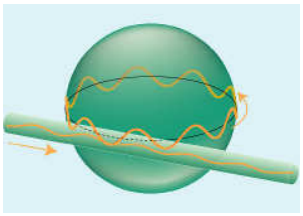
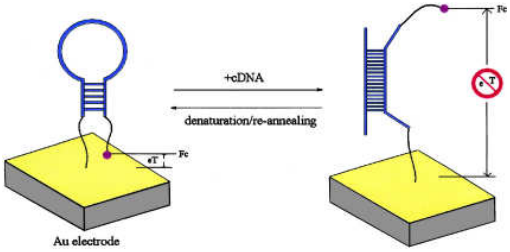
Welcome to Lecture 12

Reminder: Tomorrow "Introduction to Healthcare VC"
by Arthur Tinkelenberg

Last time we talked about some really C-O-O-L biosensors



WGM biosensor

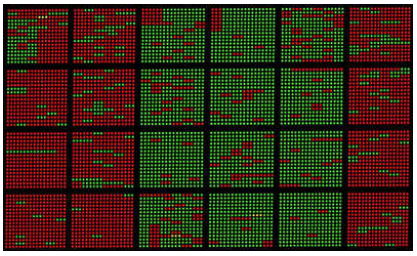


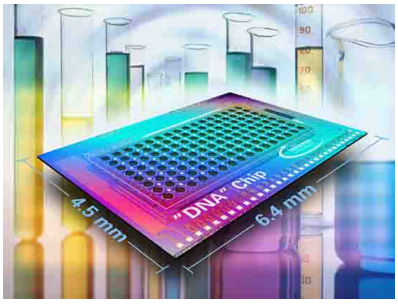
Molecular beacons

Today: Biochips

Biochips and Microarrays

Definition: Biochips (or Microarrays) are ordered arrangements of miniaturized analytical or reaction elements on a plain substrate that allow for specific recognition/binding event and subsequent qualitative or quantitative measurement or detection of this event

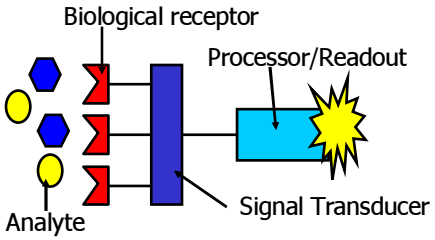


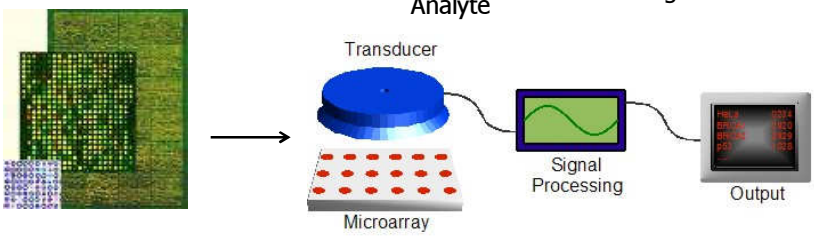


Generally speaking biochips and microarrays are a subset of biosensors

Remember the 1st lecture?

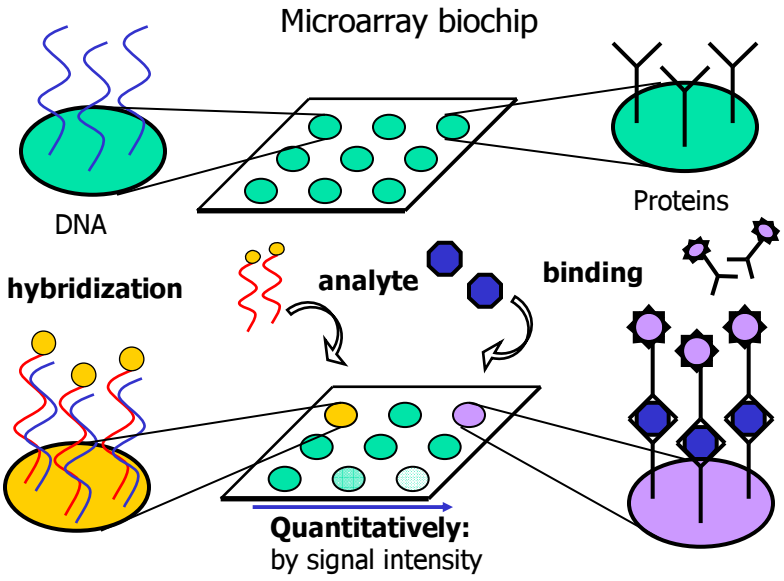
Broadly defined biosensor is a measuring device that contains a biological sensing (recognition) element





Hence, one can think of microarrays as a dense, two-dimensional grid of biosensors

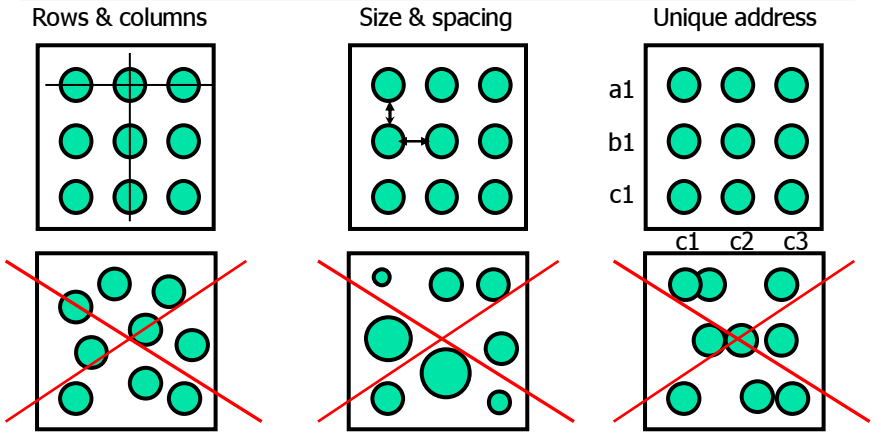
Microarrays - working principle



Microarrays – key features

• Ordered • Addressable • Specific • Planar

Ordered: a collection of analytical elements must be arranged in an easily "addressable" manner, typically as uniform and evenly spaced spots in rows and columns so that each spot has its unique address



The value of order

This design allows for rapid and inexpensive manufacturing, detection and quantification

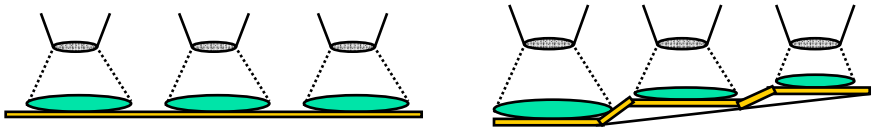
- Planar – stems from the software and hardware requirements
- Standard motion control technologies e.g. linear actuators, encoders, printers, scanner, and other hardware
- With rows and columns very little software customization is necessary
- Uniform size and spacing are essential for providing high quality analytical data
- Size matters – the smaller, the better – more can be packed on a single chip - cheaper per sample

Rapid and precise array preparation and analysis of irregular shapes would be dramatically more expensive

Quality of surface

Surface must be flat and uniform:

Flatness refers to 2D "evenness" <10µm in Z direction over X and Y

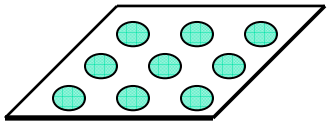


flat surface

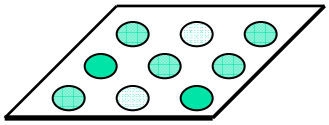
uneven surface

Ink-jet printing relies on the precise distance form the nozzle to the surface to create spots of uniform size

Problems with analysis too…



Many detectors have relatively small depth of focus: 20-30µm



identical samples on flat surface

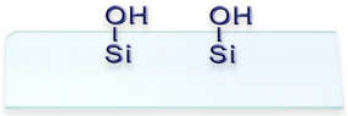
and on uneven surface

Microarray surfaces

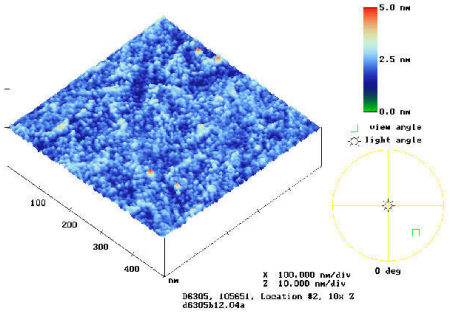
All the usual suspects:

gold, metal oxide, silicone, but the main substrate is

GLASS

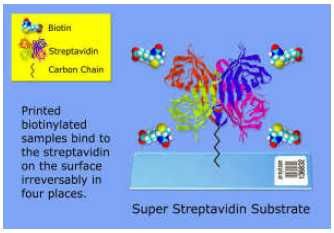


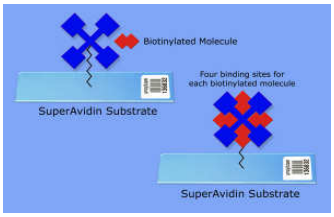
Glass is polished to "atomic" flatness

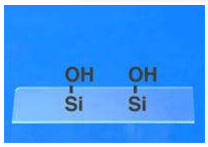


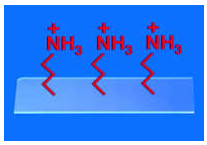
Atomic force microscopy image of a glass micro-array substrate from ArrayIt™, with flatness data represented in a color palette. The scale bar (upper right) denotes surface flatness in nanometers (nm). 1 nm = 10 Å. The glass is flat to ~ ±20 Å

