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Introduction

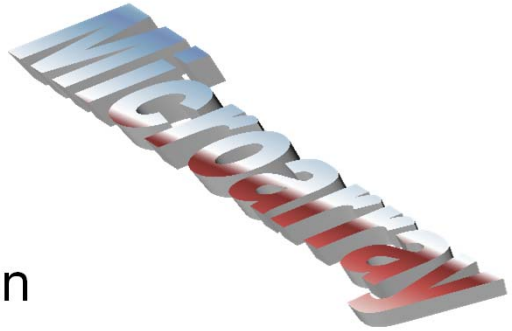
Ligand assay

=> Parallelization

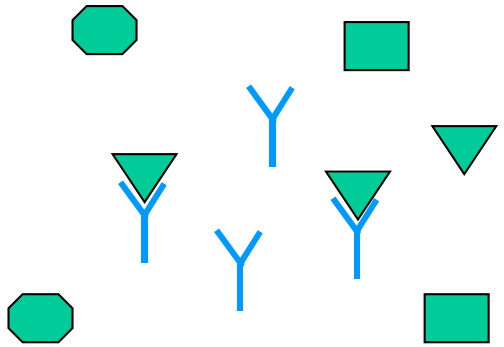
=> Parallelization

=> Miniaturization

=> Detection



- Ligand
  - Molecule interacting with specific receptor
  - Principle of complementarity
- Specific binding between
  - Probe
    - Known composition
  - Target
    - To be determined



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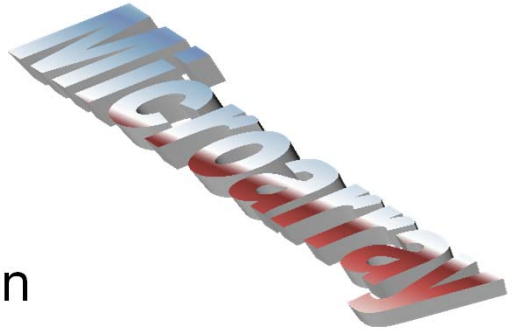
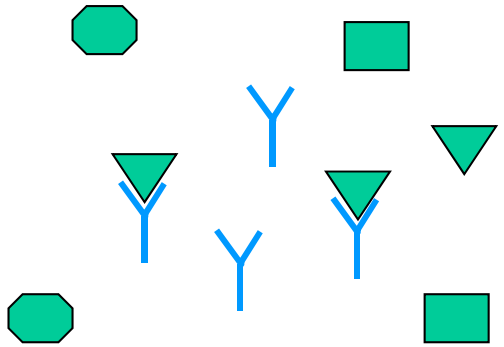


- Examples
  - ssDNA – ssDNA
  - ssDNA – RNA
  - Protein – enzyme
  - Receptor protein (drug target) - drug



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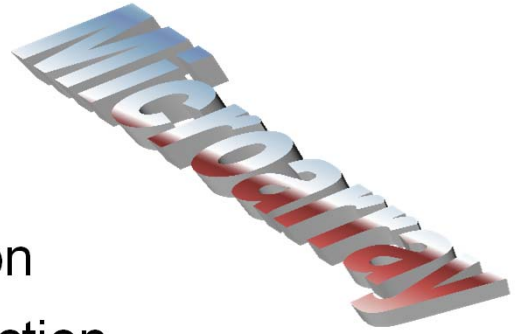
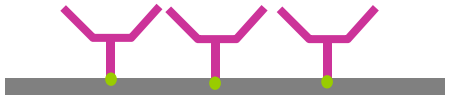
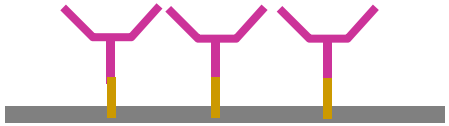


- Probes attached to substrate
  - Direct
    - Physical
      - Van-der-Waals (hydrogen)
      - Ionic
    - Chemical bond



  - Indirect
    - Linker molecule



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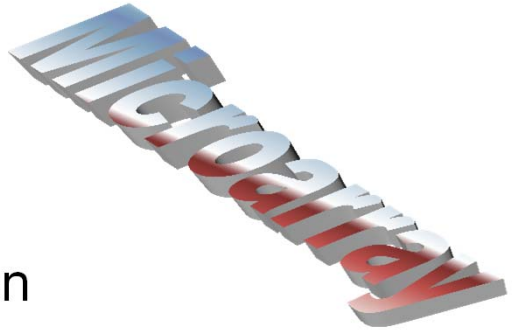
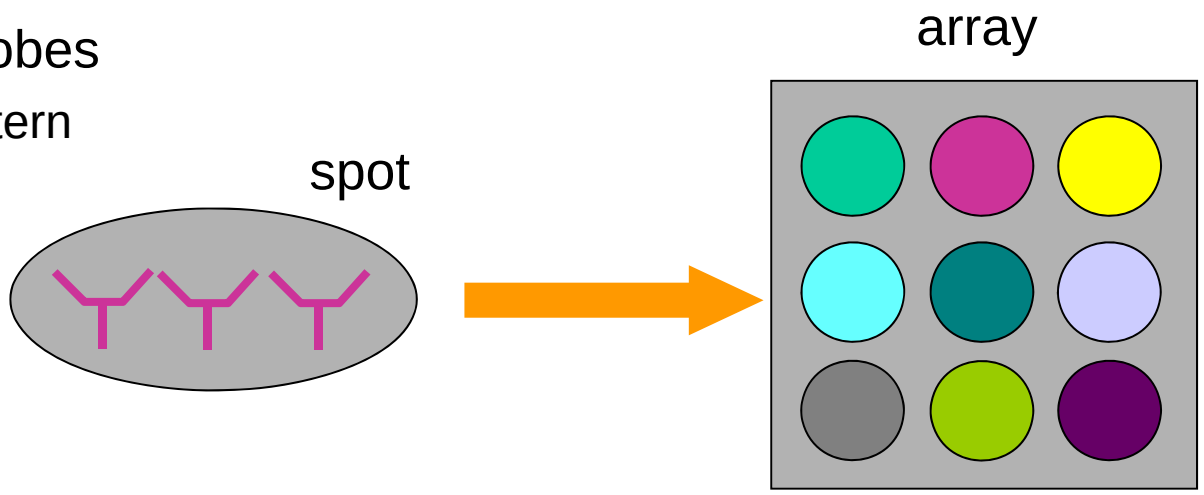


- **Sensors** (probes) represented by **spots**
- Identification of probes
  - Regular grid pattern
  - Lattice position



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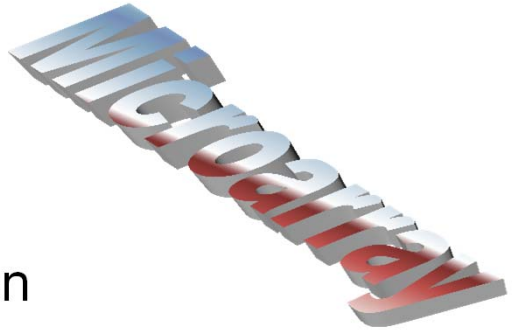
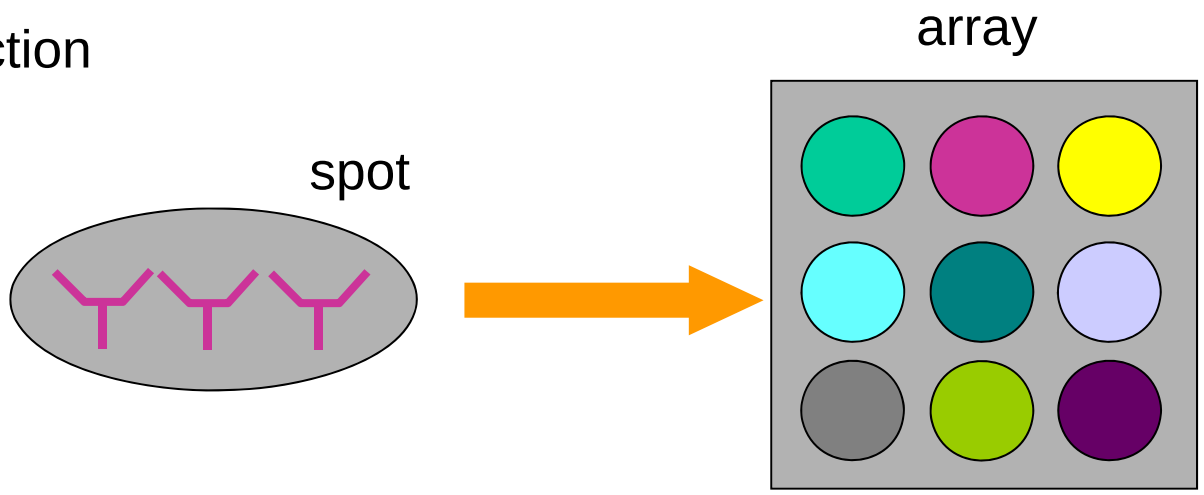


- Testing with multiple probes
- Simultaneous reaction



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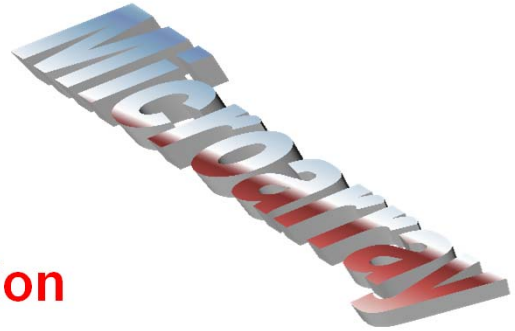
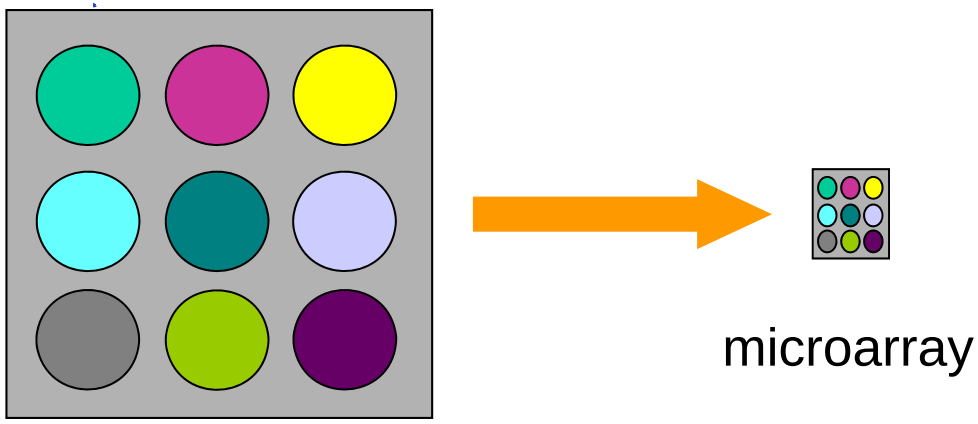


- Downsizing
  - Spot size
  - Pitch
  - Substrate surface



macroarray

microarray

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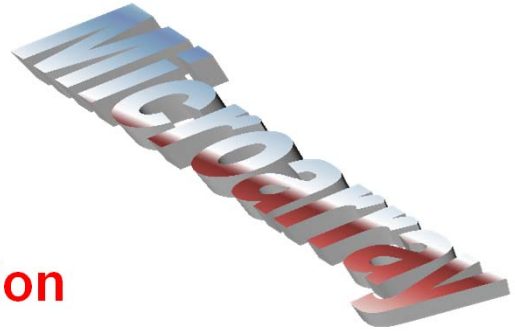
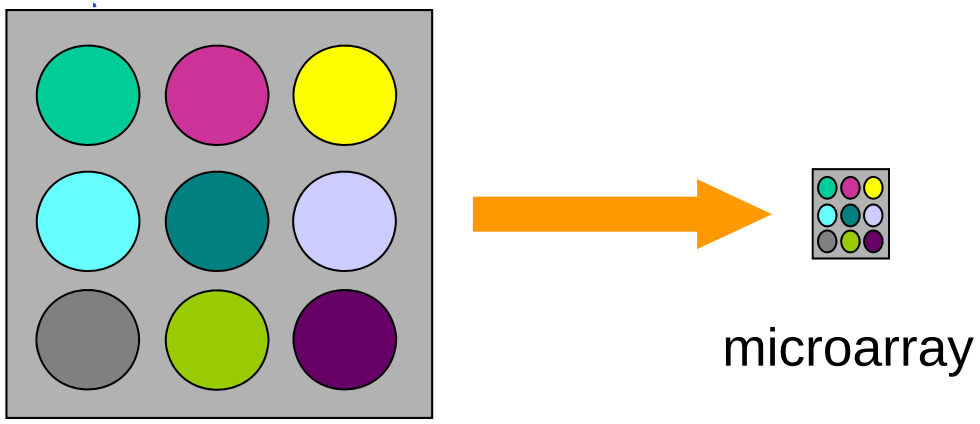


- Savings
  - Probe
  - Sample
  - Substrate
- Issues
  - Sensitivity



macroarray

microarray

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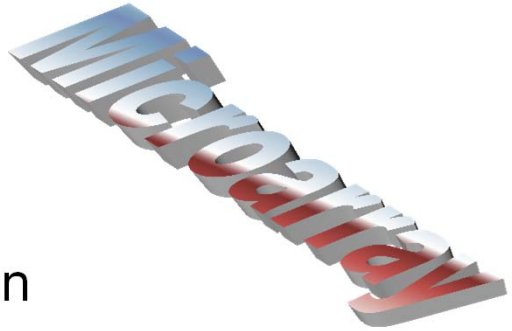
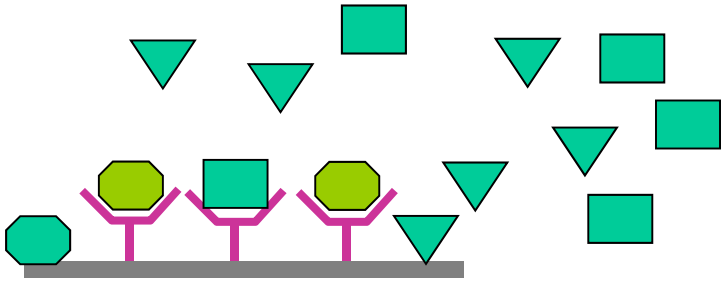


- Identification of **binding event**
  - Unbound target molecules
  - Distinction of binding
    - Specific
    - Unspecific
      - In solution
      - Adsobed on walls



spot

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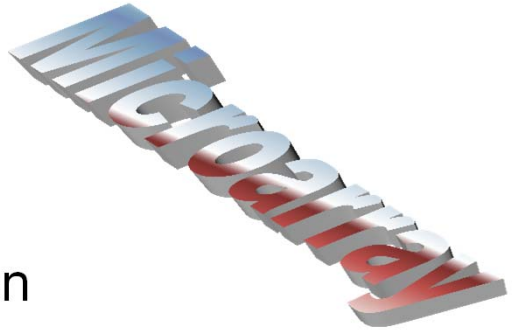
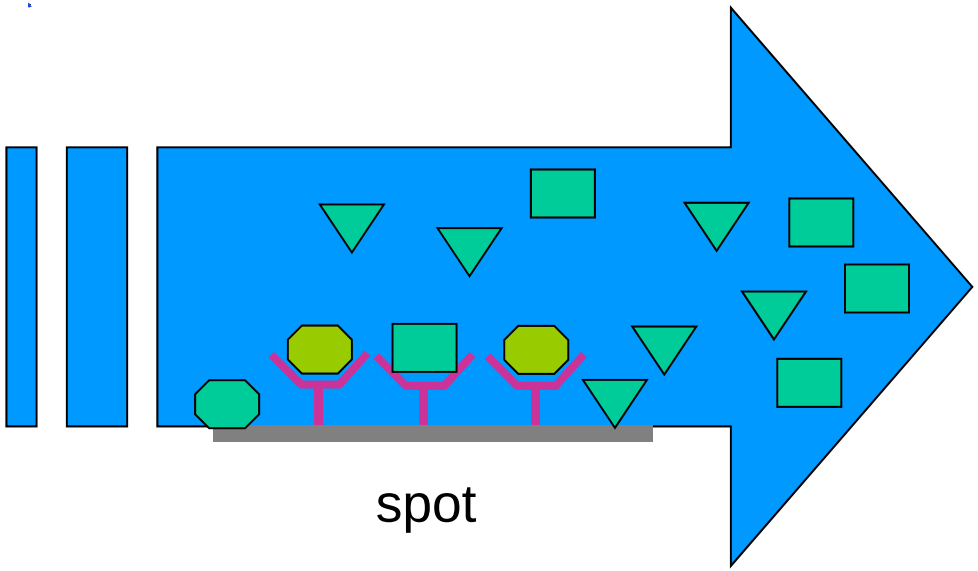


- Implementation
  - Removal
  - Washing



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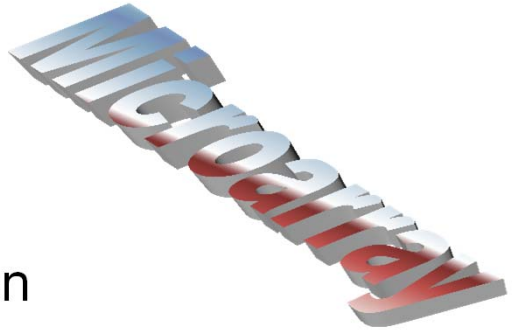

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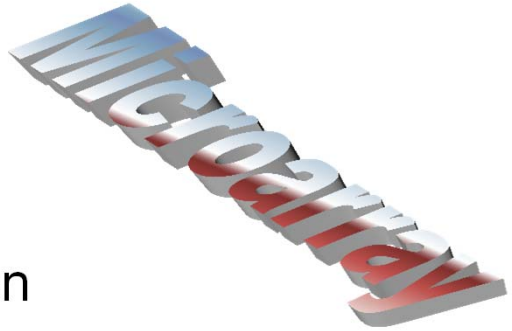
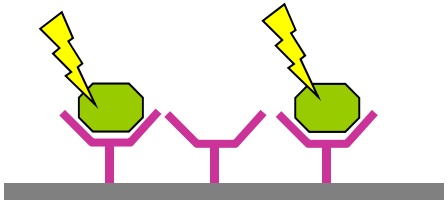


- Technological options
  - Labelling of target
    - Fluorescent
    - Bio- / chemiluminescent
    - Radioactive
  - Label-free
    - Electronic
    - Evanescent field



spot

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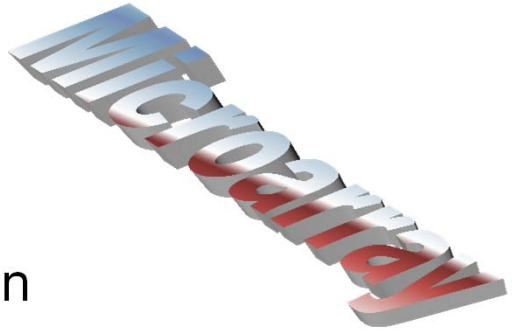
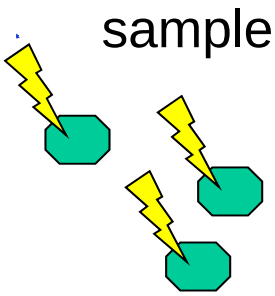
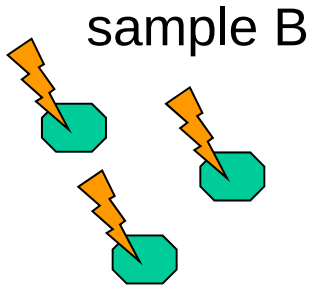


- Multiplexing
  - Multiple samples, e.g.
    - Different patients
    - Exposure to drug / placebo
  - Each sample
    - Different target label
  - Simultaneous reaction





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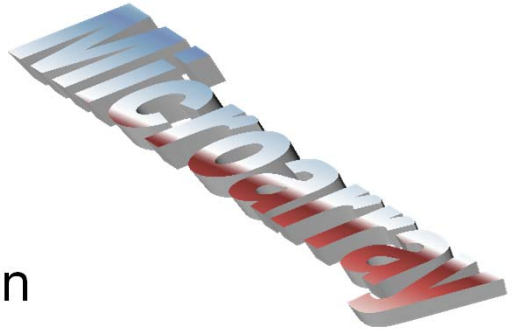
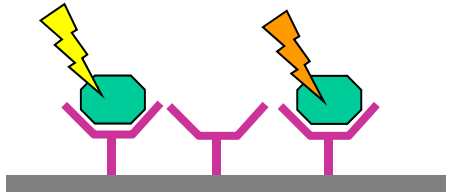


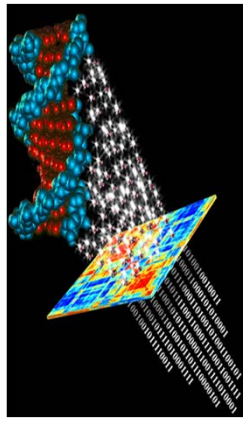
- Multiplexing
  - Objective
    - Reproducibility
      - Variable spot size
      - Experimental conditions
  - Internal standard
    - Differential measurement
    - Various errors ruled out



spot

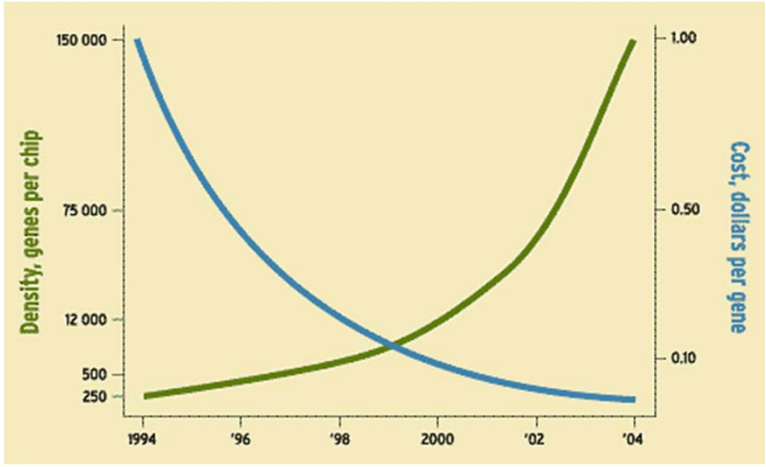
Gain of Information in Life Sciences

- Analogy to semiconductor industry
  - Microarrays CPUs of life sciences?
  - Moore's law
    - Number of transistors per chip doubles every 18–24 months
    - Similar growth for density of microarray chips



http://discover.nci.nih.gov/

- Cost
  - Shrinking array features
  - More chips per wafer
  - Less consumption
    - Sample
    - Reagents



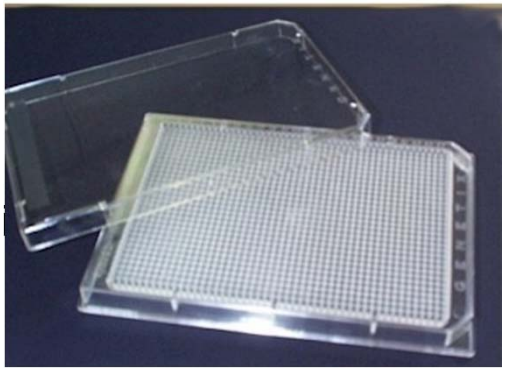
Limits?

- Technological limit of miniaturization
  - Sensitivity

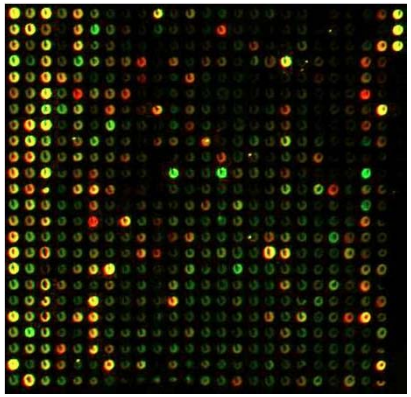
- Limit by life science applications
  - All genes of organism on single chip
    - 30,000–40,000 genes
  - Many medical application
    - Maybe 10-100 test in parallel

Array-Based Experiments

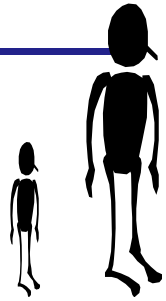
- Well plates
  - Well-established standard
  - Test-tube paradigm
  - Standardized automation schemes for liqui
  - 96-, 384- and 1536-well standards



- Standard blotting membranes („macroarrays“)
  - Spot sizes above 0.3 mm
  - Pitch ~2 mm
  - Density ~25 spots per cm^2
    - < ~1000 spots / array
  - Nylon and other membranes
  - Well plate format 8 x 12 cm^2
  - Imaging via existing blot or gel scanners
  - Large hybridization volumes, ~ some ml
  - Fabrication: manual or robotics



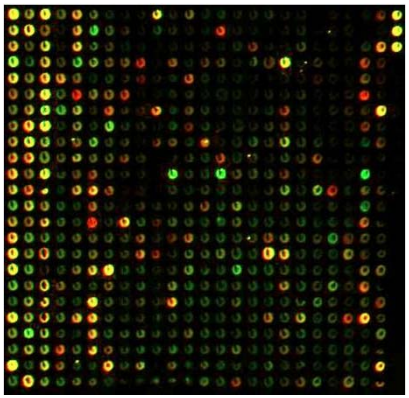
From Well Plates to Microarrays



- Downsizing of assay formats
  - Long-term trend in molecular biology diagnostics
  - Driven by economics and technical benefits

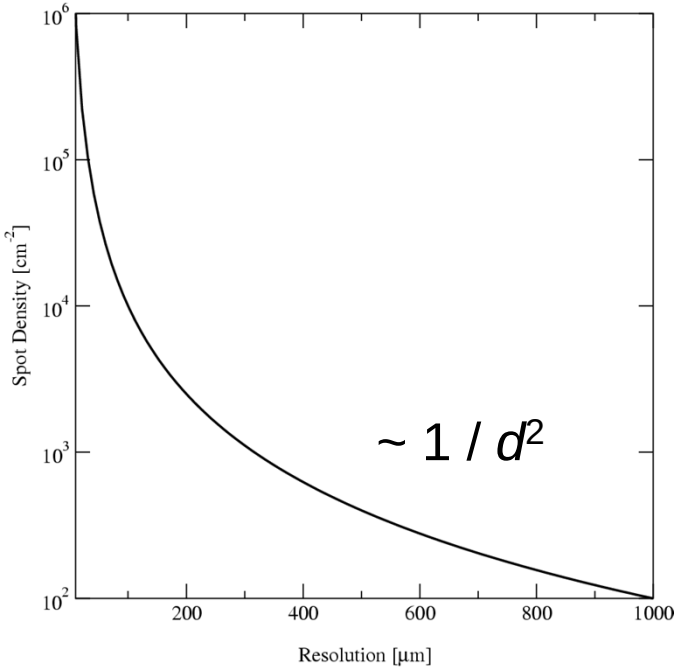
- MTPs
  - Limits of miniaturization reached?

