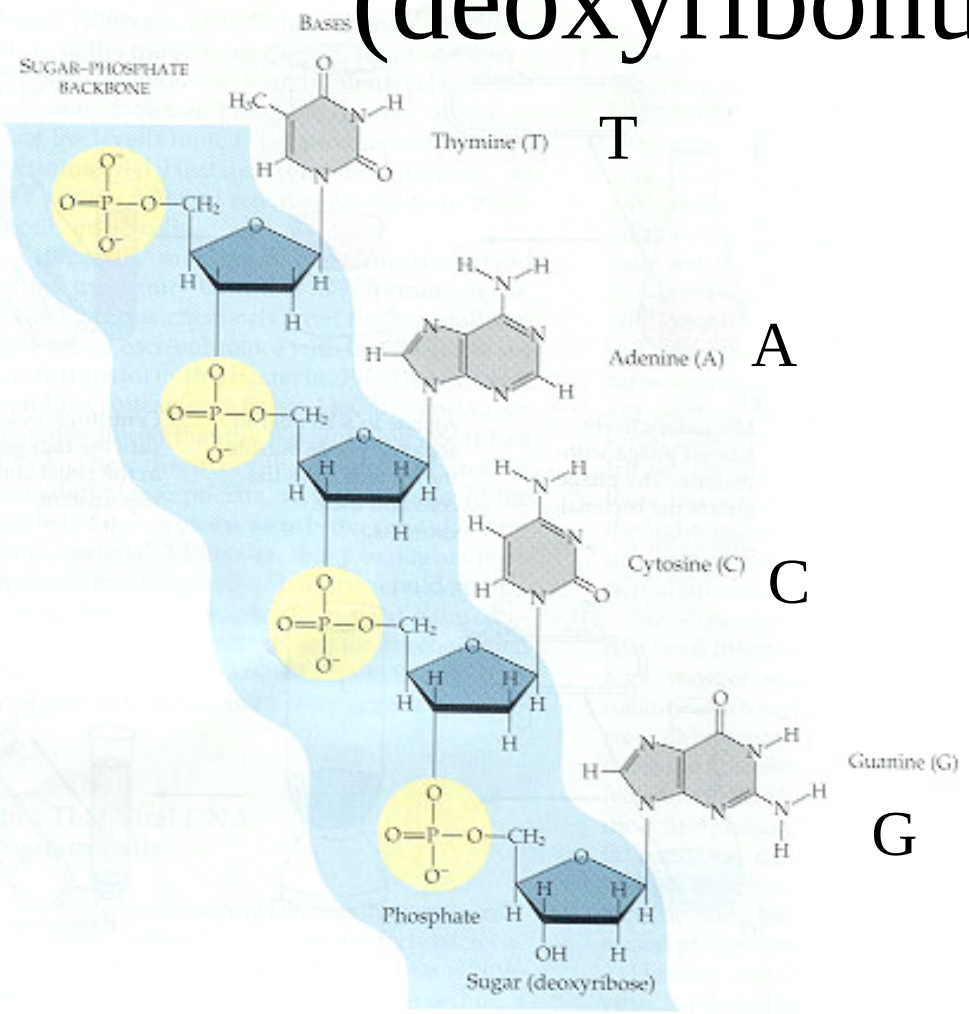
Outline

Abstract
Outline
DNA Primer
Motivation
Design and Layout
Wafer Fabrication
Testing and Yield
Probes
Hybridization
Silver Coating of DNA
Electrical Measurement of Silver Coated DNA
System Design
Summary
Acknowledgements

DNA Primer (deoxyribonucleic acid)

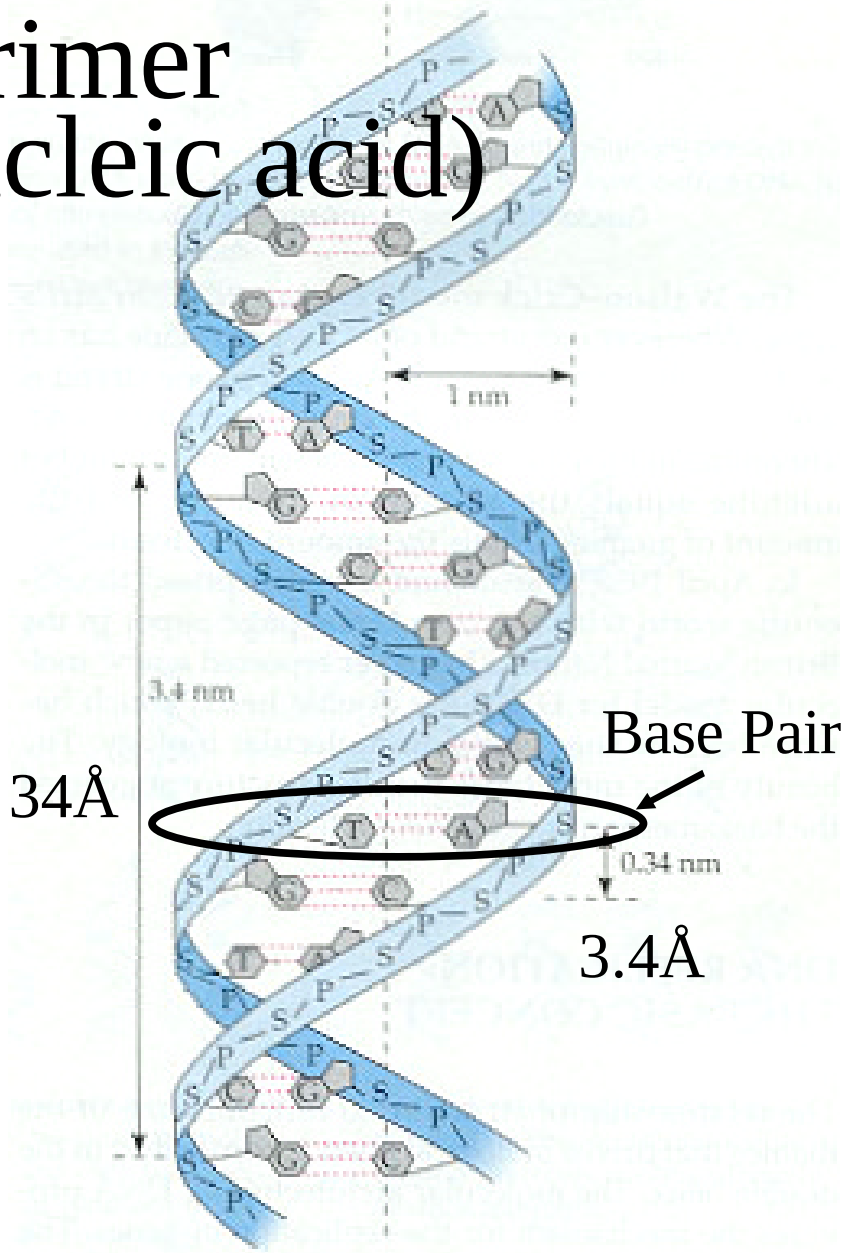


T

A

C

G



34Å

Base Pair

3.4Å

3kb Fragment is ~1μm

Terminology

DNA – deoxyribonucleic acid
Base – one of four nucleic acids adenine (A), guanine (G), cytosine (C), or thymine (T)
Base Pair – A is always bonded to T, G is always bonded to C
Molecule Fragment Length – stated in Kb (Kilo base pairs, each 0.34 nm)
PCR – Polymerase chain reaction, is a particular reaction sequence that starts with an original DNA molecule and creates an exponentially growing population of copies of fragments of that molecule.
Denaturing - at temperatures ~ 95°C double stranded DNA separates into two single stranded DNA molecules.
Primer or Probes **–** synthetically produced single strand molecule with a specific target sequence of bases.
Annealing or Hybridization – Complementary DNA molecule fragments attach to one another, done at ~65°C

PCR

1. Start with one DNA molecule
2. Denature the two bound strands of DNA at ~95°C into two single strands of DNA
3. Cool to ~65°C in the presence of a primer (several bases long) allowing primer to bind to each single strand
4. Extend the molecule at ~72°C (supply nucleodides and polymerase enzyme) so that each single strand + primer becomes full double strand DNA molecule fragment.
5. Repeat 2, 3, 4 each time doubling the number of molecules every ~60 seconds. After 20 cycles (assuming perfect fidelity) more than 1,000,000 copies are produced.

Sensor Based on PCR and Mechanical Stress

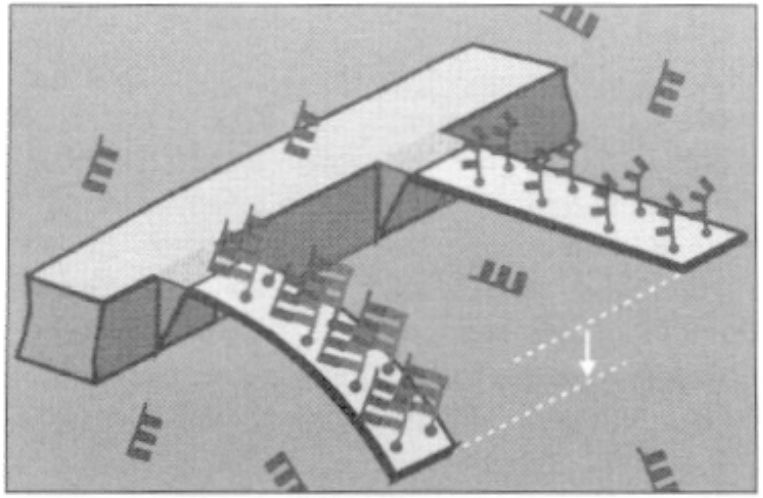


Fig. 2. Schematic illustration of biomolecule hybridization transduced to cantilever deflection. The cantilevers are functionalized on one side with different oligonucleotide base sequences. The differential signal is set to zero and a complementary oligonucleotide is injected. Hybridization with the matching oligonucleotide is shown on the left cantilever where surface stress induces bending and increases the differential signal.

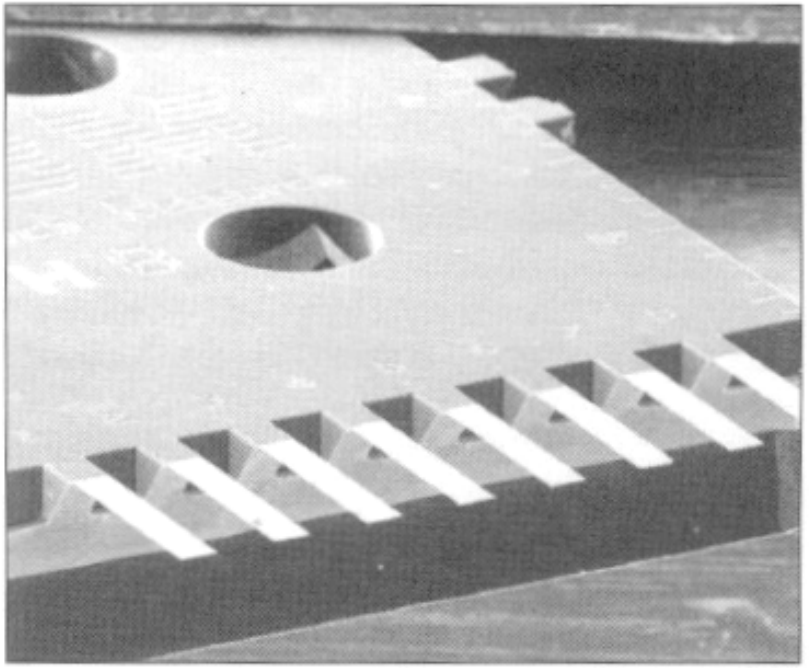
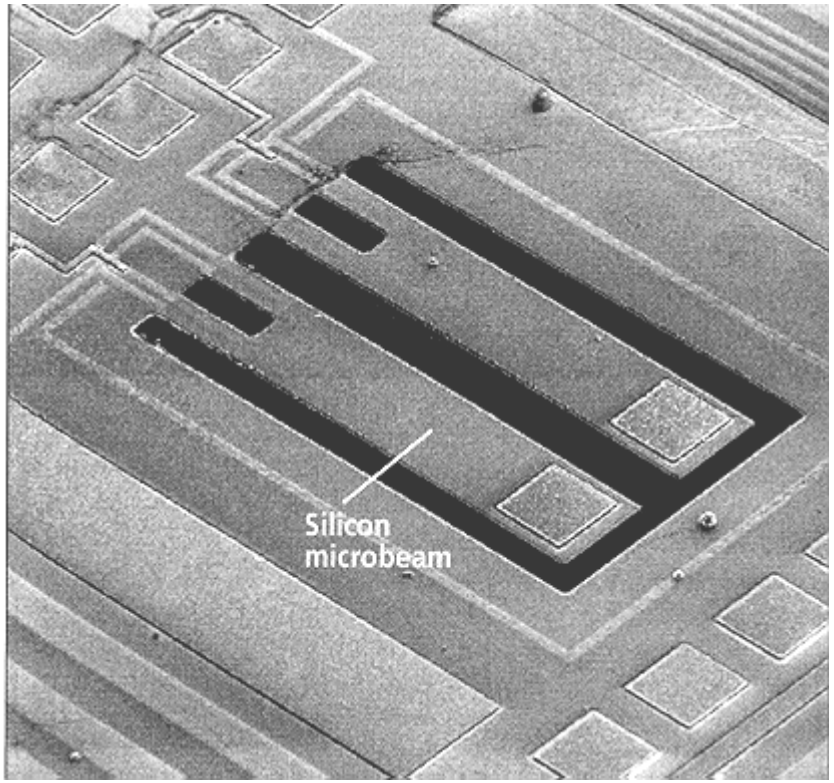


Fig. 1. Microfabricated silicon cantilever array. Each cantilever is 1 μm thick, 500 μm long, and 100 μm wide on a pitch of 250 μm.

Sensor Based on PCR and Mechanical Resonant Structures

The two cantilever structures have piezoresistive sensors to measure the resonant frequency of the beams. The beams have a DNA probes at the end of the cantilever that hybridizes with specific DNA molecule fragments. The additional mass is detected by a change in the resonant frequency. This type of detector requires PCR to have enough molecules to cause a change.



Sensor Based on PCR and Fluorescence

2003 Product of the Year
Cepheid's biohazard detector gets stamp of approval for postal deal

Cepheid Inc., GeneXpert System

By Jeff Karoub, Small Times Magazine

Can what's good for homeland security be good for the growth of small tech? Cepheid Inc. could offer the proof – now that it has the proving ground.

The U.S. Postal Service in August completed a 15-city test of the Biohazard Detection System, the first commercial system capable of detecting anthrax spores quickly and accurately. Cepheid provided the detection mechanism at the heart of the system. Postal officials, who described the test as a "resounding success," plan to begin a nationwide deployment of the system early next year.

The contract, awarded in May to Cepheid collaborator Northrop Grumman Corp., is a boon for the publicly traded, Silicon Valley-based startup. Cepheid officials said they expect to receive up to $30 million of the $175 million contract next year as part of the contract's first phase. The second phase, which would kick in after October, should be equal to or slightly larger than that.

The deal is a key to the company's planned push to profitability by late next year or early 2005. But Cepheid said it's the ultimate test bed for further development and commercialization in multiple areas of its GeneXpert instrument platform, the detector within the postal system. The DNA-based system for identifying pathogens also passed the test as Small Times' winning product for 2003, by moving into and creating its own market as well as improving society and industry standards.