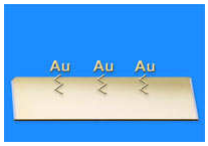
ArrayIt products

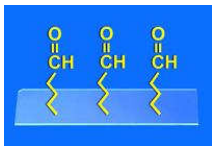


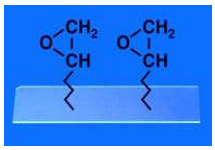






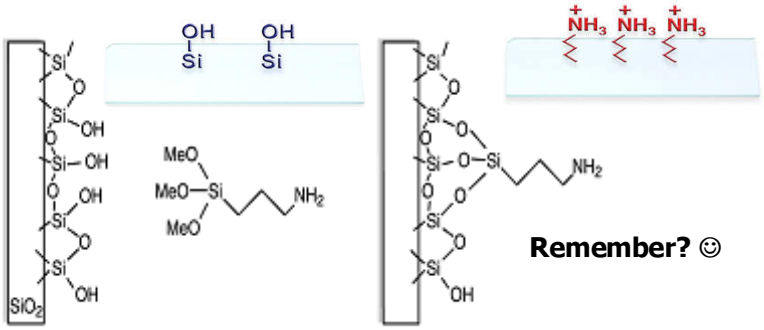






Amino-modified glass

Introduction of NH_2-groups onto glass surface



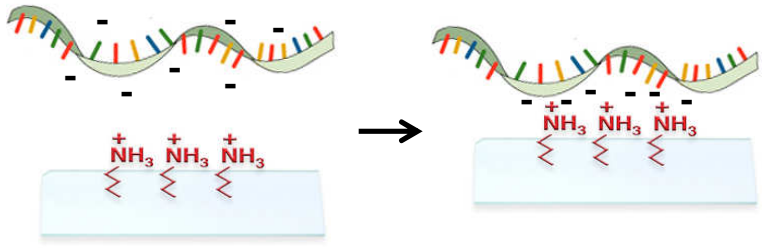
Remember? ☺

Glass is often subjected to prior harsh hydrolysis to maximize the number of OH-groups on the surface and the treated with organosilanes such as 3-Aminopropyltrimethoxysilane

A dozen of other similar reagents are available

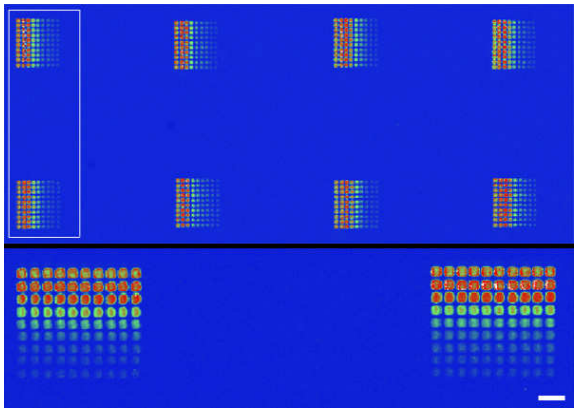
Amino-modified glass

Non-covalent electrostatic binding of DNA to amino-modified glass



- A negatively charged DNA molecule can be applied to the amino surface using contact or non-contact printing
- Dehydration of the printed sample results in the attachment of the DNA to the chip via electrostatic interactions
- Can be used for covalent attachment of proteins too

DNA array on amino-glass

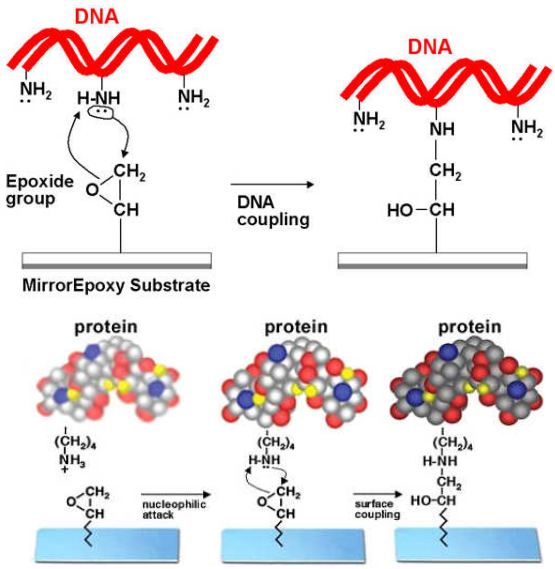


A scanned image of a microarray containing a 2-fold dilution series of a fluorescent oligonucleotide printed on a ArraIt amino glass substrate

A 25 µM oligonucleotide solution was diluted in 2-fold steps from 25 µM to 50 pM and printed on the slides at 140 µm spacing using a PixSys 5500 microarray robot and then laser-scanned

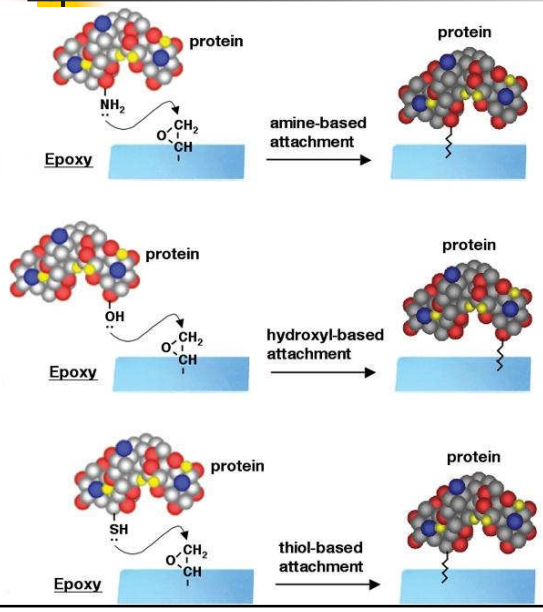
The chip contains a total of eight identical micro-arrays (top panel), the first two of which (white box) are shown enlarged and rotated 90° (bottom panel). The data are displayed in a rainbow palette and the space bar corresponds to 200 µm.

Epoxide coupling chemistry

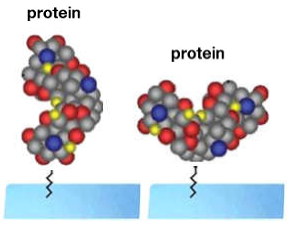


- Oligonucleotides (cDNAs and RNAs) contain primary amine groups on the A, G, and C residues
- The lone electron pair attack the electrophilic carbon on the epoxide group, forming a covalent bond between the DNA and the substrate
- The mechanism of covalent attachment of proteins to epoxy-glass is essentially the same – no shortage of amino groups there ☺

Covalent protein arrays



Epoxides react with other amino acids too

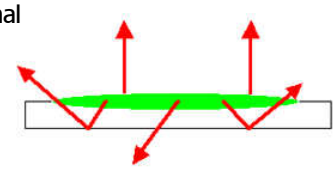


but the proteins captured by this and many other covalent reagent are NOT uniformly orientated on the surface

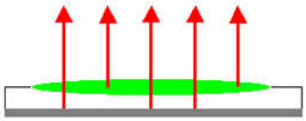
Mirror modified glass

Optical properties of modified (mirror) glass substrates

In the presence of excitation light, a microarray spot emits fluorescent signal

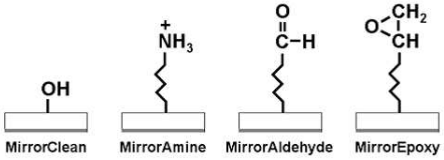


A standard glass substrate produces weaker fluorescence and elevated noise due to the loss of excitation light through the transparent back surface and light scattering

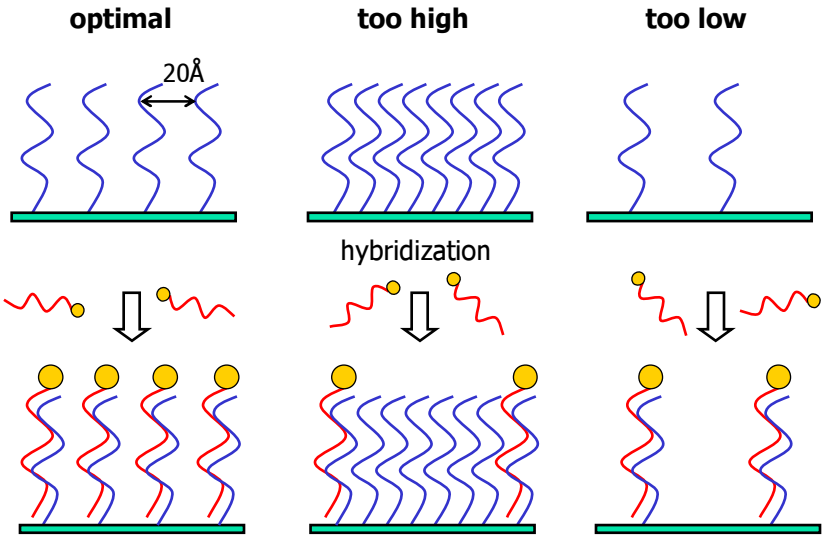


A "mirror" substrate produces a stronger fluorescent signal and reduced noise due to the capture of excitation signal off the reflective backside surface and the elimination of light scattering

Available for a wide range of surfaces →

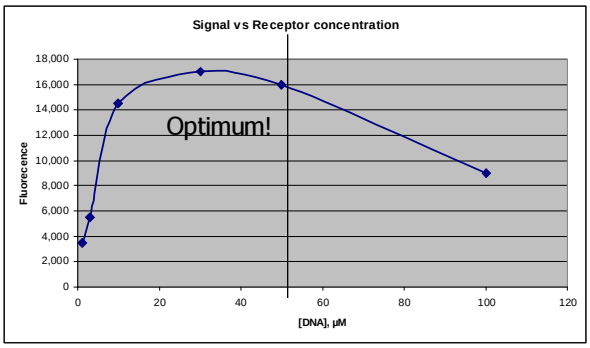


Receptor density is critical



Optimal density

Even more critical than for conventional biosensors



- A simple calculation shows that for single chain DNA the optimal surface density is ~2.6×10^5 molecules per μm^2 or about 1 molecule per 400 $Å^2$
- The min detectable coverage Γmin (i.e. sensitivity) is better for receptors with high binding constants (K) and high surface

Remember?

$$[L_{min}] = \frac{\Gamma_{min}}{K\,(\Gamma_{max} - \Gamma_{min})} \sim \frac{\Gamma_{min}}{K\ \Gamma_{max}}$$

Microarray manufacturing

Creative fusion of biology and engineering!