- Microarrays
  - Further automation on behalf of screening
  - Comprehensive libraries screened in single chemical reaction
  - Amenability to high-speed scanning
  - Enabling massive mining of biomolecular data



Microarrays

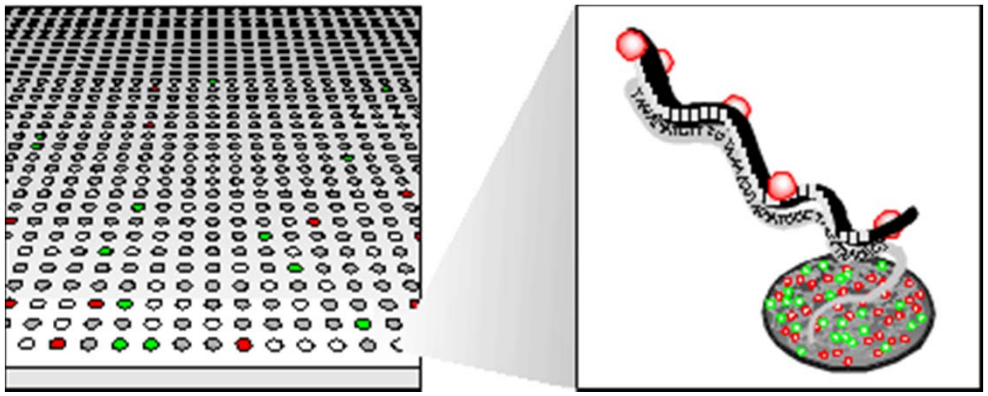
- Spot sizes of ~ 200 µm and below
- Pitch < 500 µm
- Density
  - Some 1000 spots / cm^2
  - Thousands of spots / array on 2 x 8 cm^2
- Fabrication
  - Specialized high-speed robotics
- Detection
  - Specialized imaging devices
- Dramatic increase in throughput



Spot densities as a function of the microarray pitch

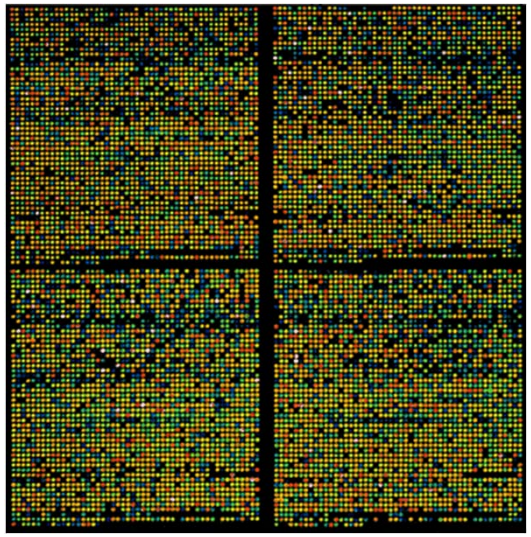
10.1. Terminology: Microarrays & Biochips

- Microarrays
  - Technically „correct“
  - DNA arrays
    - DNA chip
    - DNA microarray
    - Gene chip
    - Genome chip
- Biochips
  - Popular name, especially in marketing
  - Ambiguous definition
    - Lab-on-a-chip systems
    - Microarrays
    - Biomolecular computers
    - ...
- In this course: Microarray = Biochip



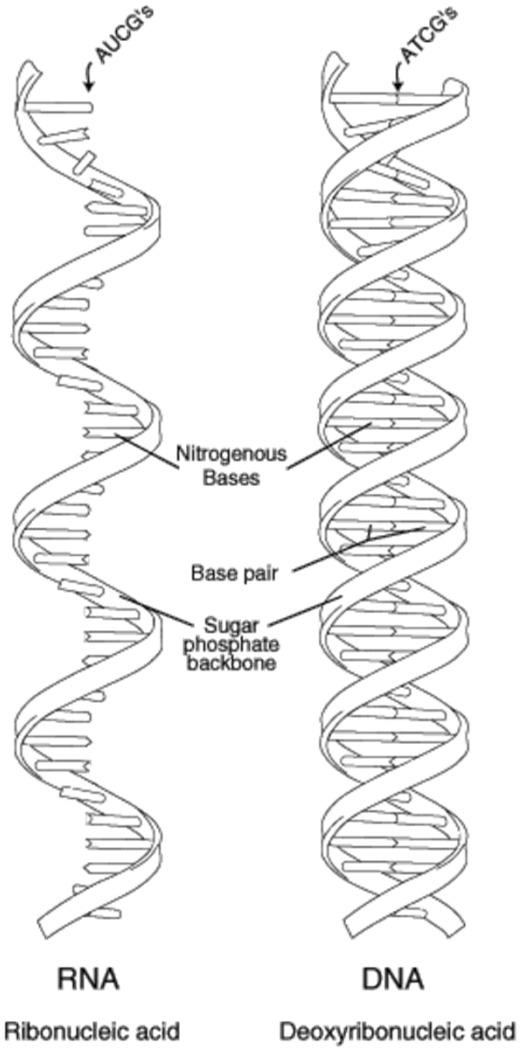
10.1. Array-Based Experiments

- Array
  - Orderly arrangement of samples
  - Medium for matching known and unknown DNA samples
- Ligand assays
  - Observation of product of binding reaction between
    - Analyte containing **target** molecules
    - Specific binding reagent (**probes**)
- Method (DNA)
  - Base-pairing
  - DNA: A-T and G-C
  - RNA: A-U and G-C
- Also
  - Protein and immunoassays
- Common array experiments
  - Well plates
  - Standard blotting membranes
  - Fabrication: manual or robotics



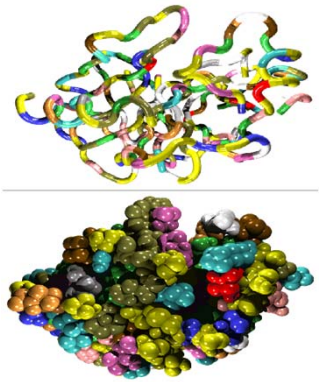
10.1. Major Classes of Microarrays

- DNA
  - ssDNA
  - cDNA
- RNA

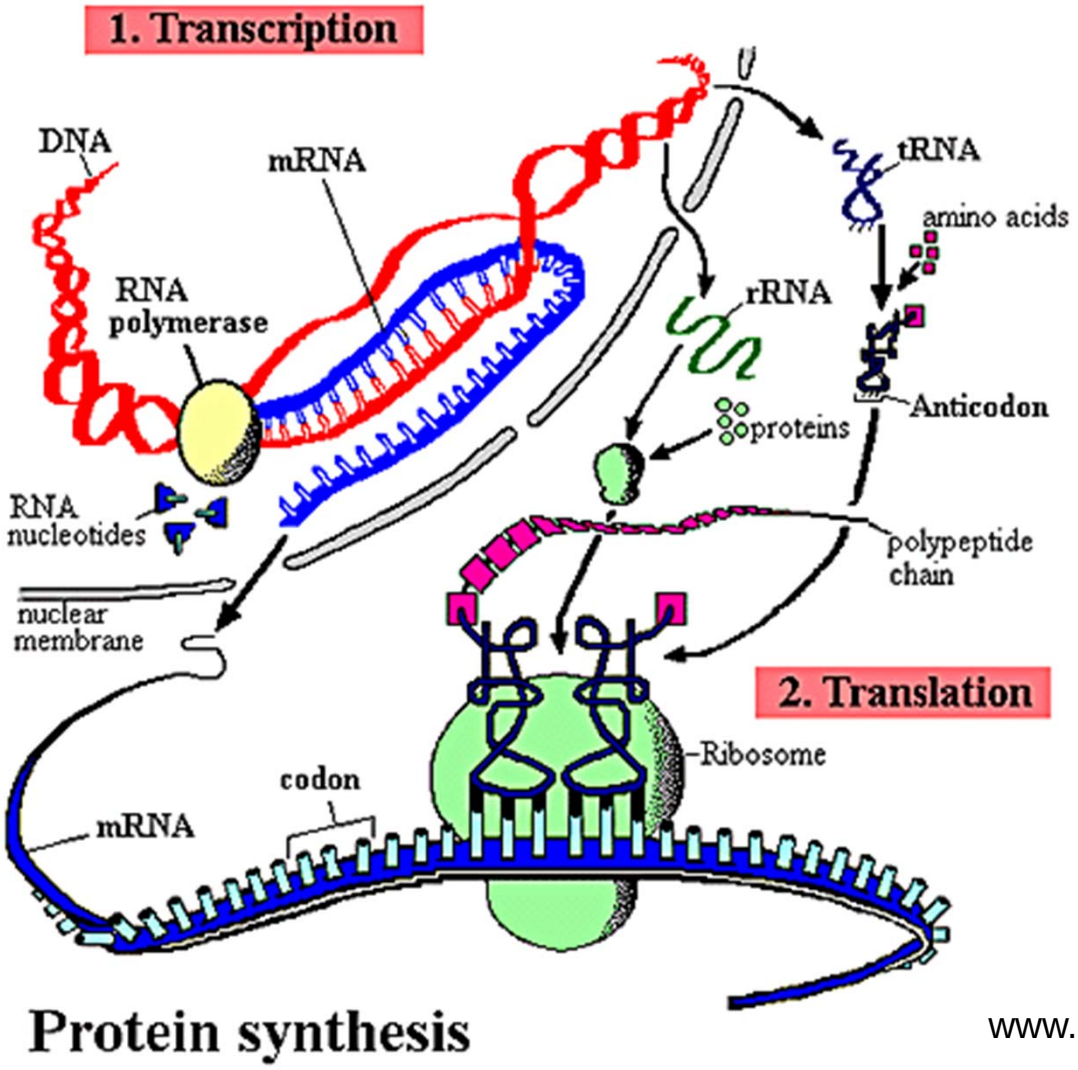


www.accessexcellence.com

- Proteins
  - ↗Antigene / antibody
  - ↗Enzymes
  - ↗Drugs
  - ↗...



10.1. Summary: Protein Synthesis



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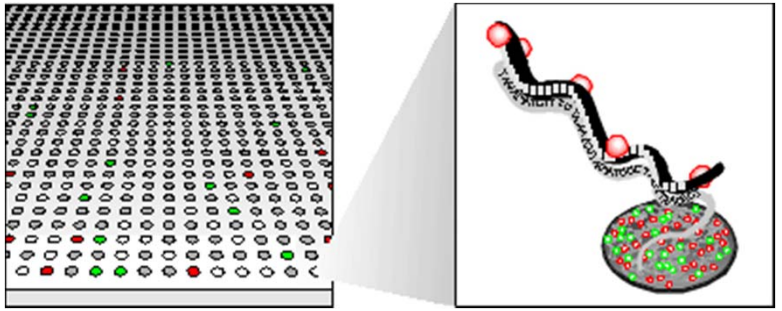
10.1. Distinction: Type of Immobilized Probe



- cDNA microarrays
  - Historical name: DNA microarray
  - DNA synthesized from an RNA template using reverse transcriptase
  - 500 – 5000 bases per probe molecule
  - Immobilized to solid support such as glass slide
  - Exposed to set of targets sequentially or simultaneously

- Oligo- and peptide nucleic acid (PNA) microarrays
  - Historical name: DNA chips
  - 20-80mer oligonucleotides or PNA
  - Fabrication
    - In-situ synthesis (e.g. by means of photochemistry, Affymetrix)
    - Conventional „offline“ synthesis followed by immobilization

10.1. Categories

- Distinction by application
  - Identification of sequence
    - Gene / gene mutation
  - Expression profiling
    - Measurement of expression level
    - Abundance

10.1. DNA Microarrays: Applications

- Gene discovery
- Drug discovery: Pharmacogenomics
  - ➢ Genetic predisposition for response on certain drrug?
    - Working fine with one group of patients
    - Highly toxic for other group
  - ➢ „Hybridization“
    - Functional genomics
    - Molecular pharmacology
  - ➢ Correlations between
    - Therapeutic responses to drugs
    - Genetic profiles of patients
- Toxicological research: Toxicogenomics
  - ➢ Hybridization
    - Functional genomics
    - Molecular toxicology

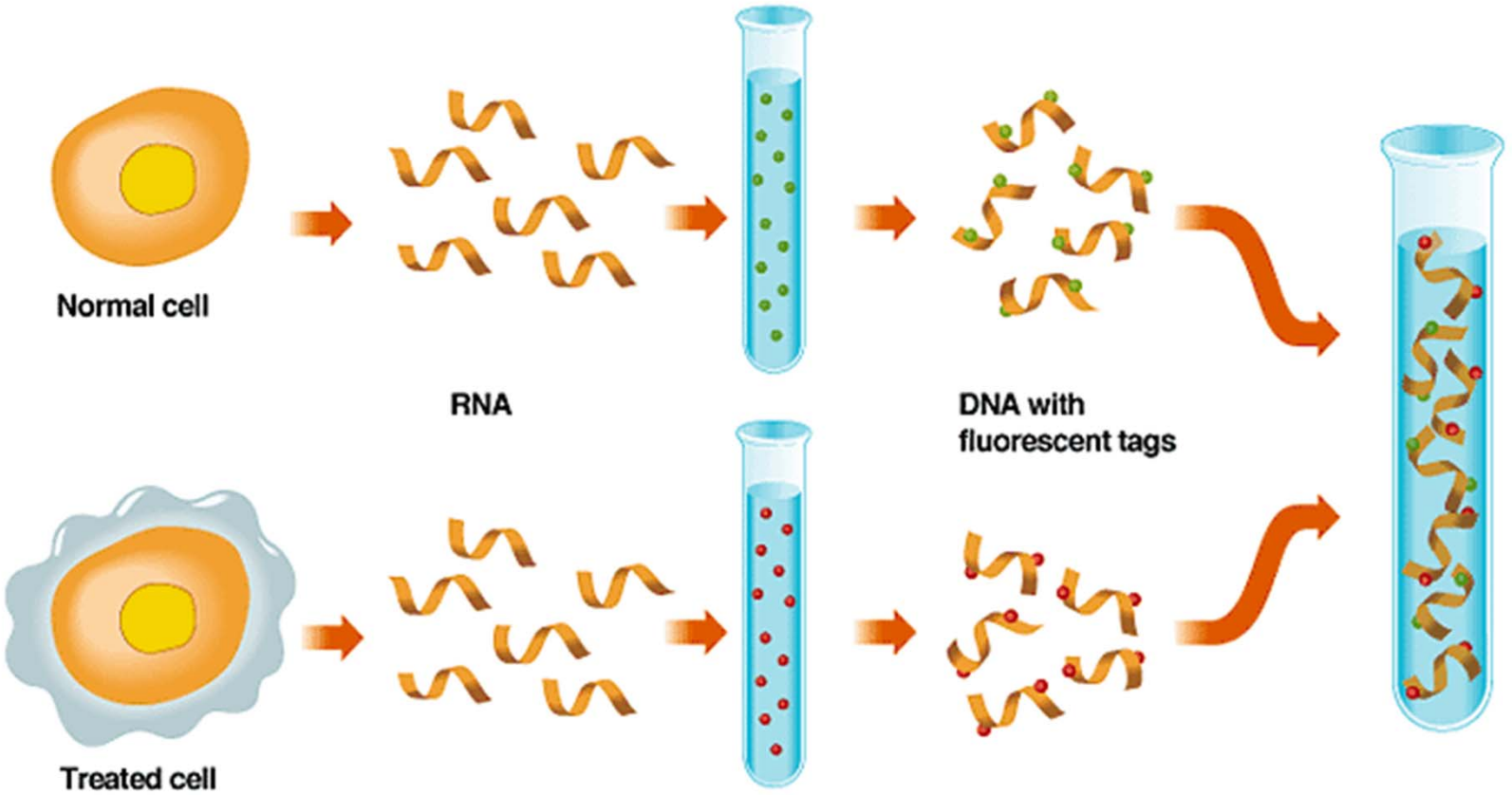
10.1. Gene Expression

Gene expression profiling

- cDNA microarrays
  - Much longer fragments

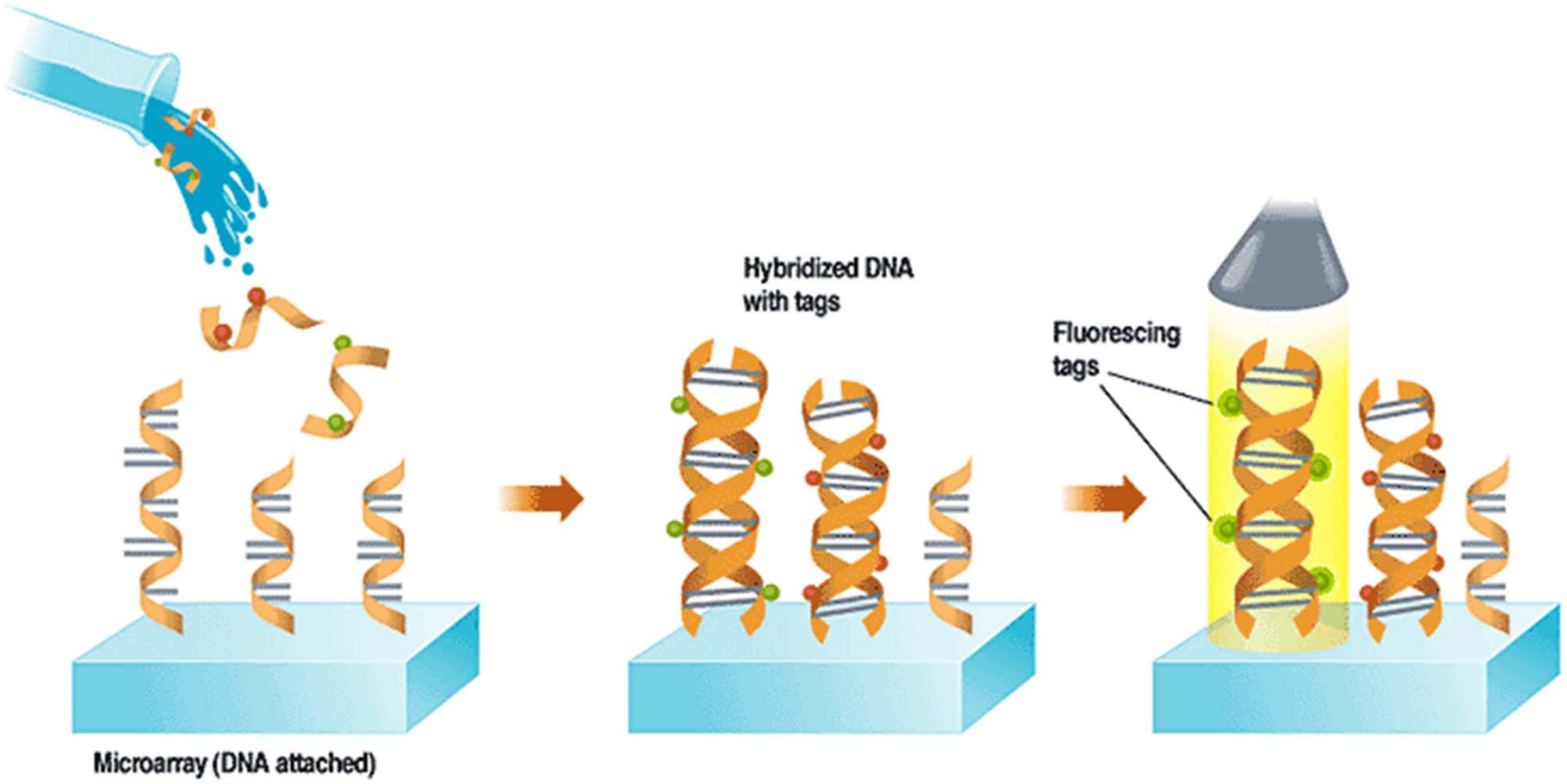
- Which genes are active
  - At certain time
  - After certain stimulus
  - In particular tissue

10.1. Gene Expresssion Profiling with Microarrays



Following slides from Moore, IEEE Spectrum online

10.1. Hybridization Arrays



10.1. Current Applications and Markets

	DNA Chips	Protein Chips	Lab Chips
Diagnostics	Used for research	Potential usage, but no development at present	Potential usage, but no development at present
Pharmacogenomics	Used for research	Potential usage, but no development at present	Potential usage, but no development at present
High Throughput Screening	Potential usage, but no development at present	Potential usage, but no development at present	Used in early access agreements
Expression Profiling	Significant usage in research and drug discovery programs	Moderate usage but in early stages of development	Potential usage, but no development at present
Toxicology Screening	Moderate usage but in early stages of development	Potential usage, but no development at present	Potential usage, but no development at present

Bioresearch Online, BioInsights 1999 study

10.1. Densities, Technologies & Applications

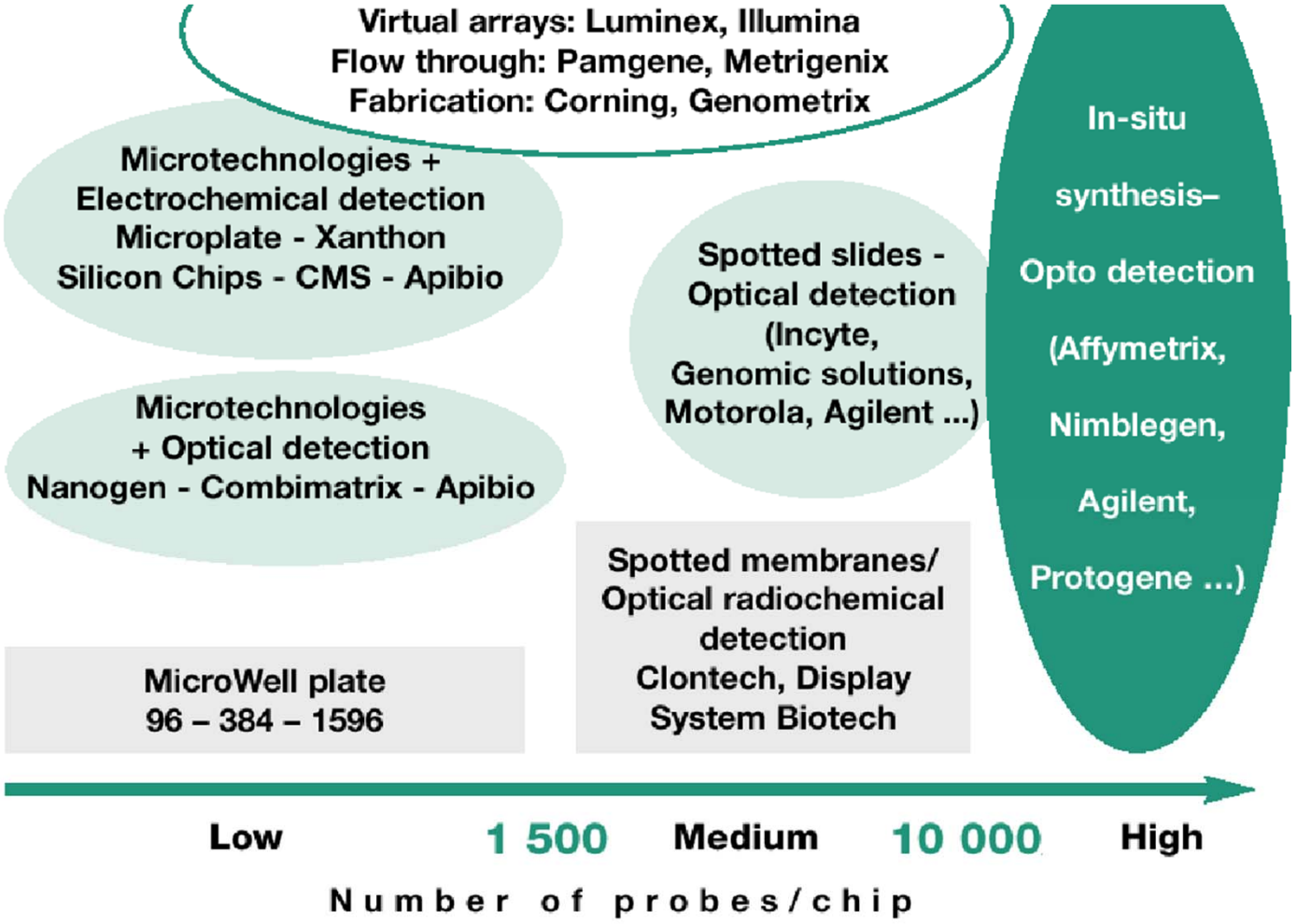
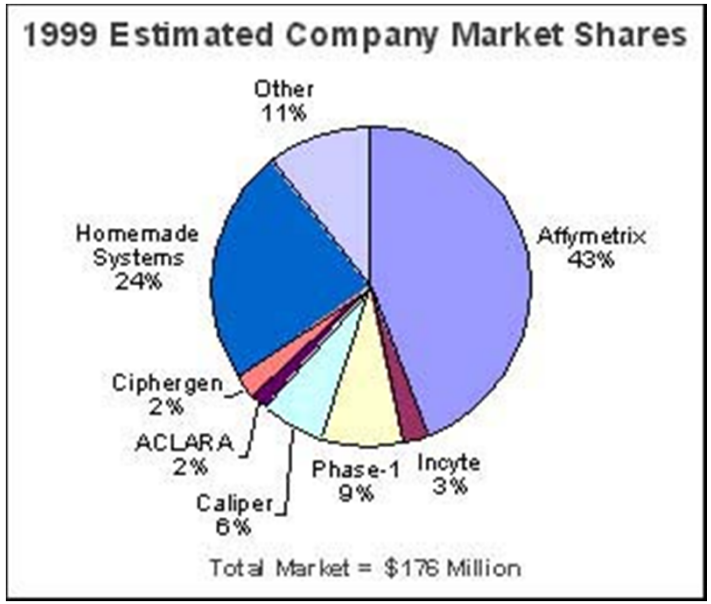
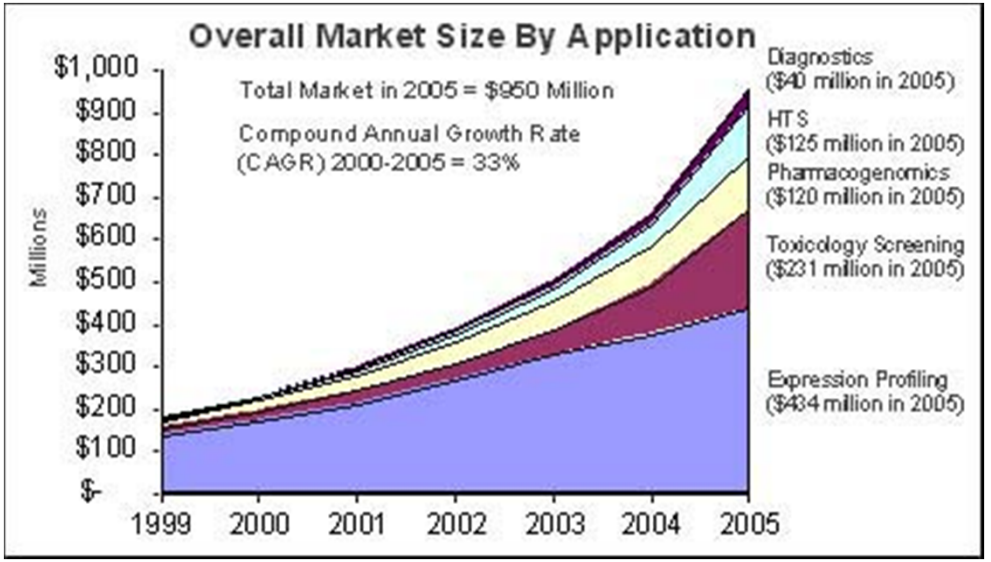


Fig. 10.2. Microarray densities and their applications

10.1. Current Applications and Markets

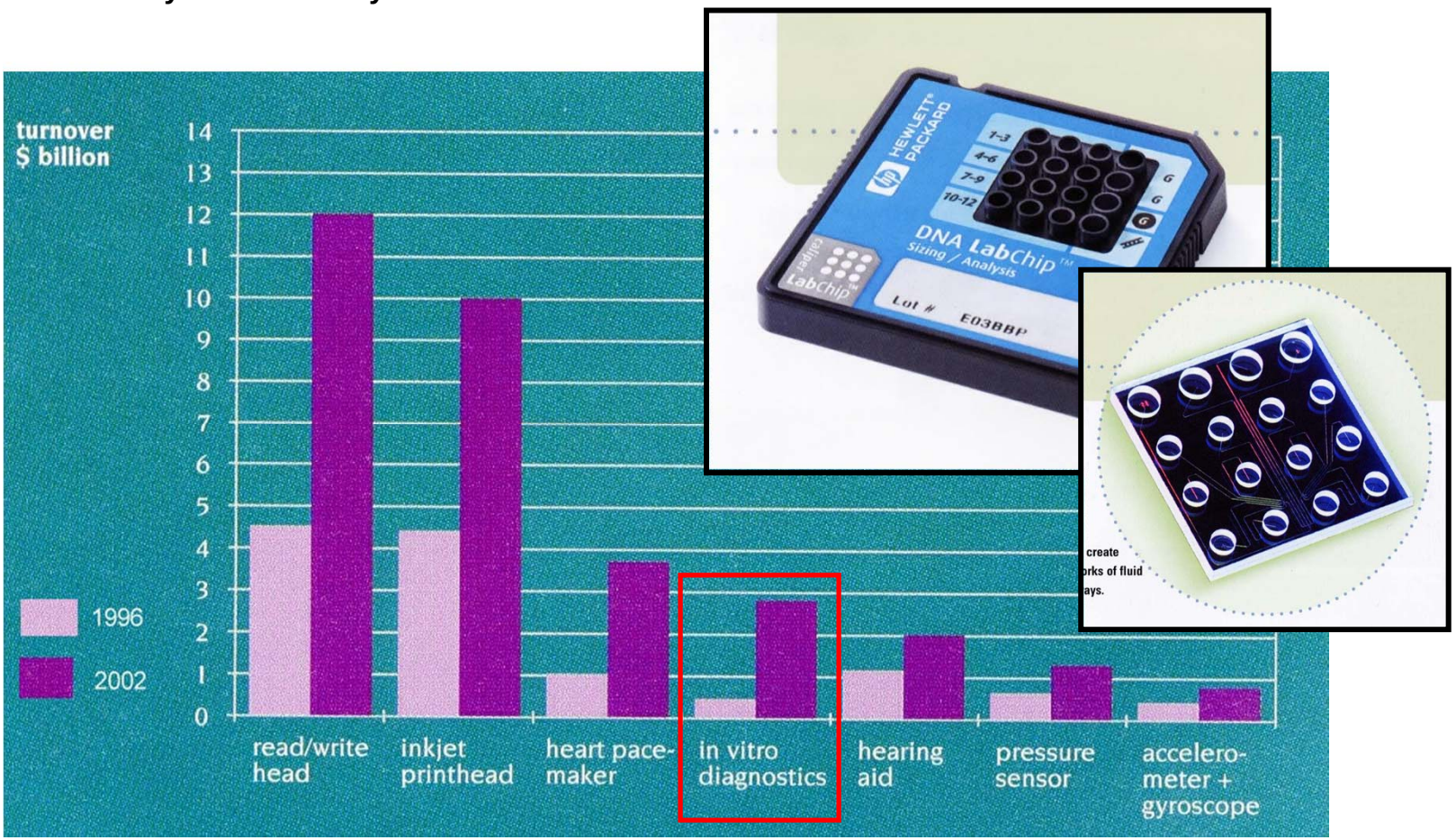




Bioresearch Online, BioInsights 1999 study

10.1. Bioanalytical Applications

NEXUS
Market analysis for microsystems 1996 - 2002



Agilent

10.1. D&MD Market Analysis

Presently:

- Highly parallel gene expression
- Highly parallel genotyping

In 2003 – 2005:

- Medium-to-low density gene expression
- Medium-to-low density genotyping
- High-throughput screening
- Infectious disease detection

Starting 2010:

- Low-density genotyping
- ...