The company projects it initially will make most of its money from the GeneXpert, but anticipates the sales of its consumable cartridge will continue to grow after the hardware is installed. The postal service has discussed installing one or more detection systems in about 290 facilities, and could conduct air-sampling tests using Cepheid's disposable cartridges as often as every half hour.

The Biohazard Detection System marks a major milestone in an effort begun in 1996, when Kurt Petersen co-founded Cepheid with the help of a Department of Defense contract. His goal: develop rapid, accurate and portable systems for detecting dangerous biological organisms.

Cepheid uses technology based on a miniaturized thermal cycler developed at Lawrence Livermore National Laboratory. The cycler performs a technique for replicating DNA called polymerase chain reaction (PCR). PCR is accurate, but getting results can take a day or two because the process requires a skilled technician who must prepare and analyze the sample in a lab. Cepheid's SmartCycler, launched in 2000, cuts that time to 30 minutes, and the GeneXpert automates the process, removing the need for specialists and the risk of human error.

Beyond the postal system, Cepheid has been working with others to develop such automated tests using the GeneXpert for detecting cancer and such diseases as tuberculosis, meningitis, group B streptococcus and antibiotic-resistant bacteria. Several scientific papers have been written on this work and commercialization will begin next year. Analysts predict these clinical applications will have greater market potential than biodefense.

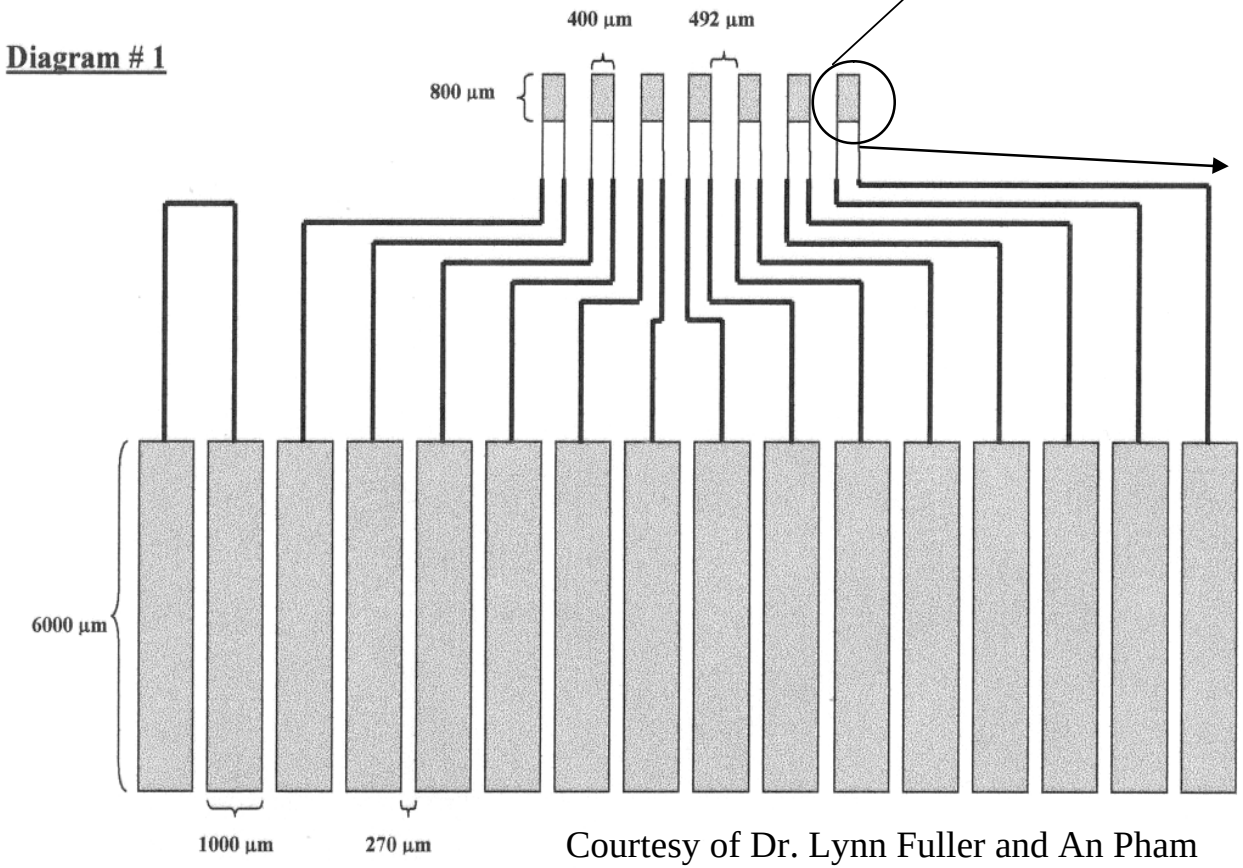
Motivation

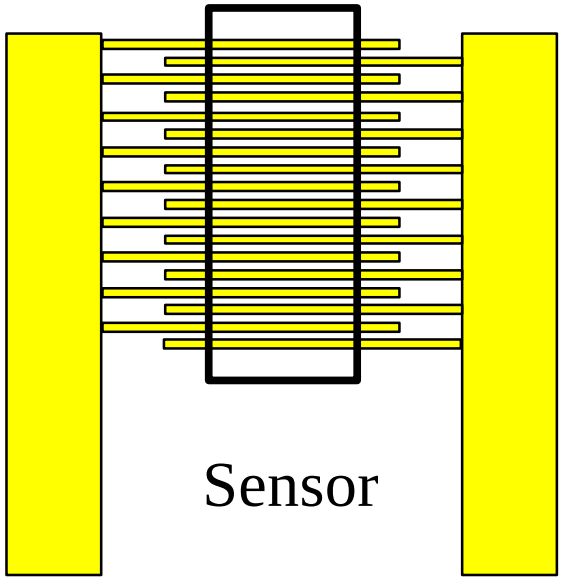
- Biological agents such as anthrax, small pox, and tularemia have been developed as weapons. Even non-weaponized forms of these agents could spread to pandemic proportions if unchecked.

- Current biosensor technologies are either PCR-based (accurate but slow and requires highly skilled operator and laboratory environment) or Assay-based (portable, rapid but not accurate or sensitive)

- The sensor described in this talk is fast, accurate and very sensitive. It does not require a highly skilled operator or laboratory environment.

Chip Design





Design Specification:

1) 200 pairs of interdigitated electrodes per sensor
2) >5000 Å Oxide on Silicon
3) 300 Å Gold on 300 Å Chrome
4) 2.0 µm line/1.0 µm space

Courtesy of Dr. Lynn Fuller and An Pham
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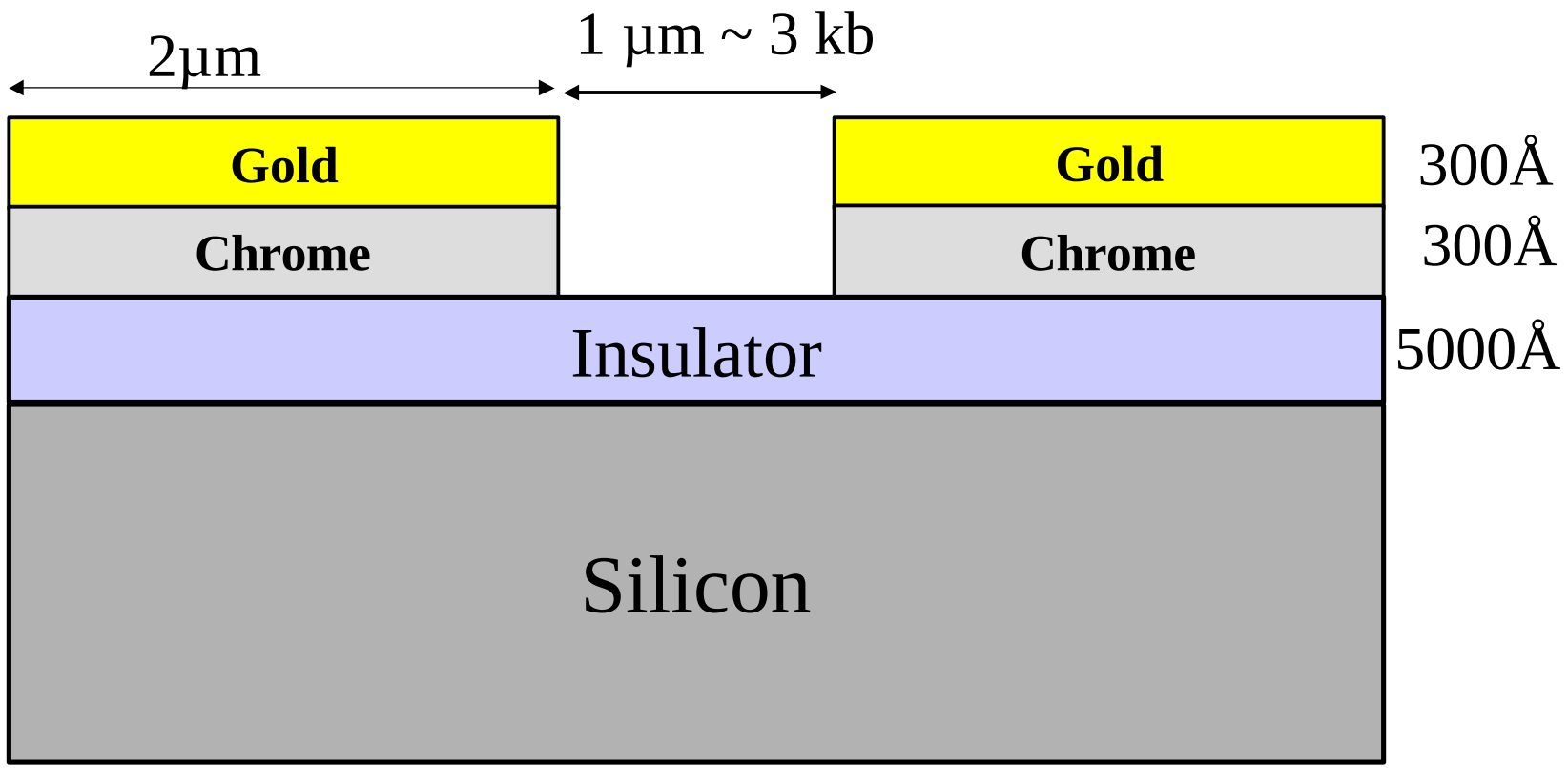
Sensor Cross Section

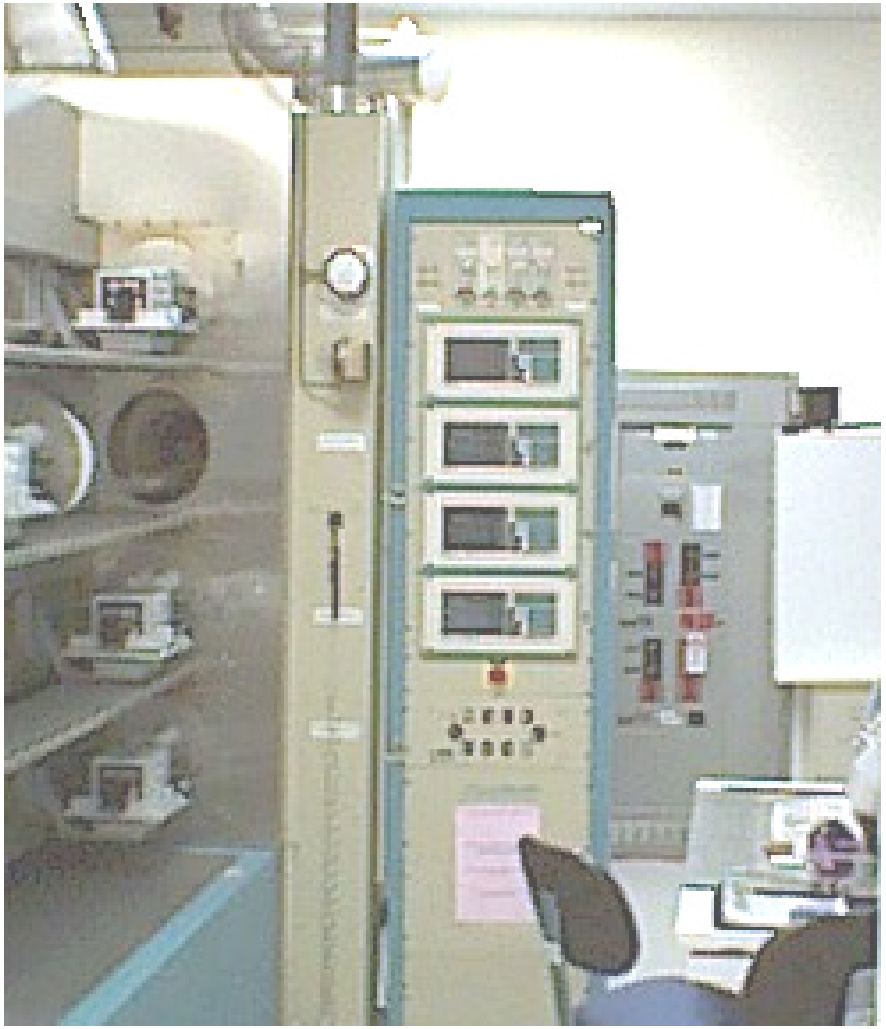


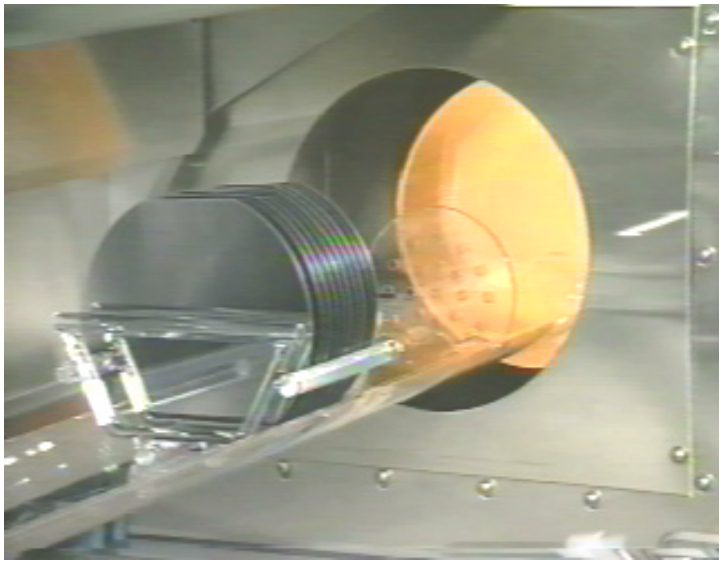
150 mm Wafer Fabrication Process

RCA Clean Wafers
Grow 5000 Å Oxide
Deposit Chrome
Deposit Gold
Photolithography
Etch Gold
Etch Chrome
Strip Resist
Inspect and Test
Dice Wafer
Attach Probes
Ship to System Level Packaging