Tools, gadgets, materials and motion control systems have been developed for applications in aerospace science, computer chip manufacturing, video projection industry, etc and have been creatively adapted for use in micro-array printing

Note the necessity for PRECIZE robotic motion (next slide)

Many small companies with AMAZING technology

Contact printing:

- Tweezers
- Capillary printers
- Microspotting and Pin & Ring

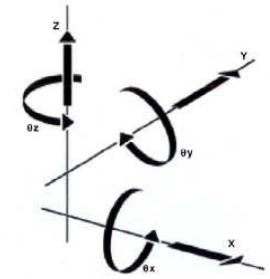
Non-contact printing

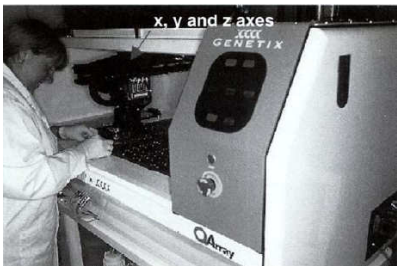
- Microsolenoids
- Piezoelectric
- Thermo bubble jet

Semiconductor Technologies

- Photolithography

Manufacturing: robotics



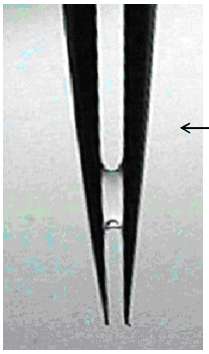


The little guy on the right: Capacity – 50 slides, 4,000 spots per each slide per h

Slide arraying video by Genomic Solutions Inc:
http://www.genomicsolutions.com/instruments/microgrid.aspx#

Tweezers and split pins

Tweezers

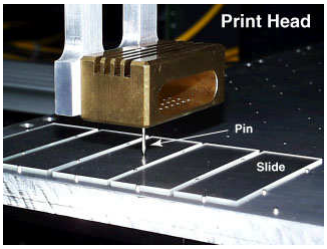


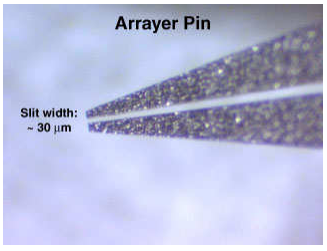
Tapping force breaks the meniscus and expel liquid sample onto the printing surface.

Tweezers, split pins, and other similar printing devices have channels holding the sample inside the device

- Using a 48-pin print-head, 6,912 solutions can be printed onto 100 slides in under 3h
- Each split pin picks up ~200 nL of stock solution and delivers 1 nL of solution to each slide on the platform. Pins are washed before each dip

Split pins technology



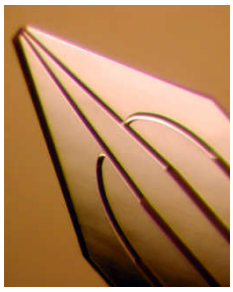


Capillary printers

early technology ↓

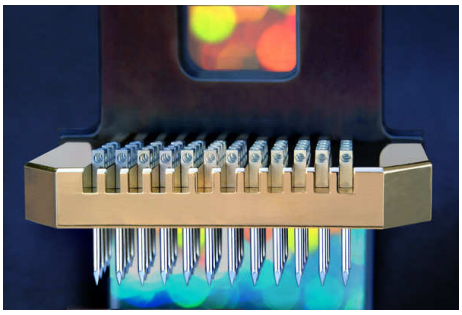
Printers equipped with conventional glass capillary were used in the early nineties

Modern capillary printers are made with high precision, e.g. very durable silicon ⟶

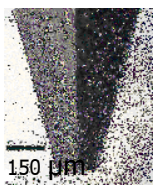


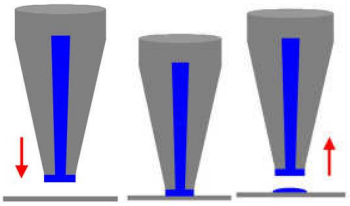
Other materials and shapes are used too

Microspotting



Pin size varies from 50 to 375 µm

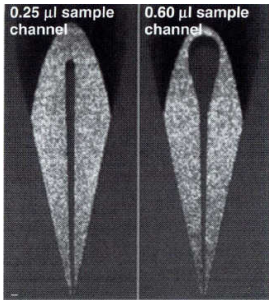




- The printing mechanism exploits surface tension and adhesion i.e. by a gentle "ink stamping" mechanism
- Sample loads into the Pin by capillary action, completely filling the sample channel to provide a pre-determined loading volume
- The flat surface of the tip allows a thin layer of sample to form at the end which contacts the printing surface during the printing down-stroke - a droplet "tug-of-war" between the substrate and the Pin
- As the Pin travels upward, the adhesive forces of the substrate pull the droplet off the end of the Pin, leaving behind a perfectly printed microarray spot

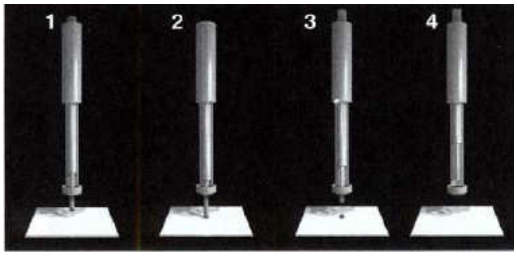
Other solid pin technologies

Microspotting



The capacity and spot size are defined by internal volume of the solid pins

Pin and Ring

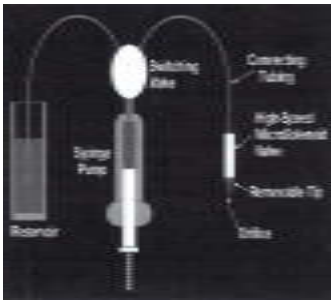


The sample is loaded into the ring by capillary action

The pin is propelled through the sample, which results in attaching a small pendant drop to the tip

When the pin touches the surface of the substrate the drop is transferred and the pin is moved back to initiate another round of printing

Non-contact printing



A syringe pump feeds the pressurized sample into the nozzle fitted with a microsolenoid valve

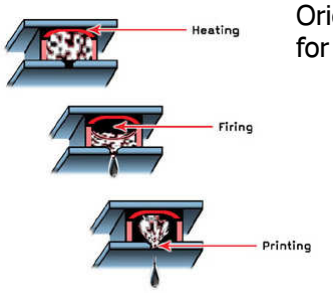
The valve is actuated by oscillating electric pulses and when it transiently opens, a small drop is released into the nozzle orifice (volume 1-10 nL)

MicroSys 5100 system developed by Cartesian Technologies (Irvine, CA)

Guess what happened to the company? ☺

How else can you do it?

Thermal bubble jet technology



Originally developed by Cannon in the US for application in inkjet printers

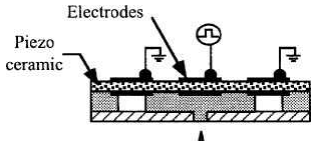
Principle: the viscosity of fluids varies with temperature

Hence, a drop can be expelled from the delivery nozzle in a precisely controlled manner

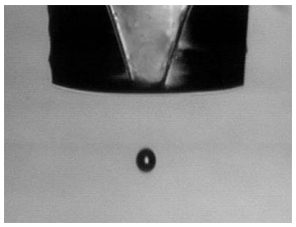
The necessity to heat the sample is a major drawback for biological applications

Piezoelectric inkjet printers

Inkjet printers deliver picoliter volumes of solutions



A shear-mode piezoelectric ink-jet design

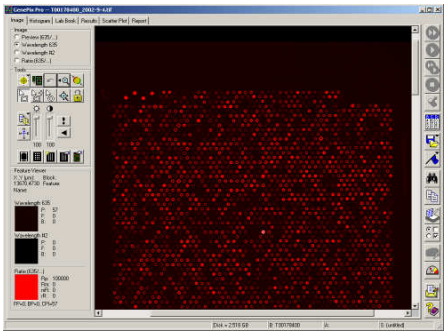


Piezoelectric heads dispense liquid with very high precision providing small and uniform spots

Commercial printed arrays

GE Healthcare (ex-Amersham) offers a commercial array platform called the "CodeLink" system where 30-mer oligonucleotide probes (sequences of 30 nucleotides in length) are piezoelectrically deposited on an acrylamide matrix

CodeLink Human Whole Genome Bioarray



Bioarrays are scanned at 635 nm and saved as 16-bit tiff images

Manufacturing criteria

- **Affordability:** self-explanatory
- **Density:** The number of target spots or features per unit area of microarray substrate
- **Feature size:** the size of spots or elements in the microarray (synthesis 10-40µm, delivery 50-300µm)
- **Regularity:** the evenness of rows and columns in the array
- **Purity:** self-explanatory
- **Reactivity:** (bio)chemical activity or efficacy at a given location in the microarray
- **Throughput:** the rate of manufacturing for a given system or device
- **Ease of implementation:** design, hardware, software, etc

Microarray detection

Fluorescence is <u>by far</u> the main method

Detector architecture:

Scanners: 1 pixel at the time

Laser excitation/PMT detection
moving stage/optics design

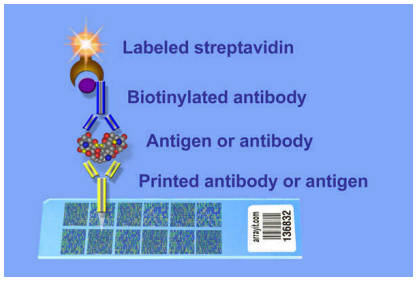
Imagers: take a picture

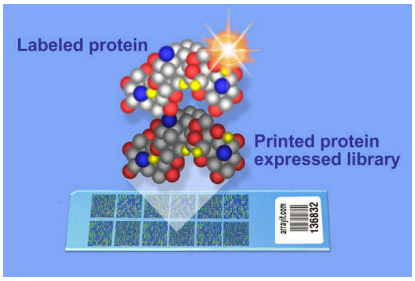
Typically, rely on a white light source and use a camera with CCD to capture the image; often use "area detectors"