10.1. Life Cycle of Microarrays

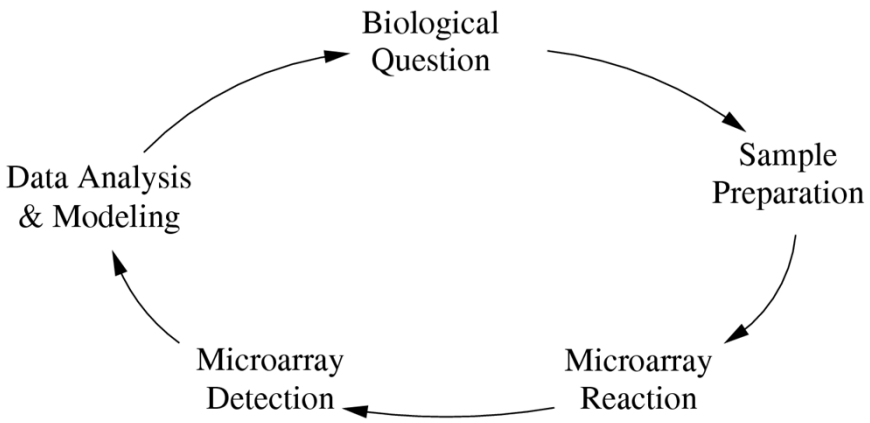


Fig. 10.2. Life cycle of a microarray

- Powerful platform
  - Biological exploration
- Precisely define biological question
  - MA experiments often generate plethora of data
  - Soundness of experimental design
    - Avoiding months of subsequent analysis

10.1. Life Cycle of Microarrays

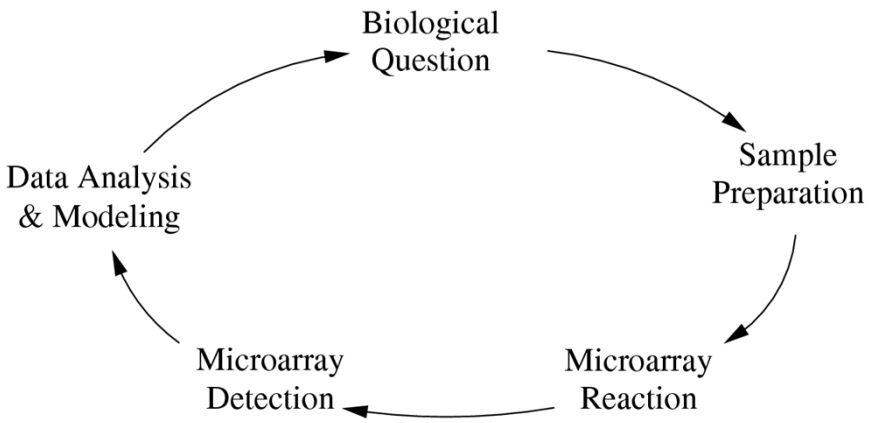


Fig. 10.2. Life cycle of a microarray

- Ratio
  - Analysis time
  - Experimental time
- Small ratio for traditional assay formats
  - Gels
  - Nylon membranes
- Large ratio for microarray-based assays

10.1. Biological Question

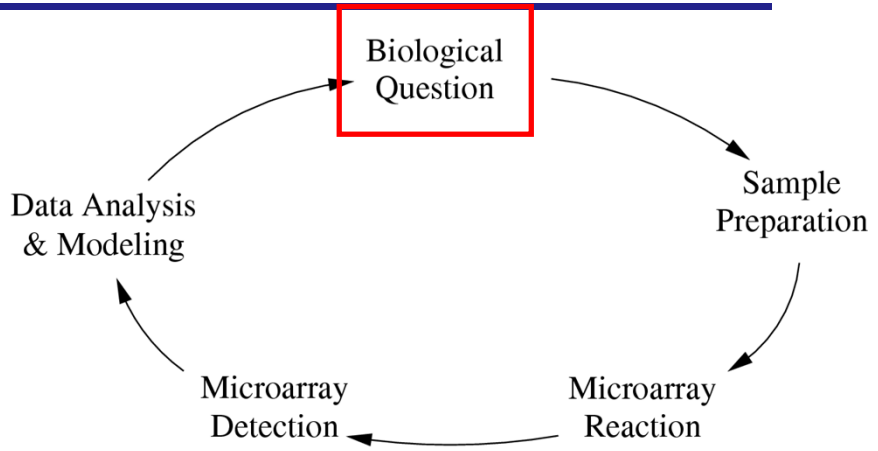


Fig. 10.2. Life cycle of a microarray

- Traditional experiments
  - Start with **hypothesis**
  - Requires a priori **understanding** of outcome

- Microarray experiments
  - Start with **question**
  - Definition of experiment
    - Focus
    - Scope
    - Intent
  - Unprecedented look at biological experiments

10.1. Sample Preparation

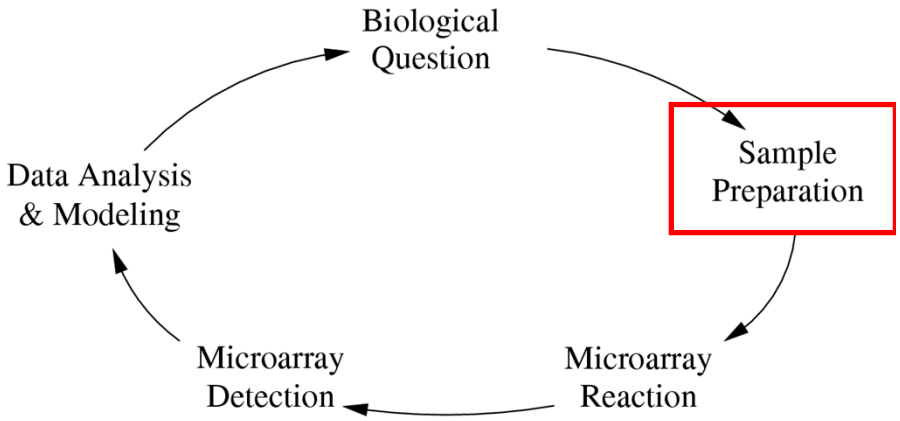


Fig. 10.2. Life cycle of a microarray

- Sample prep
  - Critical for results
- RNA sample prep
  - Rapid and complete inactivation of ribonucleases (RNAses)
  - Factors
    - Incubator temperature
    - CO_2 concentration
    - Growth media
    - Buffering components
    - Hormones
- DNA sample prep
  - Efficiency of isolating viscous, high-molecular-weight molecules

10.1. Sample Preparation

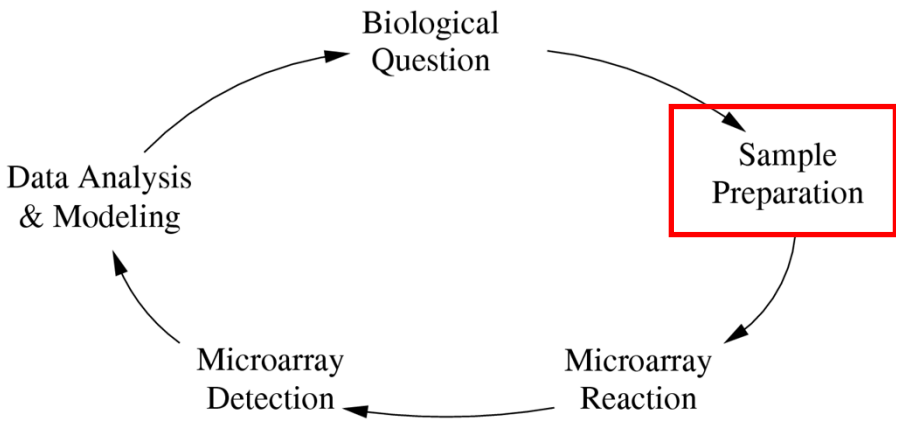
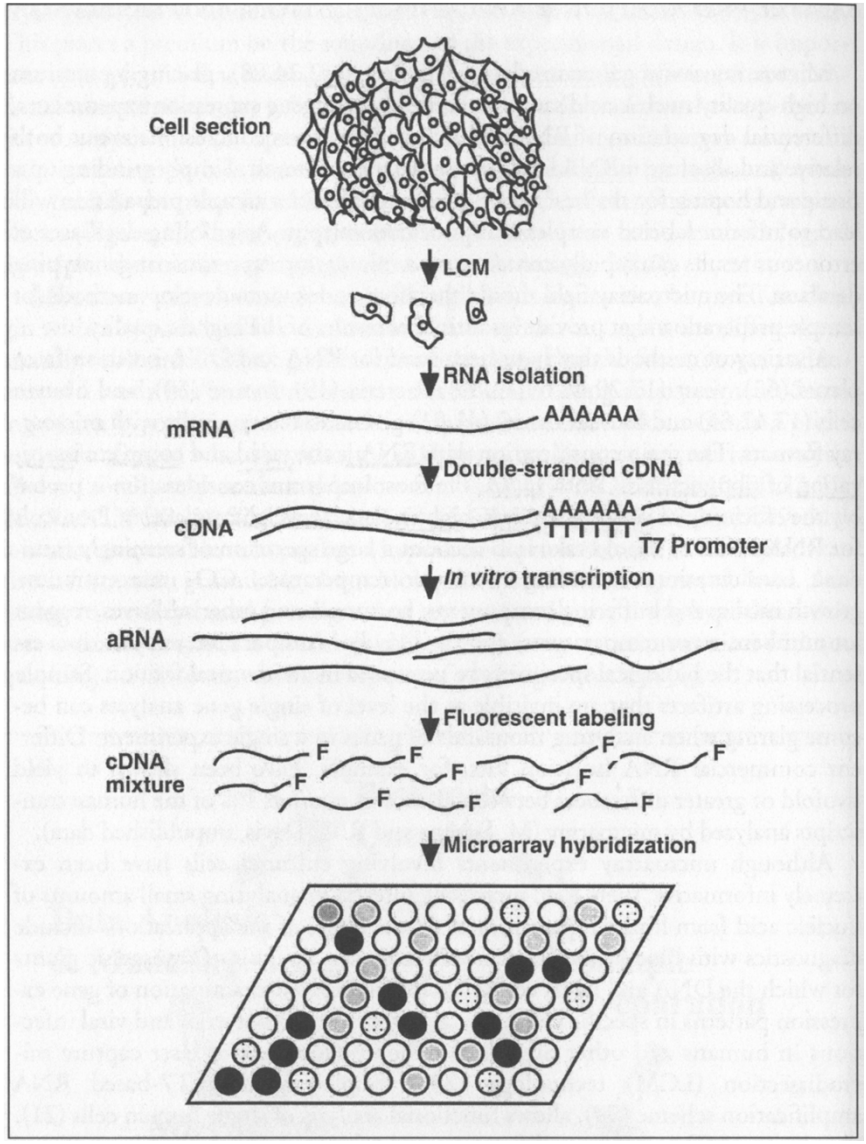


Fig. 10.2. Life cycle of a microarray

- Gene expression profiling
  - Single cells
  - Distinction
    - Space: tissue
    - Time
  - Laser capture microdissection technology
    - Capturing cells from cell section

10.1. Schematic of Gene Expression



M. Schena et al.

10. Microarrays

1. Introduction
2. **Reaction Kinetics**
3. Immobilization
4. Fabrication
5. Detection
6. Electronic Control
7. Protein Microarrays
8. Bead-Based Microarrays

10.2. Microarray Reaction: Diffusion

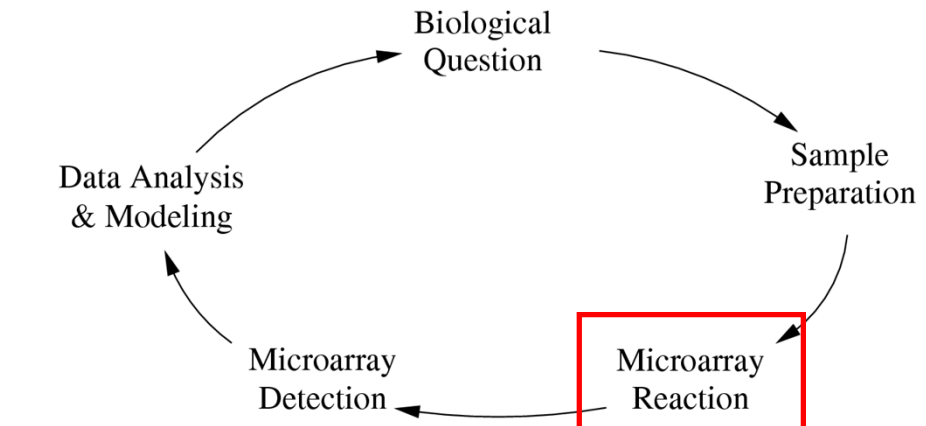


Fig. 10.2. Life cycle of a microarray

- Diffusion according to Fick‘s law
  - 1st slow step
  - Modification for steric effects
  - Spatial extension of biomolecules
  - Depletion of biomoleculas near surface

$$j_N = -D \nabla \varrho_N$$

10.2. Microarray Reaction: Hybridization

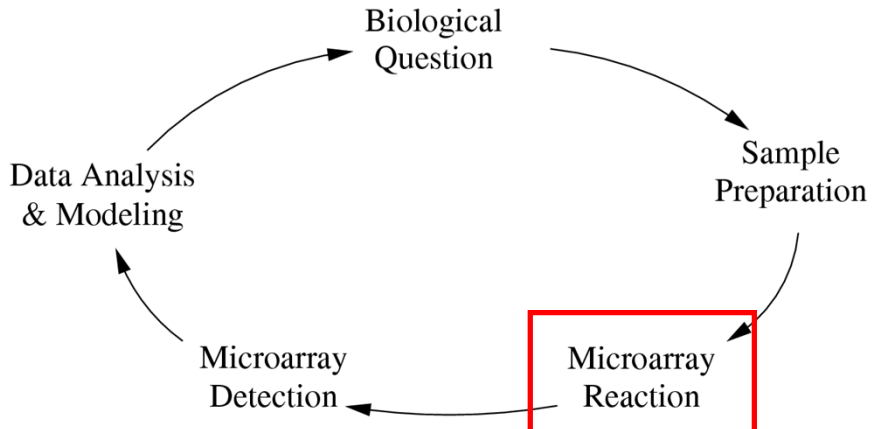


Fig. 10.2. Life cycle of a microarray

- Hybridization reaction
  - 2nd step
  - Usually fast
  - Reaction kinetics
  - Optimum probe density

$$\frac{\partial[\mathrm{AB}]}{\partial t} = k_{\mathrm{AB}}[\mathrm{A}][\mathrm{B}]$$

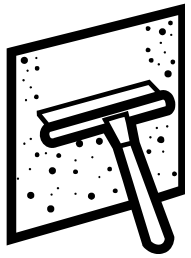
- Unspecific binding
  - Important problem

10. Microarrays

1. Introduction
2. Reaction Kinetics
3. **Immobilization**
4. Fabrication
5. Detection
6. Electronic Control
7. Protein Microarrays
8. Bead-Based Microarrays

10.3. Surface Preparation & Immobilization

- Basic requirements
  - Precision
  - Stability
  - Reproducibility

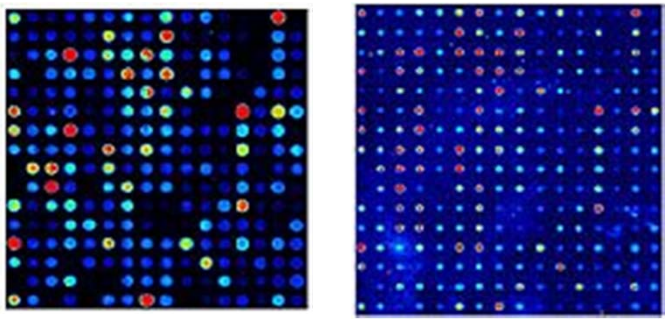


- Functionalization of surface
  - Detecting wide range of analytes via direct affinity capture
  - Covalently bound probes permit more harsh wash steps
    - Background reduction
    - Greater sensitivity
  - Higher densities on hydrophobic substrates (less spreading)

10.3. Substrates

- Characteristics
  - Spot morphology
  - High DNA retention
  - Fluorescent background
-
- Material
  - Glass
  - (Optically) flat surfaces
- Low intrinsic fluorescence
  - Sensitivity
- Bar coding

Corning



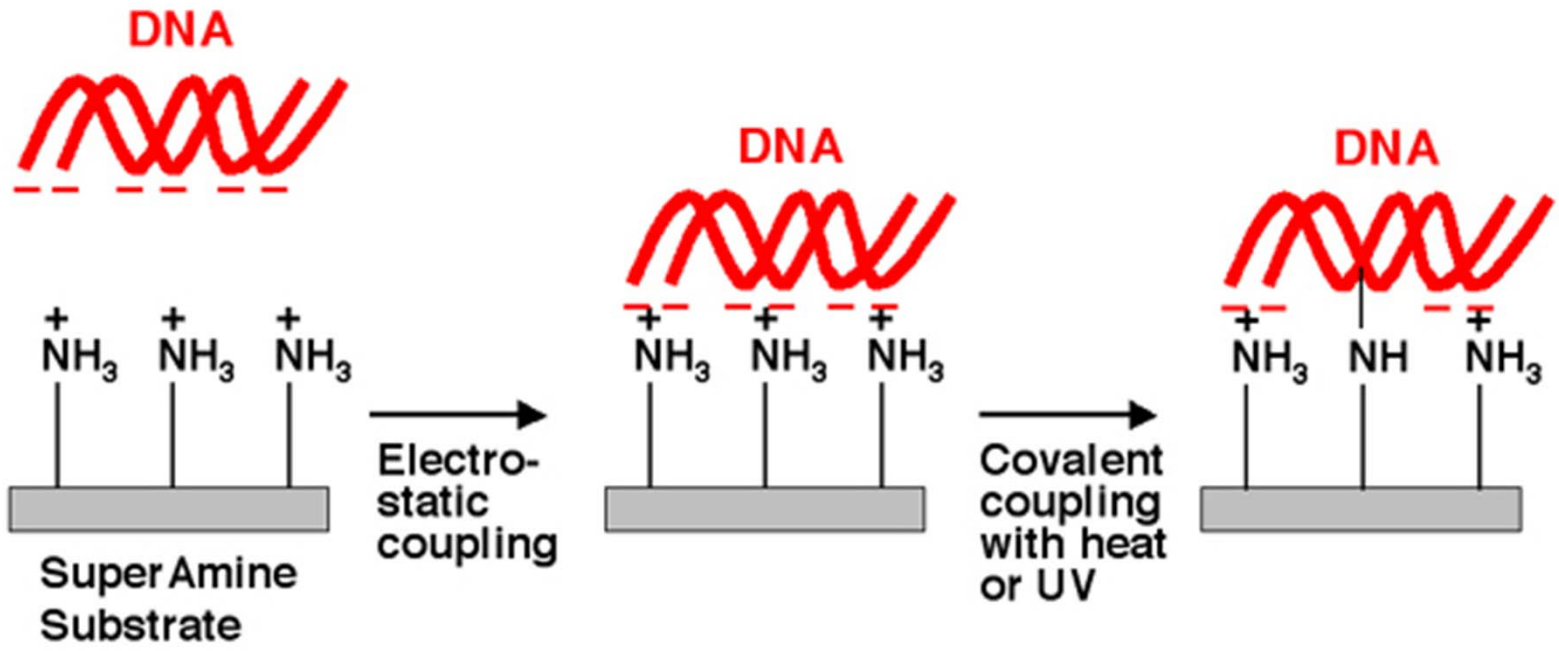
coated uncoated

10.3. Surface Coatings

Corning

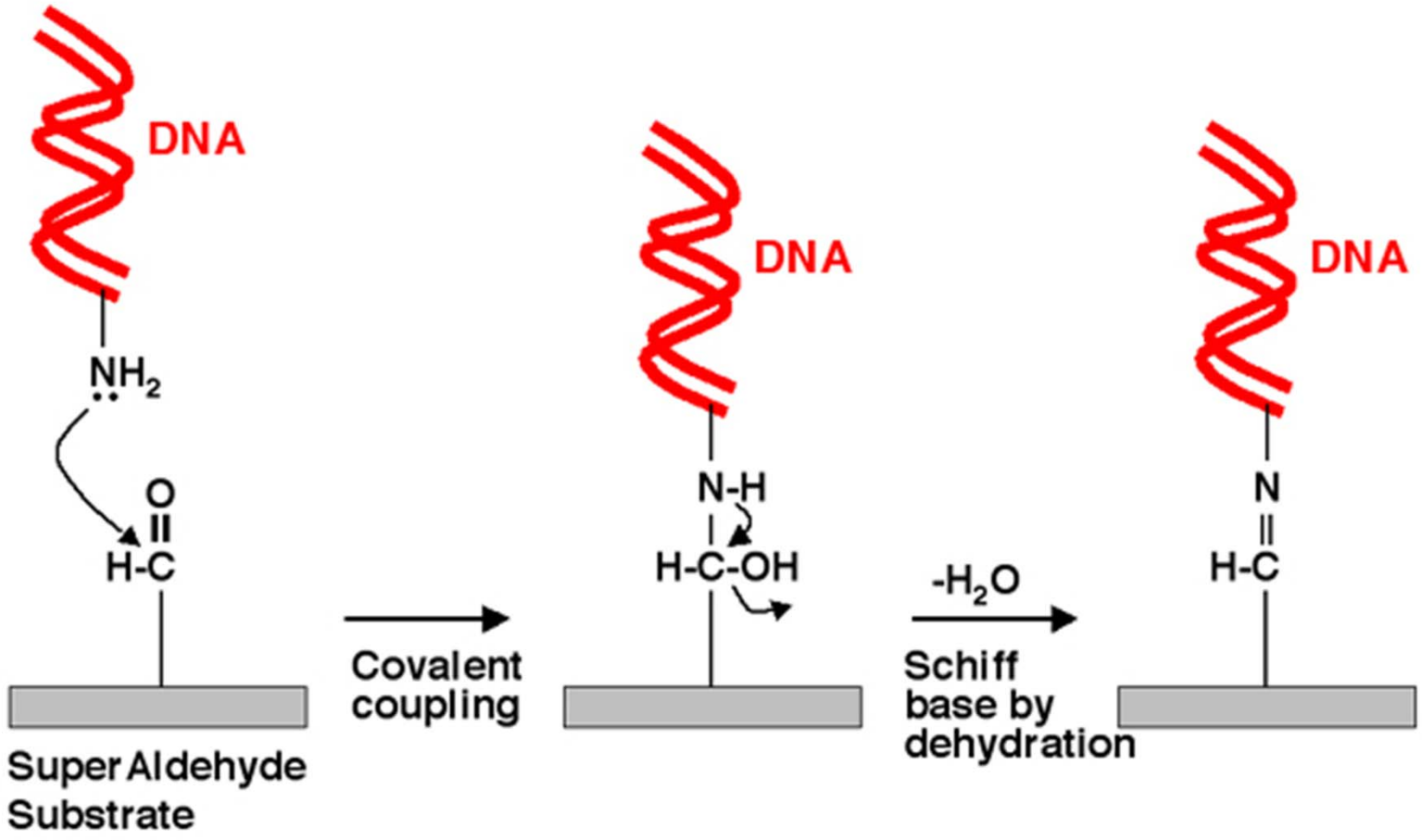
- Untreated
- Surface chemistries
  - Amino-silane
  - Non-amino surface chemistries
- Covalent amine or aldehyde groups
  - Stable attachment of nucleic acids, proteins, small molecules, extracts, cells
- Silylated slides
- Coating with microporous polymer
- Covalent immobilization of amine-terminated DNA products
- Polymer brushes

10.3. Telechem‘s Immobilization



Telechem International // arrayit.com

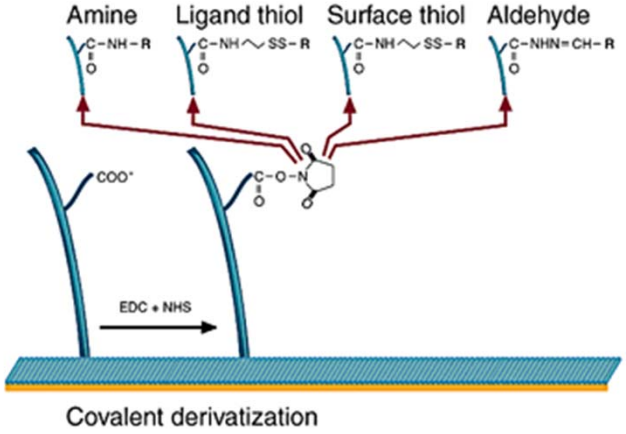
10.3. Telechem‘s Immobilization



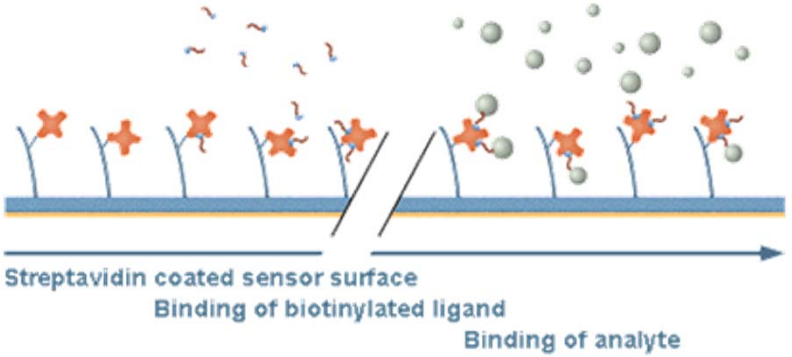
Telechem International // arrayit.com

10.3. Surface Coatings

- CM5
  - Ligand immobilization via native - -NH_2, -SH_2 -CHO and -COOH
  - 100s of scientific papers ...

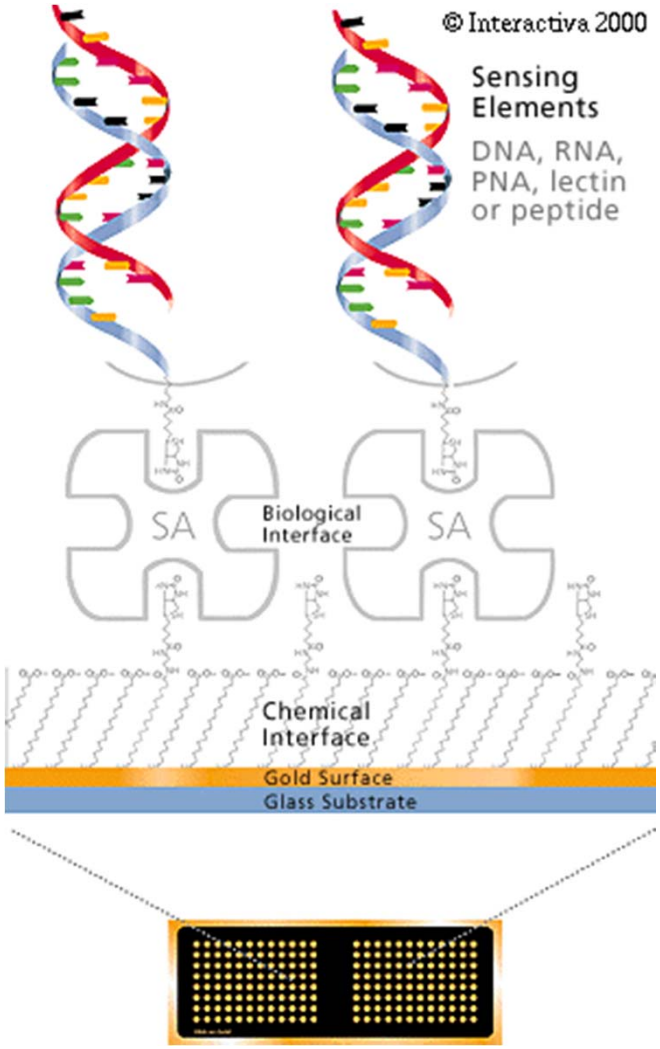


- Sensor Chip SA
  - Standard biosensor surface
  - Pre-immobilized streptavidin
  - General purpose sensor chip for capture of biotinylated ligands
  - Combination of
    - High binding capacity
    - Reproducibility
    - Chemical resistivity



10.3. XNA on Gold

- Self-assembling monolayers of long-chain thiol alkenes onto 100-nm layer of 24 carat Au
- Biotin covalently coupled to surface
- Biotin layer saturated with streptavidin
- Hydrophobic patterning of substrate to define spots



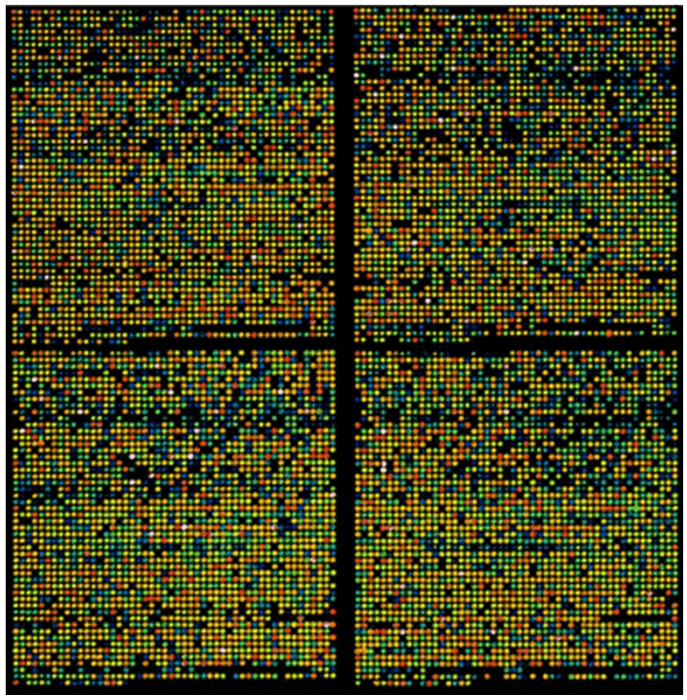
10. Microarrays

1. Introduction
2. Reaction Kinetics
3. Immobilization
4. **Fabrication**
5. Detection
6. Electronic Control
7. Protein Microarrays
8. Bead-Based Microarrays

10.4. Microarrays: Fabrication

- Introduction & Overview

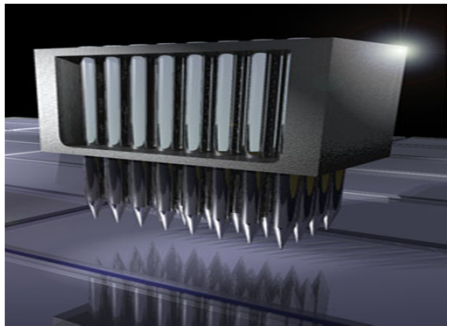
- On-Chip Synthesis
- Contact Printing (Pin-Printing)
- Non-Contact-Printing (InkJet or Piezo)
- Comparison of speed
- Electronic Arrays



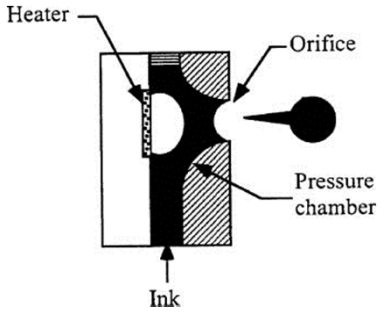
10.4. Fabrication

- Contact printing
  - Pins transfer liquid from reservoir to substrate
  - Spotting of pre-made analytes

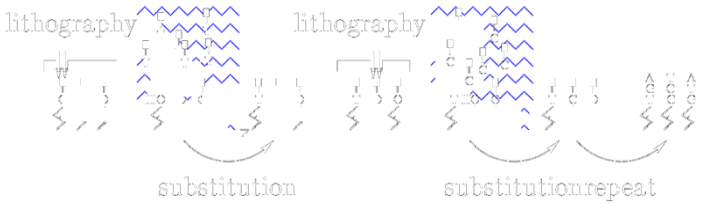
Brown Lab (Stanford)



- Non-contact printing
  - Ink-jet like principle
  - Spotting of pre-made analytes



- On-chip synthesis
  - On-chip polymerization of probe molecules



10.4. Introduction - Overview

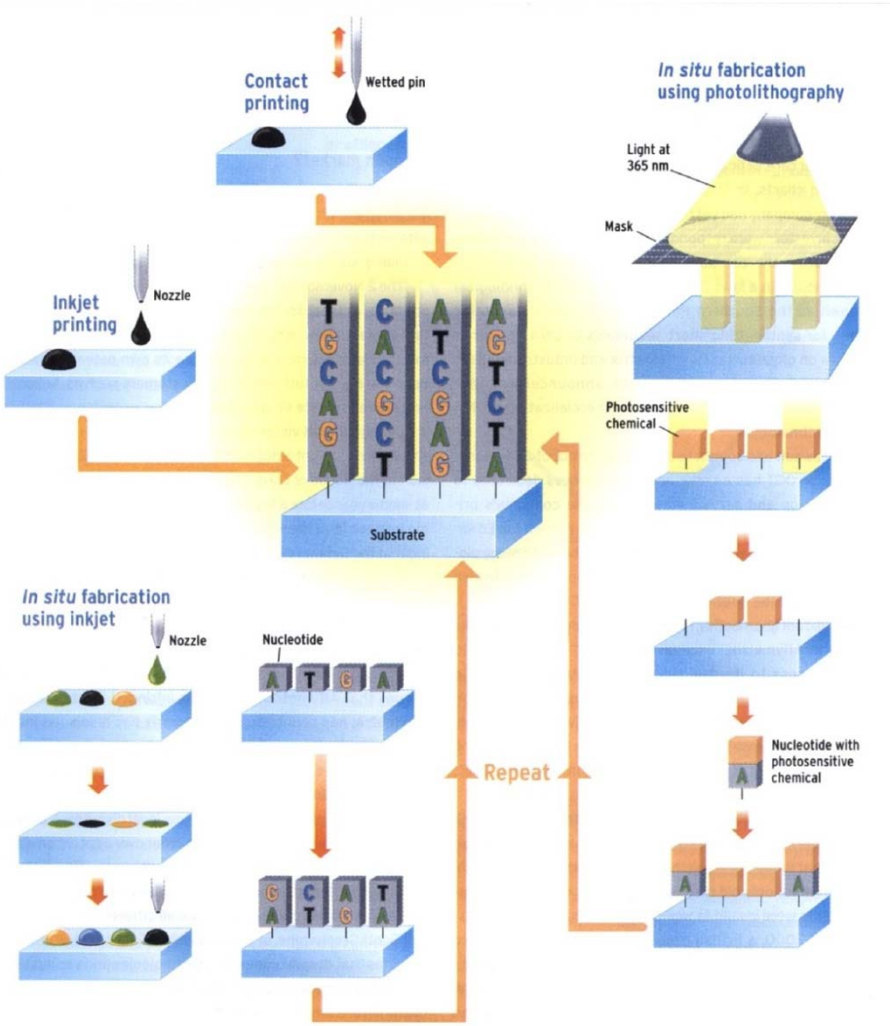
Contact Printing

- Genometrix
- Corning
- ...

In-Situ Synthesis (modified)

- Agilent
- ...