RCA Clean

Oxide Growth





5000 Å
Wet O2
1000 °C

Metal Deposition

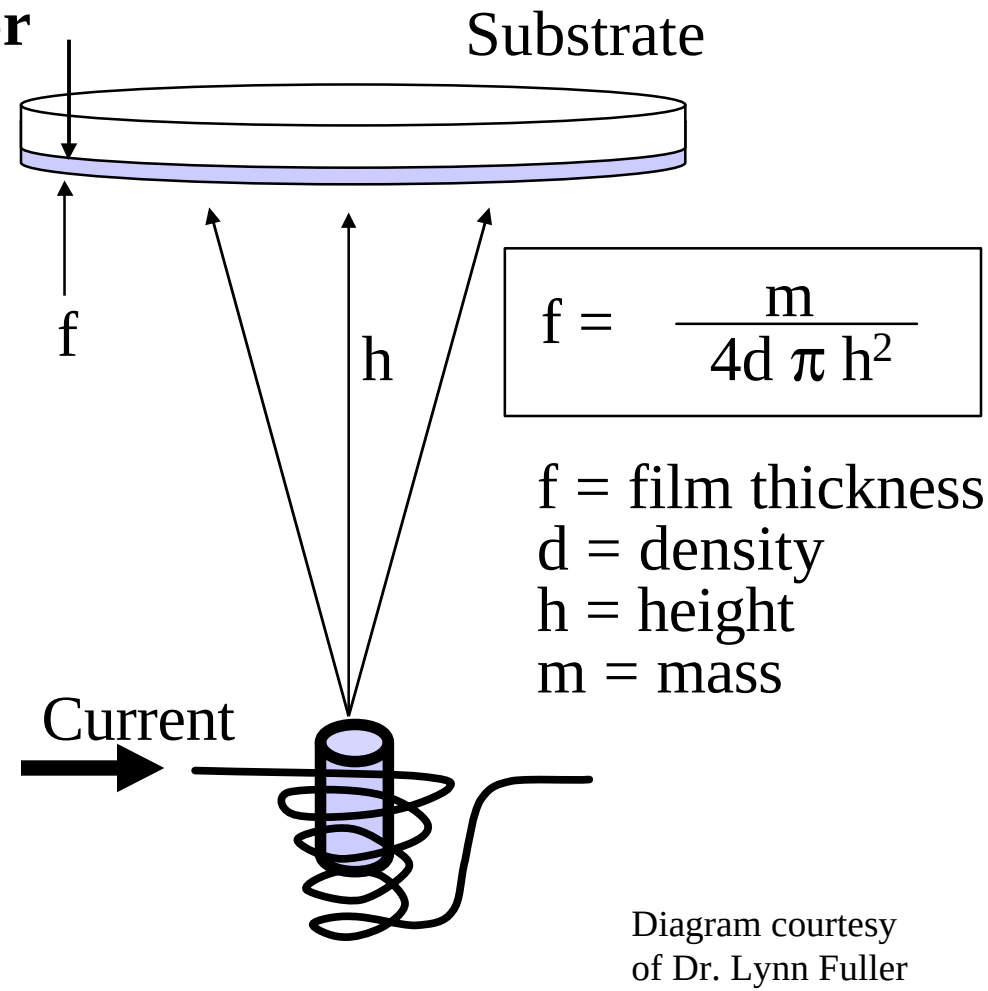
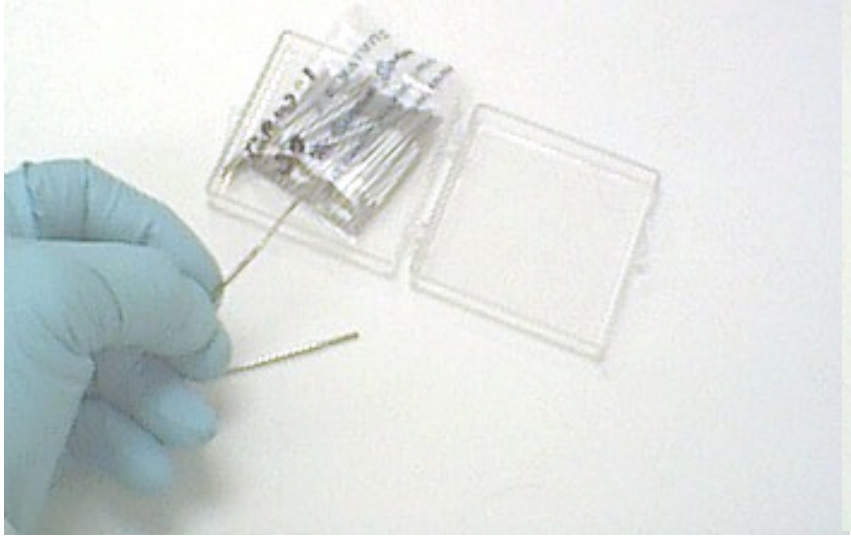


Diagram courtesy of Dr. Lynn Fuller

Evaporation Sources for Chrome and Gold

Chrome Coated Tungsten Rods

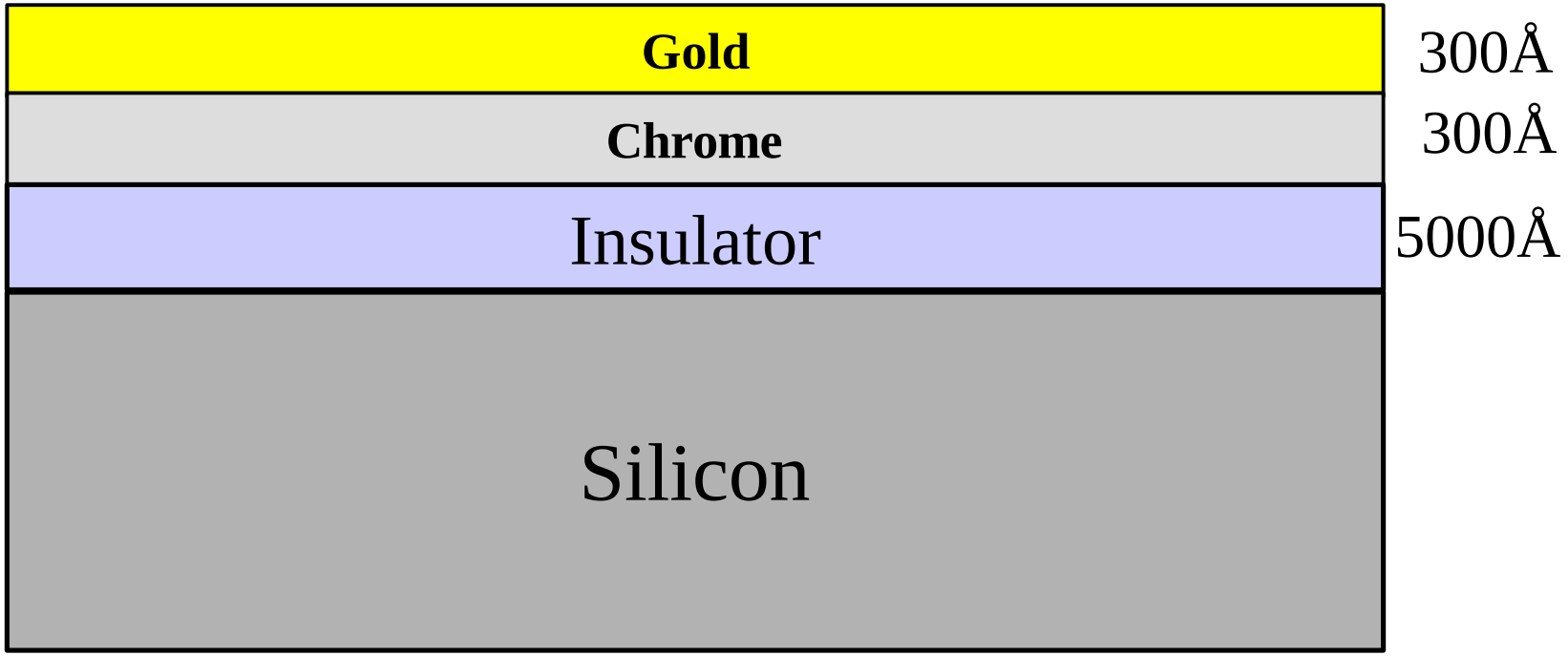


Dimpled Boats for Gold

Sputtering of Other Electrode Metals

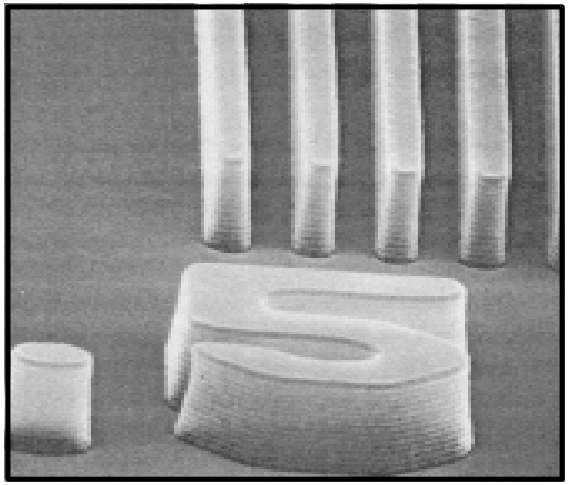
CVC 601 Sputtering Tool

Insulating Layer, Chromium and Gold



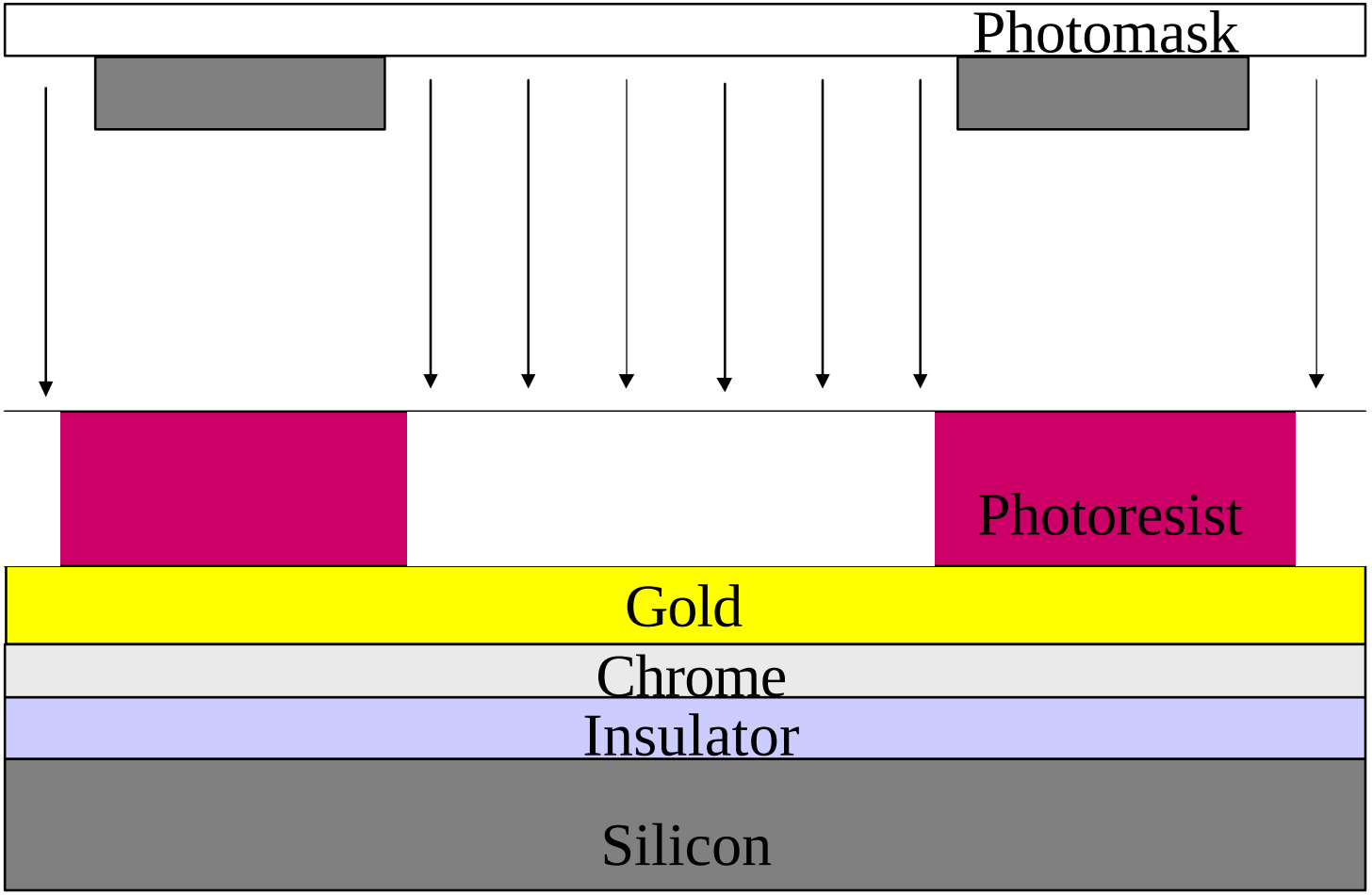
Canon FPA-2000 i1 Stepper

i-Line Stepper $\lambda = 365$ nm
NA = 0.52, $\sigma = 0.6$
Resolution = $0.7\ \lambda / NA = \sim 0.5\ \mu m$
20 x 20 mm Field Size
Depth of Focus = $k_2\ \lambda/(NA)^2 = 0.8 \mu m$



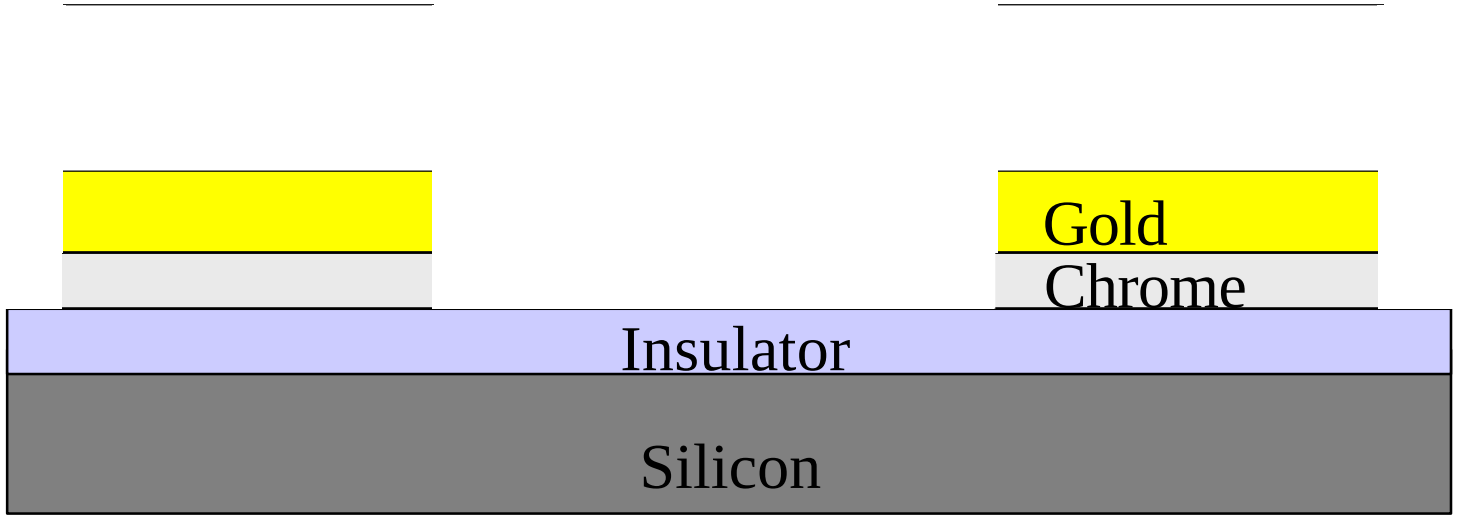
SSI Coat and Develop Track

Photolithography



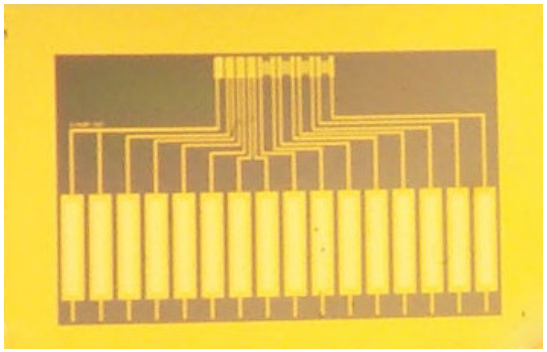
Etching of Gold and Chrome

- Gold etch (Transene TFA Gold Etchant): Potassium Iodide.
- Chrome etch (Cyantek CE8002-A): Ceric Ammonia and Acetic Acid.
- Strip resist either with plasma asher or with chemicals