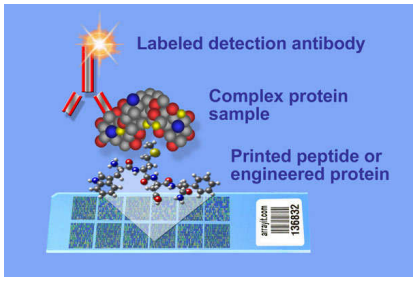
Assay formats for protein arrays

Just as with other biosensors

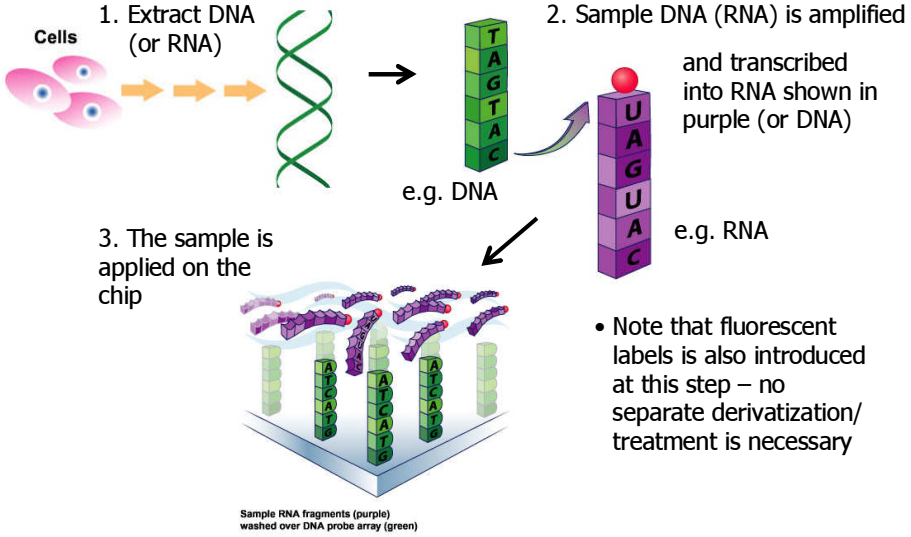
A variety of receptor-target interactions can be explored in a wide range of assay formats





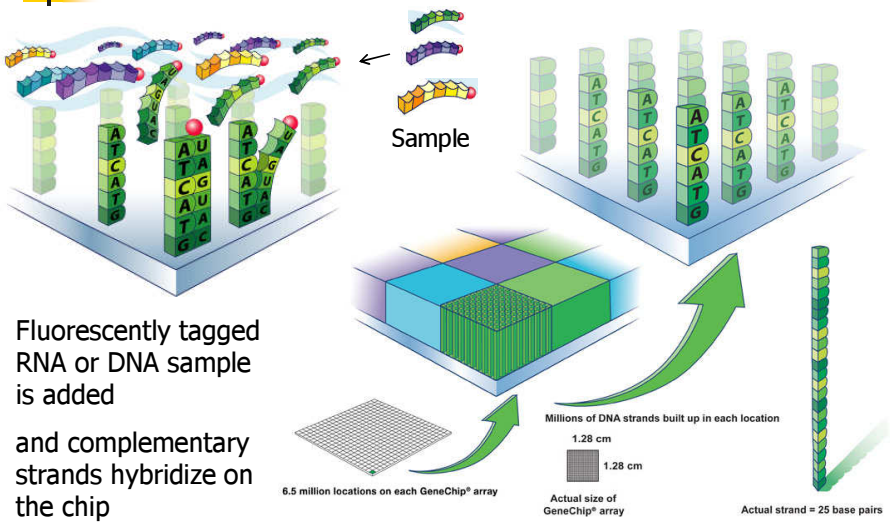


Assay format for DNA arrays



- Note that fluorescent labels is also introduced at this step – no separate derivatization/ treatment is necessary

The working principle

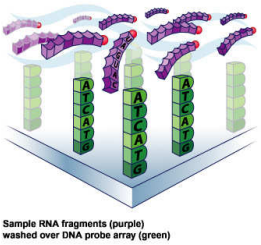


Fluorescently tagged RNA or DNA sample is added

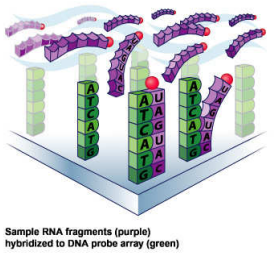
and complementary strands hybridize on the chip

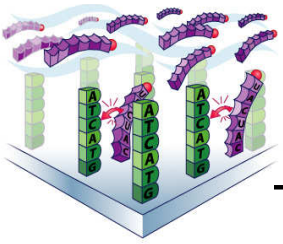
Selectivity and signal generation

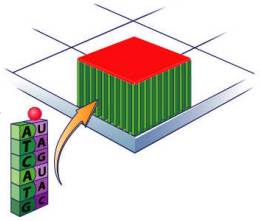
Hybridization only occurs in certain spots, depending on sequences present in the sample



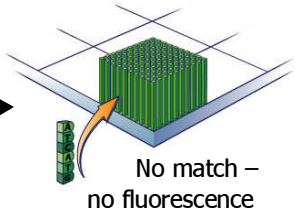
The chip is washed and fluorescence measured; the rest is bioinformatics ☺





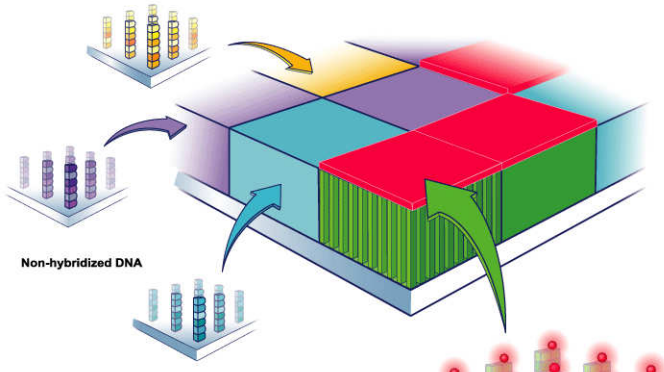


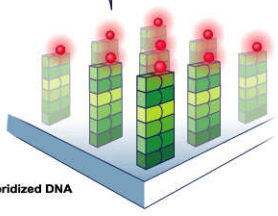
Shine a laser on the array and the square with matched DNA will **light up**



No match – no fluorescence

Massively parallel analysis

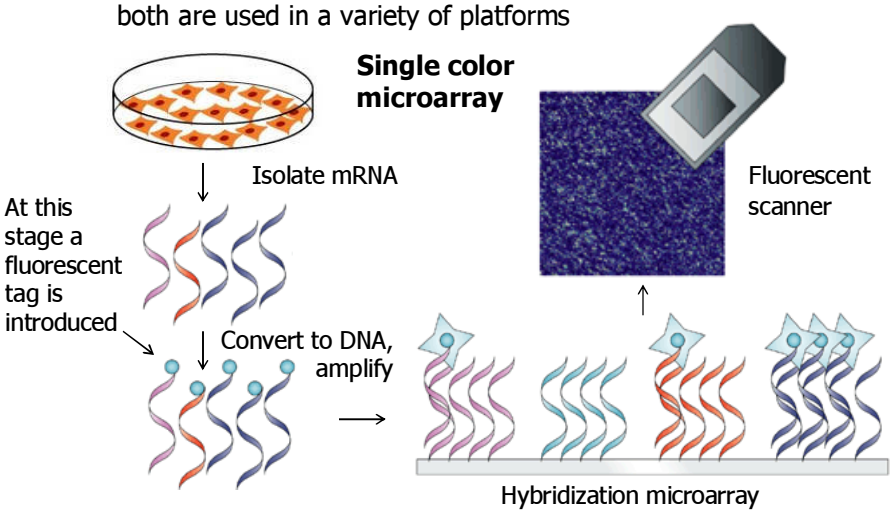




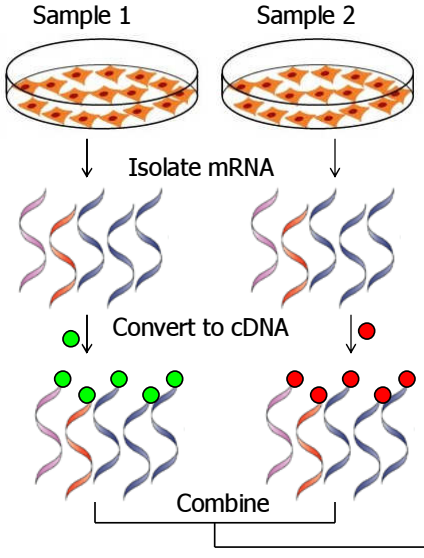
A massive amount of information can be generated in a single experiment because the sequence of oligos in each spot is known and each spot is individually addressable

Arrays come in two flavors

Labeling of targets for microarrays: single-color or two-color; both are used in a variety of platforms



Two-color assay



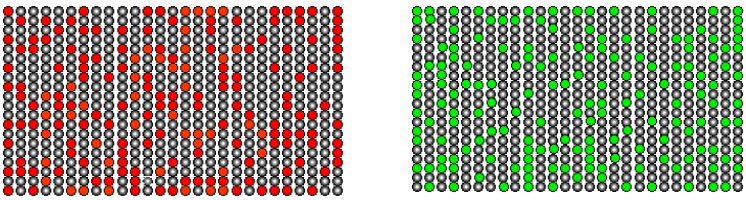
- In two color assays two different fluorescent dyes are used – often red and green
- Can be useful as an intrinsic control and in comparative experiments e.g. cancer vs normal cells
- Multi-color formats are also available now
- Analysis requires scanning for both colors

Any ideas why red and green?