Source: S. K. Moore; IEEE SPECTRUM; March 2001



Ink-Jet-Printing (Non-Contact)

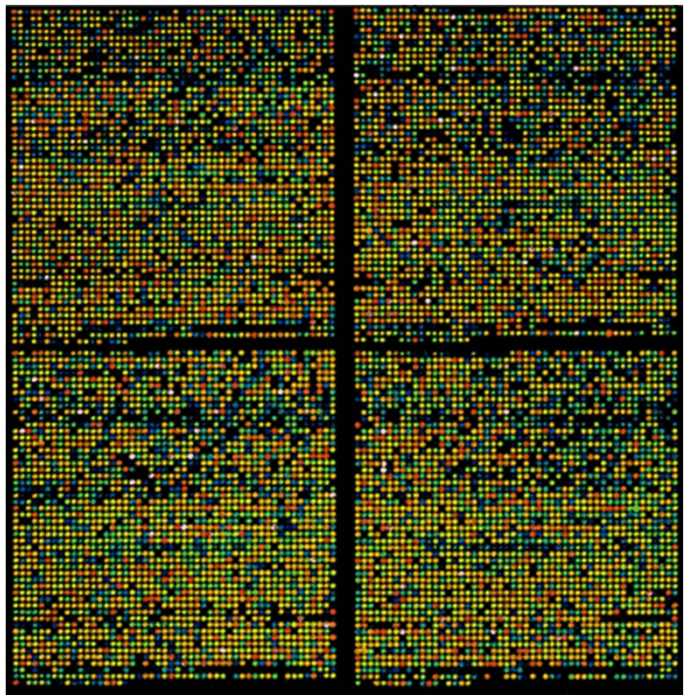
- Packard
- Agilent
- GeSiM
- ...

In-Situ Synthesis (standard)

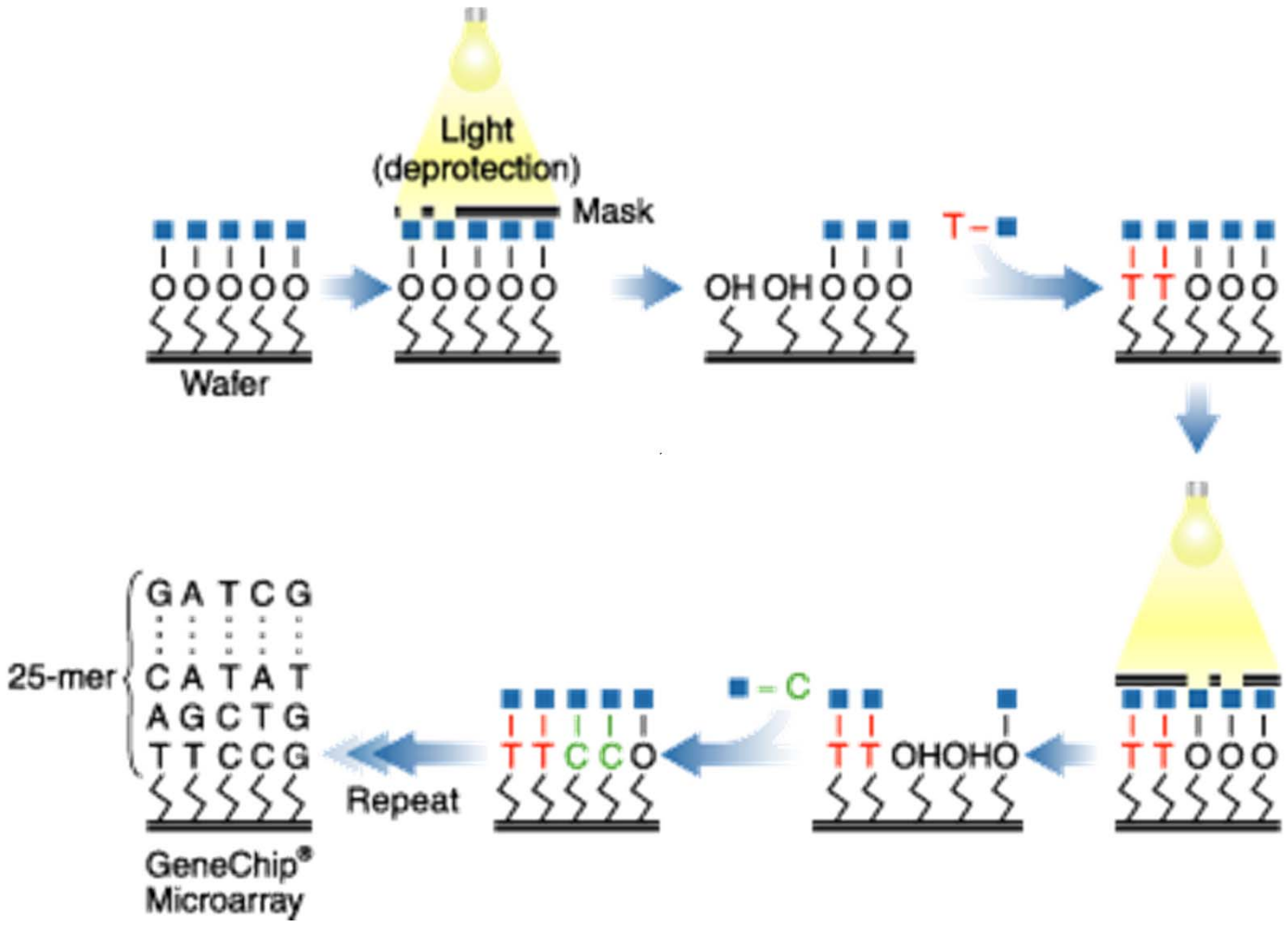
- Affymetrix
- Febit
- ...

10.4. Microarrays: Fabrication

- Introduction & Overview
- On-Chip Synthesis
  - **„Affymetrix“ procedure**
  - **„Agilent“ procedure**
  - **„FEBIT“ procedure**
- Contact Spotting (Pin-printing)
- Non-Contact Spotting (Inkjet or Piezo)
- Comparison of speed
- Electronic Arrays



10.4. Affymetrix Procedure



10.4. „Affymetrix“ Procedure

Light directed chemical synthesis

- Photo-protected glass substrate selectively illuminated by light passing through photolithographic mask
- Light deprotects certain areas (activation of these areas)
- Nucleotide incubation → chemical coupling

- Repeat until coupling of all nucleotides
- Number of steps: 4 x oligo-length

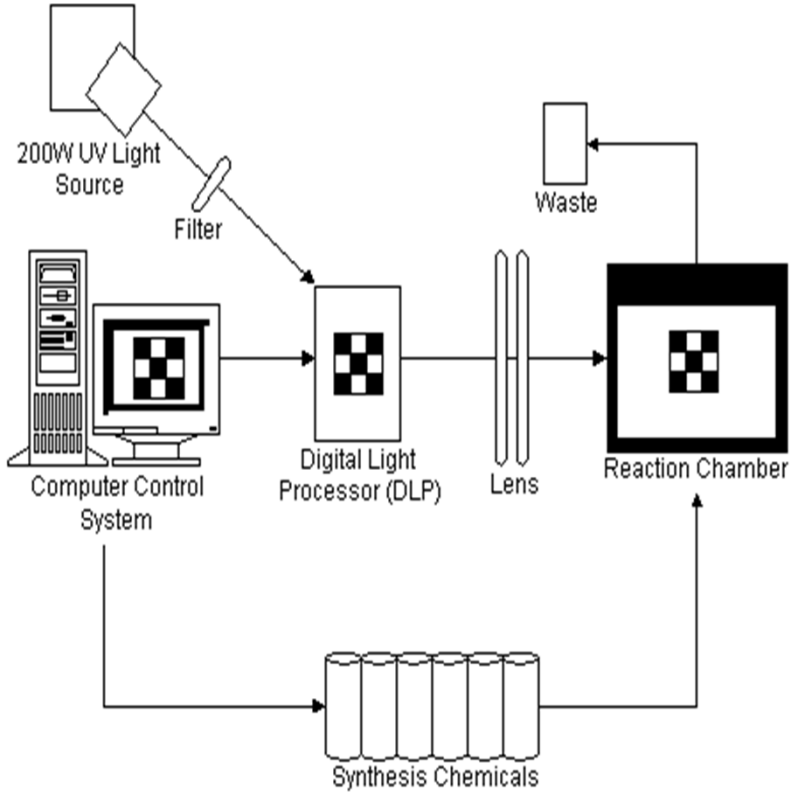
10.4. „Affymetrix“ Procedure

Light directed chemical synthesis

- High density arras
  (100,000 features/ microarray)
- Up to 25mers possible
- Quite expensive
- High redundancy necessary due to high failure rate of synthesis reactions
- Established technology (> 40 % market share)

10.4. Digital Optical Chemistry

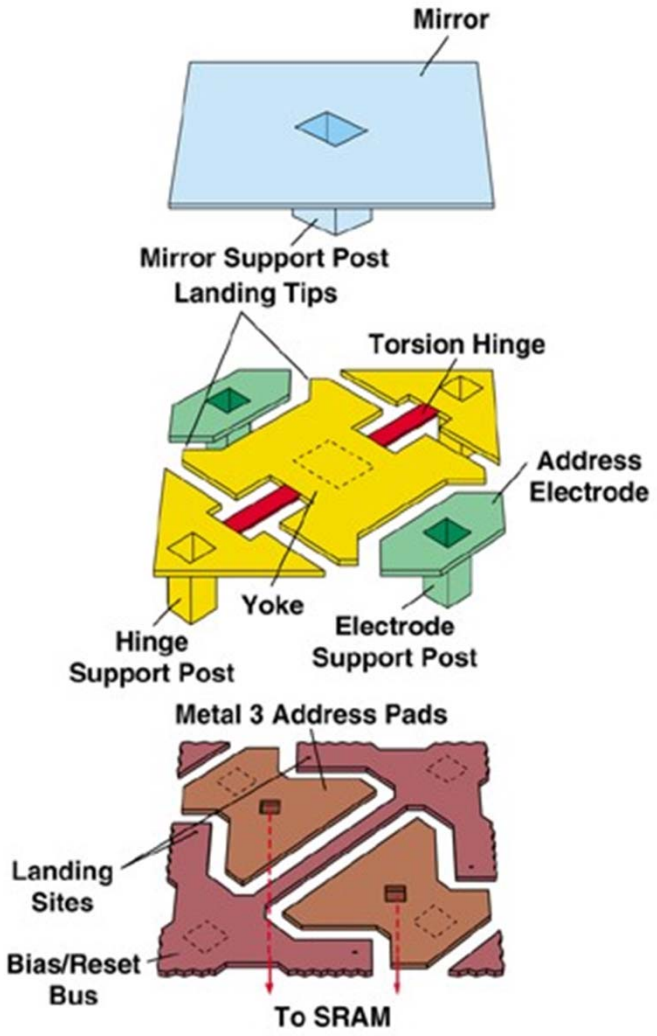
- DMD = „Digital Mirror Device“
- No need for expensive masks
- Digital mirrors for patterning
- Could be cheaper alternative to Affymetrix procedure

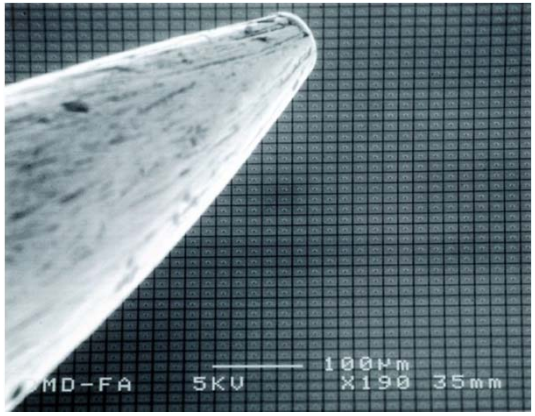


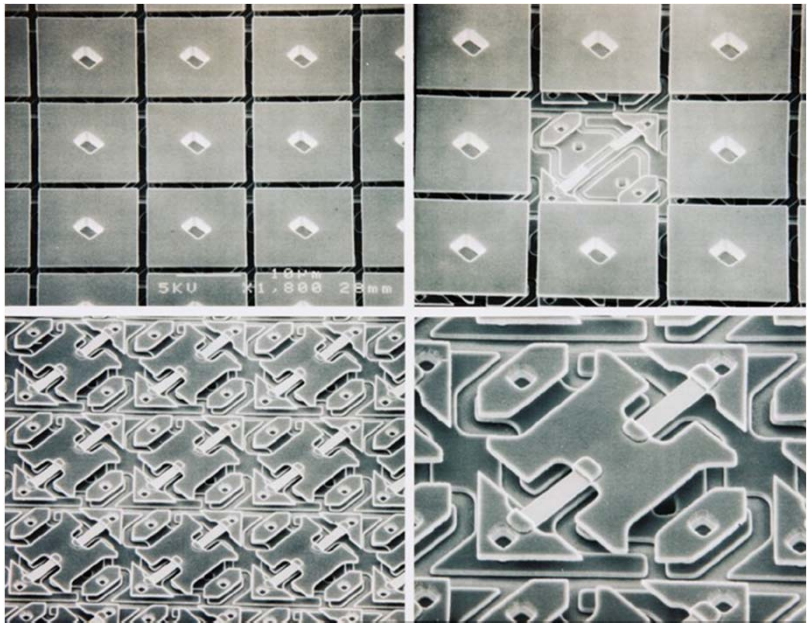
10.4. Digital Optical Chemistry

- Problems
  - Contrast not as good as photomasks
- Activities
  - Skip Garner (University Texas)
  - FeBiT (Mannheim, Germany)
  - ...

10.4. Digital Mirror Device



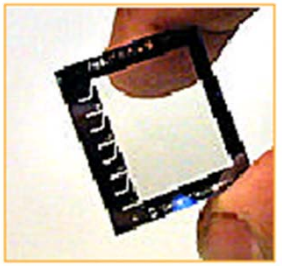




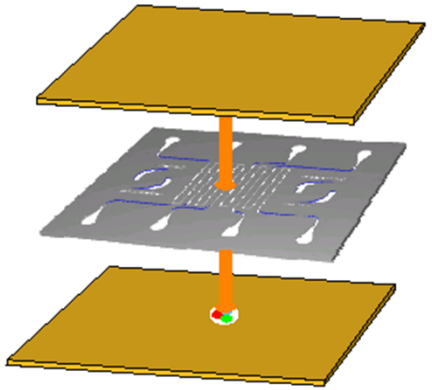
10.4. „FeBiT“ Procedure

DNA-Microprocessor

- Synthesis on fluidic chip
- 100,000 features possible
- 1-2 application specific arrays per day



Geniom® one: Sample injection



Light Source-Matrix

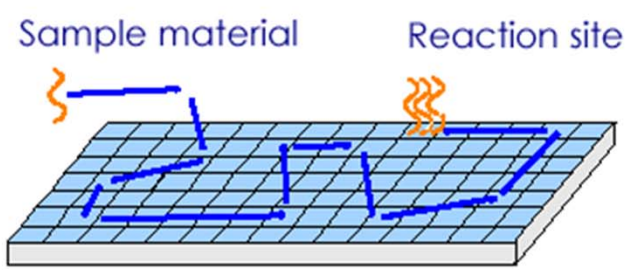
DNA Processor

Detection-Matrix

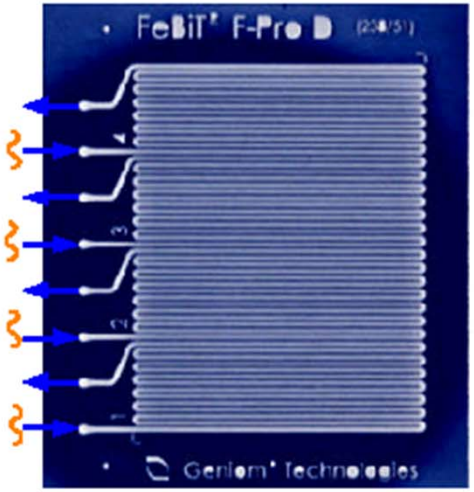
- Flexible Illumination
- Online QC
- No Moving Parts

10.4. „FeBiT“ Procedure

Planar chip compound

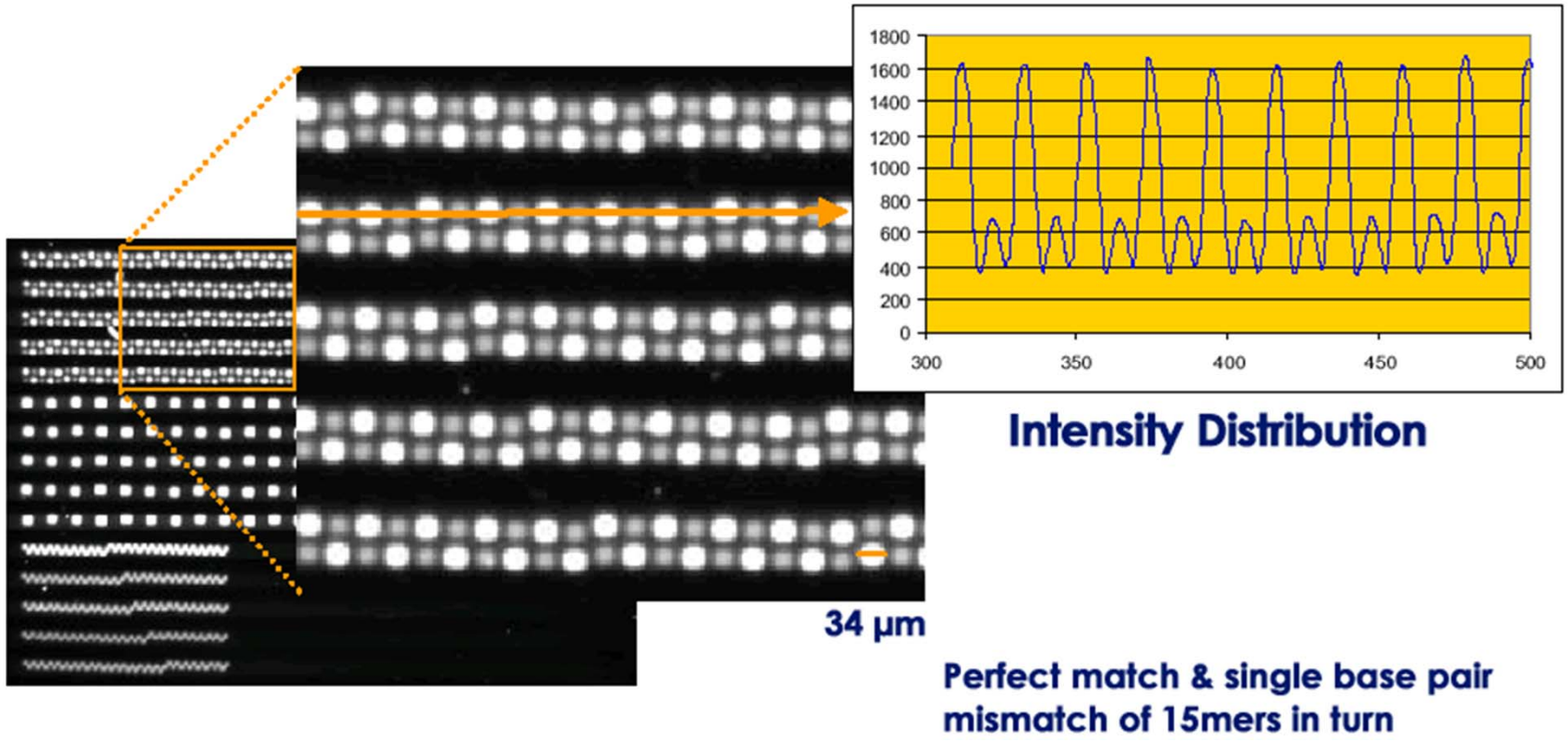


DNA Processor



- The DNA- Processor can be used for 1-4 samples in parallel
- Small chemical & sample volumes

10.4. „FeBiT“ Procedure

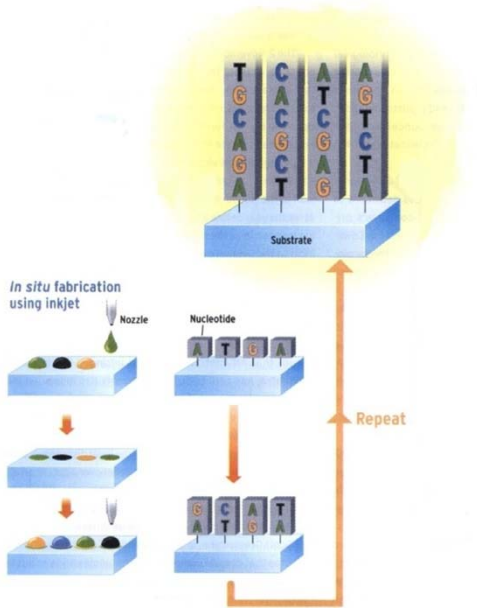


10.4. „Agilent“ Procedure

On-Chip-Synthesis by

InkJet-Printing

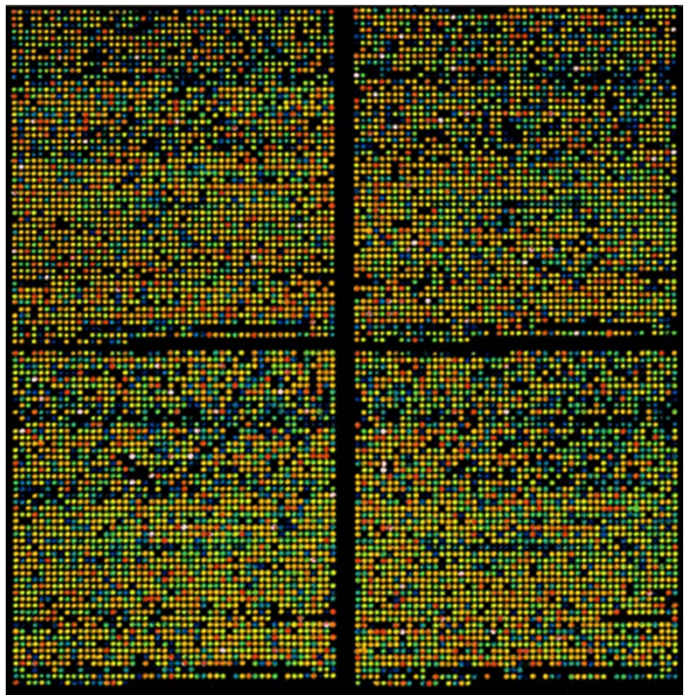
- Inkjet manufacturing process
  - In-situ synthesis
  - 70 µm pitch
  - 25– 60mers
- Up to 14,000 features cDNA or synthesized oligos
- Technology Access Program
  - Custom microarrays
  - www.agilent.com



Source: S. K. Moore; IEEE SPECTRUM; March 2001

10.4. Microarrays: Fabrication

- Introduction & Overview
- On-Chip Synthesis
- Contact Spotting (Pin-Printing)
  - **Standard PIN printing**
  - **Corning procedure**
- Non-Contact Spotting (InkJet or Piezo)
- Comparison of speed
- Electronic Arrays



10.4. Contact Printing: Principle

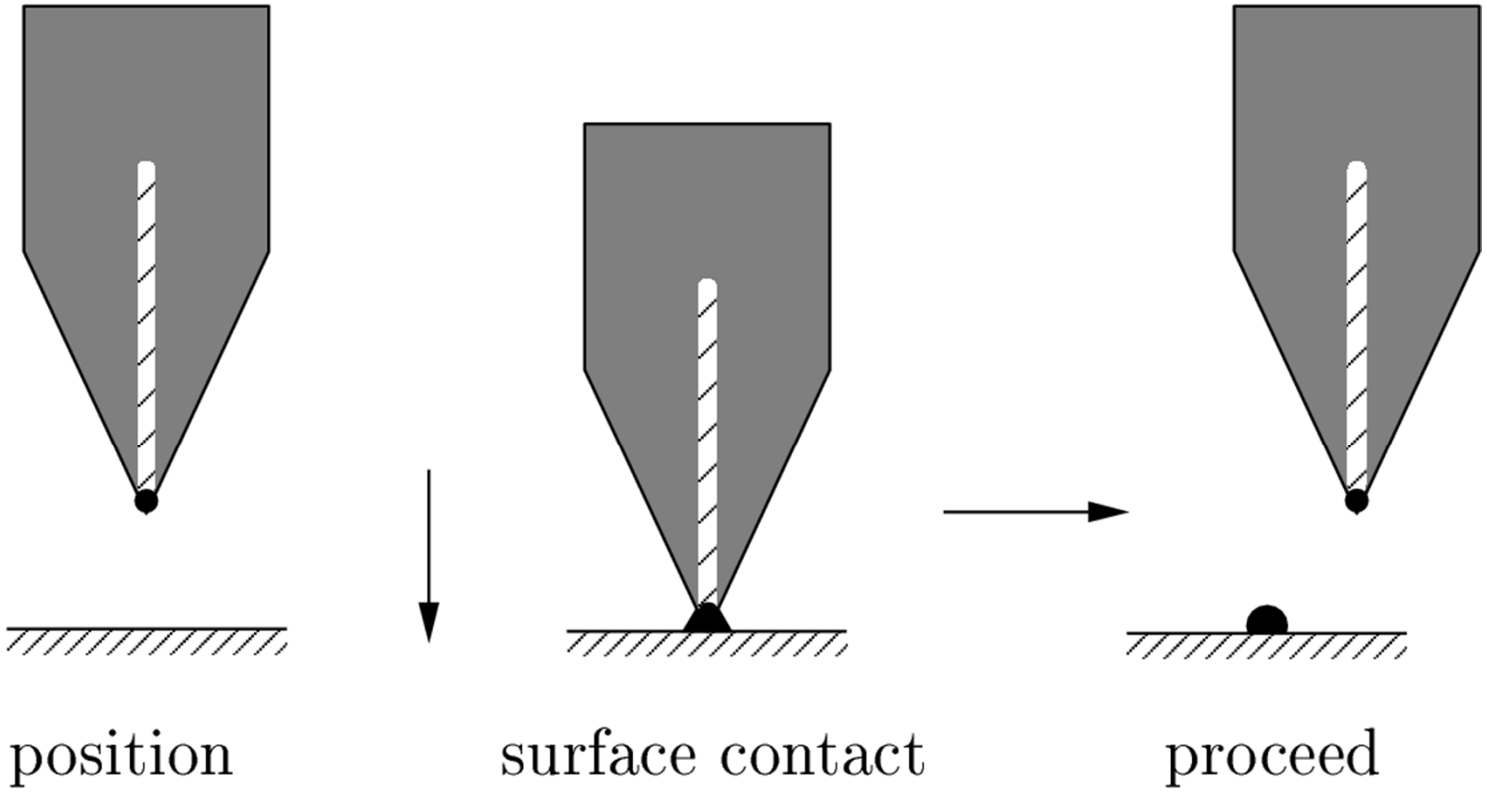
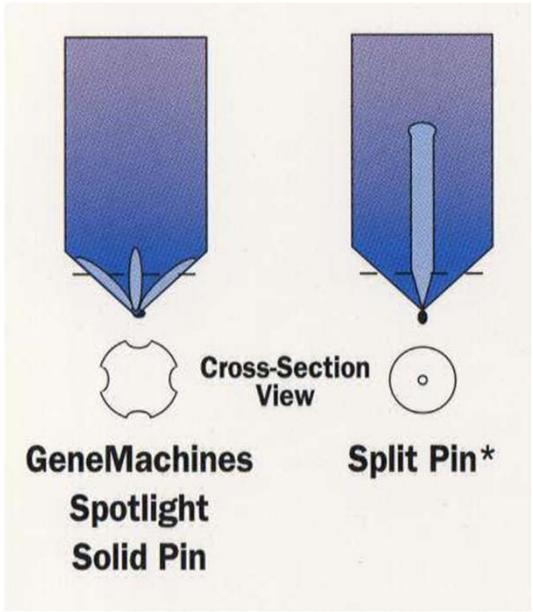


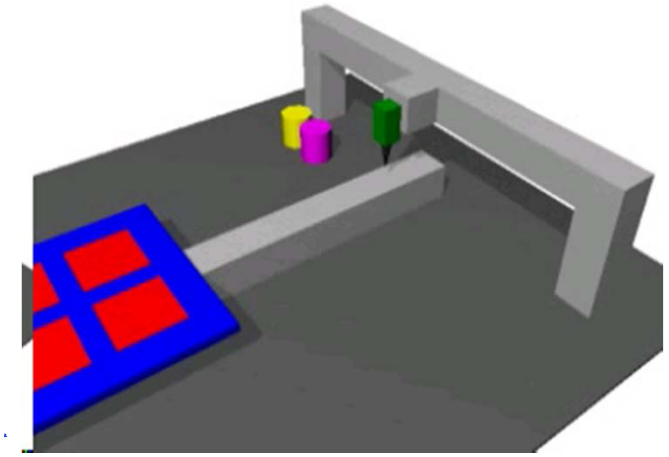
Fig. 10.12. Principle of contact printing

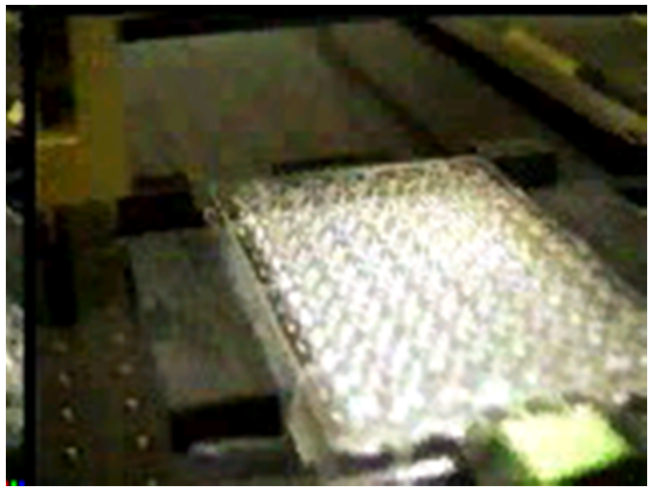
10.4. Standard Pin-Printing

PIN‘S:

- 1 – 16 Pins in parallel
- „Solid Pin“or „Split Pin“
- Slow but flexible







10.4. Standard Pin-Printing

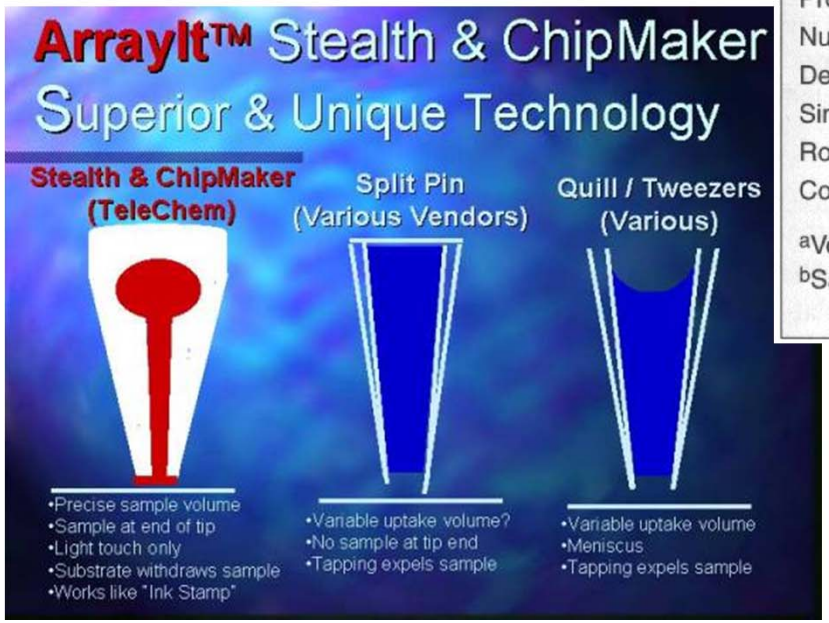
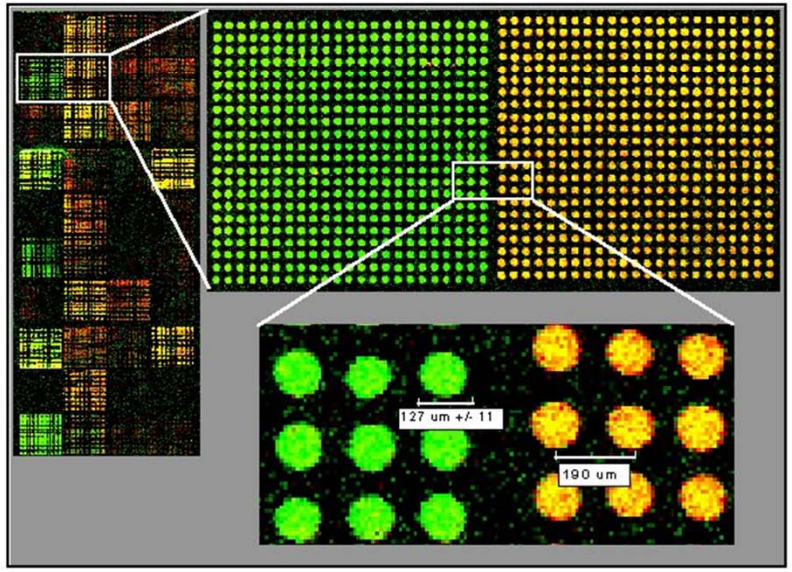