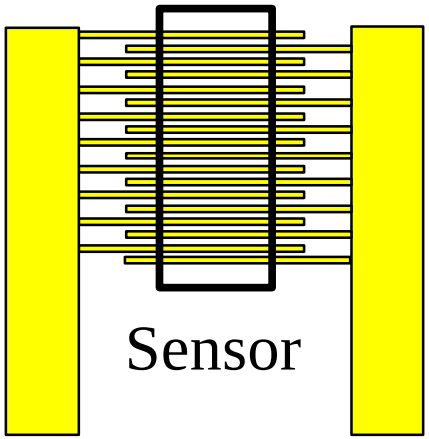
Branson Plasma Asher

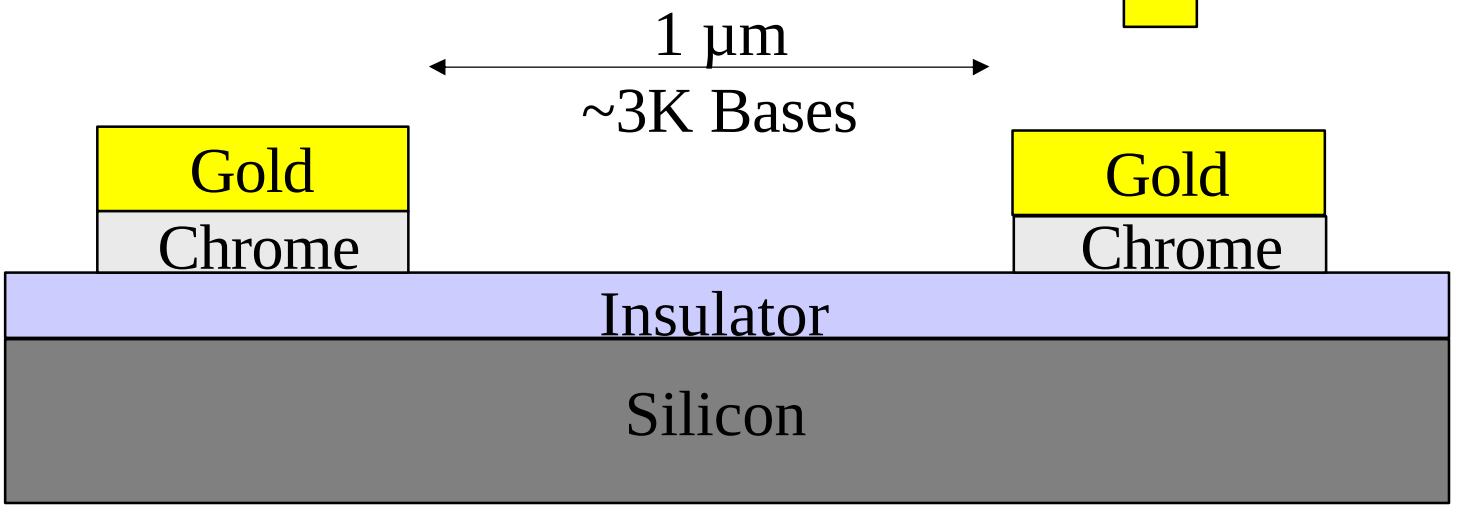
Interdigitated Gold Electrodes



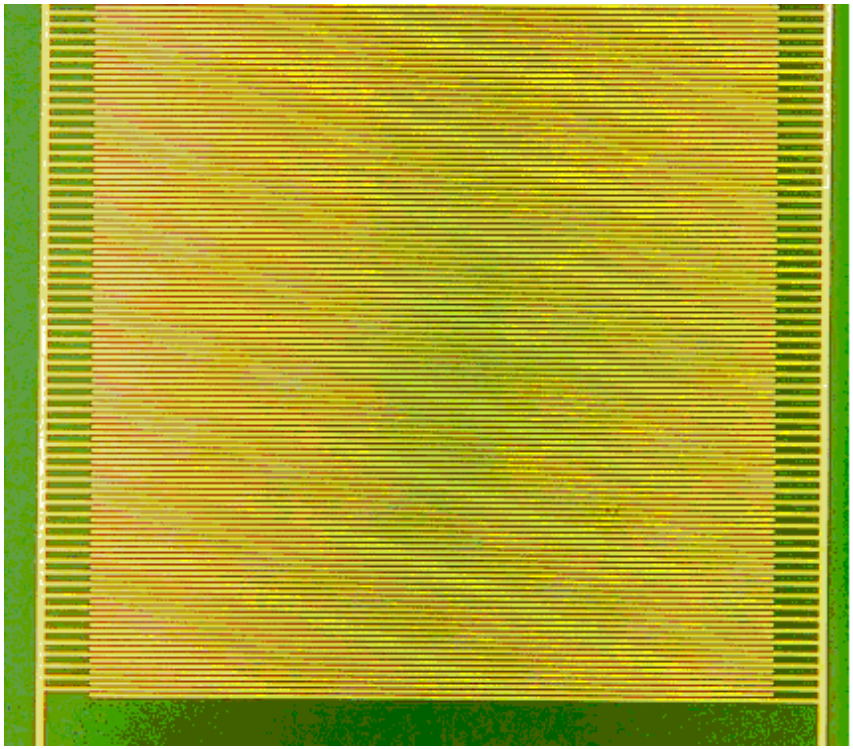
Chip with 8 Sensors

Top view

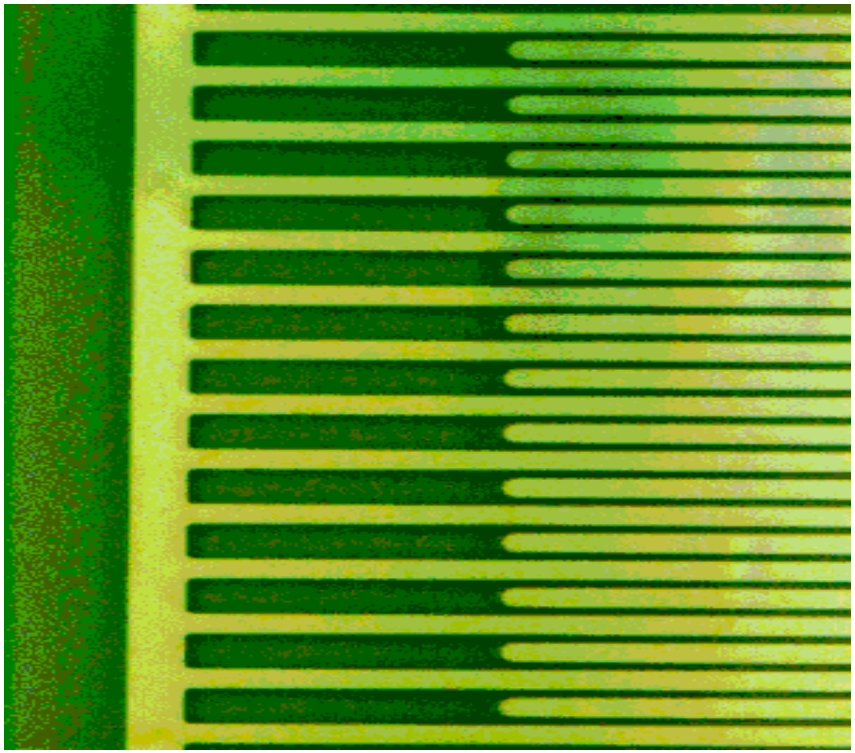




Picture of Interdigitated Electrodes



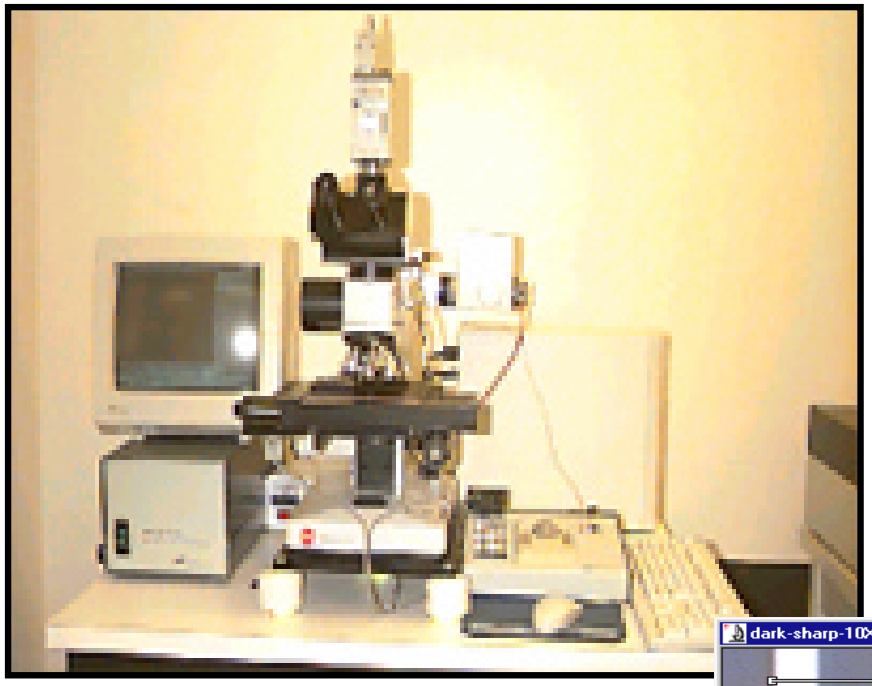
20X

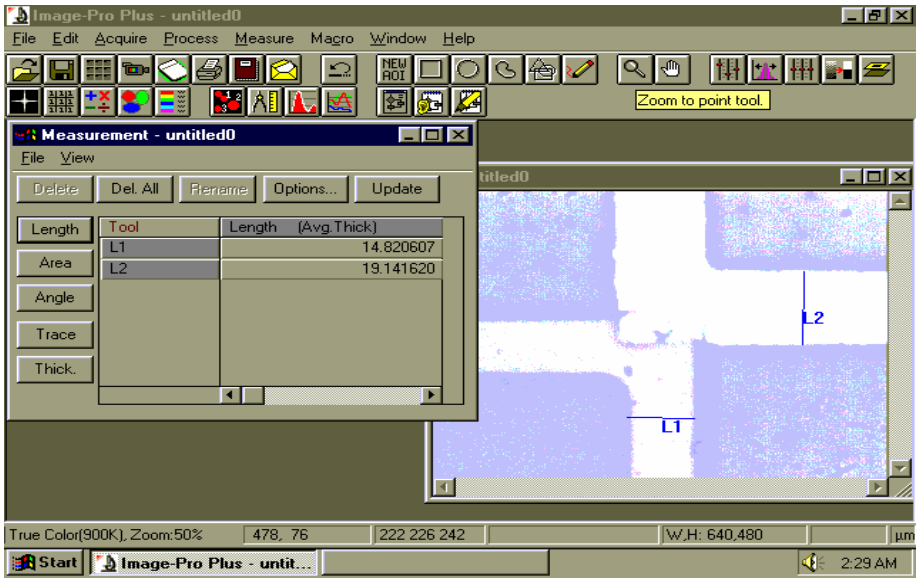


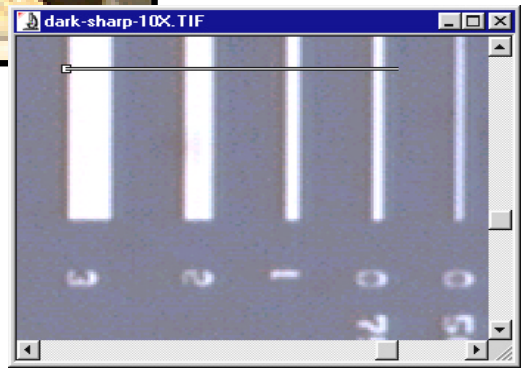
150X

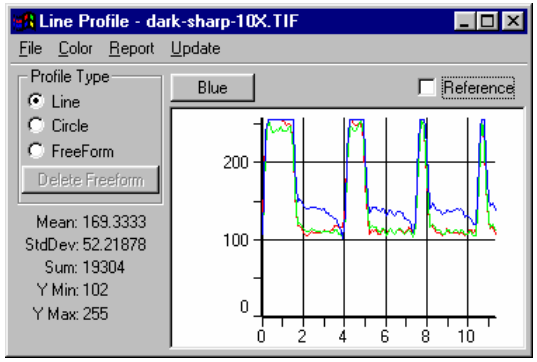
Sensors

Leitz Inspection Microscope



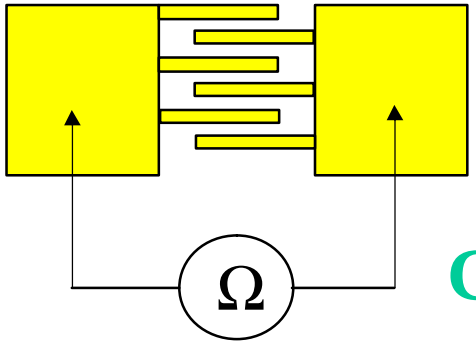






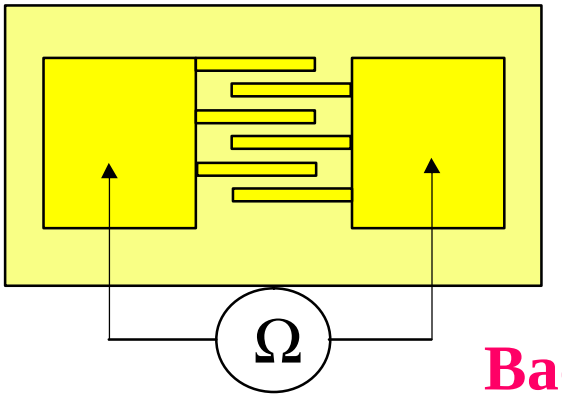
Testing

•Each device is tested both visually and electrically.