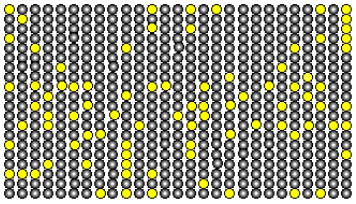
Hybridization microarray → Fluorescent scanner

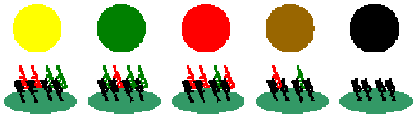
Two color arrays

Microarrays are scanned for red and green



Spots that bind to both cDNAs appear yellow

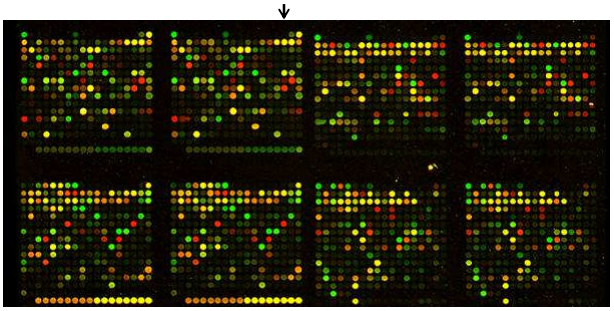




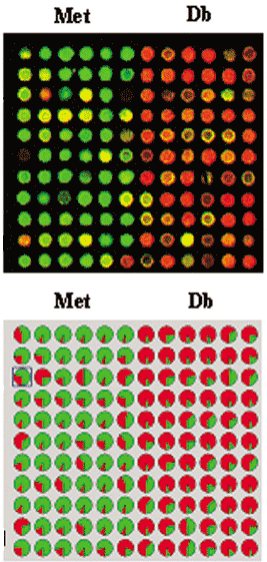
Two-color microarray principle

What it looks like

Tissues: Rat Brain (green) and Kidney (red) hybridization using gene expression arrays



Hybridization microarray of two tumor lines (Met and Db). Green represents higher expression in the Met line, and red represents higher expression in the Db line. Bottom: the same section of the array showing the relative percentage of signal intensity at each spot



After the break

We will talk about:

- Affymetrix Genechip and technology used for its production
- Applications of protein and DNA arrays

But now – it's time for the quiz

Clontech Antibody Microarray 500

Solid phase ligand binding assays using Ab's immobilized on surfaces (e.g. glass and nitrocellulose surfaces)

Highly parallelized (HTP-enabling)/miniaturized with 500 Ab's in duplicate on each chip

Antibodies are specific to proteins involved in:

- Signal transduction
- Cell-cycle regulation
- Gene transcription
- Apoptosis
- Oncogenesis

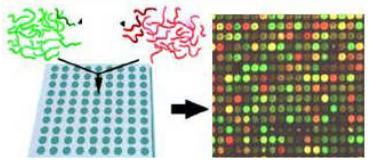
Main use in proteomics and pharmaceutical research

Clontech Microarray 500

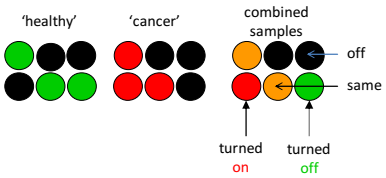
Typical experiment: Proteins extracts are prepared and labeled with fluorophores (red and green), applied to the chip, washed, scanned and the data analyzed

- Enables analysis of multiple analytes from 2 samples
- Probes (e.g. control & test samples) are labeled with alternative fluorophores for comparative purpose

Both samples incubated together

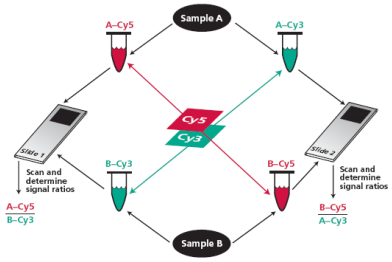


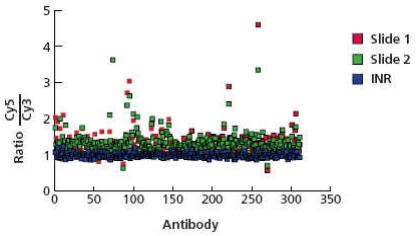
Relative differences are measured



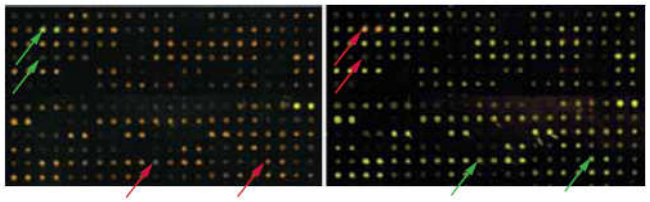
Assay with Microarray 500

Duplicate chips with reversed labeling: Fluorophores attached by alternative chemistries to control for labeling effects





Ratio: A-Cy5 / B-Cy3



Ratio: B-Cy5 / A-Cy3

Ab-chip: pros and cons

Analysis for allergens – 300 Abs on a single chip

Pro's

- Rapid, automated, highly sensitive
- High data yields, economical, low sample consumption
- Some tools shared with (more advanced) DNA chips

Con's

- Ab-Ag interactions often have different optimal binding conditions; hence difficult to optimize conditions for several Ab's on the same chip
- Relatively small numbers: only 500 different Ab on a chip to date

Protein chip from Invitrogen

8,000 GST-fusion proteins on a single chip

Gene Families:

- Kinases & Phosphatases
- GPCRs & G-protein related Ion channels
- Nuclear receptors
- Proteases
- Cytokines & Chemokines
- More

Protein kinases	378
Transcription factors	188
Membrane proteins	1,085
Nuclear proteins	830
Signal transduction	710
Secreted proteins	100
Cell communication	863
Metabolism	2,166
Cell death	145
Protease/peptidase activity	138

Used in proteomics, drug discovery and diagnostics e.g. protein-protein interaction, identification of kinase substrates, antibody specificity profiling, etc, etc, etc

Real stress-o-meter?

A PROTEIN BIOCHIP FOR THE SCREENING AND DIAGNOSING of CHRONIC PSYCHOSOCIAL STRESS

- This new protein biochip offers the first panel of markers for screening and diagnosis of chronic psychosocial stress
- Some cytokines, growth factors and the hormones testosterone and prolactin are risk factors for psychosocial stress
- Plasma samples from patients on long term sick leave for a stress-induced affective disorder, people at risk of professional burnout and healthy controls were analyzed and the markers identified were independently associated with a significant risk of being ill from stress

Randox announced the release of a protein biochip for the screening and diagnosis of chronic psychosocial stress

GeneChip by Affymetrix

Whole Transcript: The Gene 1.0 ST Array System offers a cost-effective solution for **whole-genome**, whole-transcript expression **analysis for human**, mouse and rat

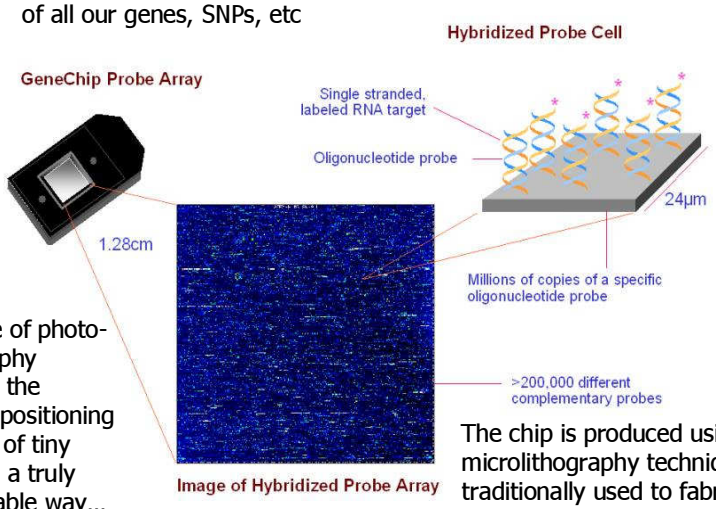
Each gene is represented by ~26 probes spread across the full length of the gene – all in specific addressable locations, providing a more complete and accurate picture of gene expression

The Affymetrix® Genome-Wide Human SNP Array 6.0 has more than **1.8 million** markers for genetic variation, including more than 906,600 single nucleotide polymorphisms (SNPs)

How is GeneChip different from the rest?

Same detection principle

Millions of individual DNA probes capable of detecting expression of all our genes, SNPs, etc



The use of photo-lithography enables the precise positioning of mlns of tiny spots in a truly remarkable way…

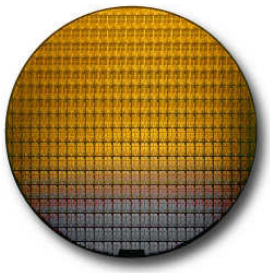
The chip is produced using microlithography techniques traditionally used to fabricate integrated circuits

Photolithography

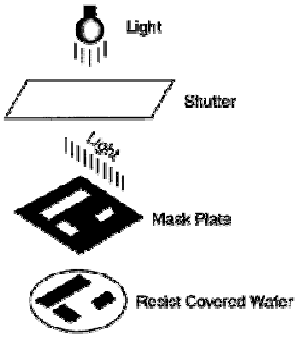
Photolithography is the process of using light to create a pattern

Light is used to transfer a geometric pattern from a photomask to a light-sensitive chemical material on a substrate

In semi-conductors industry photolithography (or optical lithography) is used in microfabrication e.g. manufacture of CPUs by Intel, AMD, TI, etc - to selectively remove parts of a thin film or the bulk of a substrate



Silicon wafer with CPUs



Semiconductor manufacturing

Clean rooms

Entering and working in CRs

Sony →

Intel ↓

Clean Rooms

A work area in which the air quality, temperature and humidity are highly regulated and the air is repeatedly filtered to remove dust particles and any other impurities

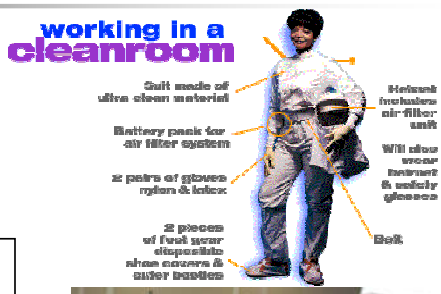
There are 6 classes of CR – 1, 10, 100, etc – defined as the number of particles <0.5µm/ft^3

←

DuPont™ Tyvek®

The miracles of science™

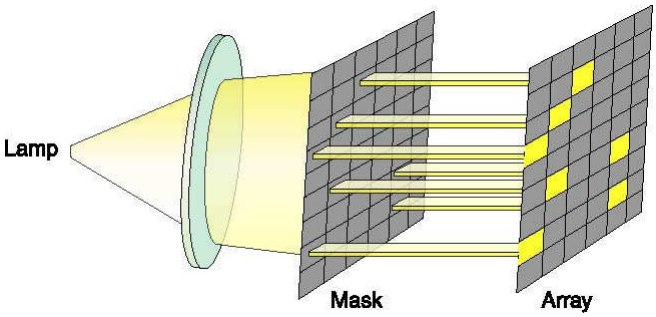
Special non-woven polyethylene fabric for bunny suits