Table 1. Comparison of Printing Technologies

Parameter	Printing Technology: Piezoelectric	Syringe-Solenoid	Microspotting Pin
Minimum sample volume (µL)[a]	20–50	20–50	5
Loading volume (µL)[b]	5–10	5–10	0.2–1.0
Print volume (nL)	0.05–10	4–100	0.5–2.5
Spot size (µm)	125–175	250–500	75–360
Spot density (spots/cm^2)	500–2500	200–400	400–10 000
Programmable volume	Yes	Yes	No
Number of nozzles or pins	4–8	8–16	1–64
Delivery speed (spots/s)	100–500	10–50	64
Simplicity	✔	✔	✔✔✔
Robustness	✔	✔✔	✔✔✔
Cost per spot	$$$	$$	$

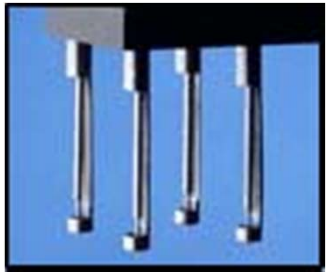
[a]Volume of sample in the 384-well source microplate
[b]Sample volume of the dispensing device

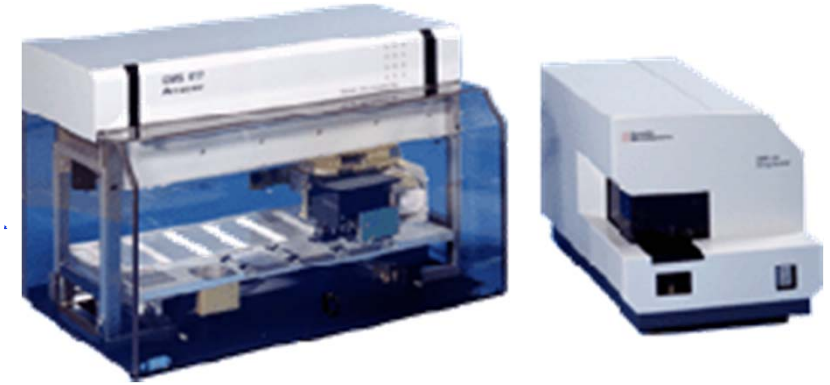
10.4. BioRobotics

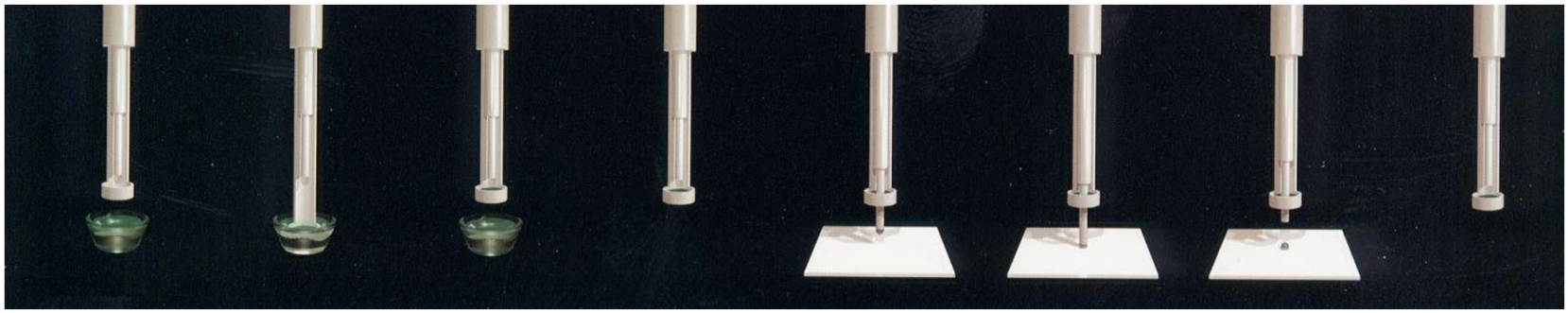


10.4. Affymetrix (Genetic Microsystems)

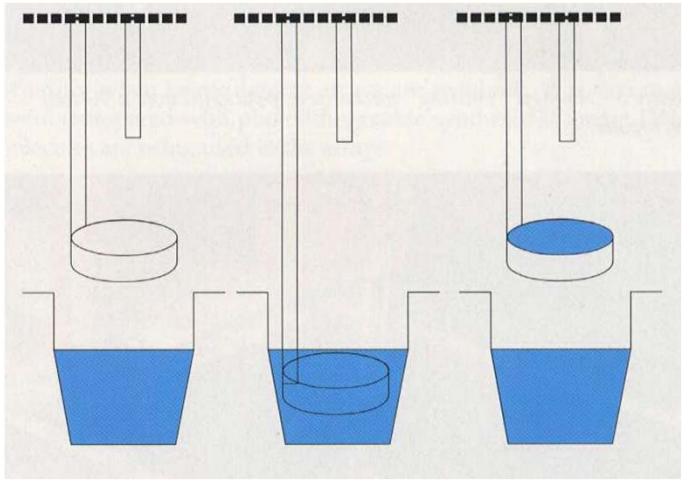
- GMS 417 arrayer
- Costs : about 80,000 €
- Pin-and-ring tool
- 4-pins simultaneously

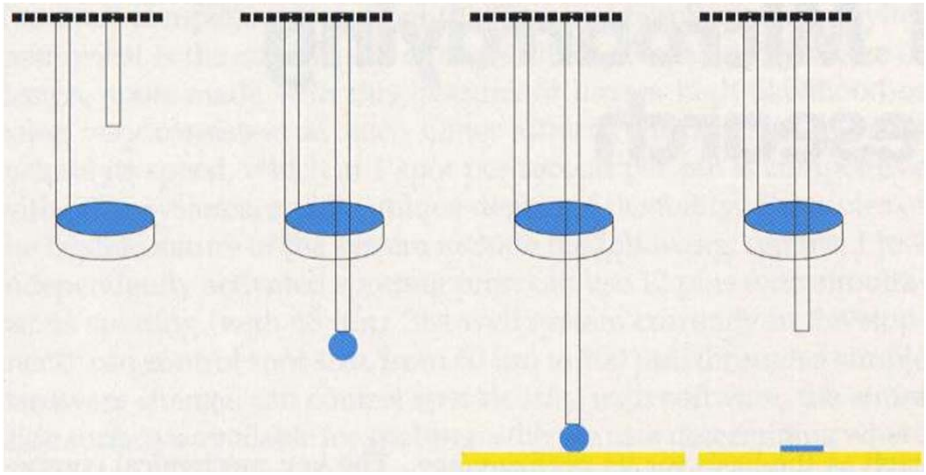


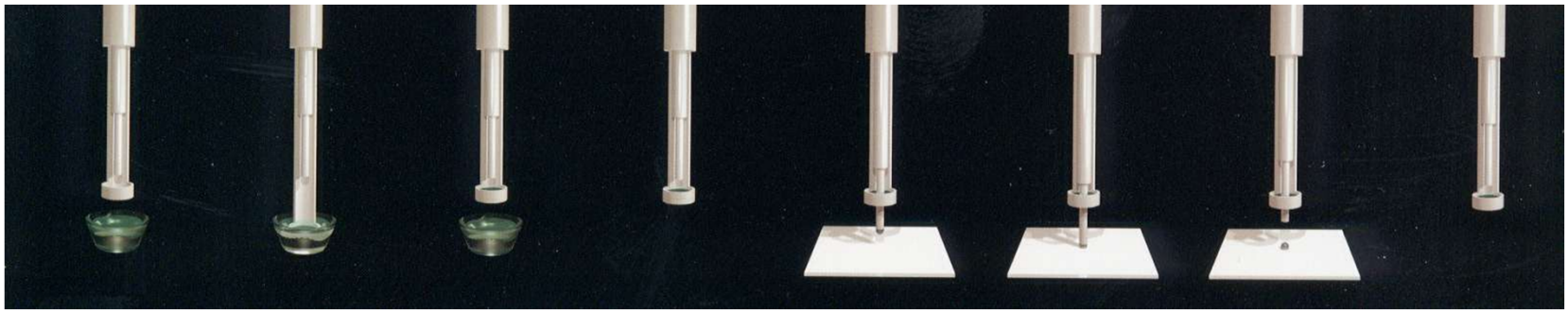




10.4. Affymetrix (Genetic Microsystems)

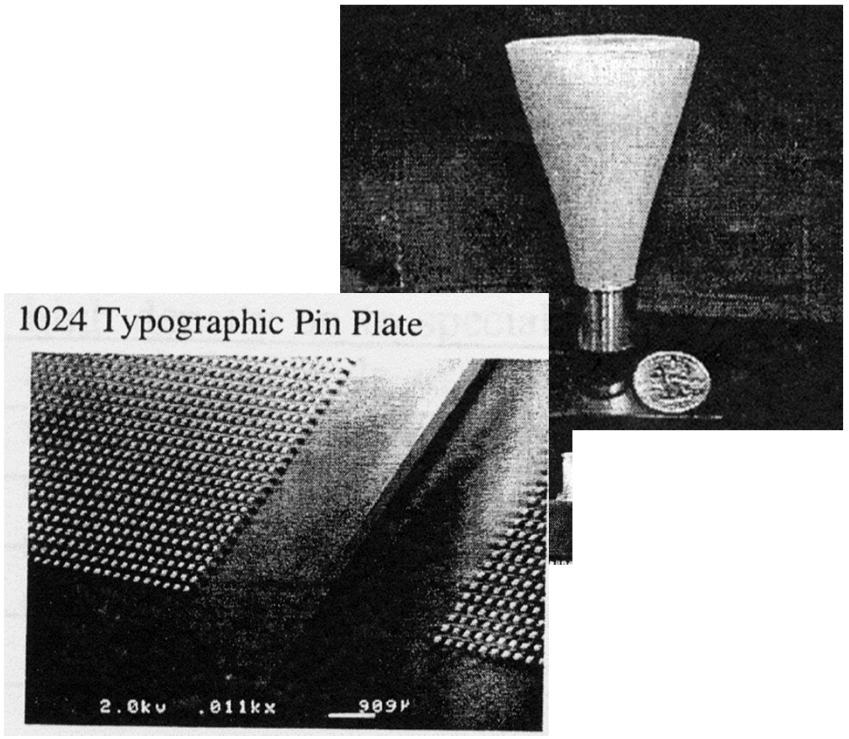






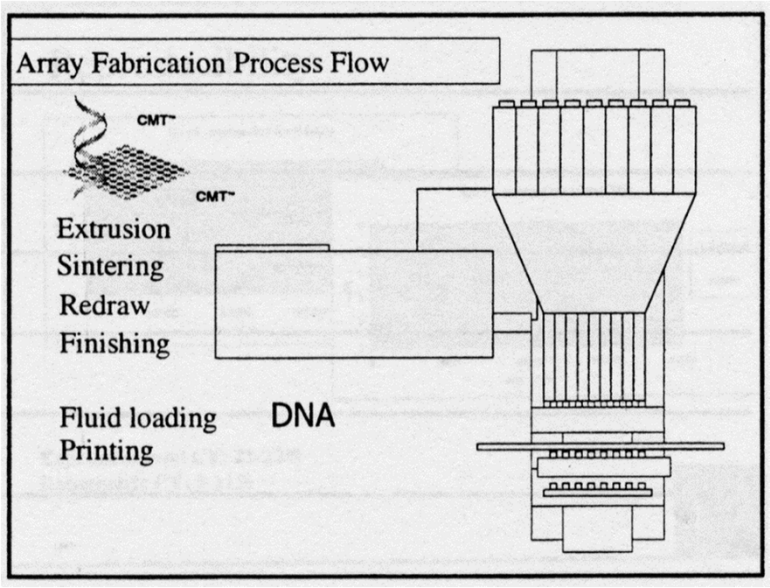
10.4. „Corning“ Procedure (1)

- 1,024 pins in parallel
- Cycle time 5 - 6 s
- Printing technology not commercially available → Customized Chips
- www.corning.com/cmt



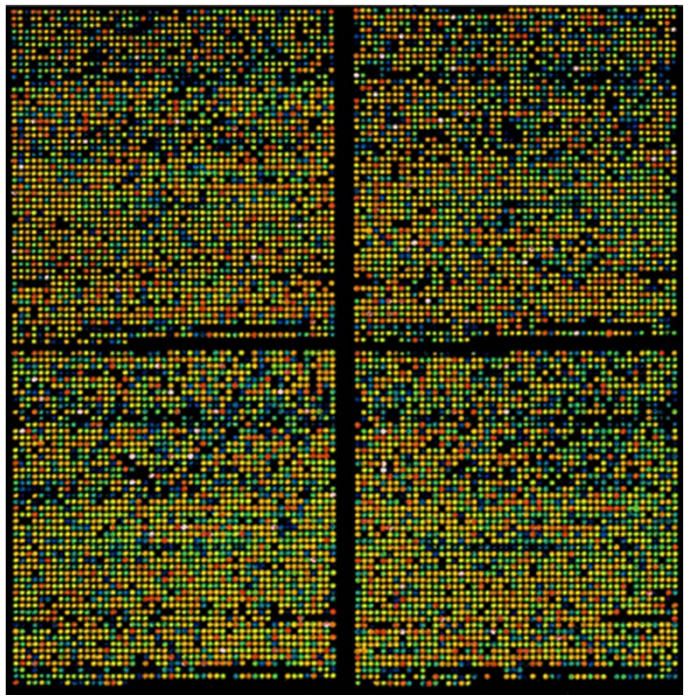
10.4. „Corning“ Procedure (2)

- 1,024 pins in parallel
- Cycle time 5 - 6 s
- Printing technology not commercially available → Customized Chips
- www.corning.com/cmt



10.4. Microarrays: Fabrication

- Introduction & Overview
- On-Chip Synthesis
- Contact Spotting (Pin-printing)
- Non-Contact Spotting (InkJet or Piezo)
  - **Packard Procedure**
  - **GeSim Procedure**
  - **IMTEK & HSG Procedure**
- Comparison of speed
- Electronic Arrays



10.4. Non-Contact Printing: Principle

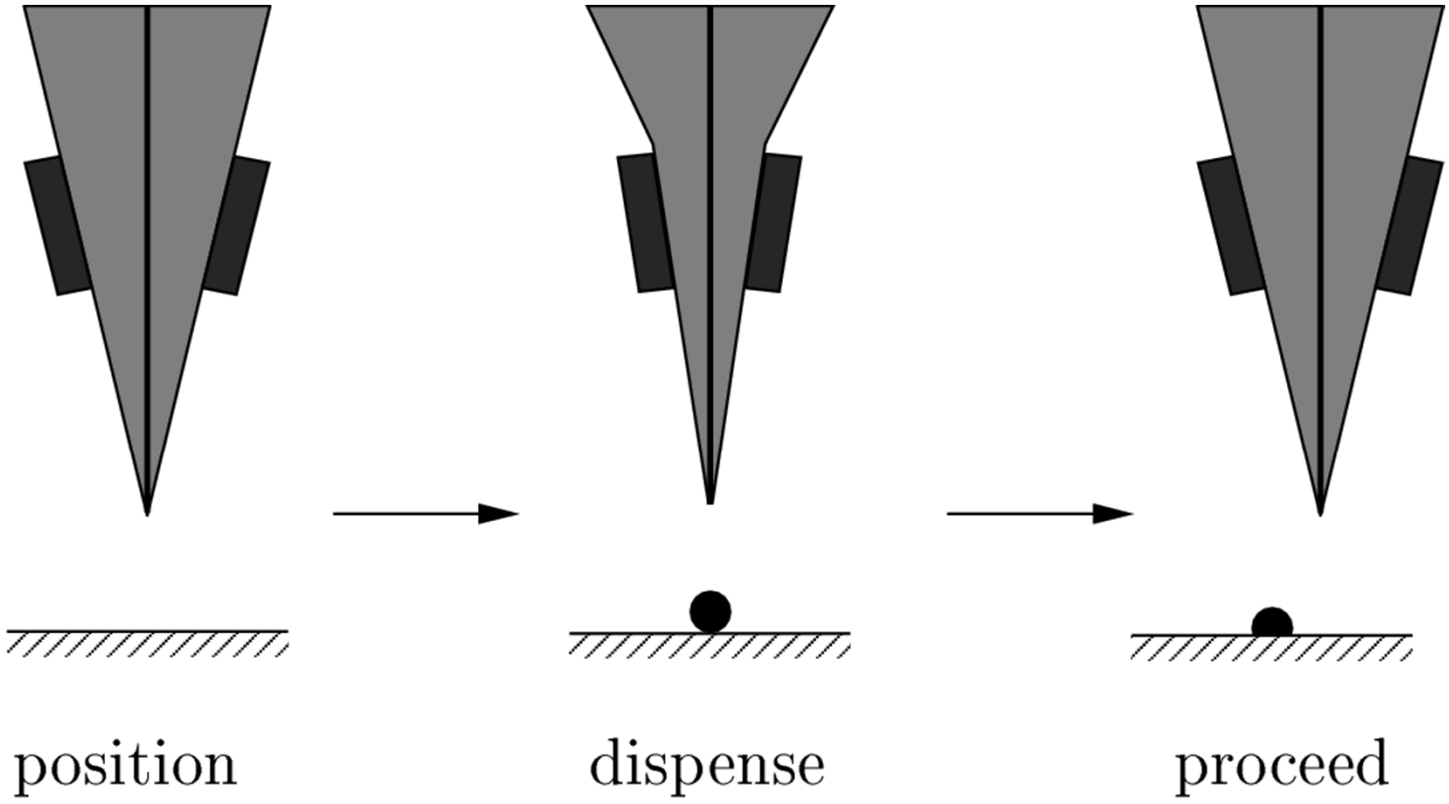
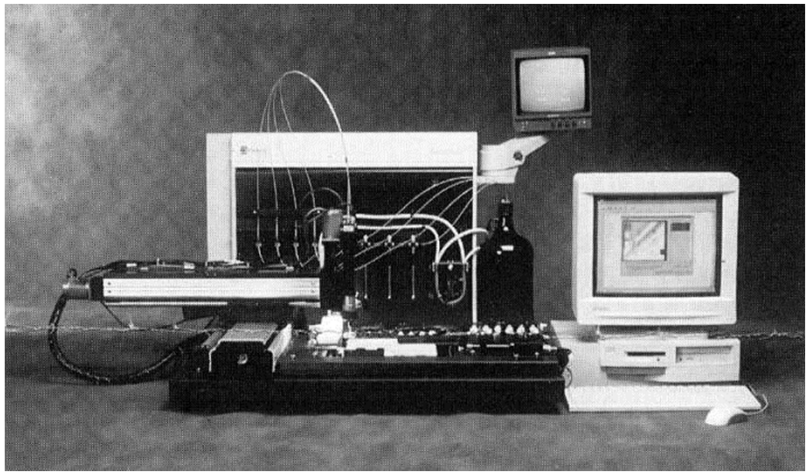


Fig. 10.16. Principle of non-contact spotting

10.4. „Packard“ – Procedure

- BioChip Arrayer
- Costs: about 120,000 – 300,000 €
- PiezoTips
- 8 dispensers
- Concept can be expanded



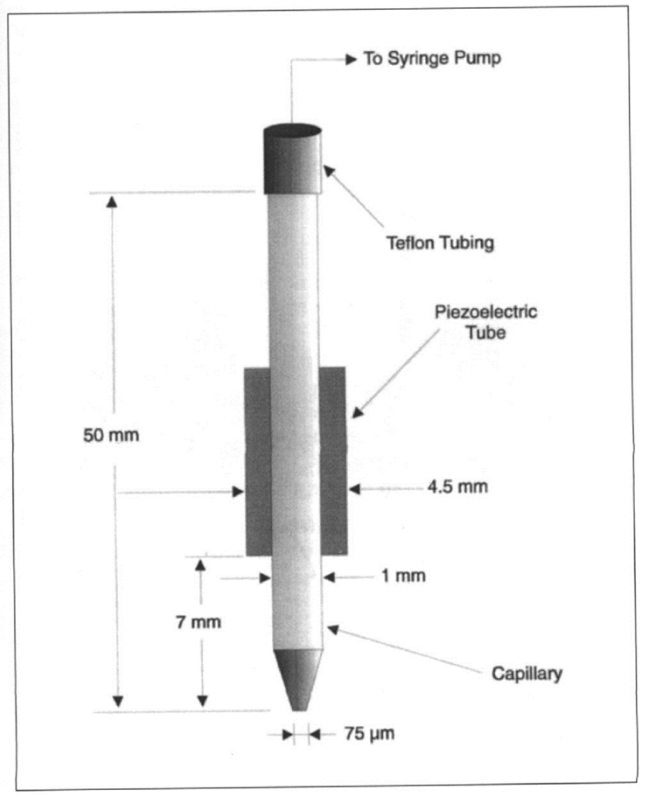
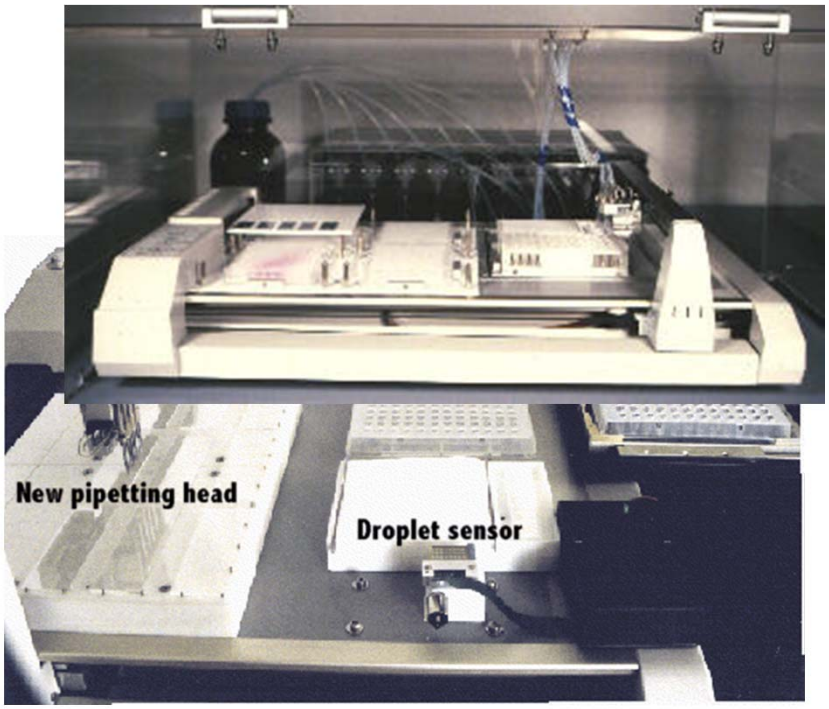


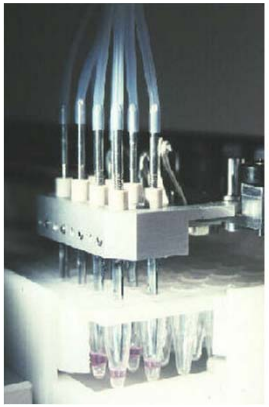
Figure 1. Diagram of a piezoelectric dispenser used on the BioChip Arrayer.

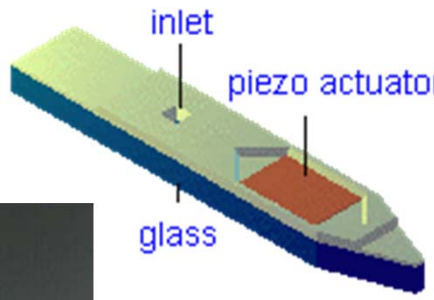
10.4. „GeSiM“ - Procedure

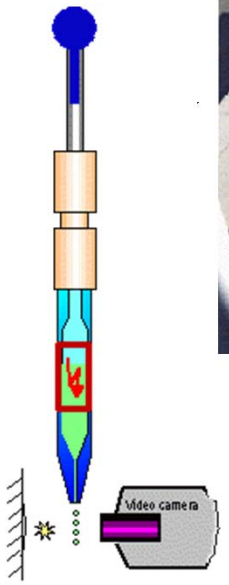
- Nanoplotter
- Costs: starting @ 20,000 €
- Up to 8 heads available

  (ca. 60,000 €)



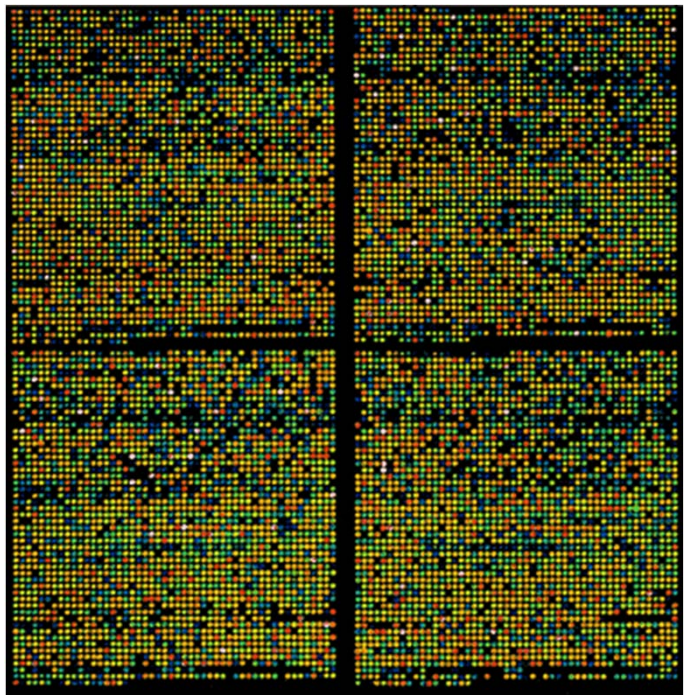






10.4. Microarrays: Fabrication

- Introduction & Overview
- On-Chip Synthesis
- Contact Spotting (Pin-printing)
- Non-Contact Spotting (InkJet or Piezo)
  - **Packard Procedure**
  - **GeSim Procedure**
  - **IMTEK & HSG Procedure**
- Comparison of speed
- Electronic Arrays