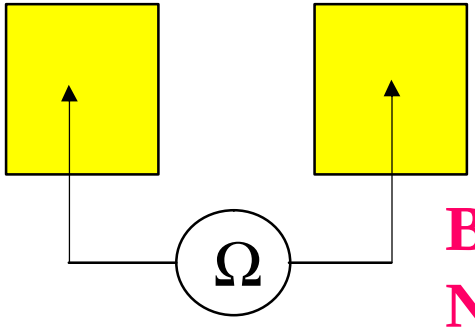
R = ∞

Good



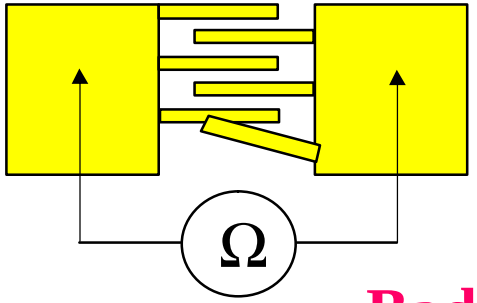
R = low

Bad
(Under Etch)



R = ∞

Bad
No Electrodes
(Over Etch)



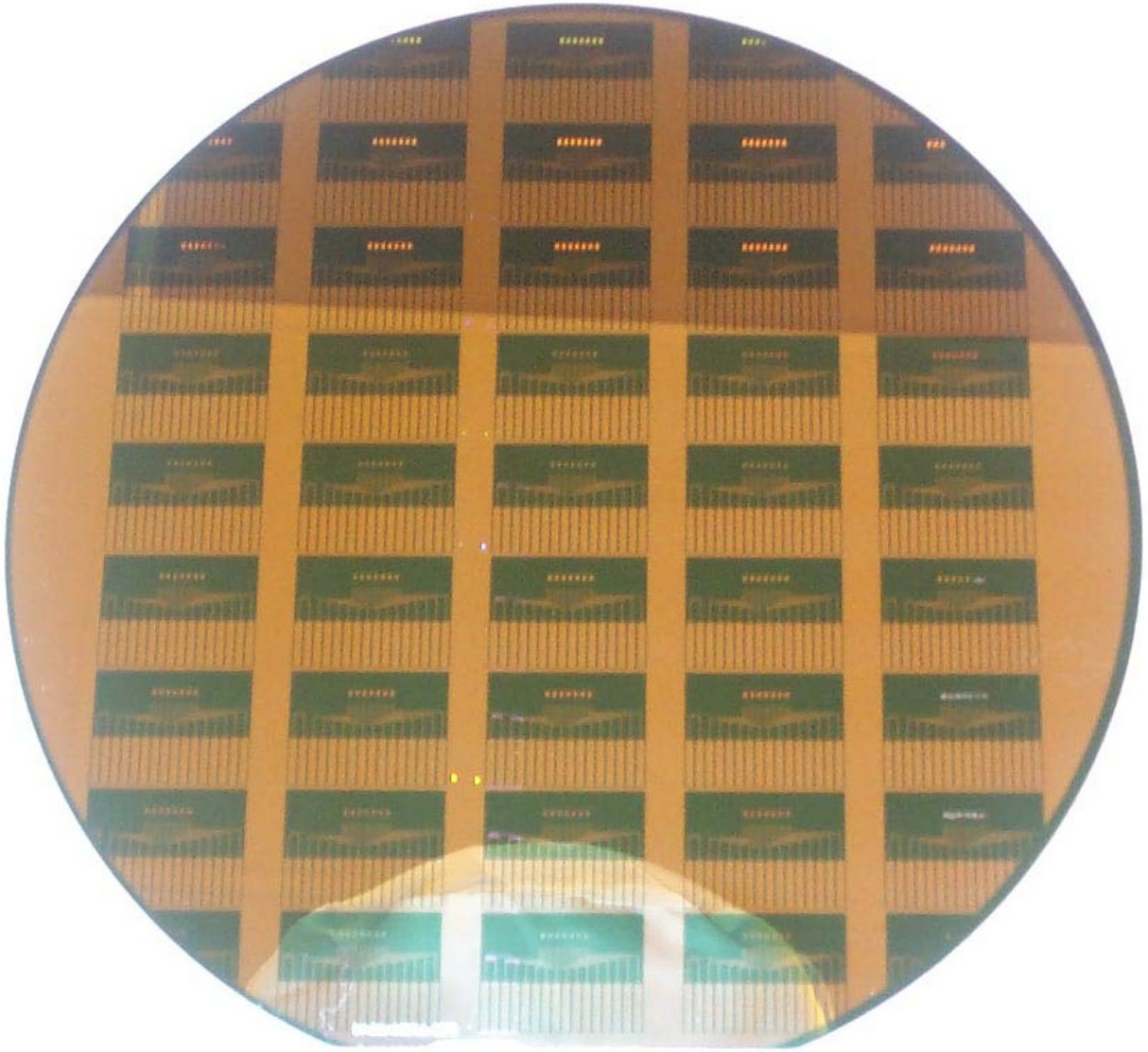
R = low

Bad
(Poor Adhesion)

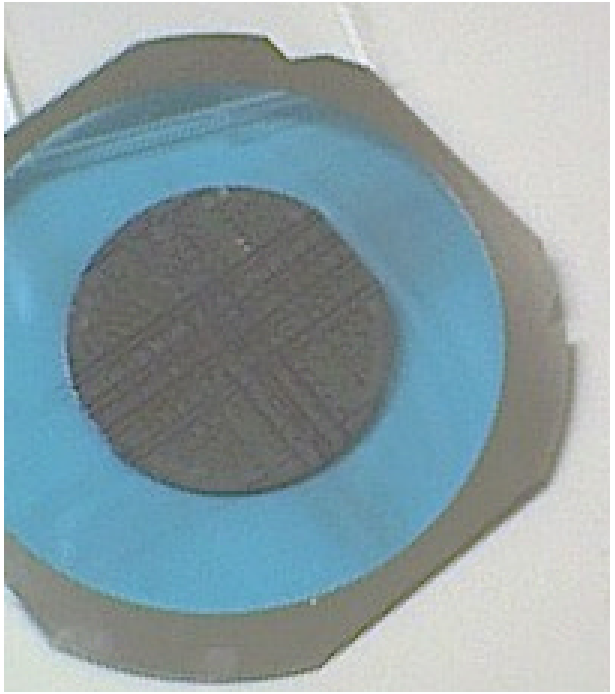
Yield

- Define die yield as percentage of die with all sensors working.
- Initial die yields were less than 2% giving only 1 working die/wafer
- Experimentation with etch time, etch technique and gold thickness brought die yield up to around 10%, only 5 working die per wafer
- Experimentation with chrome thickness brought die yields above 50%, only 26 working die per wafer
- Experience over 3 months increased the yield to over 80% giving 41 working die per wafer
- Reduction in die size can give us 100 working die per wafer

Completed Wafer



K&S Wafer Saw



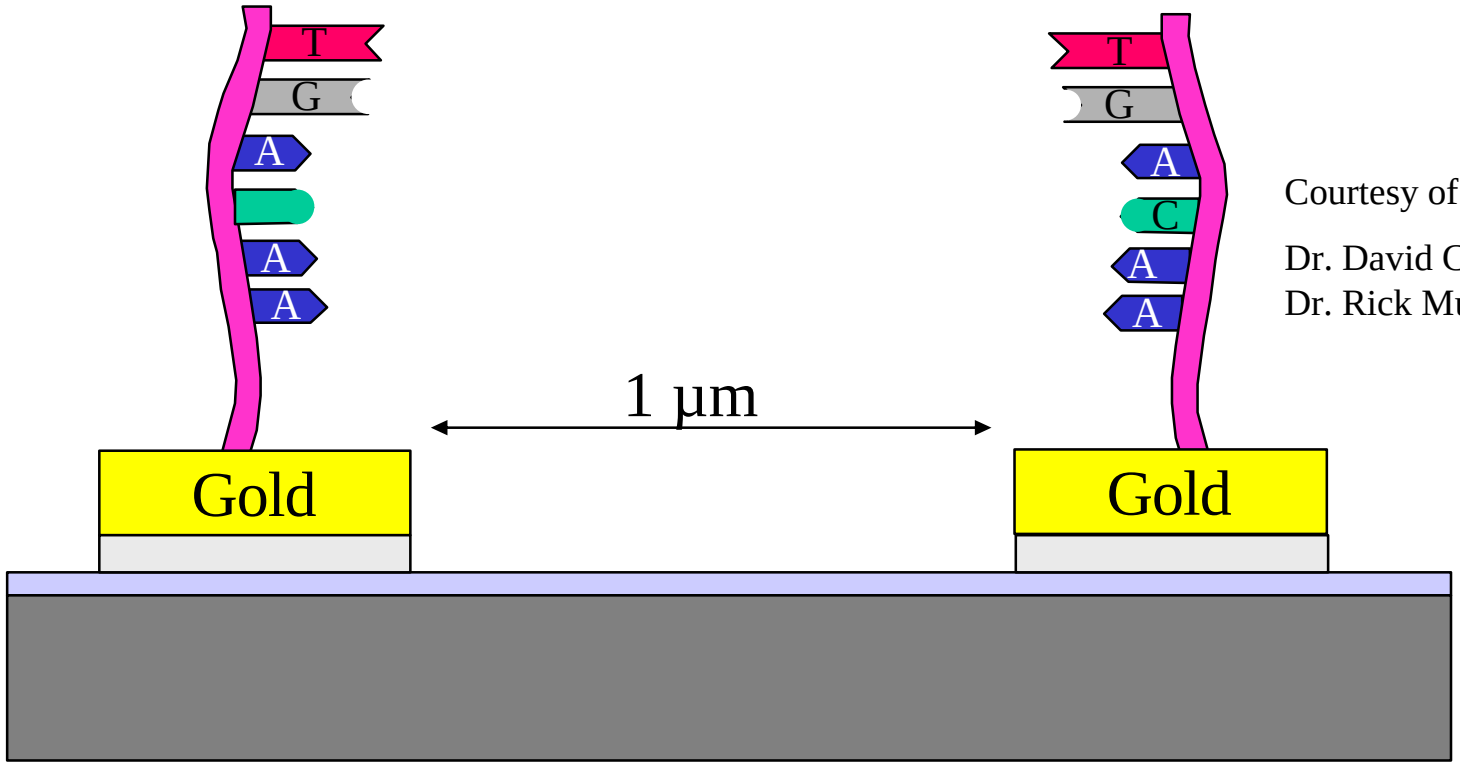
Probe Design

	Probe Name	Probe Sequences
Bacillus subtilis 5-7 kb fragment	ComP	AAG CCC TGA CTC TCT CCT TAA TGC CAA A – hexamethyl thiol
	yuxH	TAC ACG TTC TCT TCG CTT TCT CTA TAA – hexamethyl thiol

Many different probes can test for different DNA molecule fragments of the same bacteria or virus. These probes are purchased from commercial sources.

So with a microchip with 8 sensors we could do 8 different tests for one bacteria or we could repeat one test 8 times for one bacteria or we could do one test each for 8 different bacteria or virus

Probes Attached to Gold Electrode

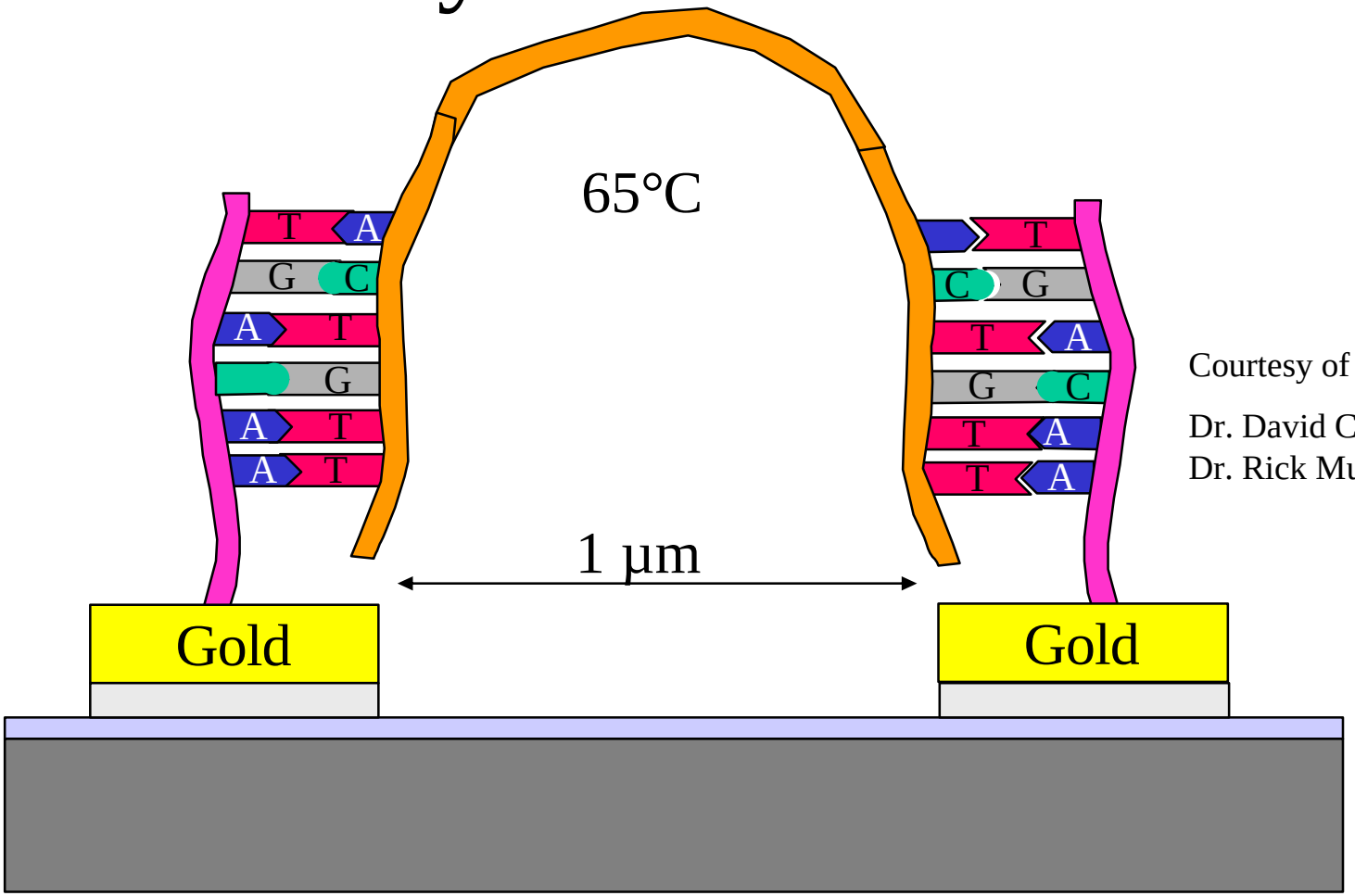


Courtesy of

Dr. David Chafin and
Dr. Rick Murante

Probes are attached to the gold surface using standard thiol chemistry (Bain, 1989, Herne, 1997, Kelley 1997, Takenaka, 2000, and others

Hybridization



Courtesy of

Dr. David Chafin and
Dr. Rick Murante

Because the DNA is hybridized to a probe DNA with 15 matching base pairs, the probability that the attached DNA is the desired DNA is one billion to one or better. (i.e. 4^{15})