10^4 times cleaner than in hospitals

Photolithography and GeneChip

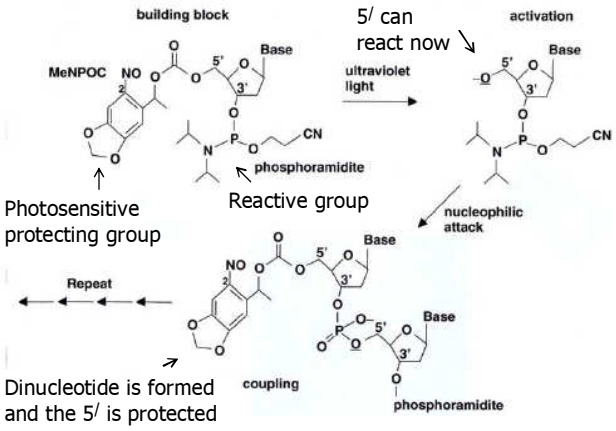
By shining light on particular parts of the chip, while masking the rest, it is possible to do chemistry – oligonucleotide synthesis – only in predetermined spots



Mask application enables parallel synthesis of different nucleotide sequences on the same chip

Coupling chemistry

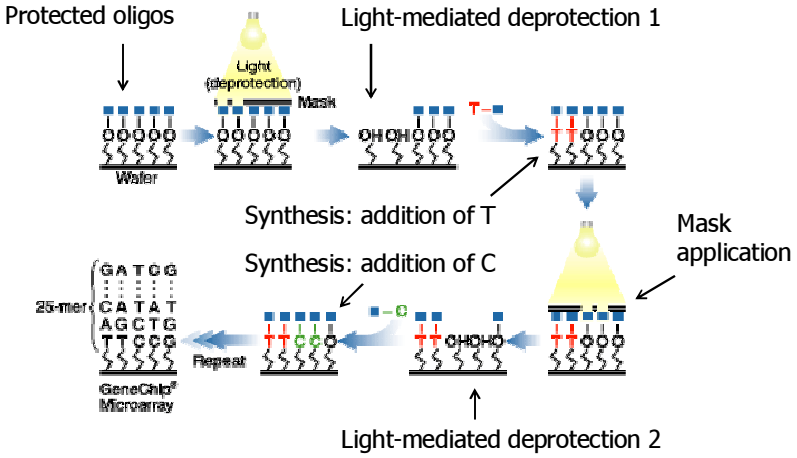
Example of photo-activated coupling chemistry



- The GeneChip high-density oligonucleotide arrays are fabricated by using **in-situ** preparation of short oligonucleotides on a small glass chip using **light directed synthesis**
- This allows for the precise construction of a highly ordered matrix of DNA oligomers on the chip…

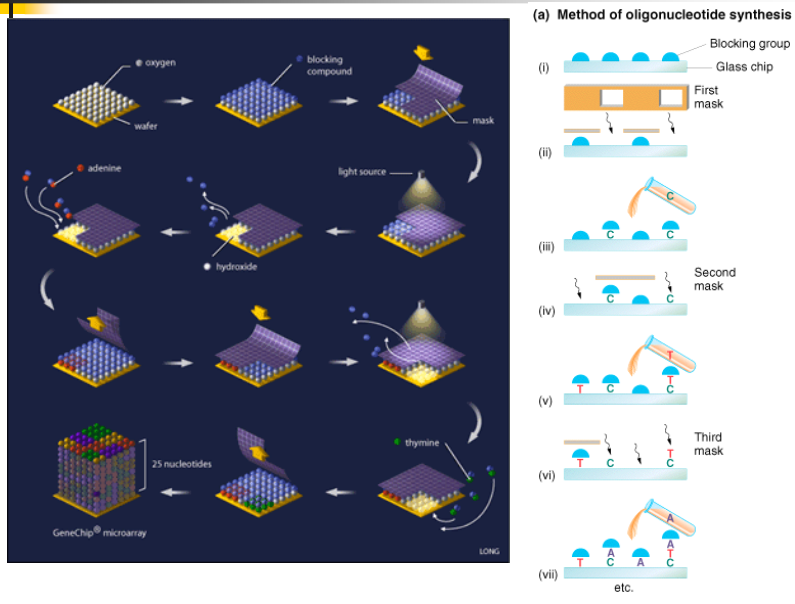
This is just one step of multi-step synthetic protocol

Mask photolithography



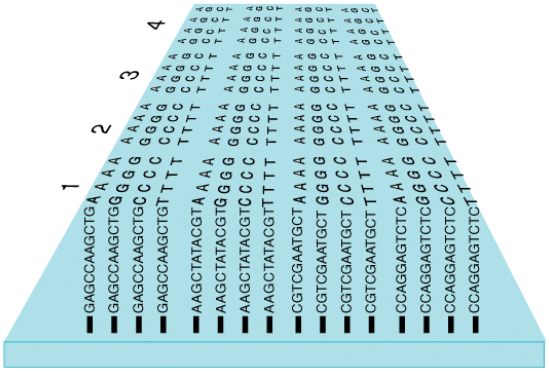
<u>The key point</u>: using the mask you can add T or C to millions of spots on the chip simultaneously i.e. effectively in one step

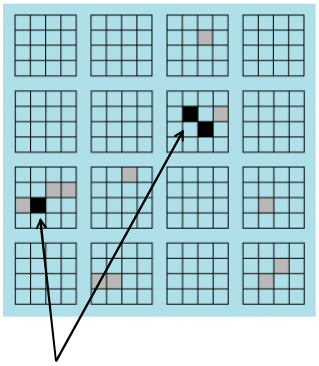
Affymetrix process



Resulting oligonucleotide array

Dense Ordered Planar Specific



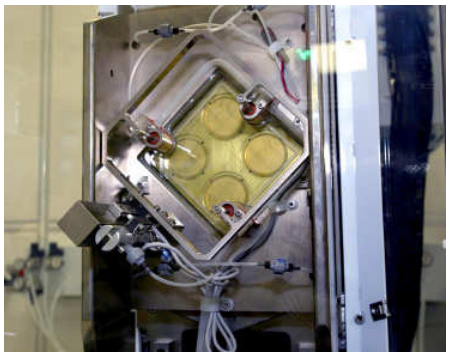


and addressable: the location of spots where hybridization occurred

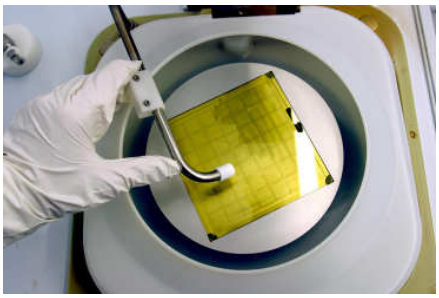
Any questions?

Oligonucleotide synthesizer

This technology enables making millions of individual probes by doing just for reactions per nucleotide in the probe – i.e. 100 reactions for a 25-mer, and all you need is just four reagents

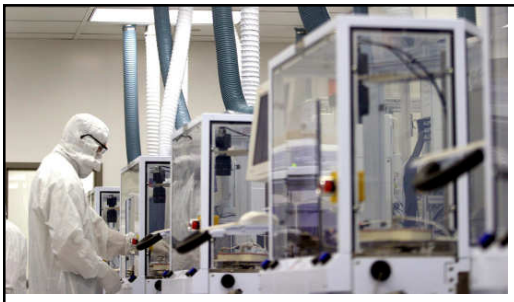


Almost like a conventional DNA synthesizer ☺

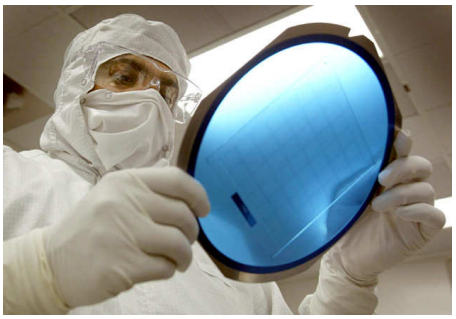


Transfer of wafers at the cleaning wetbench and application of antireflective coat

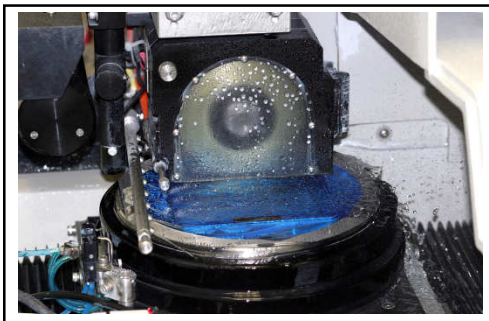
Transfer and inspection of the coated wafers



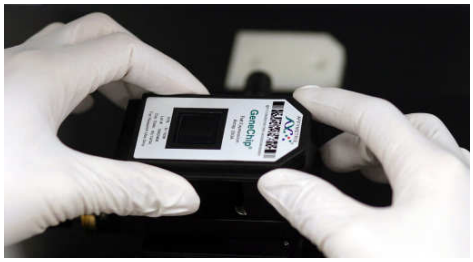
Wafer loading on the oligonucleotide synthesizer and removal on completion



Inspection of the wafer prior to dicing
and wafer mounting on the dicing saw

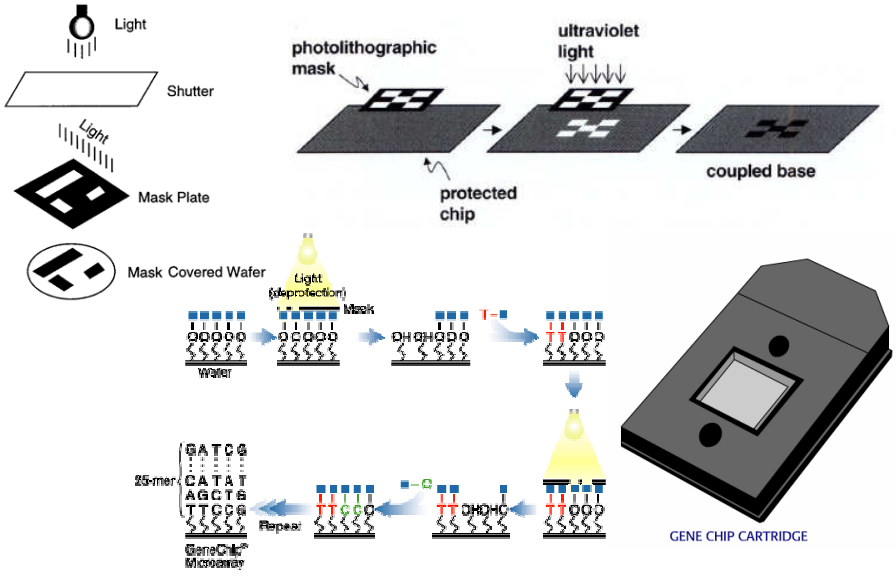


The wafer is diced and individual chips are mounted into plastic GeneChip cartridges