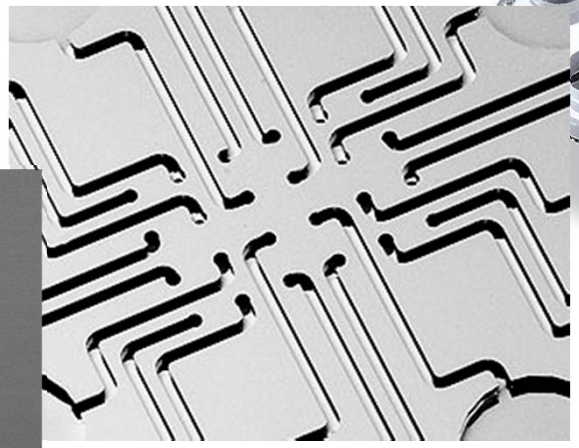


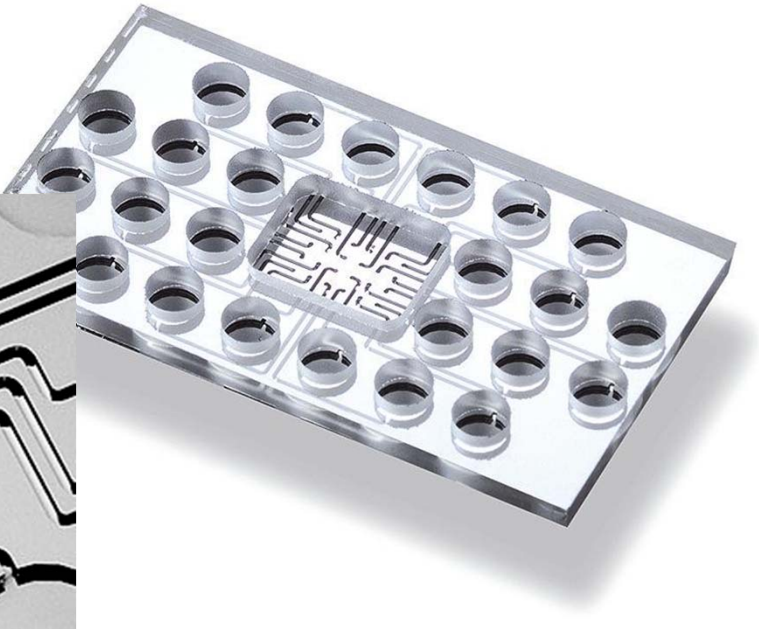
10.4. IMTEK & HSG-Procedure

TopSpot

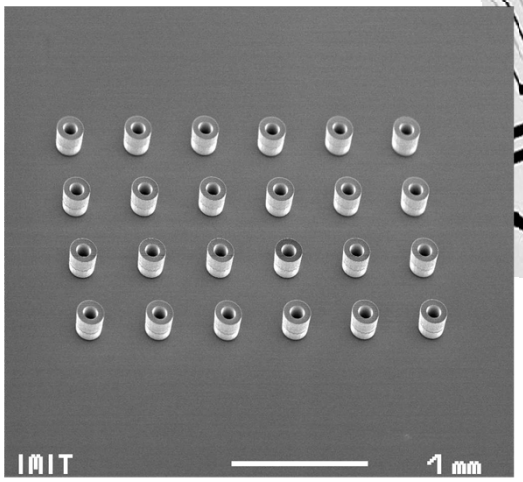
top view

top view:
flow channels for
conversion of format





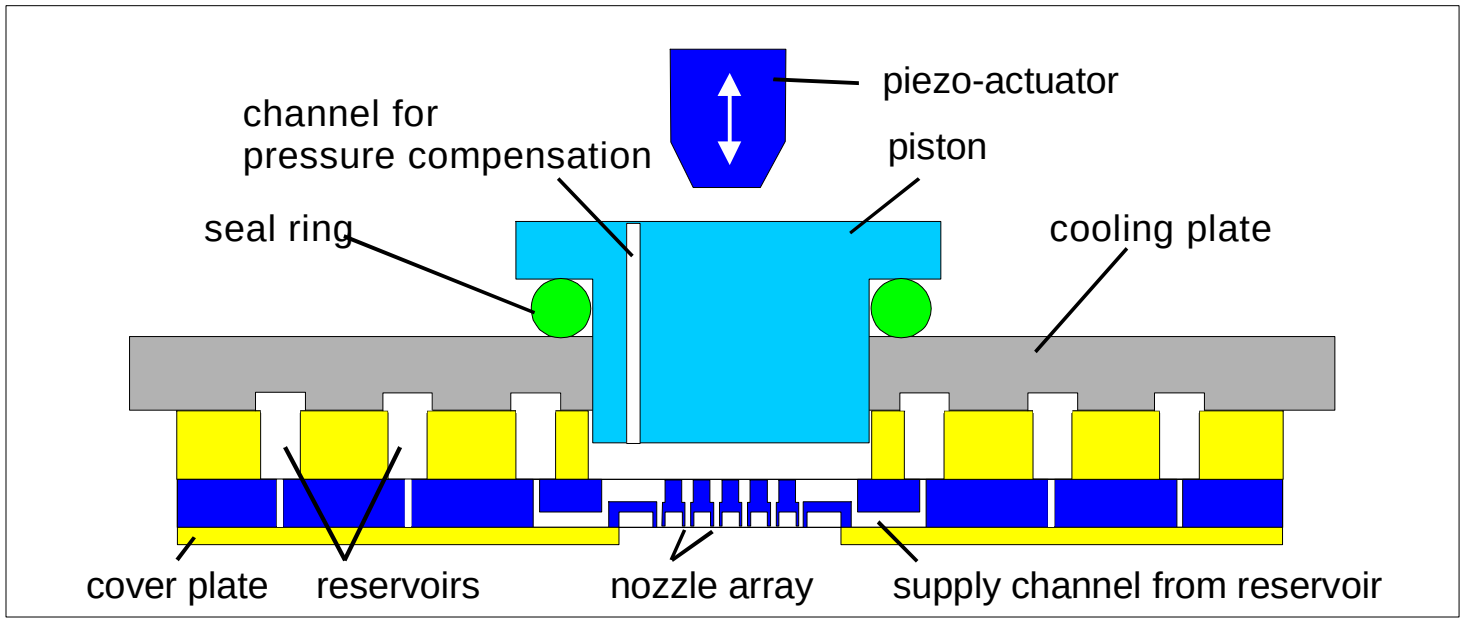
bottom view



Reformatting is done
by capillary forces

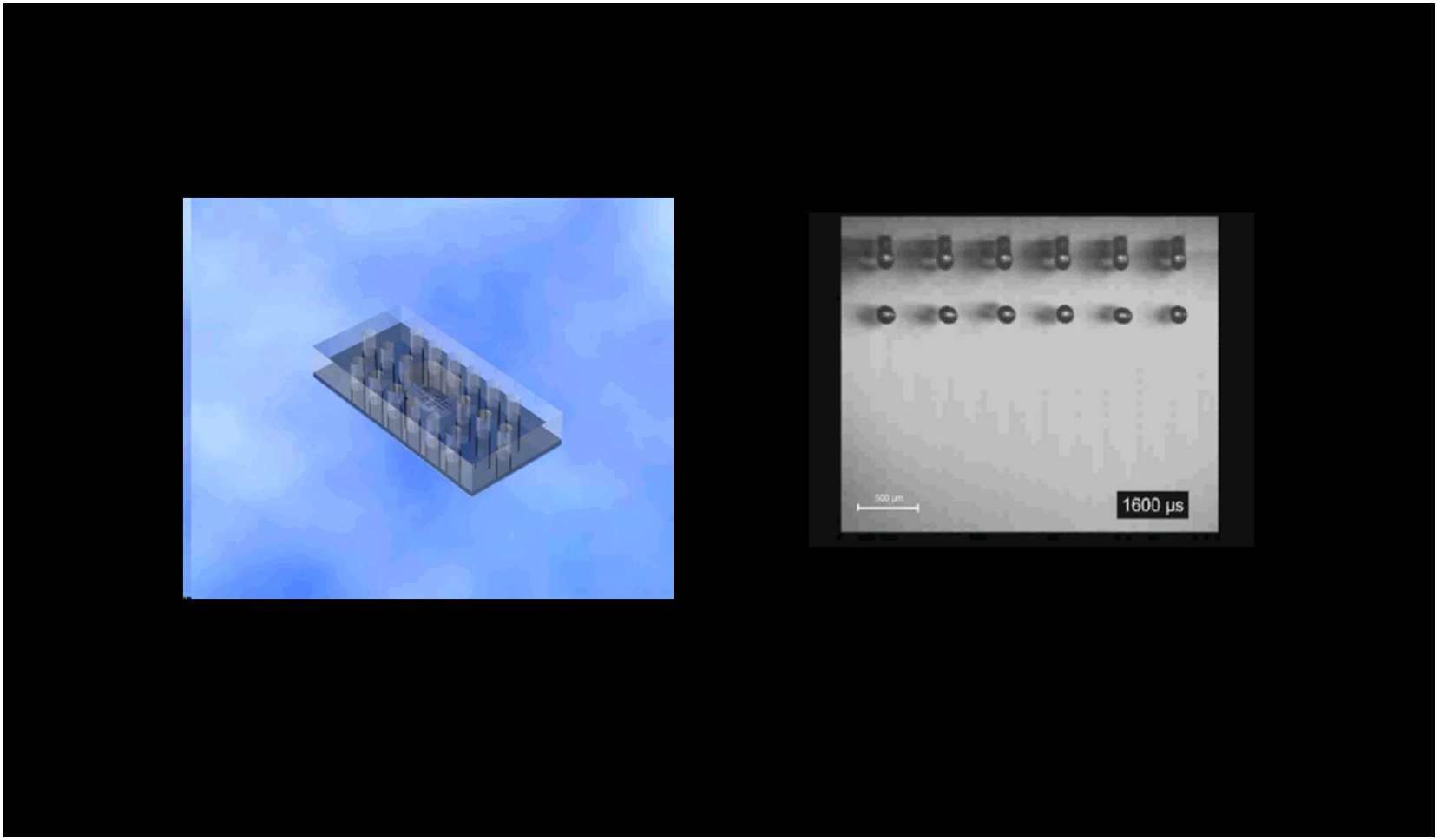
10.4. IMTEK & HSG-Procedure

TopSpot



Actuation is done by a
pneumatic pressure pulse

10.4. TopSpot – Principle



10.4. TopSpot – Printheads

Customized printheads

- 24 media: 500 µm / 2.25 mm
  (pitch of nozzles / reservoirs)

- 96 media: 500 µm / 2.25 mm
  (pitch of nozzles / reservoirs)

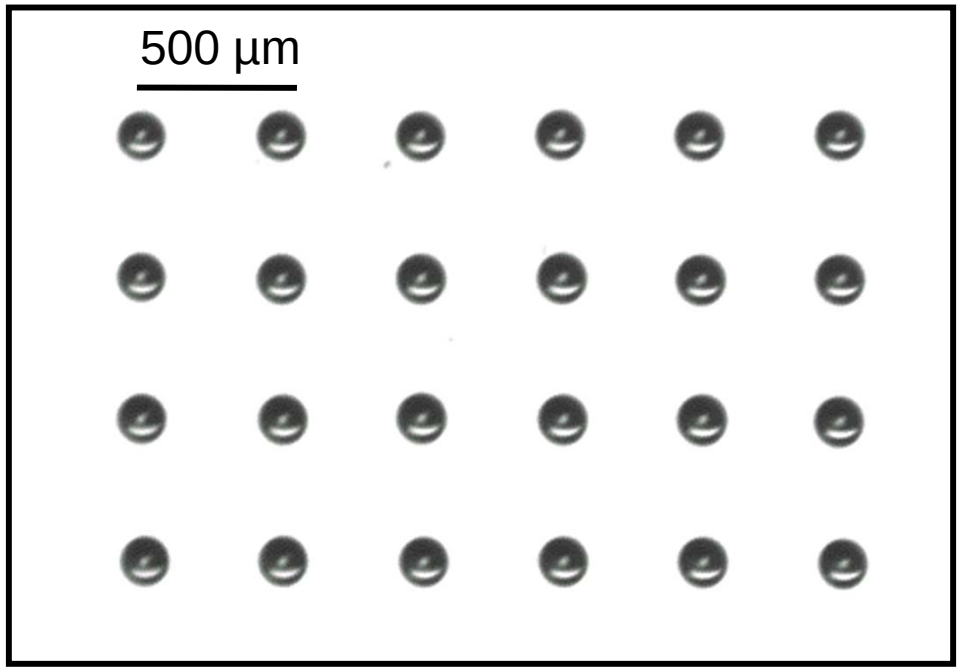
- 96 media: 2.5 mm / 4.5 mm
  (pitch of nozzles / reservoirs)

- 384 media: 500 µm pitch
  (planned)

10.4. TopSpot - Printmodule

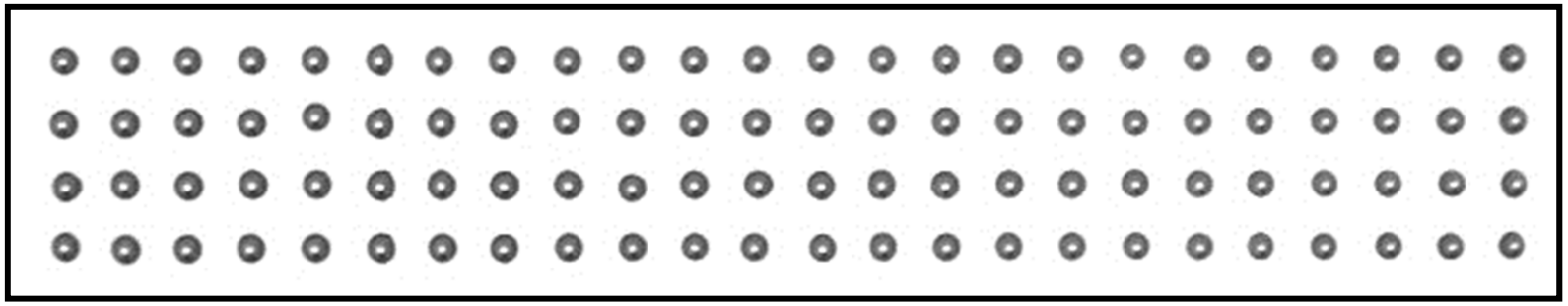
- Different adaptors for 24 / 96 channel printheads
- Integrated temperature control (dependent on humidity) for reduction of evaporation
- High-performance piezostack actuation
- Manual *z*-adjustment of print-module
- Manual *z*-adjustment of actuation system

10.4. TopSpot - Quality Control



24 channel printhead

Quality control done by image processing system



96 channel printhead

10.4. Spot Quality

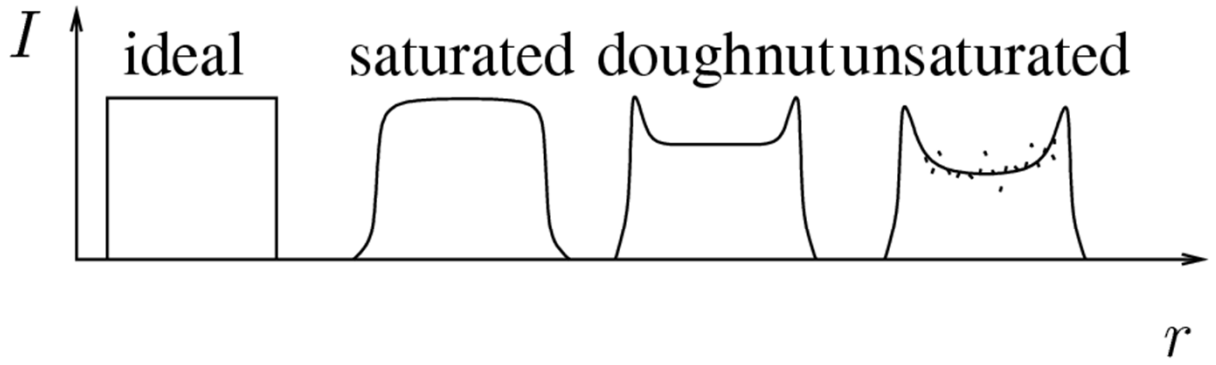
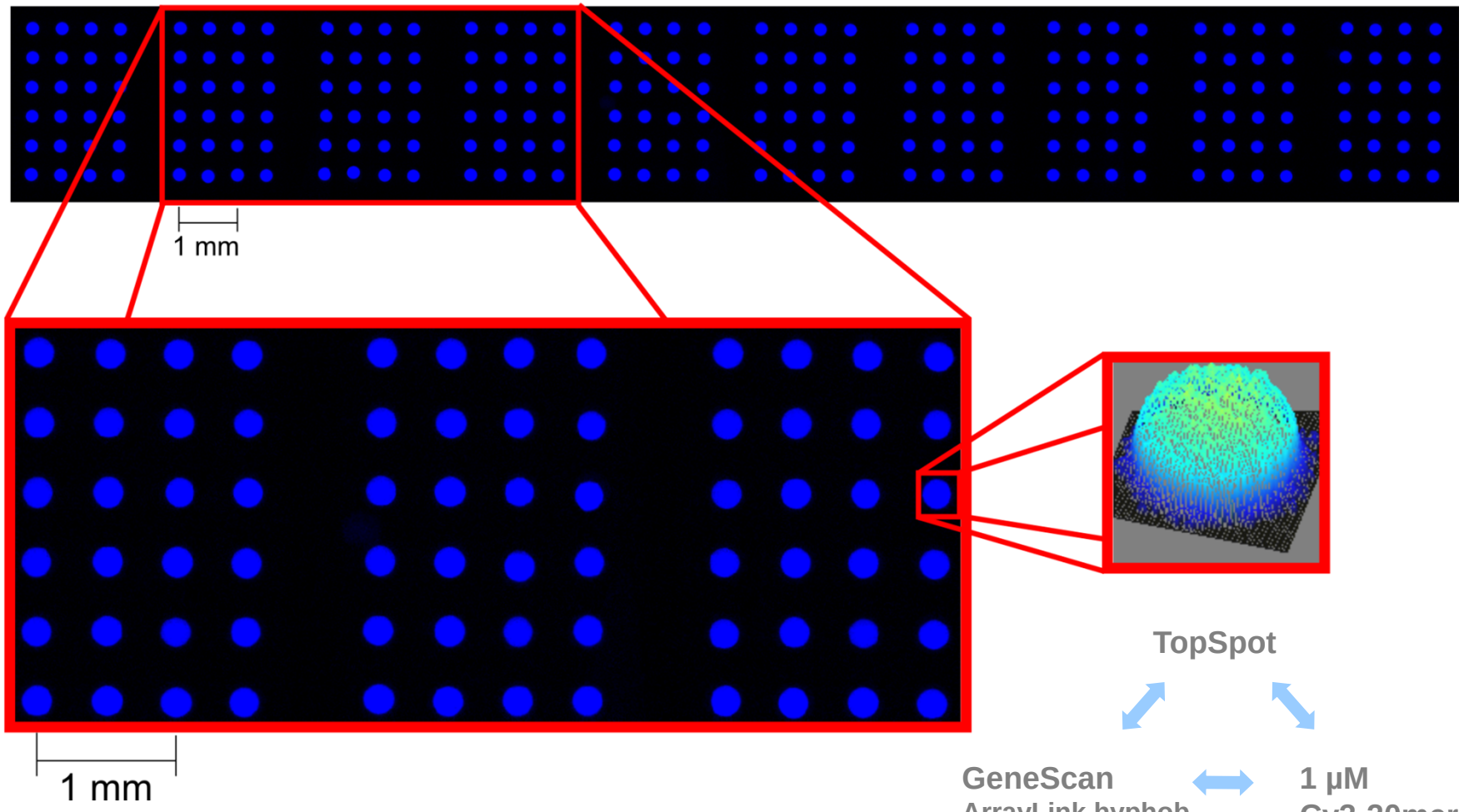


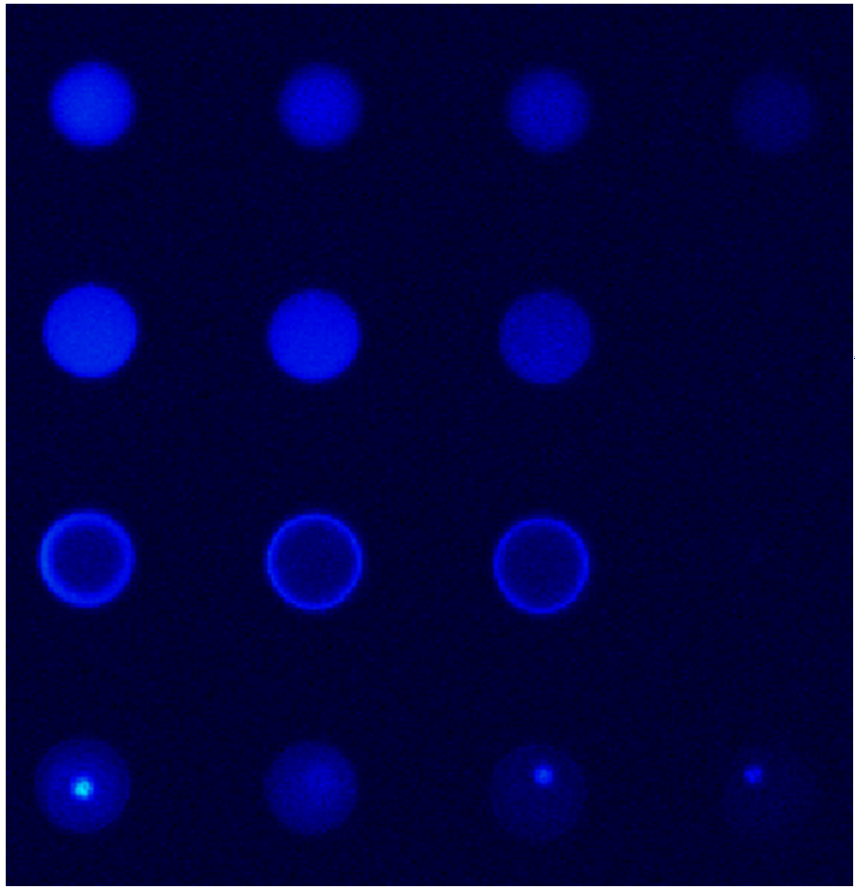
Fig. 10.22. Typical spot intensity distribution (JD: check with Martina or Bas)

- Common effects
  - Spot-to-spot variations
  - Internal inhomogeneities
- Impact factors
  - Substrates
  - Coating
  - Droplet volume
  - Drying process
  - Type of probe, its concentration and buffer

10.4. Printing Microarrays with TopSpot



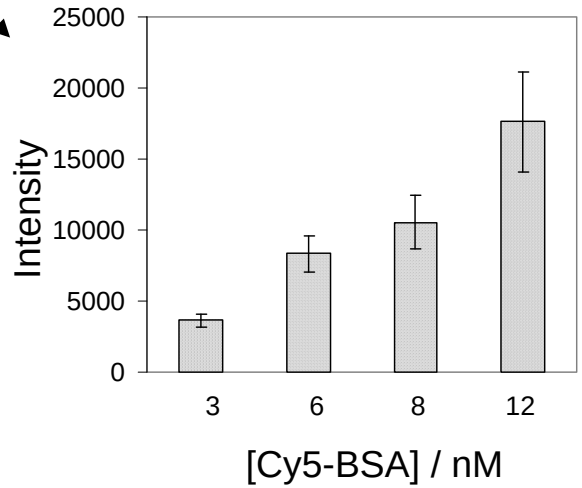
10.4. Buffer & Spot Quality



TopSpot

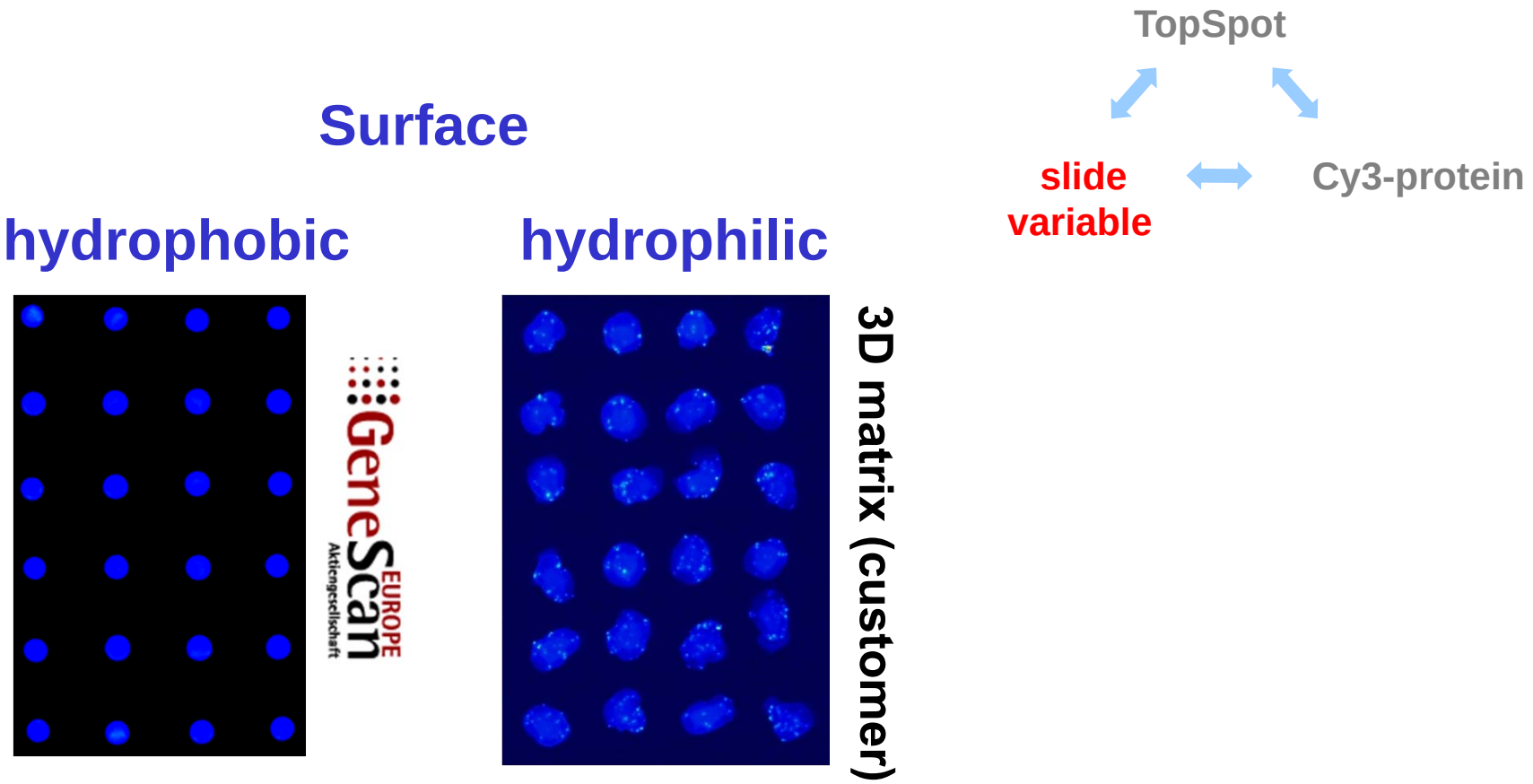
GeneScan
ArrayLink hyphob

Cy5-BSA
div. buffers



zeptosens
Bioanalytical Solutions

10.4. Surface Coating & Spot Quality

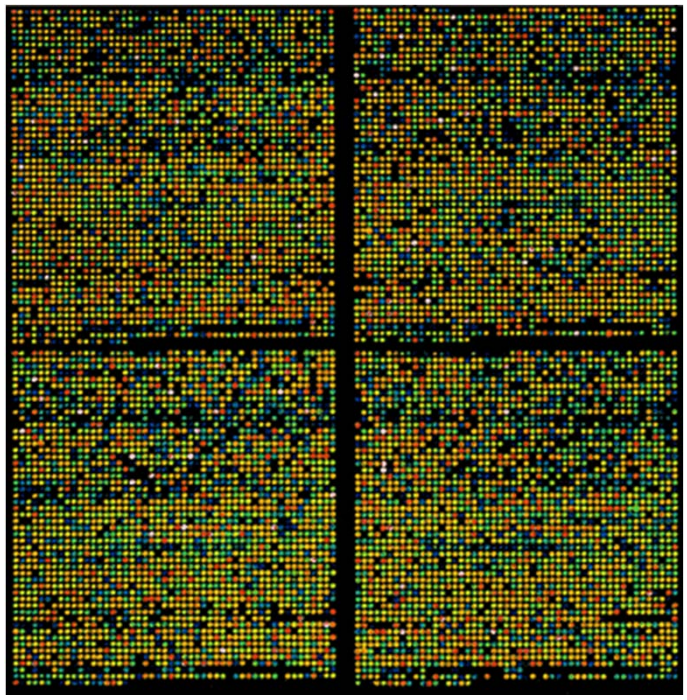


10.4. TopSpot Family

	TopSpot /E	TopSpot /M	TopSpot /P
			
Data:			
Biochips / h:	**200**	**400**	**300**
Reagenzien:	**96**	**480**	**1.440**

10.4. Microarrays: Fabrication

- Introduction & Overview
- On-Chip Synthesis
- Contact Spotting (Pin-Printing)
- Non-Contact Spotting (InkJet or Piezo)
- Comparison of speed
- Electronic Arrays



10.4. How to measure speed?

- **Time is money …**
  - ➢ Increasing throughput → Lower production costs
  - ➢ Lower production costs → Higher earnings or …
  - → Accessing new markets

- **Measure of speed:**

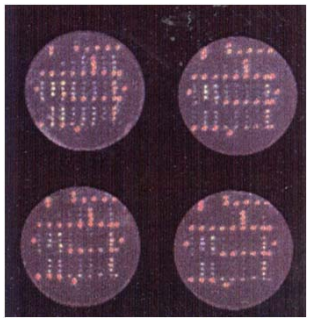
$$\text{speed} = \frac{\text{different analytes}}{\text{slides}} \times \frac{\text{slides}}{\text{time}}$$

10.4. Examples of Throughput

- **Commercial available pin printers**
  - 1 – 2 analytes / second / pin
  - Typically 1 – 16 pins
  - **~ 1 – 30 analytes / second**

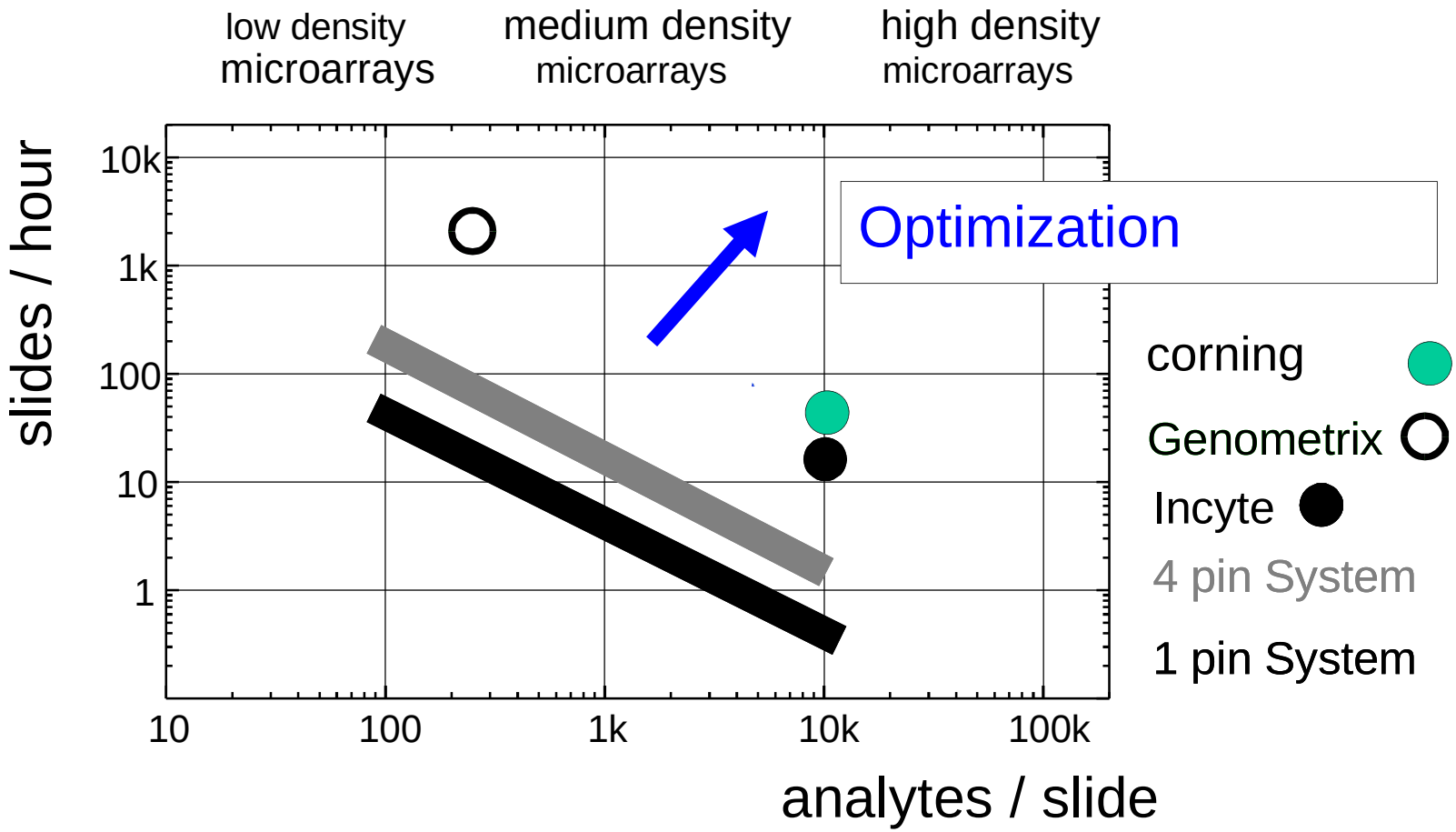
- **Incyte Pharmaceuticals (not commercial available)**
  - 10,000 analytes / slide x 1,200 slides / 48 hours
  - 16 ceramic pins; 280 slides / tray
  - **70 analytes / second**