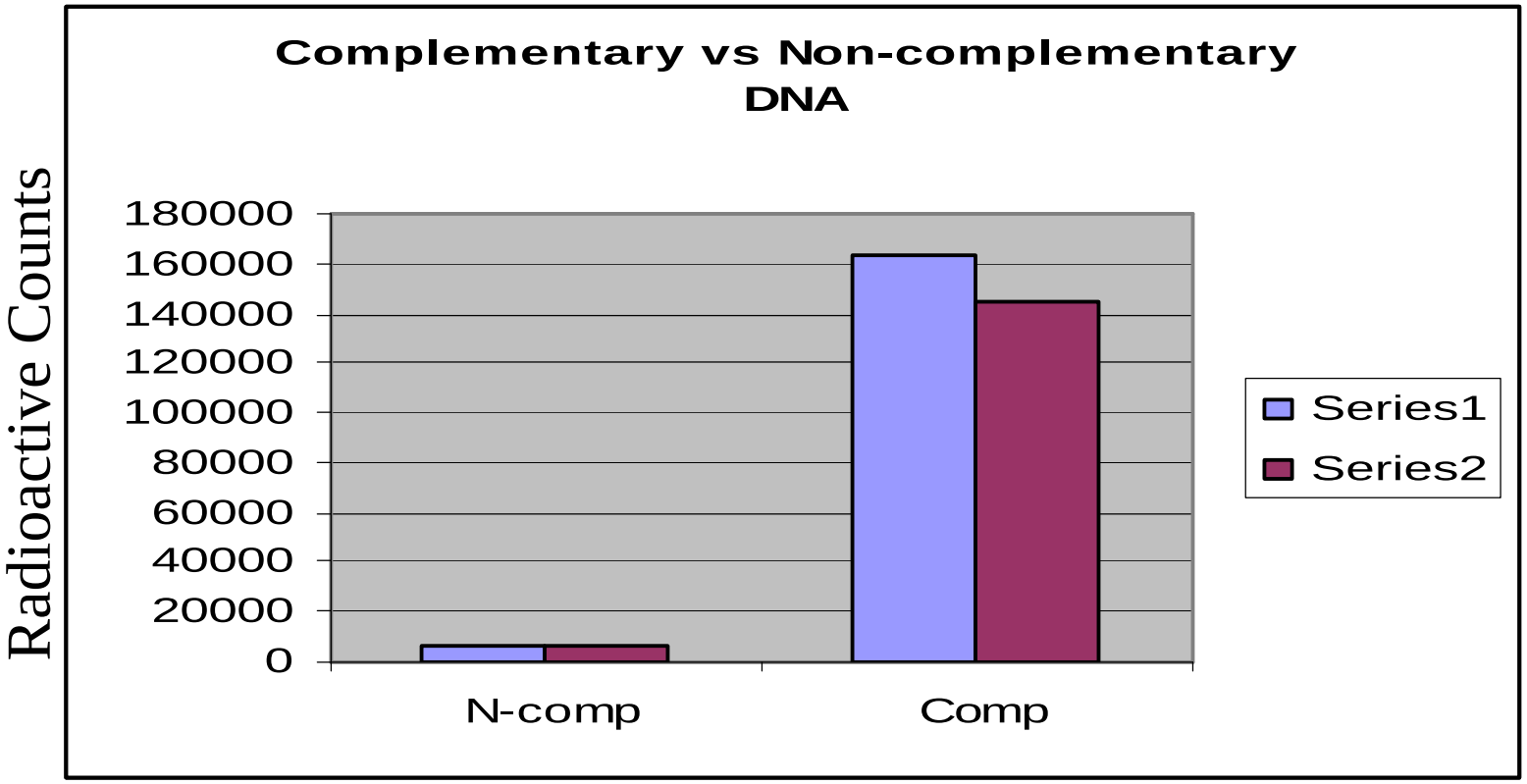
Verification of Hybridization

A DNA Molecule is only ~2 nm in diameter or 0.002 µm
it can not be seen with an optical microscope or with any Scanning Electron Microscope (SEM)

Radioactive Labeled DNA
(we could measure the difference between complementary and non-complementary DNA)

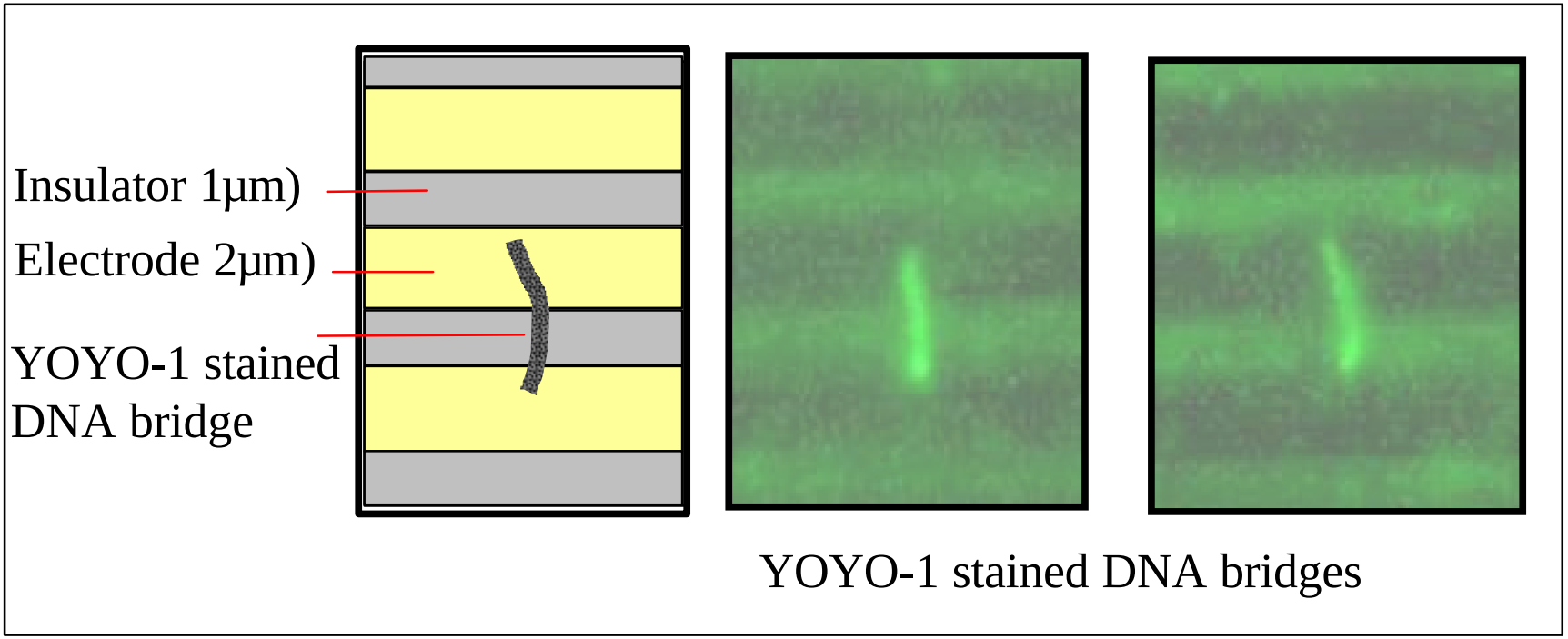
Fluorescent Labeled DNA and Fluorescence Microscopy
(we could see the Fluorescence from DNA attached to the probes)

Radioactive DNA Test Results



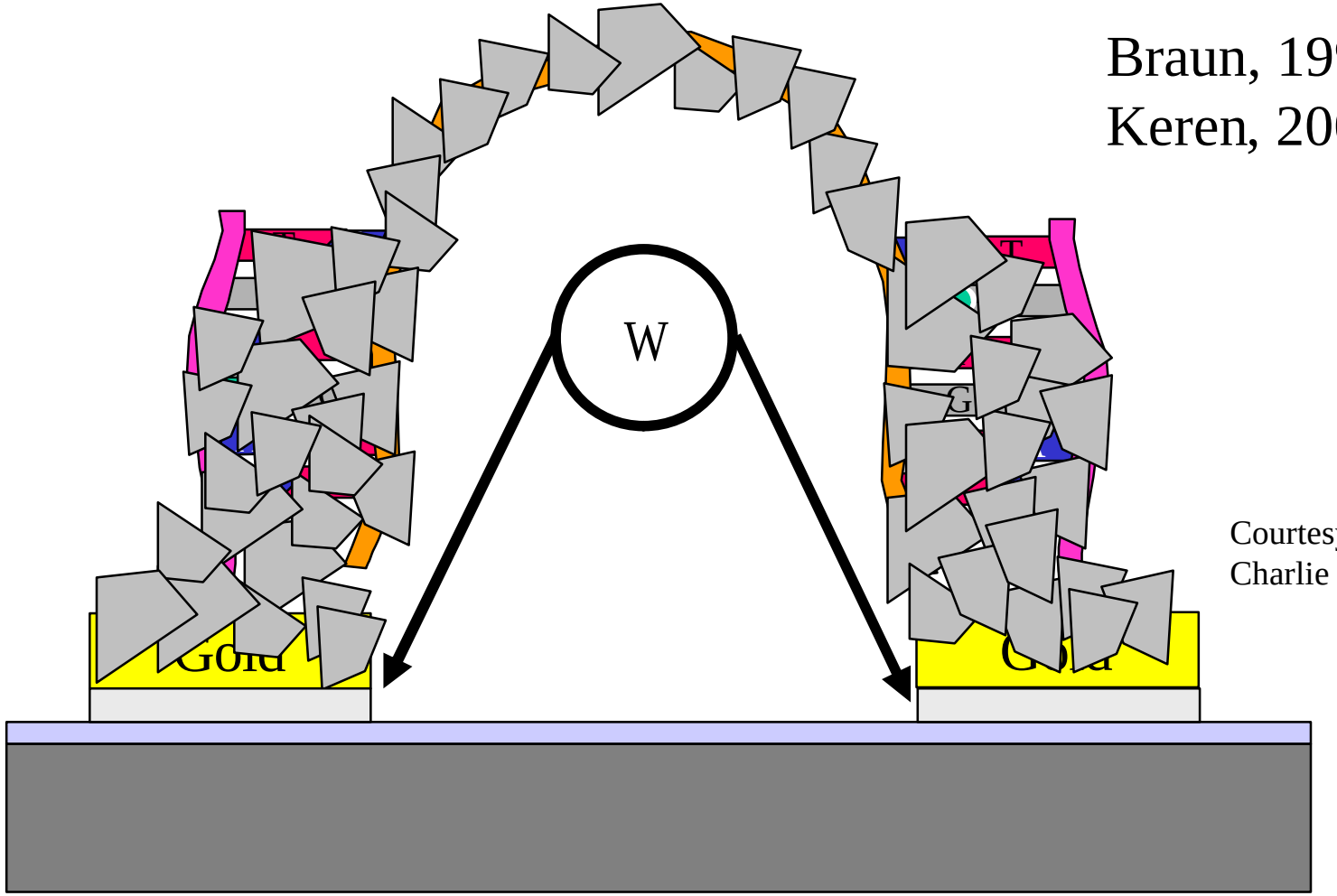
Microchips presented with complementary DNA showed higher radioactive counts than non-complementary DNA.

Still Photographs of Fluorescent Labeled DNA



Movie of Fluorescence Labelecd DNA Bridge

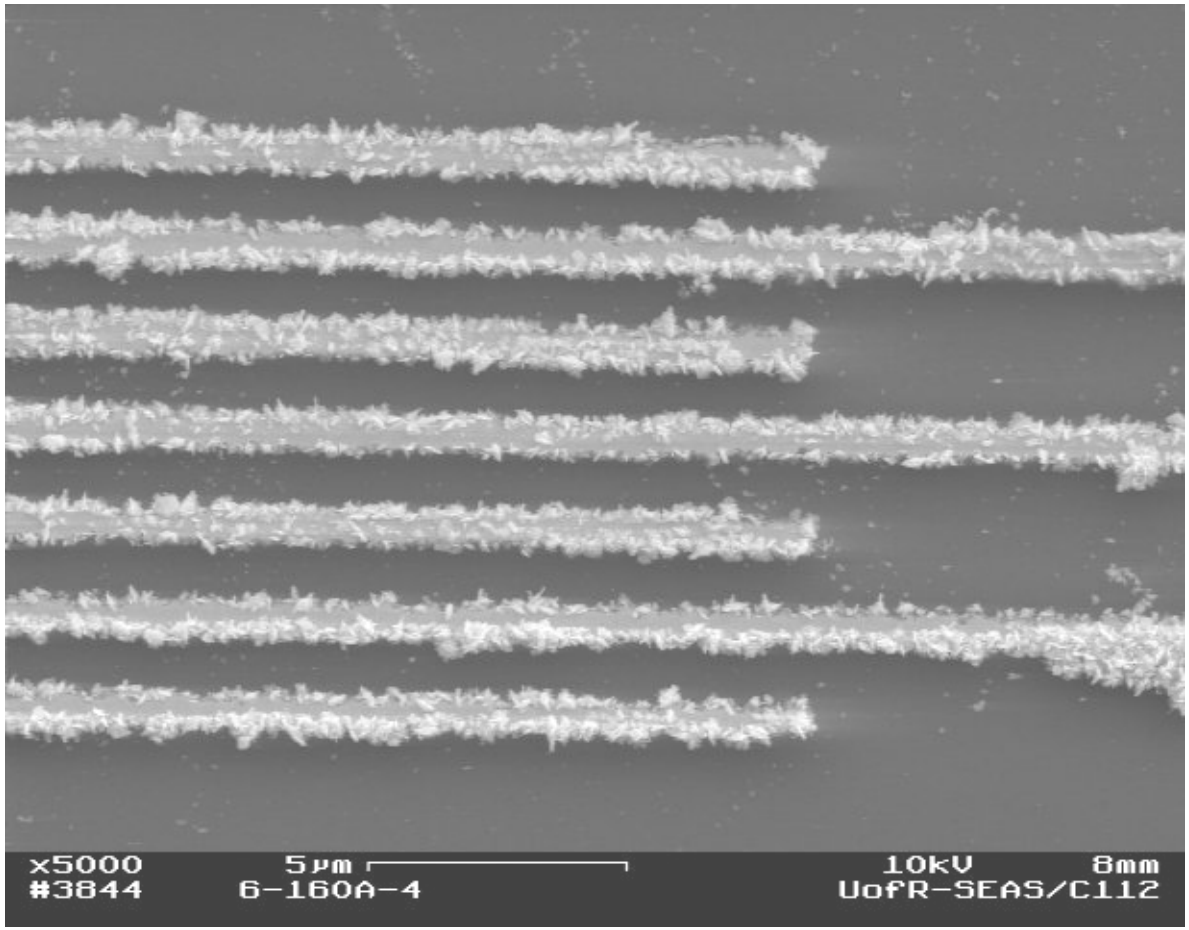
Coating of DNA Bridge with Metal



Braun, 1998
Keren, 2002

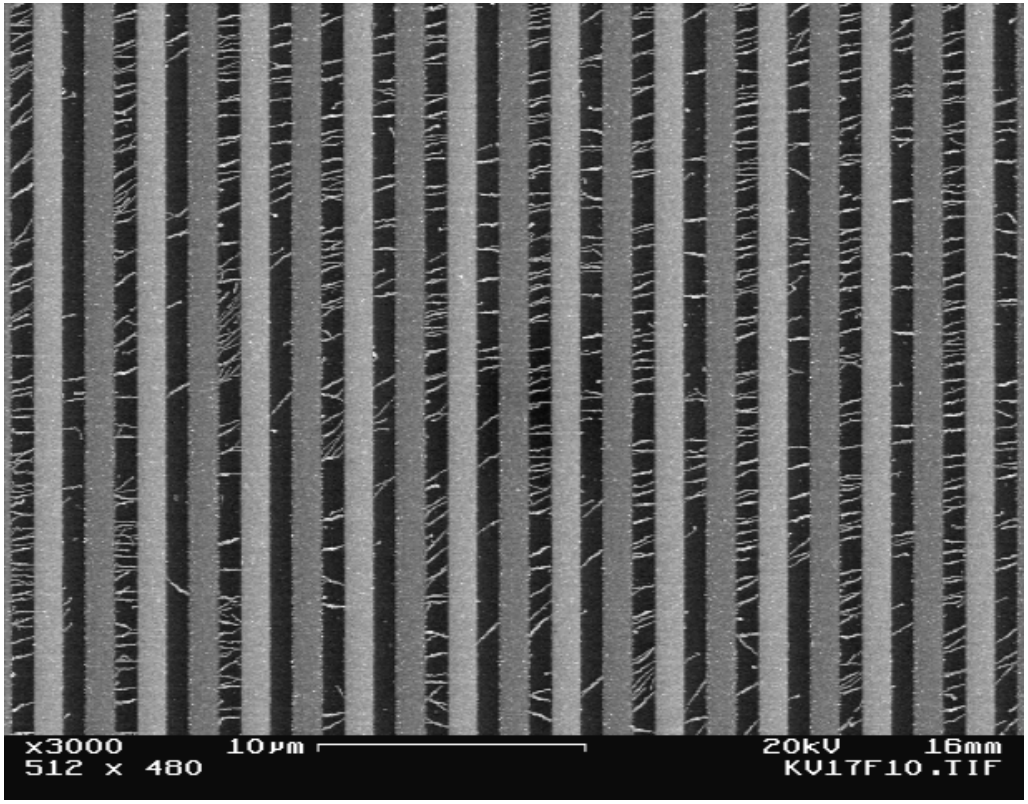
Courtesy of Dr.
Charlie De Boer

Silver On Probes (No DNA)