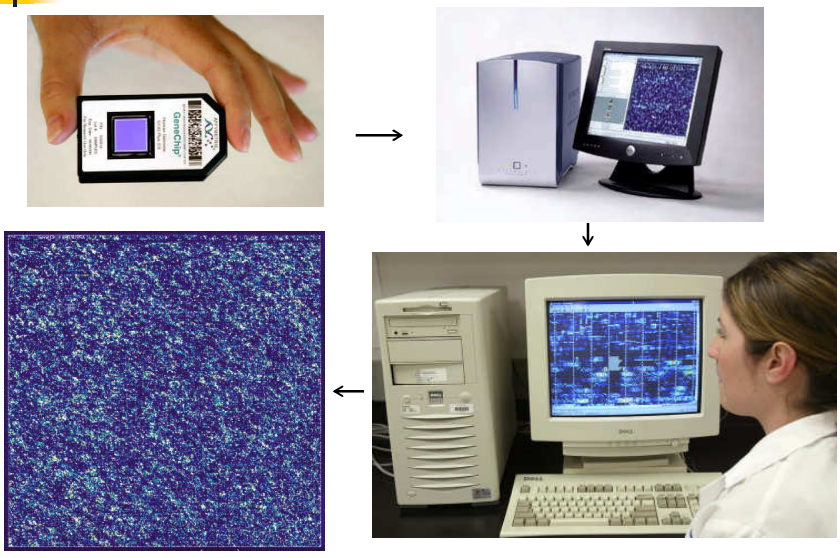


GeneChipas are labeled, barcoded, quality controlled and sold to customers

Photolithography: summary



Analysis: genechip scanner



Microarray technology

Affymetrix GeneChip –
insitu oligo synthesis by mask photolithography
oligo length: 25 mers feature size: 11um
strength: comprehensive coverage (plus genome scanning)

Agilent Technologies –
insitu oligo synthesis by piezoelectric dispense
oligo length – 60 mers
strength: flexibility

Amersham Biosciences –
presynthesized oligo by piezoelectric dispense
oligo length – 30 mers
strength: sensitive & reproducible

NimbleGen –
insitu oligo synthesis by micromirror photolithography
oligo length: 25-60 mers
strength: comprehensive coverage & flexibility

Self Spotting –
oligo or cDNA spotting by pin contact dispense
oligo length: typically 60mers
strength: cost

Printing vs photolithography

Two methods are different:

- **Delivery** – prepare the target molecules "off-line" and then spot them (print) on the substrate
- **Synthesis** – create target elements directly on the substrate by joining monomers together

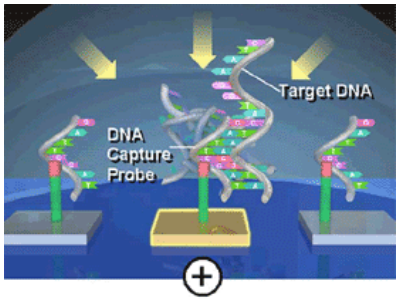
	Delivery	**Synthesis**
Technology	Printing	Photolithography
Implementation	Easy-moderate	Moderate-difficult
Density	Up to 10,000/cm^2	Up to 500,000/cm^2
Complexity	Any	Limited
Cost per chip	Fn of complexity	Less so…

The printing technology can be used with any bioreceptors

Electrochemical detection?

Nanogen's NanoChip™ applies an electric current to individual test sites on an electronic microchip

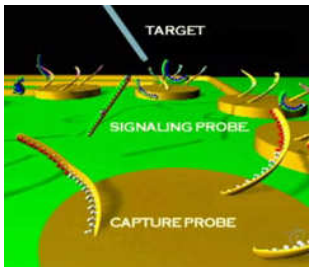
- The electric field enables rapid movement and concentration of molecules
- This feature can accelerate molecular binding on the microchip up to 1000 times that of traditional passive methods
- It also enhances hybridization, the pairing of separated strands of DNA with complementary DNA strands that act as probes



100 or 400 electrodes per chip

Motorola: eSensor™

- **The eSensor™ DNA detection technology** relies on bioelectronics - forming electronic circuit elements using assemblies of organic molecules on surfaces
- Complementary binding of DNA or RNA to the probe immobilized on an electronic circuit element creates a detectable electronic signal, when the target is present
- The **eSensor™** technology is not inhibited by common components of blood, serum, plasma and urine, and is compatible with all of the major technologies used to amplify DNA
- The **eSensor™** detection technology makes DNA testing more convenient, more economically feasible and ultimately, more widely used

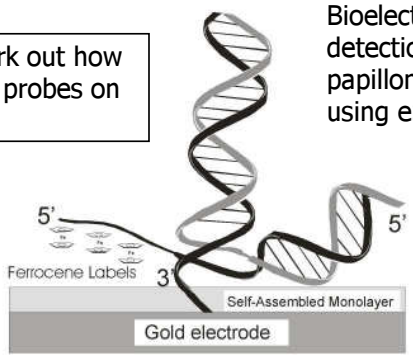


Motorola eSensor

Only 36 electrodes, but it was the first to be successfully used in clinical trials

The problem: to work out how to position millions of probes on the surface

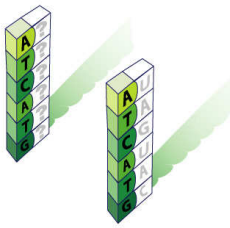
Bioelectronic DNA detection of human papillomaviruses using eSensor™



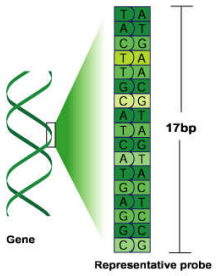
OK, let's see what DNA biochips can do…

FoodExpert Array

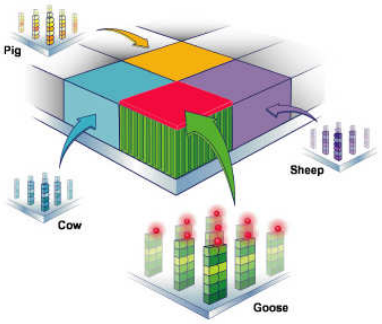
Food adulteration: Allows genetic identification of the presence or absence of 33 different species of animals in any food product with 17 bases long gene probes specific to particular genes in 33 target animal species - 12 different species of mammals, 5 species of poultry, and 16 species of fish



For example, if the product is supposed to be just goose, only goose DNA will produce a signal and the probes for DNA from any other of the 32 species represented on the array, will remain "silent"



Affymetrix FoodExpert Array



Just kidding

Let's simplify it down to just 4 different species: goose, sheep, cow, and pig

Each square on the array is filled with probes that will detect either:

Goose DNA (green)
Sheep DNA (purple)
Cow DNA (blue)
Pig DNA (yellow)

Bingo! foie gras (goose pate) is actually made of a mixture of chicken and pork ☺

No DNA (thus, no fluorescence) is detected for either of the three alternate organisms, meaning no sheep, cow or pig went into making the goose foie gras

Biomedical applications

Microarrays function as biological microprocessors enabling the rapid and quantitative analysis of many key biological functions, such as:

- Patients genotypes
- Disease diagnostics, progression, and prognosis
- Gene expression patterns
- Drugs action and mechanism
- Various other research applications

and much more!

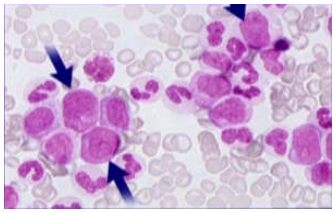
Diagnostics and prognostics

Leukemia

A cancer of bone marrow characterized by abnormal cell proliferation

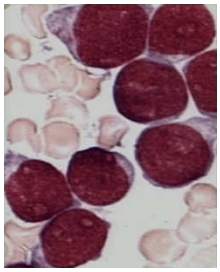
Normal

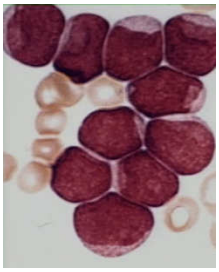
Leukemia



Acute leukemia: a rapid increase of immature blood cells

AML: Acute myeloid leukemia →





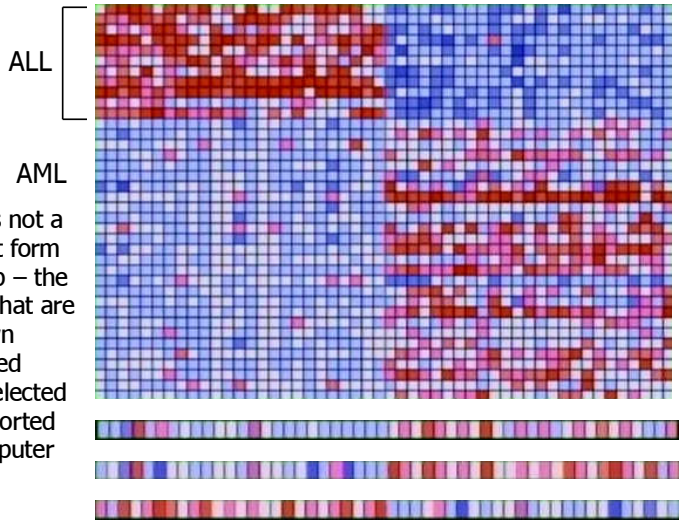
US estimate:

New cases: 44270
Deaths: 21,710

ALL: Acute lymphocytic leukemia - ←

ALL and AML

Genome analysis and selection of diagnostic genes*



*This is not a readout form the chip – the genes that are up/down regulated were selected and resorted by computer

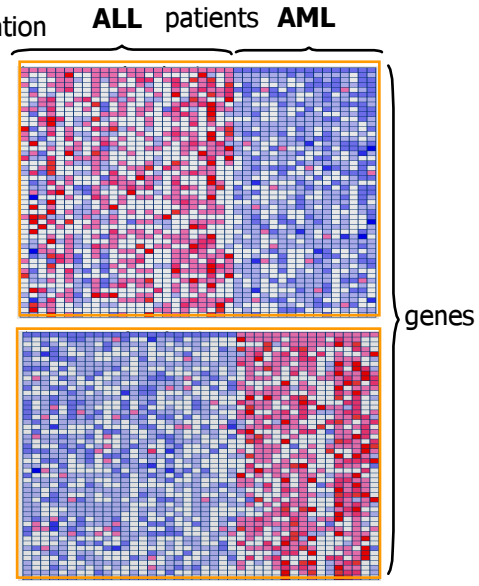
And diagnostics

Genes sorted according to correlation with ALL/AML distinction*

Gene Expression Correlates of Leukemia Genes sorted according to correlation with ALL/AML distinction

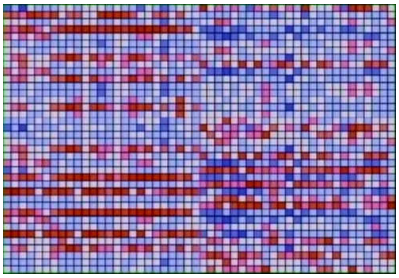
*This is NOT a reading from chip but a computer re-arranged selection of genes that show significant differences

Golub et al., Science 1999

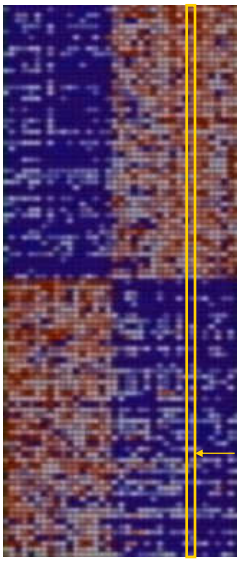


And there is more

ALL - not all the same



Re-sorted

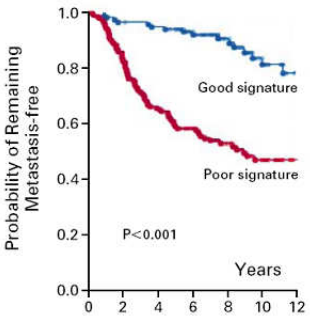


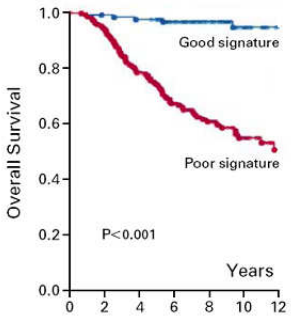
Flt3 is a tyrosine kinase receptor expressed on hematopoietic cells

and there is a good inhibitor which is being tested as a drug…

Metastasis prediction test

MammaPrint – a diagnostic test to assess the risk of metastasis in breast tumor patients; based on 70 genes "signature"

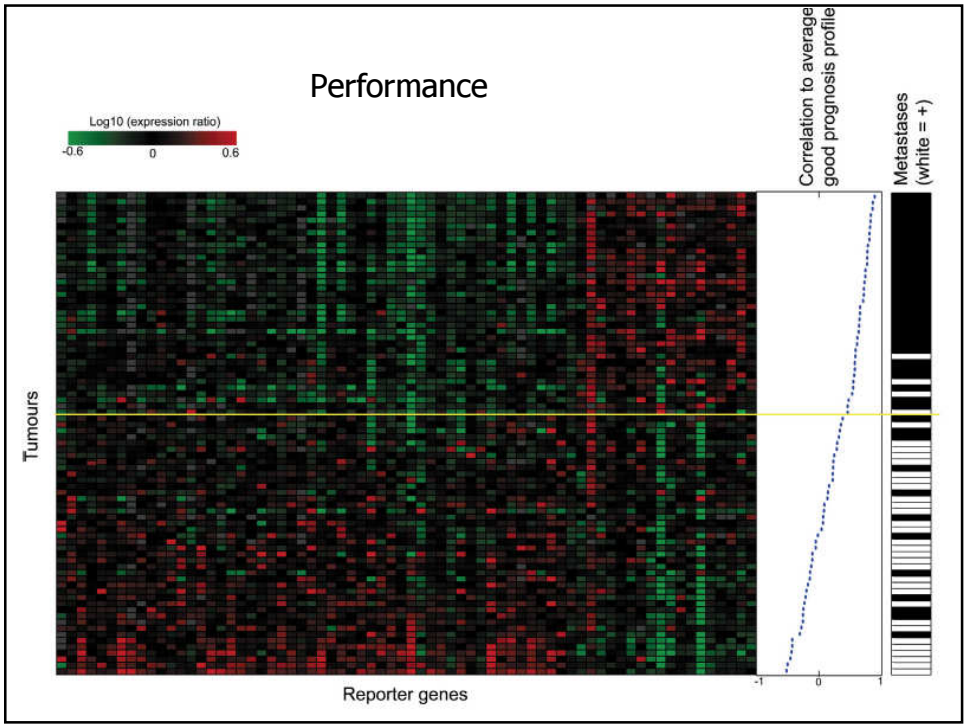


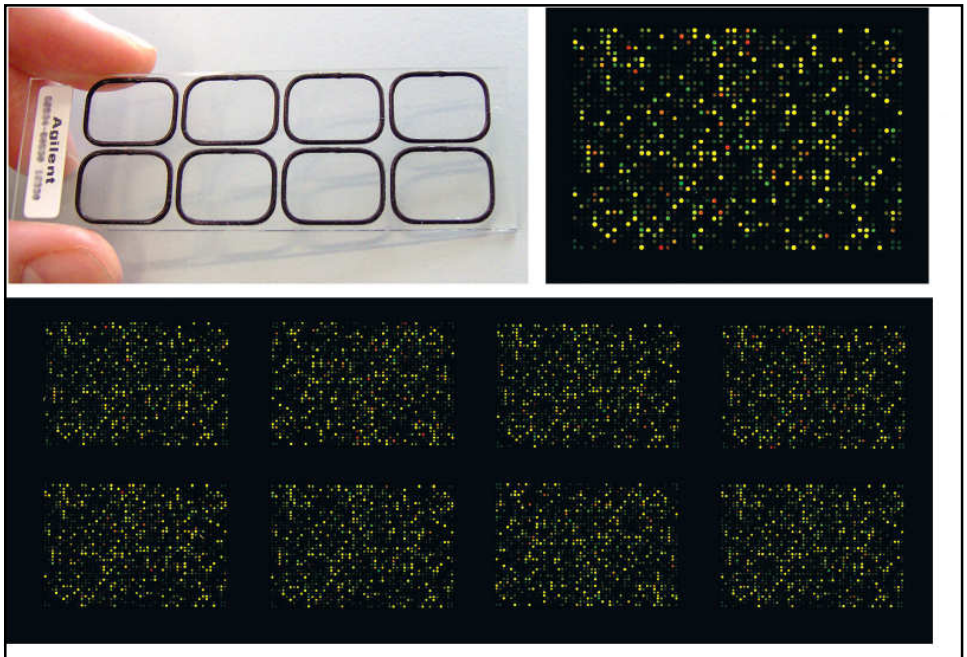


- More accurate that other prognostic assessments
- Enable doctors to adjust the treatment regimen

Approved by FDA in 2007

Van De Vijer (2002) NEJM 347, 1999



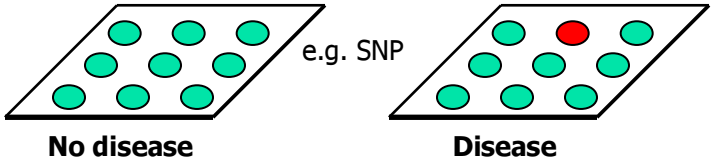


MammaPrint 8-pack, a single 1" × 3" slide containing 8 mini-arrays with 1,900 60-mer oligonucleotide probes, allowing for eight individual hybridizations simultaneously

Patients genotypes

Genetic screening and diagnostics

Risk assessment e.g. predisposition to disease or disease progression



No disease **Disease**

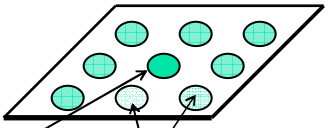
Two color code

no disease disease

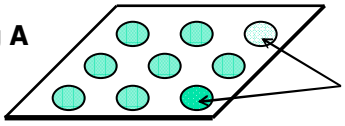
Drug discovery

Many drugs impart their activity by binding to specific cellular targets (e.g. enzymes, receptors) and inhibit their activity; they often alter expression of certain genes too

Patient

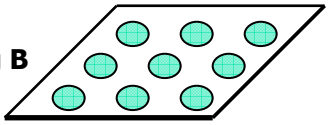


+ drug A



side effects?

+ drug B



Normal pattern restored

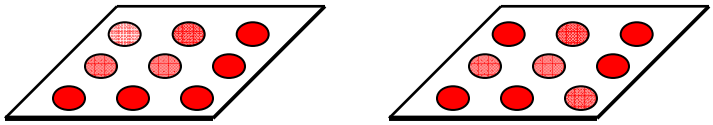
normal

repression activation

Signal intensity

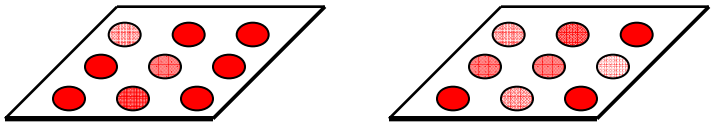
Comparative tissue analysis

Function of cells and tissues



Liver Heart

Onset and development of disease



Normal Diabetes

low high

Signal intensity: e.g. gene expression level

In conclusion

Amazing technology which has already impacted healthcare and other areas, and will probably completely change medical diagnostics in the XXI century

Have fun and

see you next week