- **Genometrix (not commercial available)**
  - **256 analytes / second**

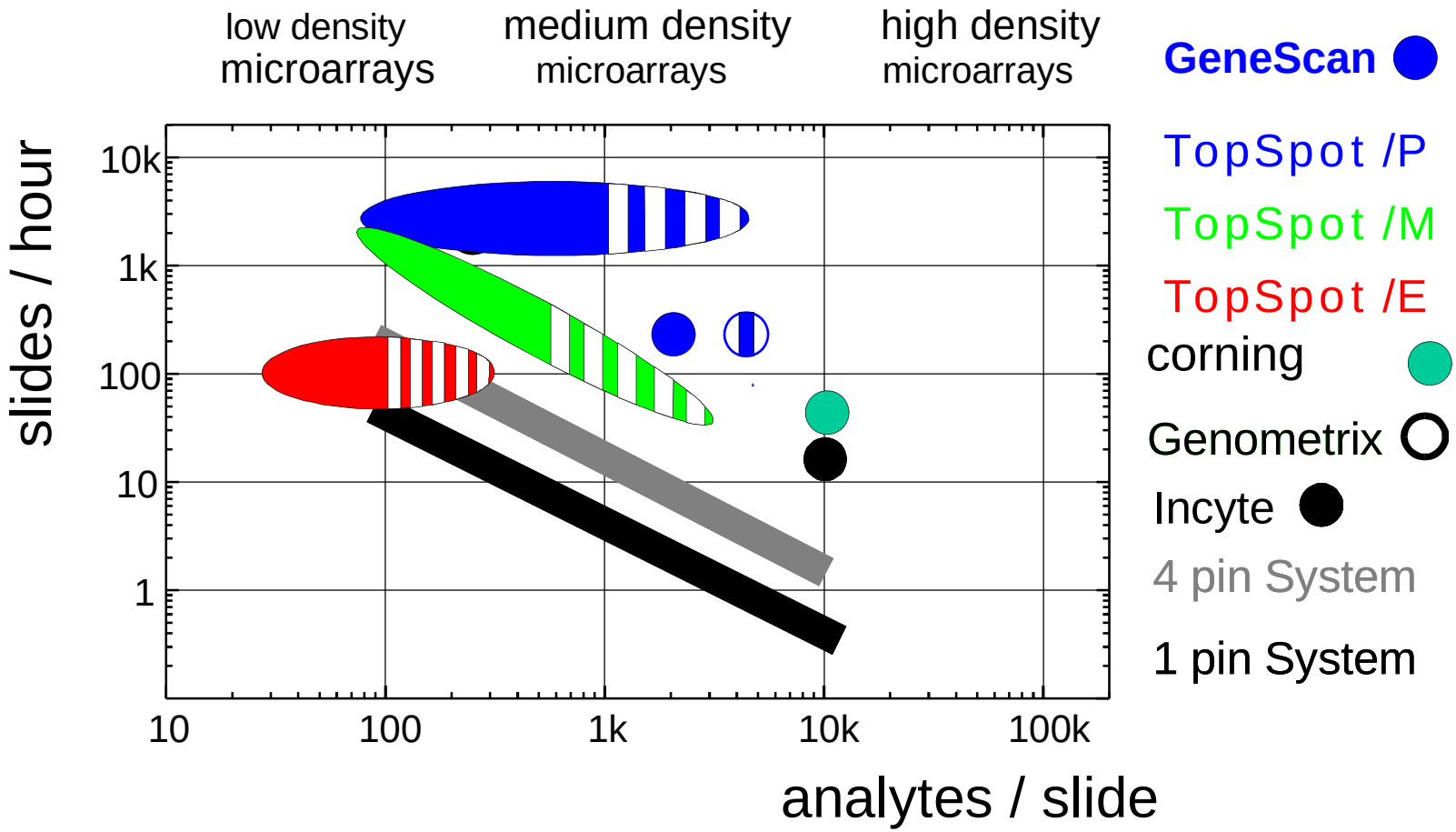


- **Corning (not commercial available)**
  - 1,024 channels with a cycle of 5-6 seconds
  - **~ 200 analytes / second**

10.4. Examples of Throughput



10.4. Examples of Throughput



10. Microarrays

1. Introduction
2. Reaction Kinetics
3. Immobilization
4. Fabrication
5. **Detection**
6. Electronic Control
7. Protein Microarrays
8. Bead-Based Microarrays

10.5. Microarray Detection

- CCD
  - Image capture
  - Pixels read out in parallel

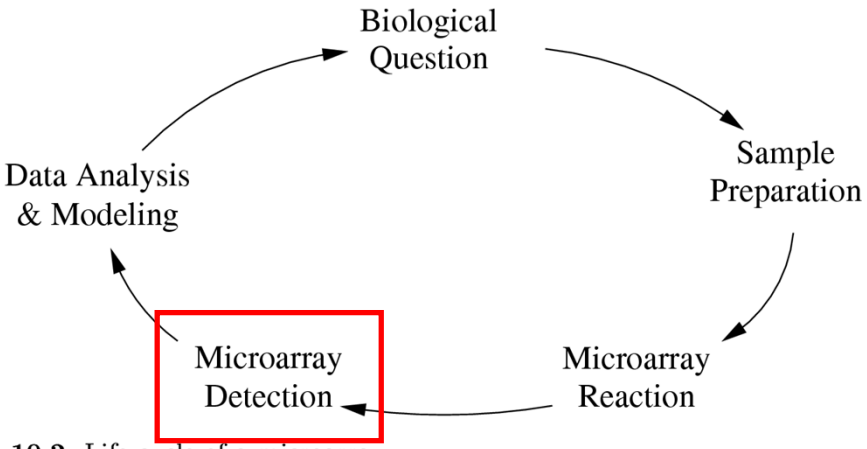


Fig. 10.2. Life cycle of a microarray

- PMT
  - Scanning technique
  - Pixel-wise
  - High sensitivity
  - Multiple channel with filters

10.5. Microarray Detection

- Pixel size
  - Physical „bin“
  - Guideline
    - Pixel size < 1 / 10 of spot diameter
  - CCD with 1 M pixel
    - Area about 1 cm x 1 cm
    - Pitch of about 10 µm
- Photobleaching
  - Incoming photons induce degradation of fluorophores
  - Decreasing image quality in subsequent scans
  - Differential photostability between dyes
- Cross-talk
  - Dual-labeling
  - Rather broad emission spectra
  - Primarily from short into long wavelengths (Stokes shift)

10.5. Fluorescent Detection

- Nucleic acid detection technologies
  - Secondary detection methology required
  - Nucleic acids exhibit no intrinsic marker suitable for direct, ultrasensitive detection
- Currently no universally employable label available
- Criteria distinguishing a good label
  - Stability
  - Sensitivity of detection
  - Speed
  - Convenience of detection
  - Overall cost of the label
  - Detection reagents
  - Detection system
  - For non-separation assays, properties of label should change after binding of complementary molecules
- In the 1990s, detection limits of zeptomole (10^{-15}) and even single molecular detection reported

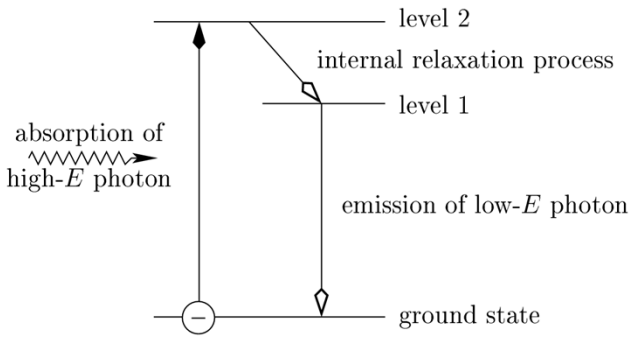


Fig. 2.31. Principle of laser-induced fluorescence. In a three-level atomic system, an electron is elevated by absorbing a high-energy photon from the coherent laser field to the upper level 2. Part of the energy is released by an internal process. The final relaxation step proceeds under emission of a low-energy photon which is used as the fluorescence signal

10.5. Labeling

- Signal generating labels (of very high specific activity)
- Fluorophores:
  - According to forecasts, fluorescence methods will be feasible in more than 90% of HTS assays

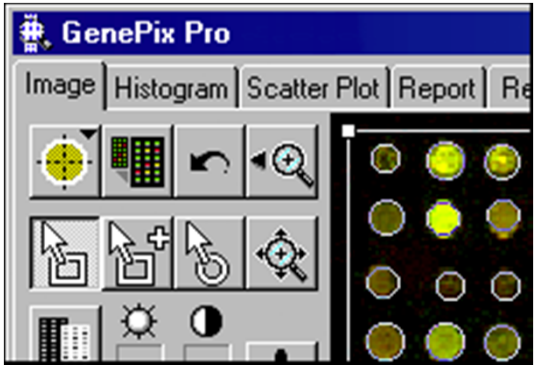
- Chemiluminescent labels

- Multicolor fluorescence
  - Differential measurements of several samples on a single chip
  - Comparative analysis facilitated by canceling problems of
    - Chip-to-chip variation
    - Deviating reaction conditions

10.5. Array Scanning

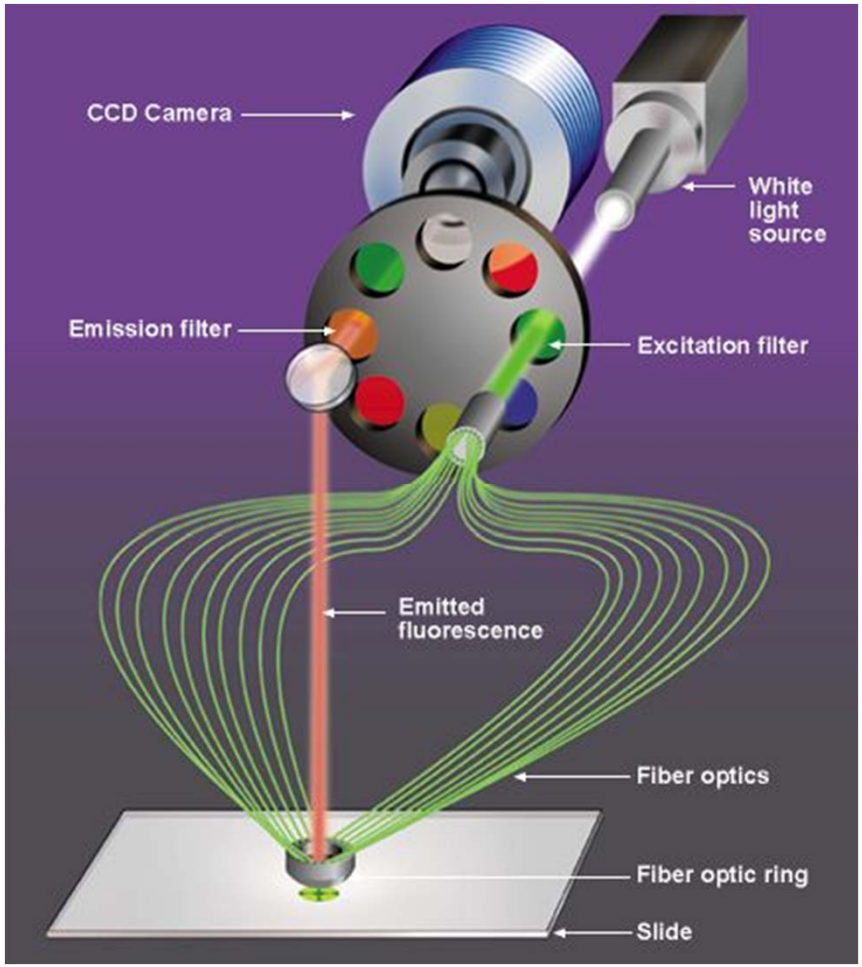
- Physical scanning
  - Laser-scanning confocal microscope
  - CCD camera

- High-Troughput Screening (HTS)
  - Large number of assays
  - Requirements
  - Small volumes per assay
  - High sensitivity
  - Many assays per unit time
  - Low costs per assay
  - Automation



Axon

10.5. Array Scanning



Genescan Europe AG
AppliedPrecision

10.5. Array Reader

- Typical specs (ScanArray, Packard)
  - Lasers with an excitation range from 488 nm to 633 nm
  - Up to six emission filters with a detection range from 500 nm to 700 nm
  - Five micron pixel resolution
  - Sensitivity to < 0.1 molecule fluor / μm^2
  - 22 mm x 73 mm scanning area
  - Scan speeds (< 5 minutes for 22 mm x 73 mm)

10.5. SPR - Kretschman Geometry

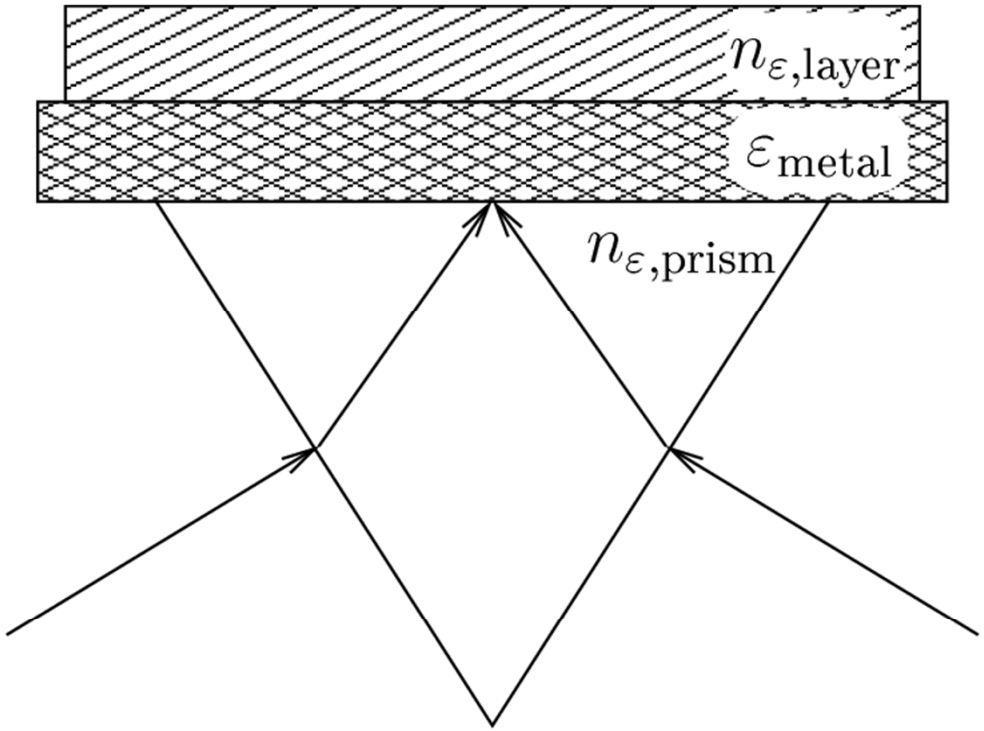
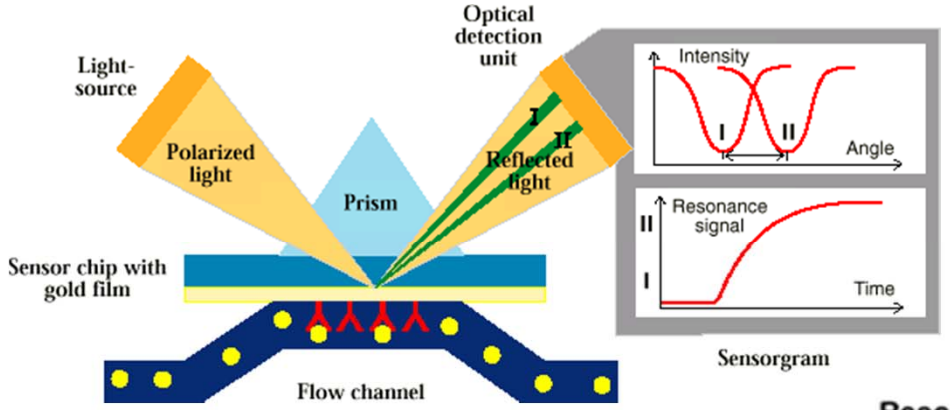
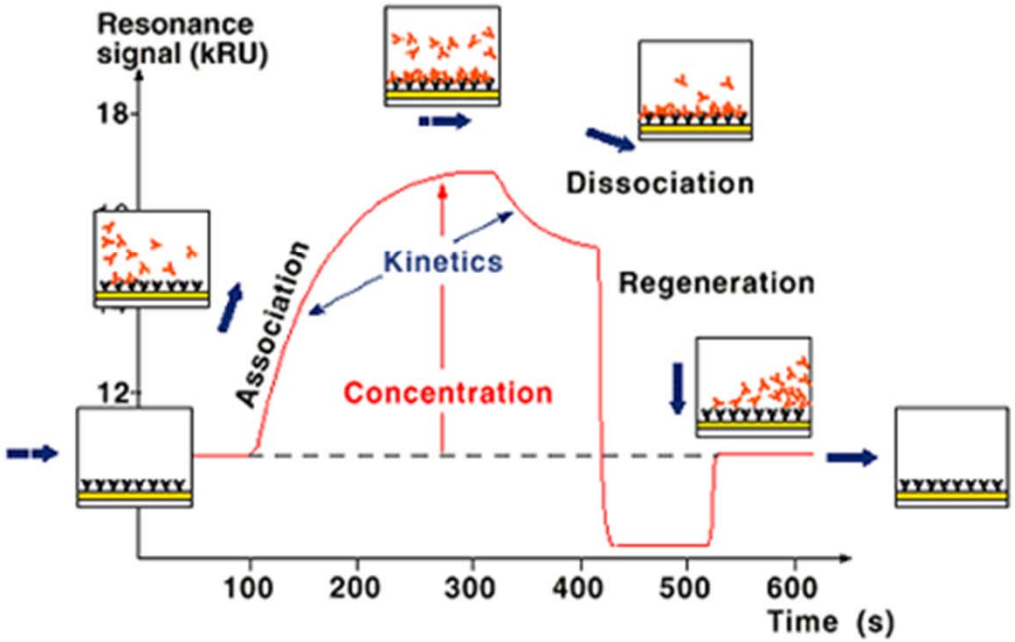


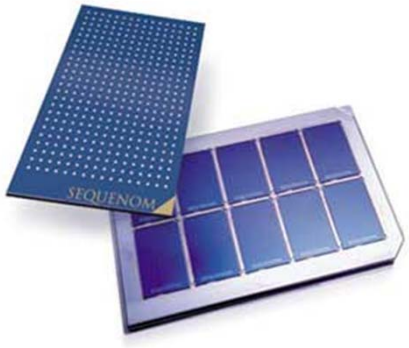
Fig. 10.23. In the Kretschmann geometry, light from the evanescent field penetrating the thin metal film couples to surface plasmons if the corresponding wave vectors match

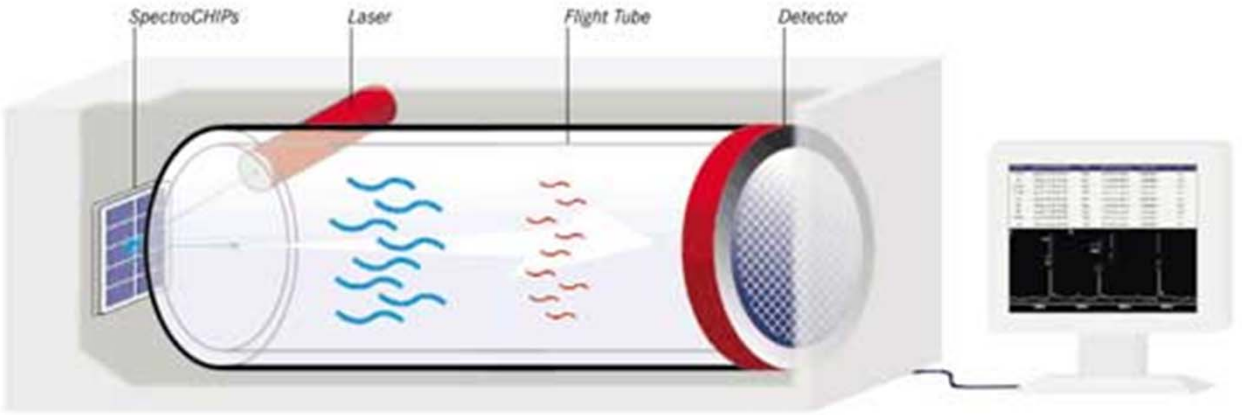
10.5. SPR Detection by Biacore





10.5. Mass Spectrometry





- MALDI-MS
  - Matrix Assisted Laser Desorption / Ionization
  - Time-of-Flight mass spectrometry
- Specifically to address the need for gentle analysis of biomolecules
- Combination of MALDI-TOF with
  - Proprietary SpectroCHIP
  - MassEXTEND reaction, SEQEUNOM
- Analysis of SNPs in truly automated, rapid and accurate model

10.5. Data Analysis & Modeling

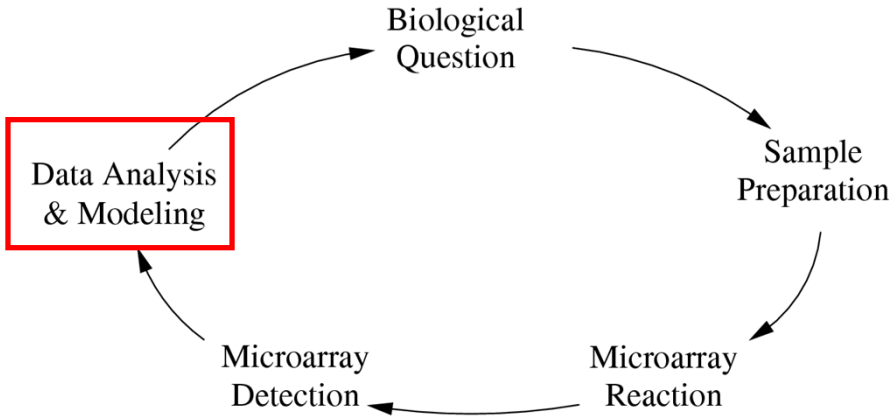
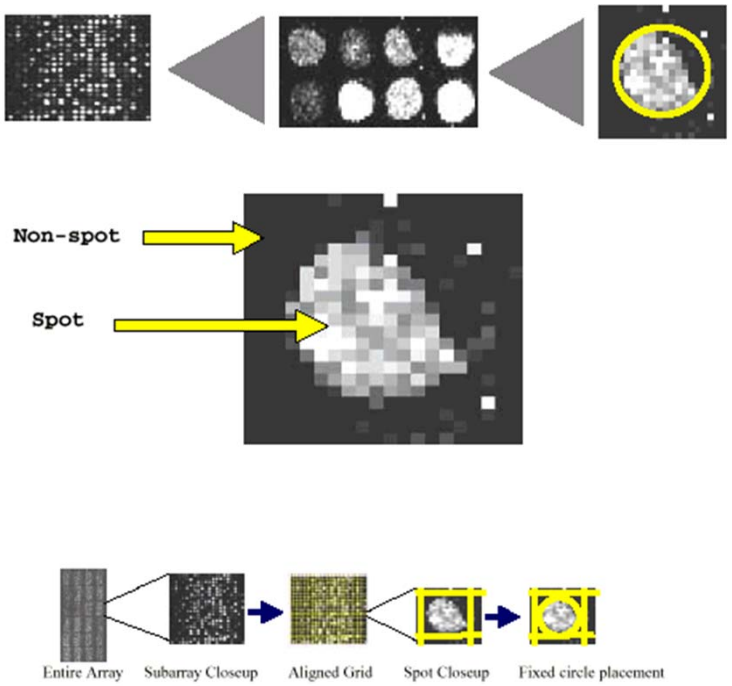


Fig. 10.2. Life cycle of a microarray

10.5. Digital Image Analysis

- Typical cDNA microarray
  - Laser scanned pixel image
  - Two 16-bit TIFF image files
    - Reference sample dye-channel
    - Experimental sample dye-channel
- Goal of image analysis
  - Reducing large number of pixel values in image files
  - Small set of summary values representing each printed spot on array
  - Typical scope of data reduction
    - 40-MB of image file data
    - 150-KB of summary quantitation data



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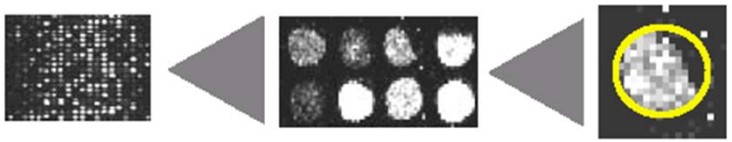
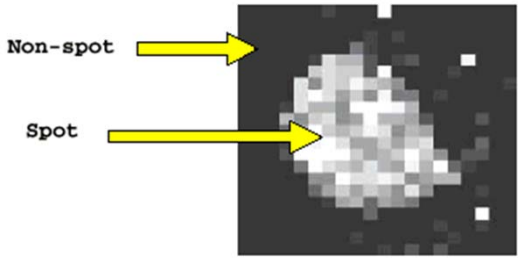
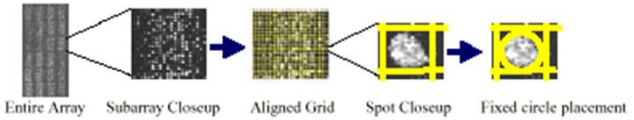
10.5. Digital Image Analysis

Fundamental operations for quantitation of microarray image files

- **Spot Discrimination**
  - Localization of small region occupied by each printed cDNA
  - Grid placement
    - Known geometry or approximate geometry of cDNA printing procedure enhances the spot-finding procedure
- **Spot Summarization**
  - Statistical procedure condensing pixel values within each spot to single number or small set of numbers representing intensity of spot







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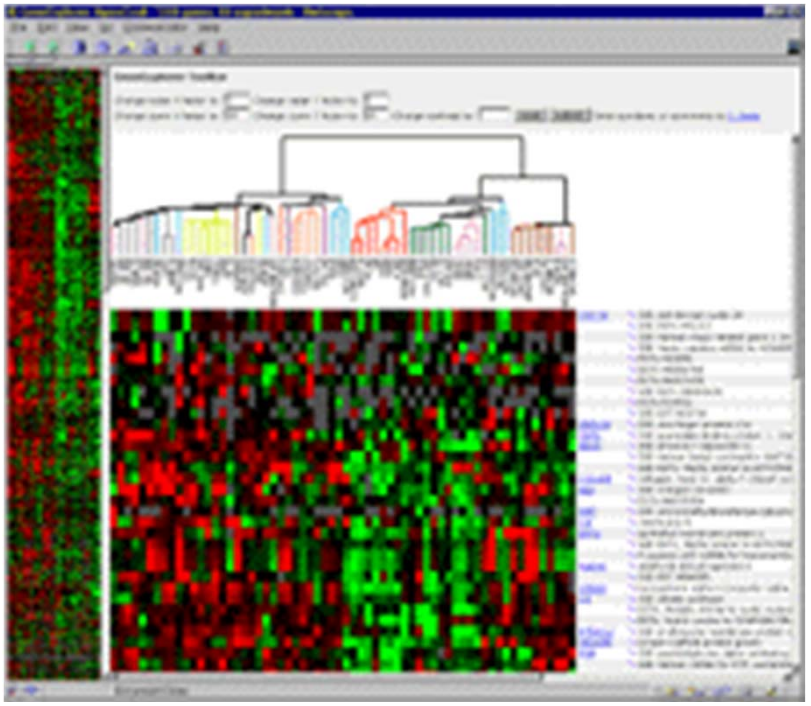
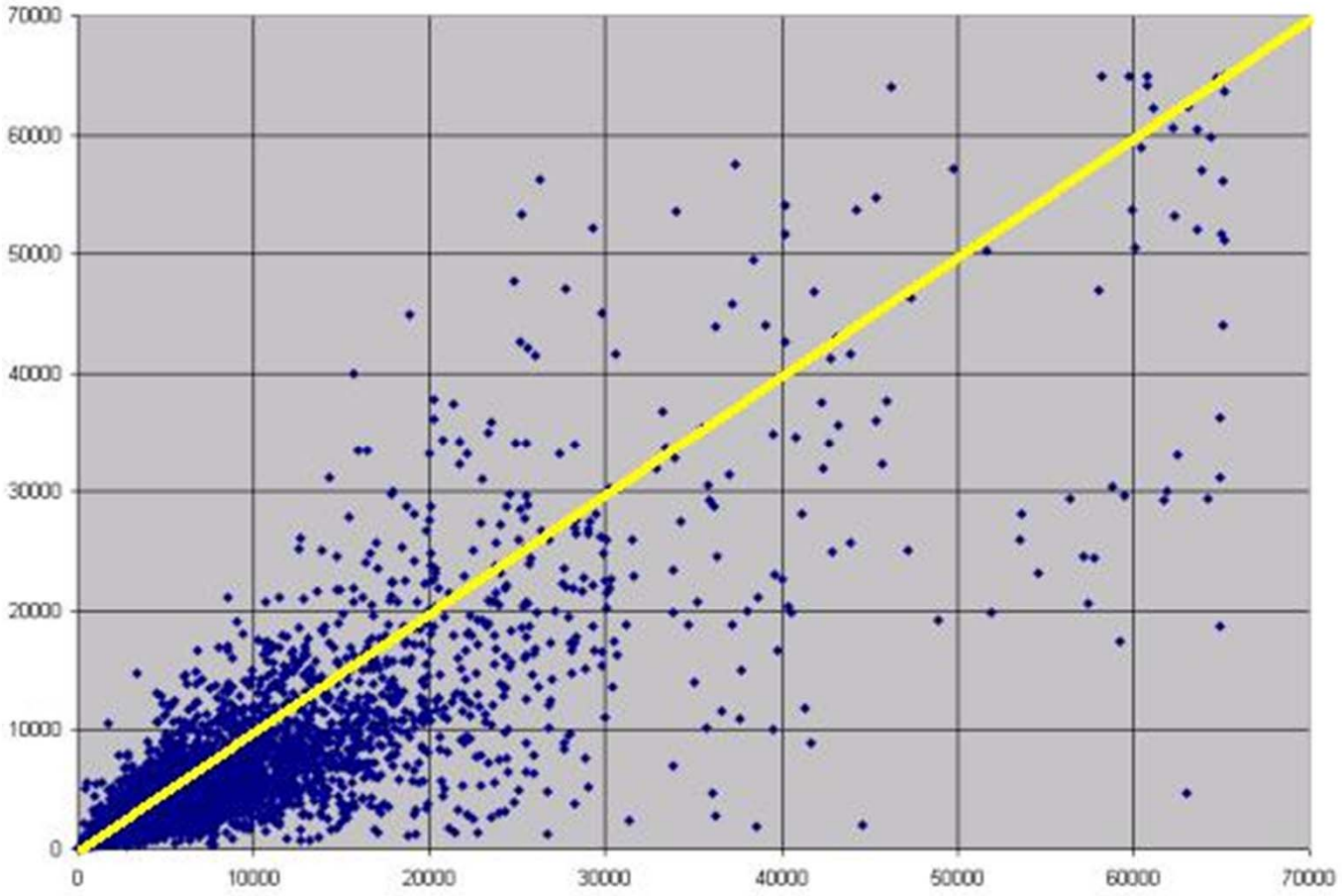
10.5. Bioinformatics

- Setting up microarray
- Automatic readout
- Data base
  - ➢ Assignment
    - Barcode – type of carrier
  - ➢ Assignment
    - spot – probe molecule
  - ➢ Assignment
    - Color – sample (for multiplexing)
- Digitized intensity
- ...