Courtesy of
Dr. Charlie De Boer

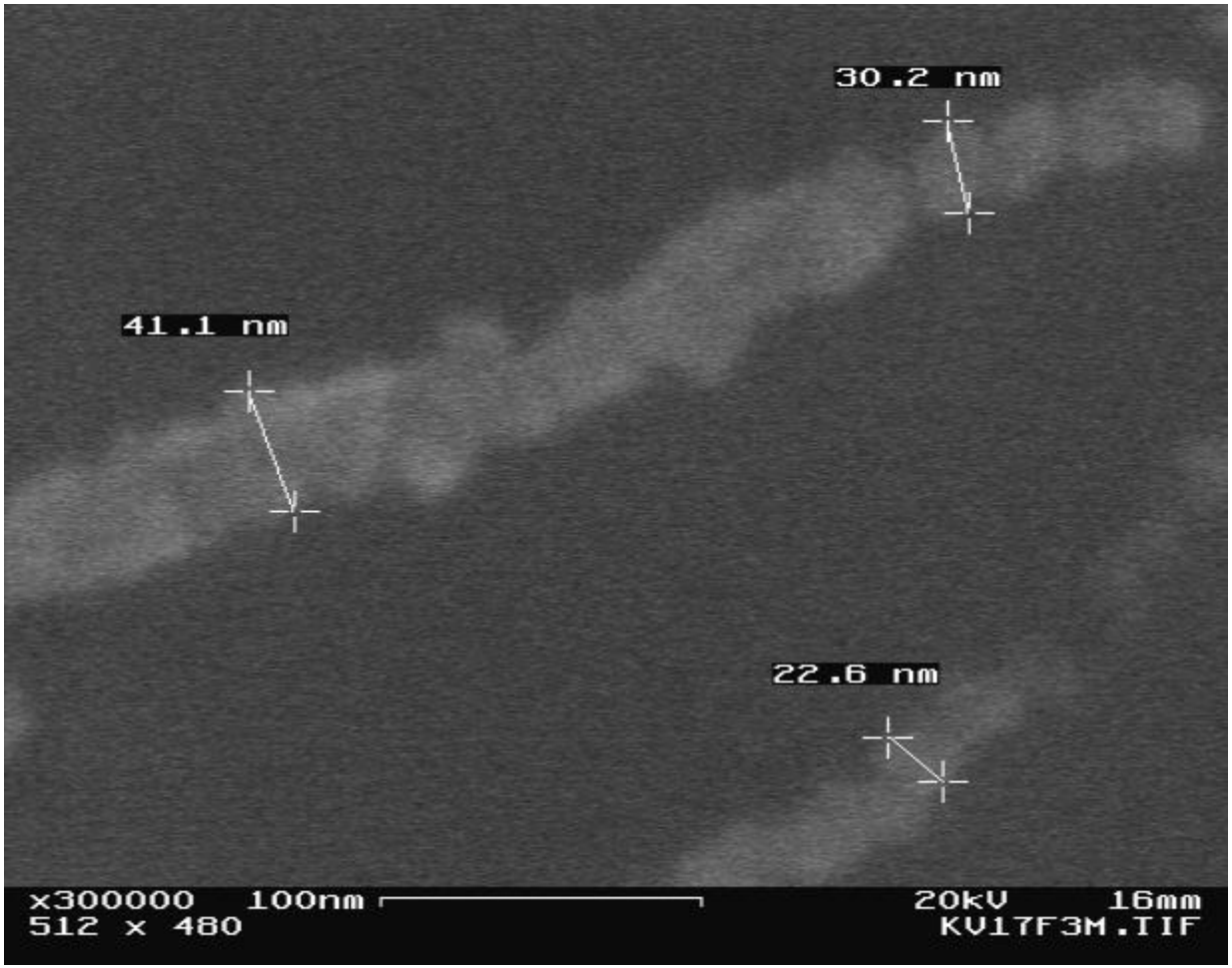
Silver-Coated DNA



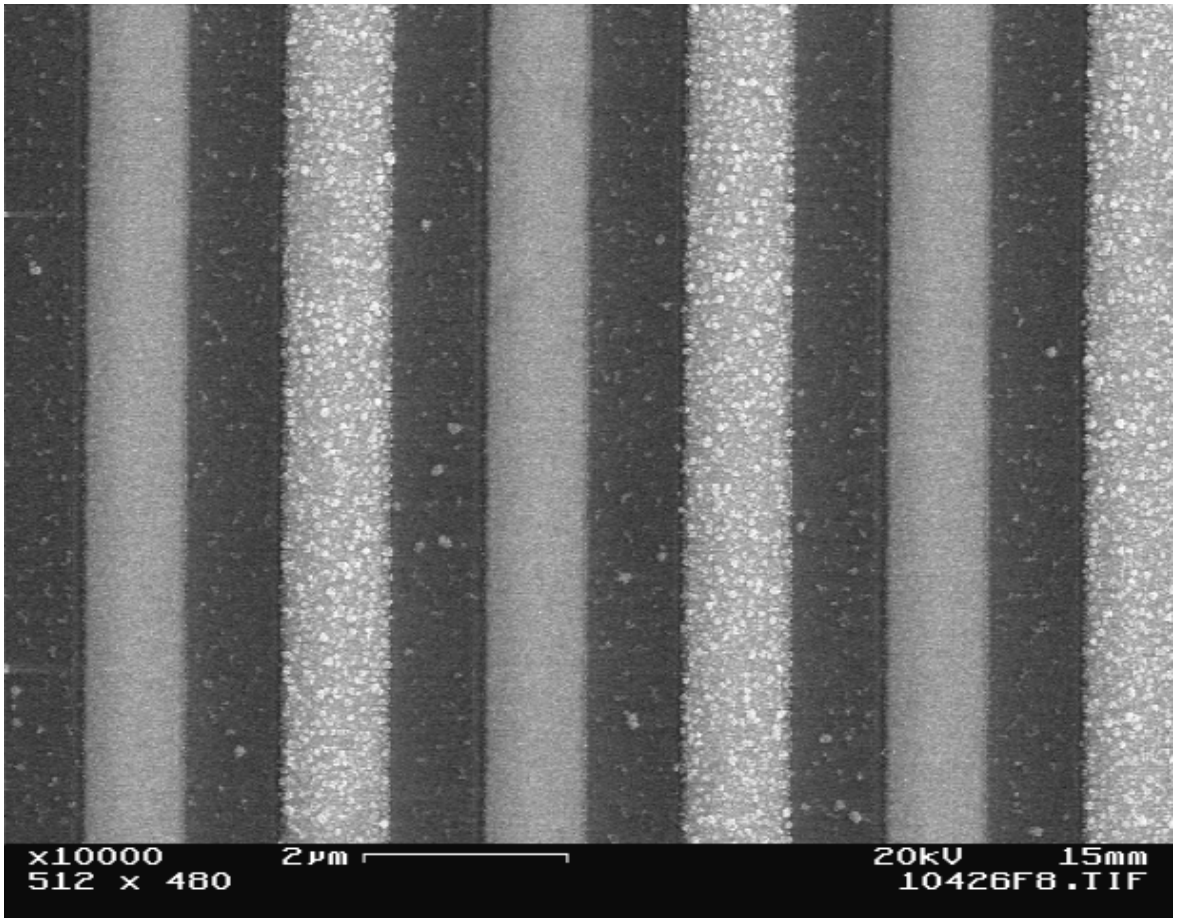
Courtesy of
Dr. Charlie De Boer

The picture above shows two metal lines 1 micrometer apart with many DNA bridges coated with metal. The measured electrical resistance between the metal conductors dropped from infinity to ~1 thousand ohms, making electrical readout of the detection of a DNA very simple.

High Magnification Image of Metal Coated DNA



Non Complementary DNA



Measuring the Resistance of the Metal Coated DNA Bridge

Problem: The metal coated DNA bridge is very fragile and can not be measure reliably with an ohm meter. The sensors are wet and false shorts can be created (electromigration) by applying a few volts for a short time (few seconds).

A custom computer controlled (Lab View) measurement system was created to measure with the lowest possible voltage and complete the measurement in a short time (few msec)

Auto Ranging Pulsed Resistance Measurement

First resistor

Apply 200 µV, 20 µs if R~20 ohms find I = 10 µA

If I > 1 µA or V> 2 V, then stop calculate R=V/I

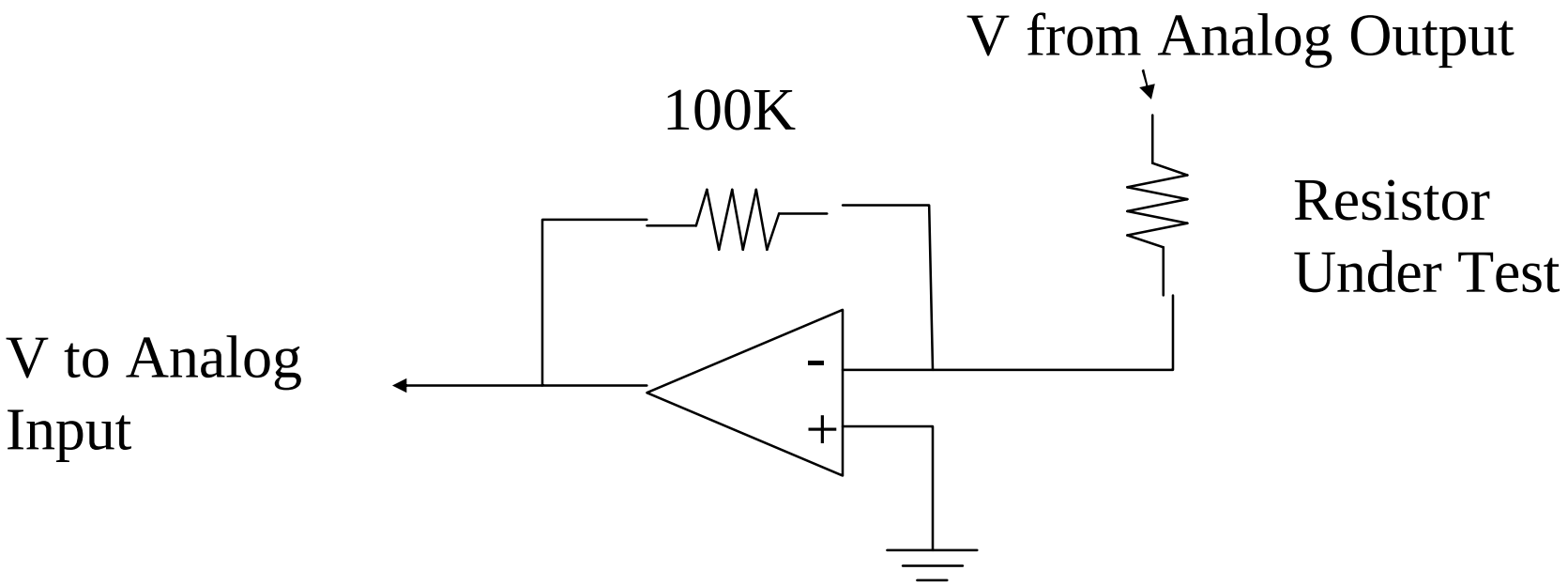
Next resistor

Apply 200 µV, 20 µs	if R~2 MEG ohms	find I = 100 pA
Apply 2 mV	if R~2 MEG ohms	find I = 1 nA
Apply 20 mV	if R~2 MEG ohms	find I = 10 nA
Apply 200 mV	if R~2 MEG ohms	find I = 100 nA
Apply 2 V	if R~2 MEG ohms	find I = 1µA

If I> 1 µA or V> 2 V, then stop calculate R=V/I

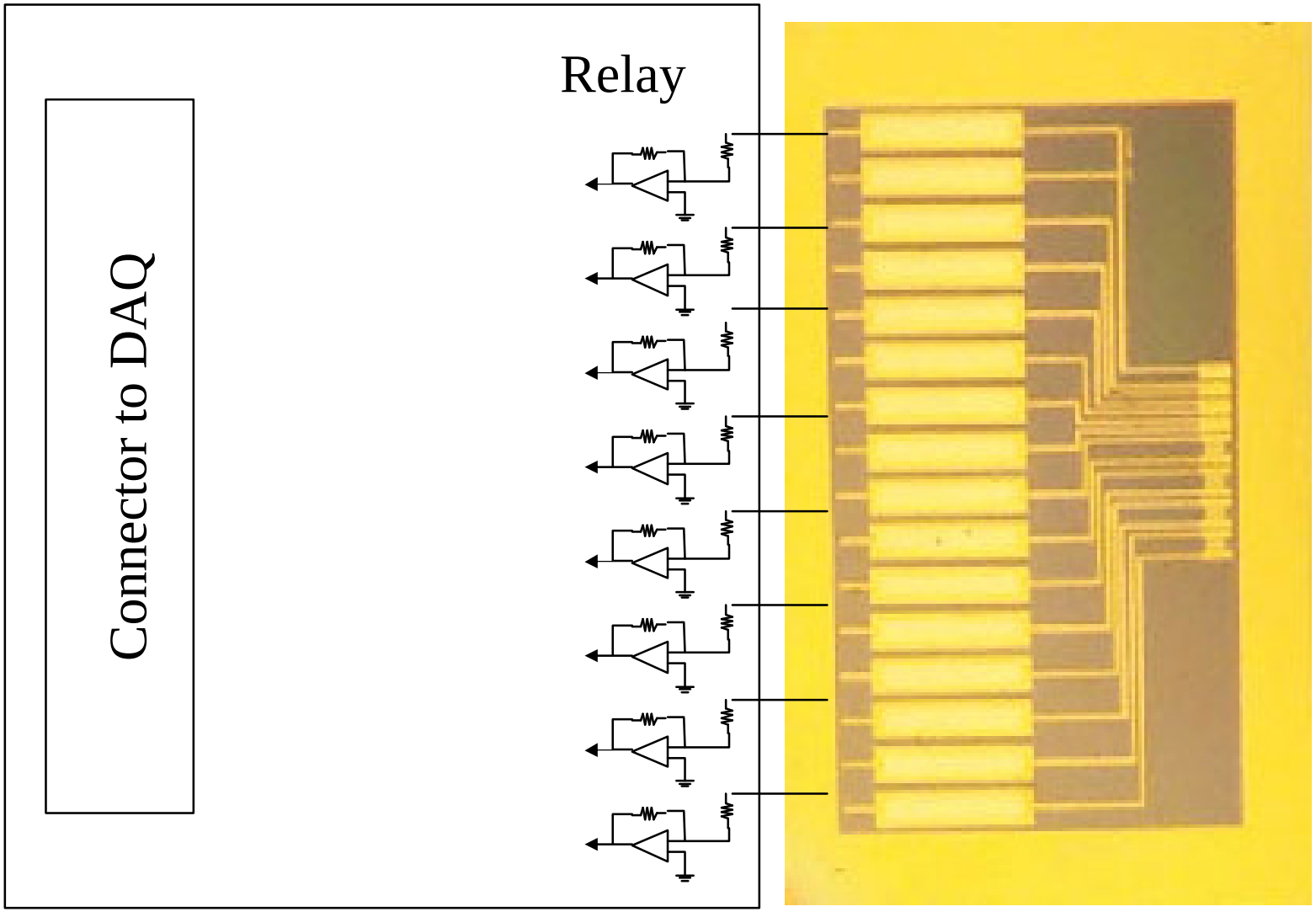
Next resistor

Signal Conditioning
(for each of the eight resistors)

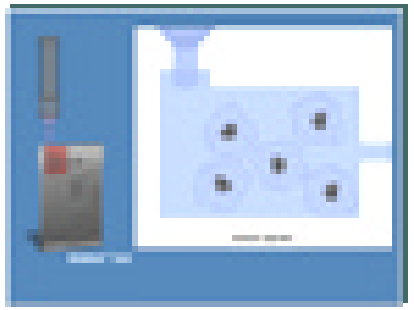


I to V conversion and Current Limiting

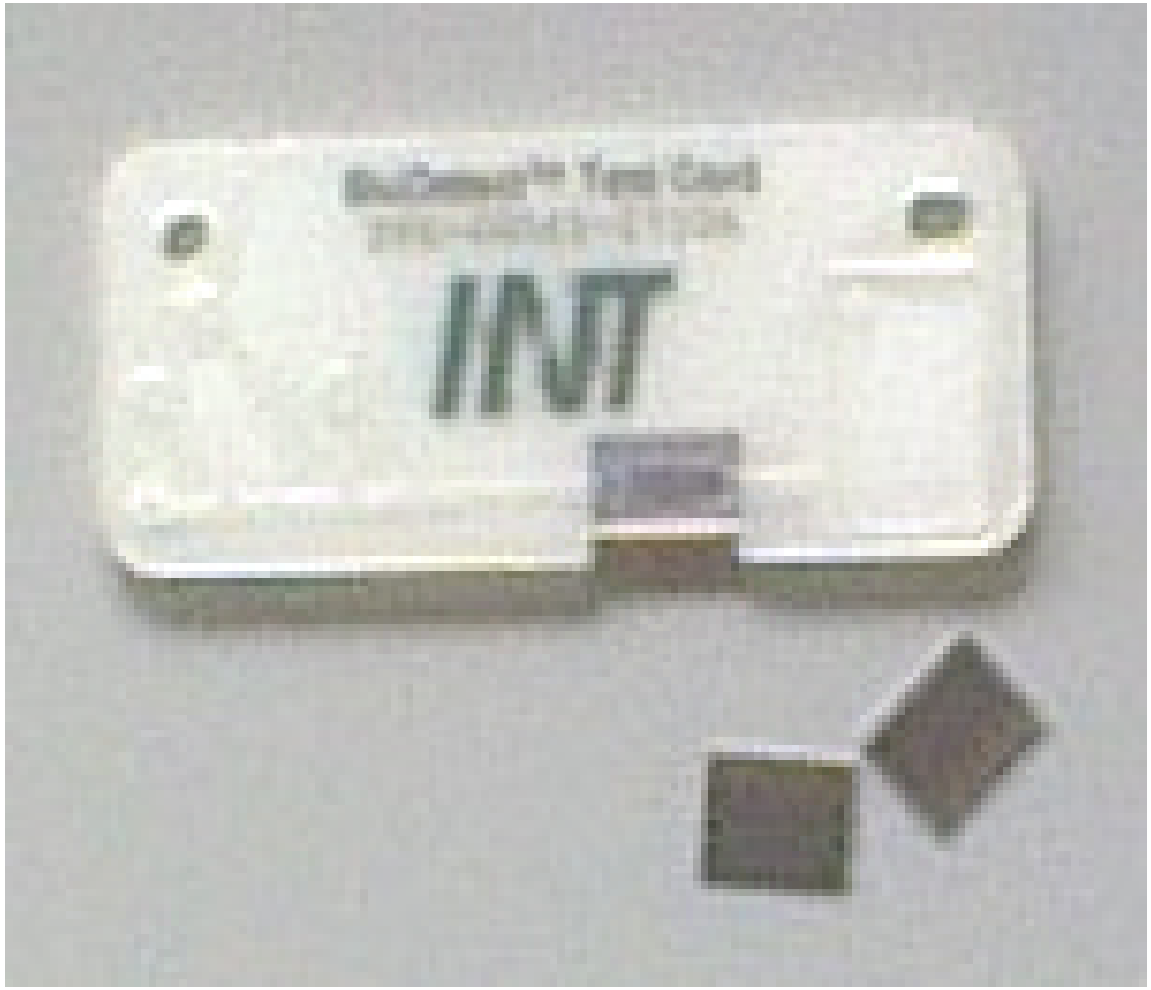
Custom Interface Board



System Design



INT Bio-Detect Card



Summary

Chip was Designed and Fabricated
Probes were Attached
Specific DNA was Hybridized, Visualized
DNA was Silver Coated
Electrical Measurements were Made
Desk Size System Exists
Miniaturization to a Laptop Computer Size System
is in process

Acknowledgements

Special thanks to:

Integrated Nano-Technologies, Employees (Some are listed below)

- Dr. Michael Connolly, President and CEO
- Dr. Charlie De Boer, Senior Research Scientist
- Dr. David Chafin, Director, R & D
- Dr. Rick Murante, Research Scientist
- Dr. Samina Jafri, Research Scientist
- Ms. Roberta Greco, Chemist
- Mr. Scott Seabridge, Electrical Engineer

AIMEE K. WILES staff photographer

Integrated Nano-Technologies' management team includes Director of Operations Pamela A. Duprey, front, Director of research David Chafin, left, Chief Financial Officer Emilio DiCataldo, back, and founder D. Michael Connolly.

DNA detectives

Local firm fuses electronics and genetics to create a new, faster way to diagnose serious disease.

BY STAFF WRITER
MICHAEL WENTZL

Imagine a hand-sized device that could tell a doctor, in just minutes, whether a patient has a strep infection and if the bacteria are resistant to specific antibiotics.

No lab. No cultures to grow. No waiting 24 to 48 hours.

Imagine if the same system, packaged in something like a Palm Pilot, could accurately and quickly detect hepatitis, sexually transmitted diseases, dangerous E.coli bacteria in water or food, biological agents on the battlefield or lethal anthrax.

At Integrated Nano-Technologies LLC in Henrietta, company executives believe they are close to producing what could be a revolutionary device.

"We think we have a big lead in this technology, but we have to get it to market before someone else we don't know about does," said D. Michael Connolly, company president.

The technology — based on electronic detection of DNA binding on a computer chip — has attracted the attention of the U.S. Army, which has a research and development agreement with the company.

DNA, PAGE 8E

Sensors using strands of DNA "search" for similar strands in the microscopic environment of a computer chip designed by Integrated Nano-Technologies. For a step-by-step explanation of the process, turn to Page 8E.

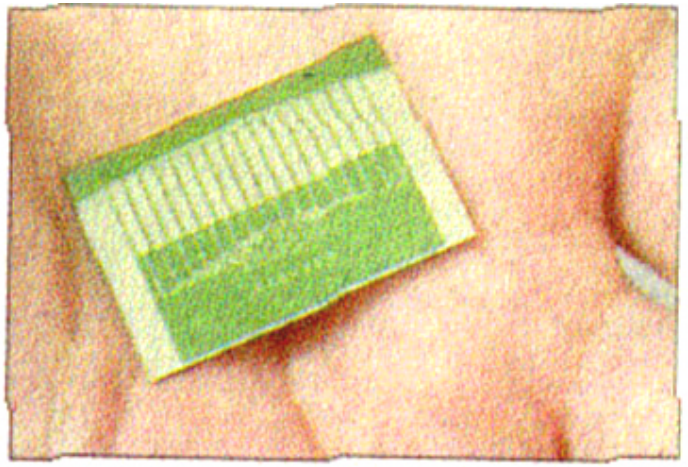
KEVIN M. SMITH staff artist

How it works

1 Use BioDetect System test card prepared with probes composed of DNA unique for suspect cells, which could be, for example, hepatitis virus, anthrax bacteria or an unknown fungus.

2 Collect small sample of suspect cells. Suspend cells in liquid chemical that breaks open cell walls, releasing DNA, a molecule in cells carrying the genetic information that determines the structure and characteristics of an organism.

3 Inject cells in liquid into test card where it is pushed through a filter that removes most particles larger than DNA. Liquid then passes through a tube that shreds DNA from suspect cells into pieces.



INT's sensor chip

4 As liquid passes over probes, DNA present from suspect cells binds to a complementary DNA probe, which sits on circuits of a computer chip.

5 When complementary DNA bind, a change in electrical properties occurs and is detected by a sensor. Customized software reads the sensor and converts the information to a diagnosis.

DNA

FROM PAGE 1E

INT has raised enough money from private investors to support doubling the number of its employees to 40 by the end of the year, primarily by hiring molecular biologists, chemists, system engineers and computer scientists.

INT plans to launch the first generation of its BioDetect System, a device about as big as a shoebox, by the middle of 2003. Getting the device to a size easily held in the hand, Connolly said, is a matter of engineering and design that could take just another year.

"The technology is to a point now where not much more invention is needed," said Connolly, a 37-year-old patent lawyer who also has a doctorate in molecular biology.

Independent evaluation of the technology is difficult because INT, carefully avoiding the limelight since it was founded two years ago, has not revealed it to many people. Connolly and the company have received two patents on the technology and applied for 20 additional patents.

Connolly agreed to discuss the company only after the second patent was awarded this month.

"From what I know of the technology, it is exciting, unique and possibly revolutionary," said Donald Boyd, a software engineering expert and emeritus provost at Rochester Institute of Technology, who has overseen collaboration between the college and INT.

Thomas Flaten, an analyst who follows pharmaceutical and biotechnology instrument companies for RBC Capital Markets in Minneapolis, said there would be an instant market for INT's product.

"Labs are always looking for a better test that's more accurate, more sensitive, quicker and requires less user intervention," said Flaten, who has not reviewed INT's technology. "But this is not an easy road. They're not alone. They'll have to gain acceptance. Just because your technology is cool doesn't mean you won't have an uphill struggle."

The target of INT's technology is DNA, a molecule in cells carrying the genetic information that determines the structure and characteristics of an organism.

Take as an example a test for strep throat, a bacterial infection.

In INT's system, probes composed of DNA with unique codes for strep would be placed on circuits on a computer chip, Connolly explained. Other probes could have a sequence specific for genes that would reveal resistance to an antibiotic.

The chip with the probes would be enclosed inside a disposable card the size and shape of a credit card.

In the case of suspected strep, cell samples swabbed from the throat would be suspended in a liquid that would be injected into the card. The liquid, combined with ions, breaks open the cell walls of the samples, releasing the DNA. It then would be pushed through a filter inside the card that would remove most particles larger than the DNA molecule. The fluid would flow through a tube where the DNA would be shredded into pieces. The sample then would pass over the probes. If a target DNA molecule is present in the sample, it will bind to a complementary DNA probe.

"When the DNA from the sample binds to the DNA probe, there is a change in electrical properties at the sensor site," Connolly said.

Customized computer software would read each sensor, gather the information and convert it to a diagnostic analysis. It could show that strep is present and whether it is resistant to specific drugs.

"A doctor could determine what drug will work up front," Connolly said. "That's where we see a lot of the value. We can get a lot of information so the doctor can prescribe the right antibiotics tailored to the problem, prevent repeat doctor visits and reduce sick time."

The BioDetect System would operate in a similar fashion with samples of blood or any bacteria, virus or organism with DNA.

BioDetect offers additional advantages over current systems that read electrical charges in DNA, such as those from Nanogen Inc. of San Diego and Cepheid Inc. of Sunnyvale, Calif., Connolly said. Those systems use fluorescent markers, for example, that require processing before testing and production of a higher volume of DNA.

"Our system can detect even a single molecule binding to the sensor. That is what really sets us apart. It could be used to count the number of molecules present," Connolly said. "It is the first system that does not trade reliability for speed. It won't require a lab and won't require a high degree of specialty training. The system really does the work."

Connolly developed the initial idea that led to BioDetect while working as a patent attorney at Nixon Peabody LLP. He had continued to read science journals even after his interest in business took him to the practice of law.

"I just saw some things in the scientific literature and saw some ways to expand on them," he said.

Connolly put together a business plan early in 2000 and started looking for financing. Connolly, who left Nixon Peabody in July 2000, raised about half the money from Rochester-area investors and most of the remainder in his hometown of Omaha.

"We needed a team and a facility put together right away to move this along," Connolly said. "You can't do this on the side or in a garage. We required a fair bit of capital."

Connolly declined to say how much INT has raised.

These early "angel investors" got a percentage of the company.

"When you need money to grow the company, you have to be willing to work out deals that benefit both sides," said Connolly.

INT researchers work in a building almost hidden from those driving on Lehigh Station Road. The company's relaxed atmosphere allows executives and scientists to take breaks to play foosball in the INT game room.

Connolly describes INT as a broad, collaborative effort involving scientists from several fields — the idea behind the word "integrated" in the company's title. Smallness implied the use of the word "nano-technologies" because "nano" means one-billionth and often refers to working at the molecular level.

INT began in August 2000 as a company of three people. Pamela A. Duprey, a Nixon Peabody personnel manager, joined Connolly at INT as the company's director of operations. David Chafin, a University of Rochester scientist who is INT's director of research, knew Connolly from their children's baseball teams.

About two month ago, Emilio DiCataldo, former chief financial officer of electronics manufacturer CVC Inc., became INT's CFO.

INT hired a former Xerox Corp. engineer, researchers from RIT and UR and retired Eastman Kodak Co. chemists. Charles DeBoer, an award-winning chemist who had more than 100 patents at Kodak, already has applied for a half-dozen for INT.

"We have people from multiple disciplines working side by side and learning from each other," Duprey said. "That's why we've been able to get as far as we are."

The challenges now for the INT team are to develop the technology to handle more complicated samples, to make the system more sensitive and make some parts smaller.

The computer chip with DNA probes is about the size of a postage stamp. The goal is a chip about 80 percent smaller. The system now has eight sensors, each less than half the thickness of a human hair. The next version will have 64.

Computer chips for product development are being made at RIT's chip fabrication facility under the direction of two INT employees. In the future, INT will manufacture the sensors but contract with other local and national companies to provide other elements of BioDetect.

The company expects to charge from $25 to $50 for each test card. The electric analyzer initially will cost about $40,000, but Connolly expects the price to drop to $5,000 to $10,000 within a few years.

The company has no immediate plans for a public stock offering. But, with job contracts and government grants, Connolly expects positive cash flow by the end of 2003.

"We have a lot of potential here," Connolly said. "We will double in size by the end of the year, and that's just the beginning." ❑

E-mail address: **mwentzel@DemocratandChronicle.com**

About the company

What: Integrated Nano-Technologies is researching and developing a device to detect and identify DNA as a way to diagnose disease or discover hazardous biological agents.

Where: 999 Lehigh Station Road, Henrietta.

Founded: August 2000.

Officers: D. Michael Connolly, president; Emilio DiCataldo, chief financial officer; Pamela A. Duprey, director of operations; David Chafin, director of research.

Employees: 20