10.5. Differential Expression Profiling

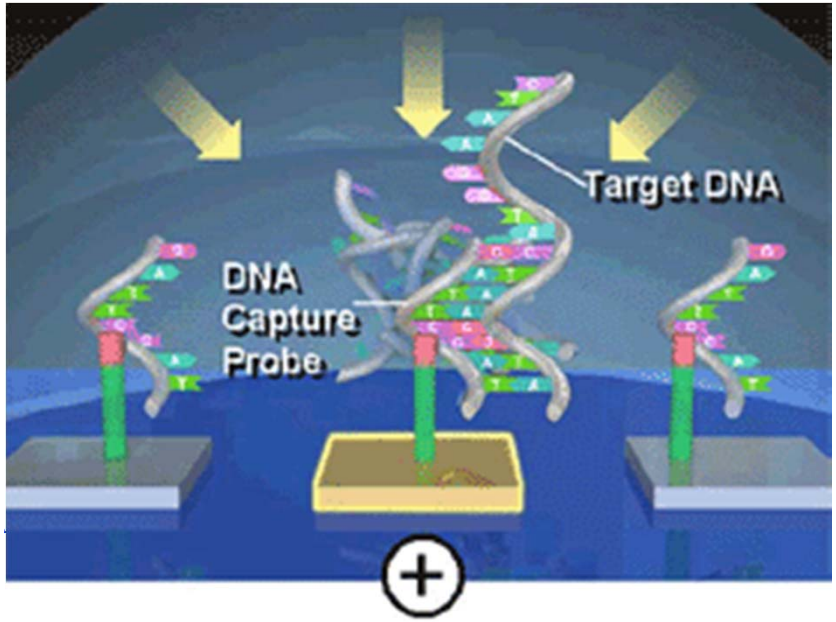


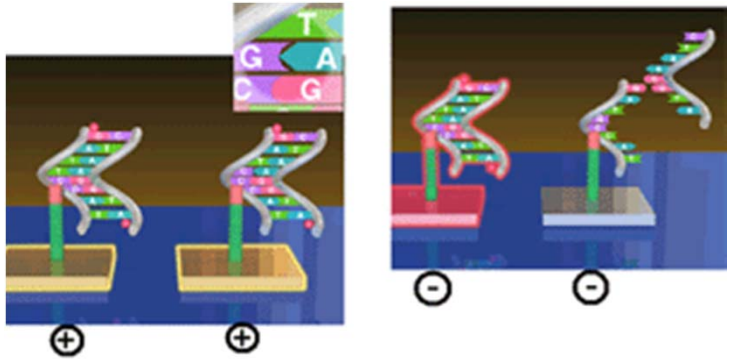
10. Microarrays

1. Introduction
2. Reaction Kinetics
3. Immobilization
4. Fabrication
5. Detection
6. **Electronic Control**
7. Protein Microarrays
8. Bead-Based Microarrays

10.6. Electronic Arrays

- Most biological molecules possess natural positive or negative charge
- Applying electric fields results in movement of molecules
- Can be used for attracting or pushing away molecules
  - Could be 1,000 times faster compared to diffusion
- Method can be used for highly specific hybridization experiments





10.6. Electronic Arrays

- Working principle
  - Microchip hosts array of individually addressable electrodes
  - Biomolecules normally charged in solution
    - DNA negatively charged
- Advantages
  - Movement of molecules by electric fields
    - „Acceleration“ of otherwise merely diffusive process
  - Manipulation of hybridization kinetics

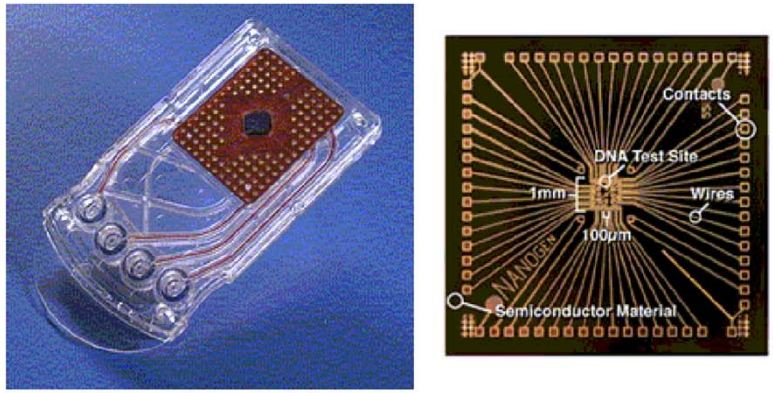


Fig. 10.25. Nanogen microarray technology. The figure shows the disposable cartridge with the fluidic ports and an enlarged view of the proprietary semiconductor microchip

10.6. NanoGen Technology Platform

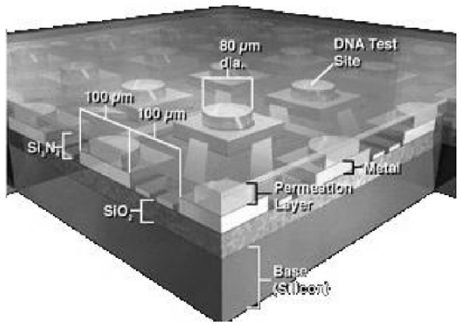
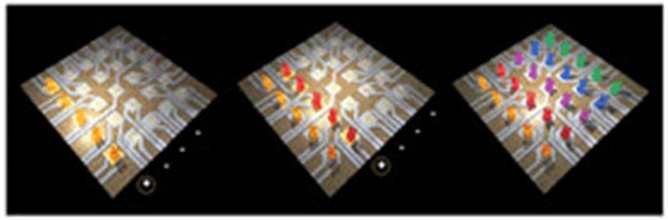


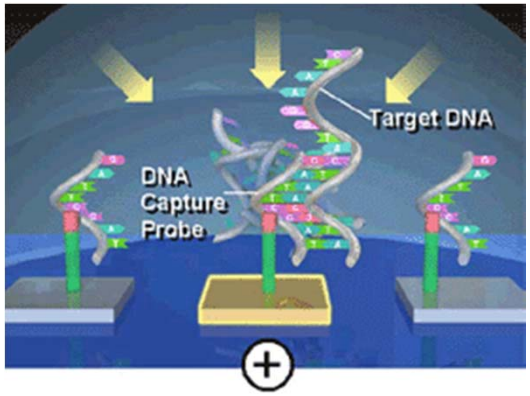
Fig. 10.26. Different layers and structures of the Nanogen chip. The permeation layer is crucial for the operation of the chip. It protects the biomolecules from the harsh electrochemical environment of the electrode layer and assures proper attachment of the probe molecules

10.6. Electronic Control

- Electronic addressing
  - Directing of molecules to specific site



- Concentrating and Hybridization
  - Electronic activation of specific site



- Stringency control
  - Removal of unspecific binding

- Electronic multiplexing
  - Multiple test on single sample

10. Microarrays

1. Introduction
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7. **Protein Microarrays**
8. Bead-Based Microarrays

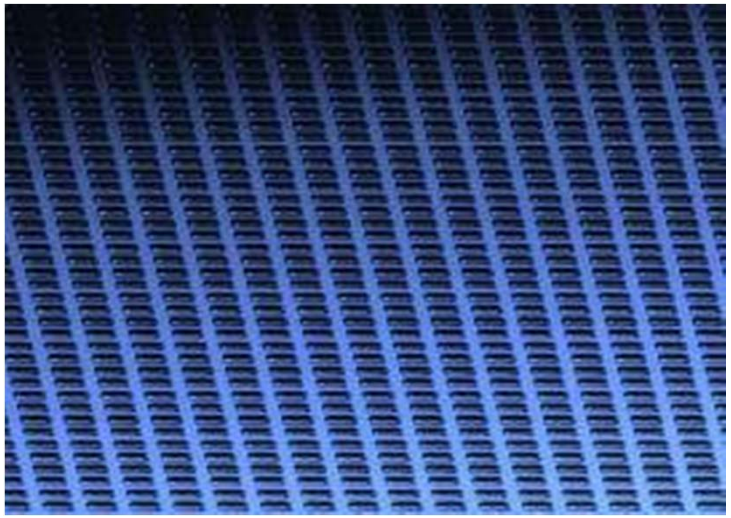
10.7. Immunoassays: Fractional Occupancy

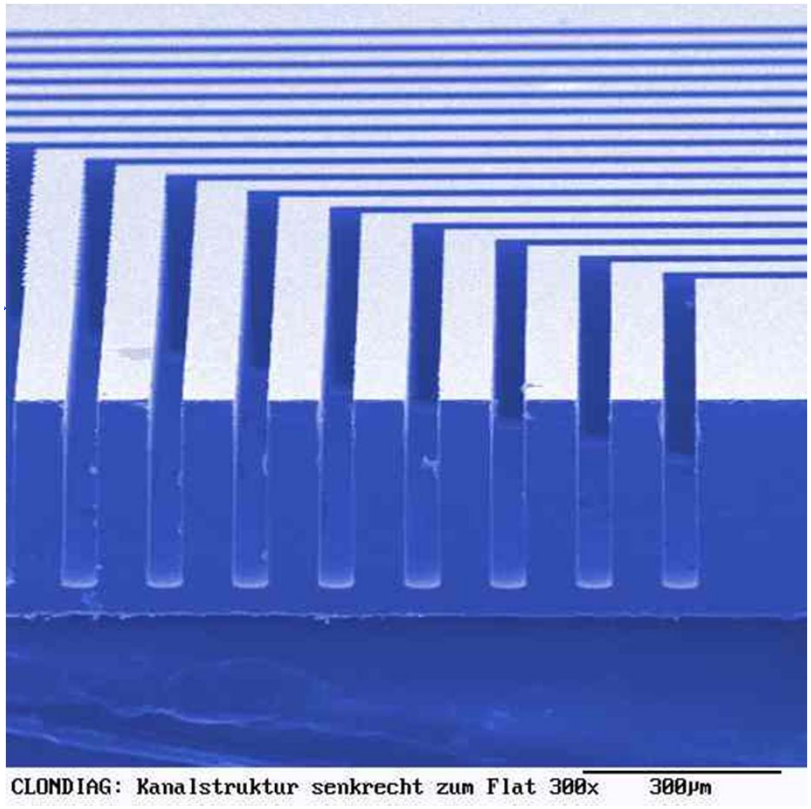
- Surprising:
  - *As the amount of a binder located in the form of a spot on a surface is reduced, one reaches a situation where the fractional occupancy" (i.e. the fraction of binding sites occupied by target molecules) becomes independent*
  - a.) of the sample volume
  - b.) of the amount of binder
- within the spot.
- Under this circumstance, fractional occupancy of binder sites in the microspot is dependant
  only on the concentration of target molecules in the test solution to which the spot is exposed.
- Analogy: thermometer influencing temperature of vessel
- (R. Ekins)

10.7. Clondiag

- „Micro Wet Printing“

- Bases positioned similar to screen printing technology





10.7. μ-Contact Printing

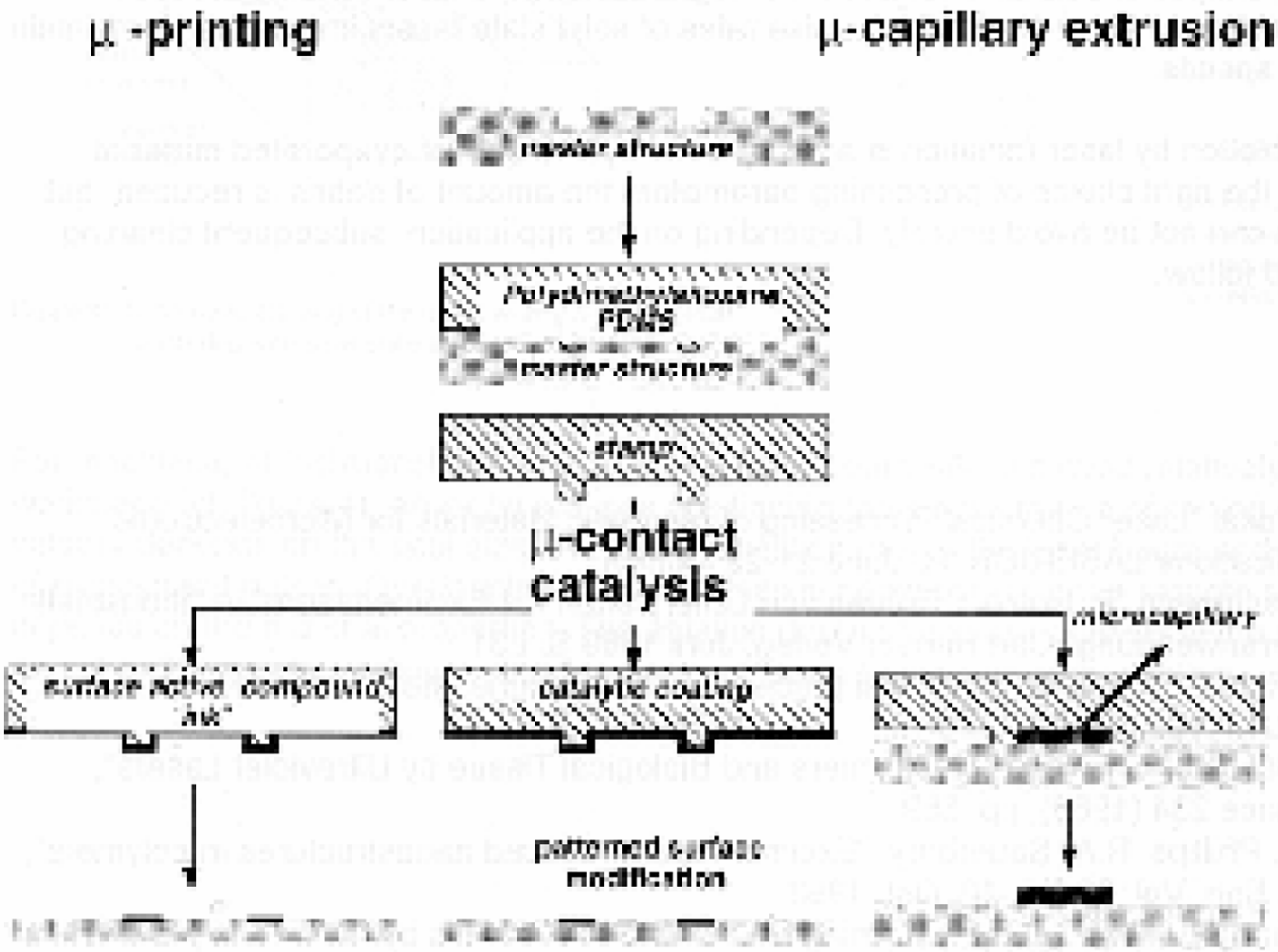


Fig. 10.30. Master fabrication and μ-contact printing

10.7. Printing Protein Arrays

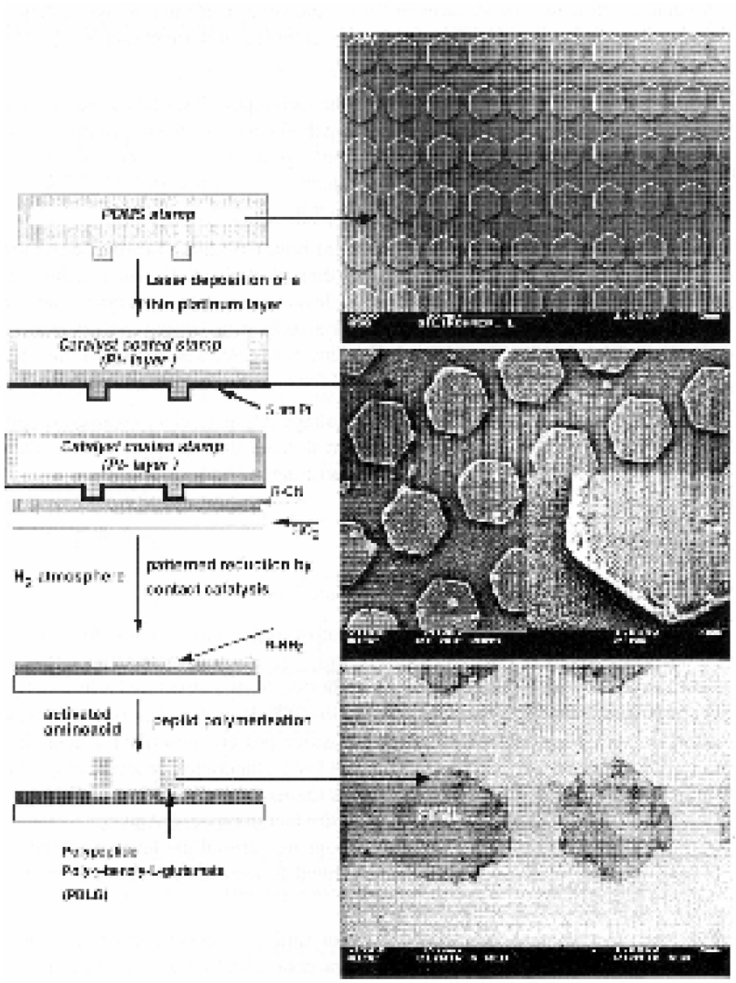


Fig. 10.31. Process steps of printing biological arrays (JD: ask Roland for source)

10.7. µ-Contact Printed Array

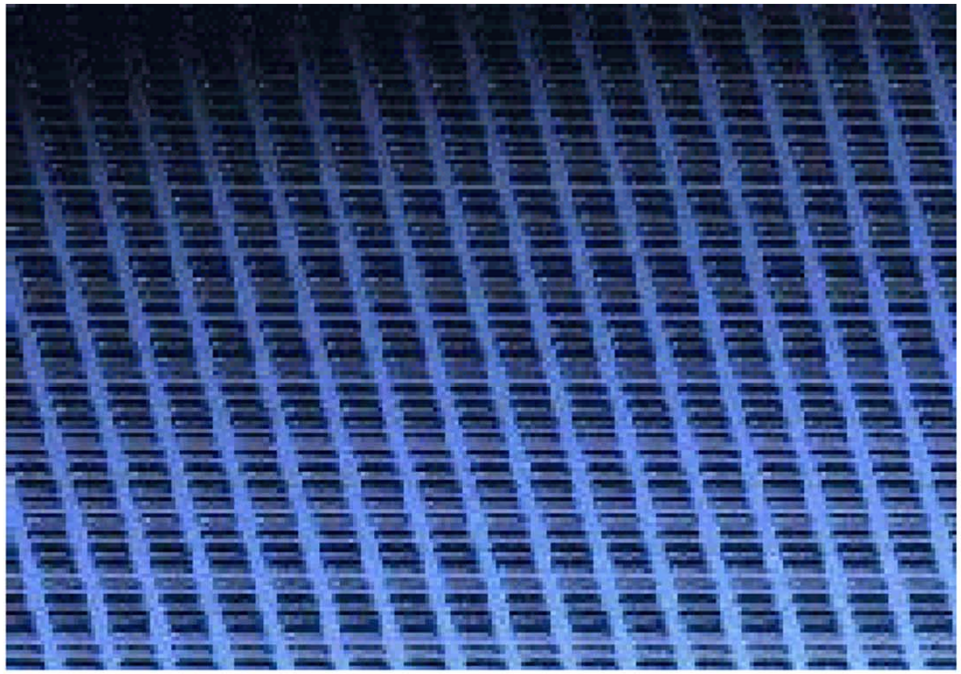


Fig. 10.32. Microarray generated by micro-wet printing (µWP)

10.7. Compound Microdispenser

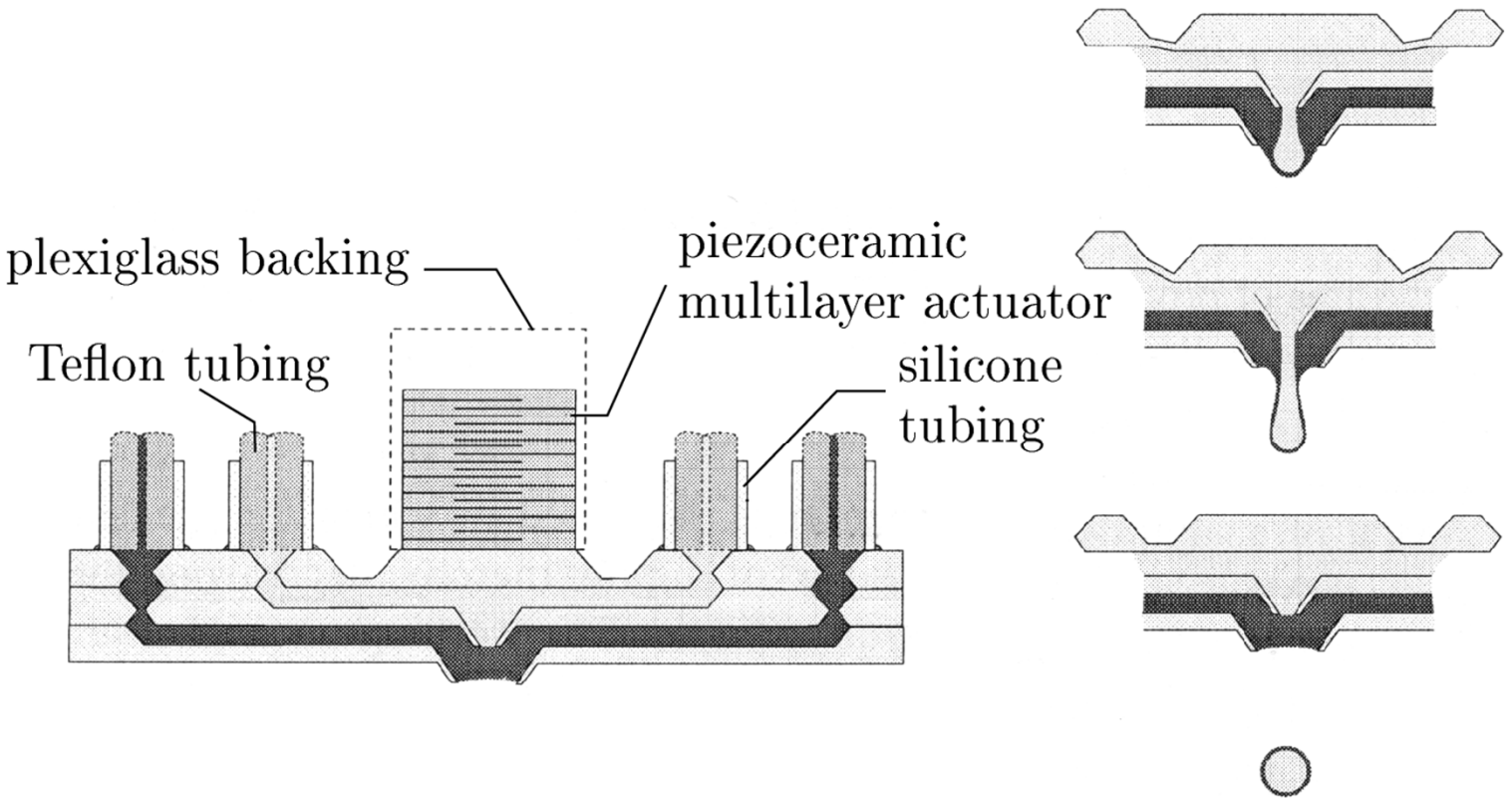


Fig. 10.33. Compound microdispenser

10. Microarrays

1. Introduction
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7. Protein Microarrays
8. **Bead-Based Microarrays**

10.8. Bead-Based Technologies

- Micro transponders
  - Code transmitting cubes
  - Circuits transmits unique signal when exposed to e/m radiation
  - Probes immobilized in surface
  - Receiver identifies bypassing cubes by their signal
- Color-coded spheres
- Optical fiber arrays

10.8. Capillary Filter

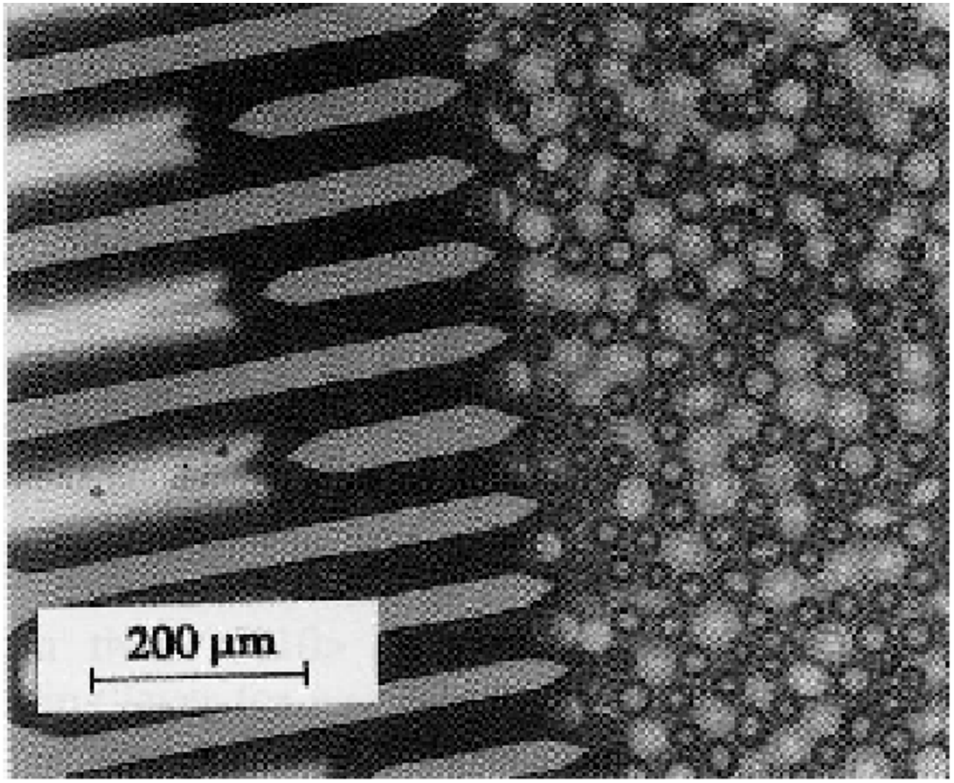


Fig. 10.34. Capillary filter holding back microbeads in the reaction chamber while fluid can pass the structure (JD: Ask Roland for picture source)

10.8. Weir-Confined Packed Chamber

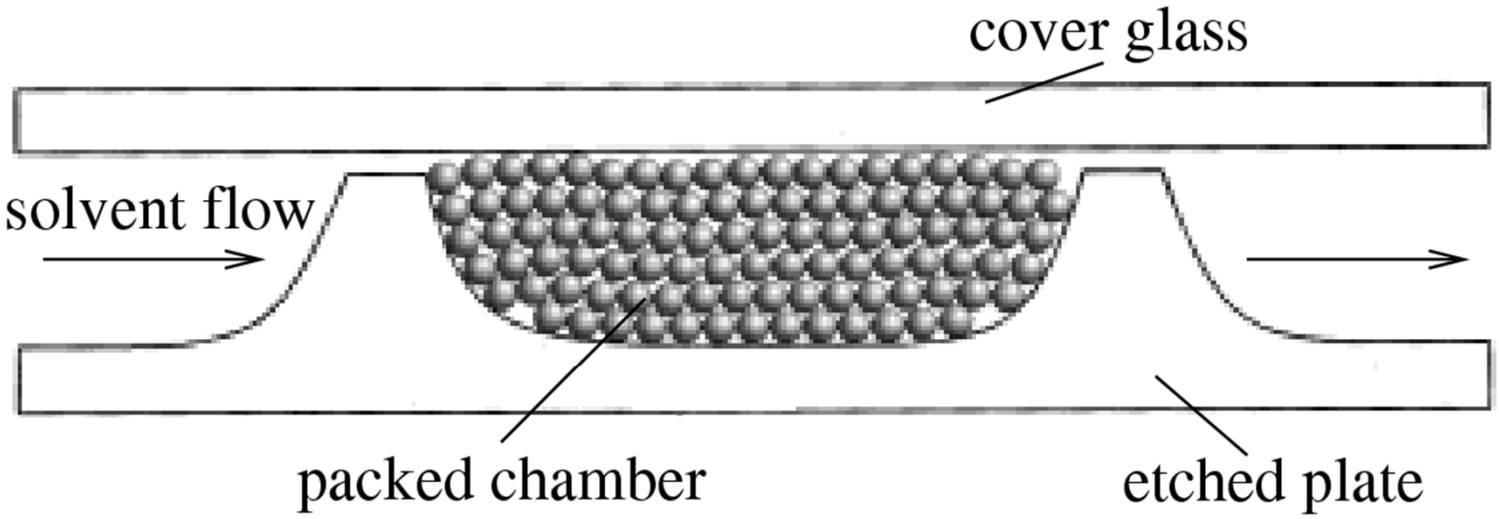


Fig. 10.35. Weirs retaining microbeads in a chamber. Electroosmotic flow is driven by walls and by free silanol groups on particles. The solvent flow direction is indicated for preconcentration step

10.8. Bead-Array Technology

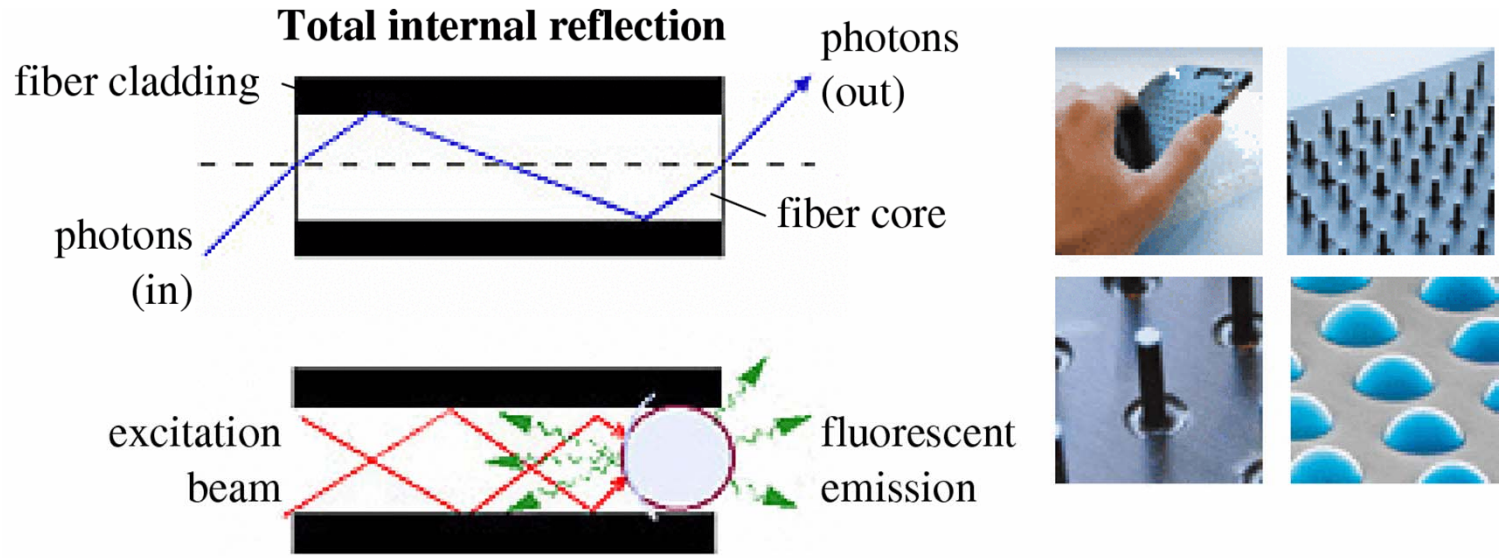


Fig. 10.36. Optical detection of beads with fiber bundles

10.8. Bead-Array Technology

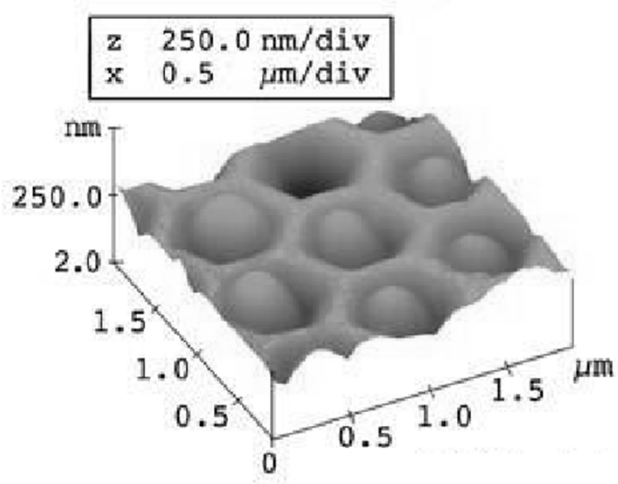


Fig. 10.37. BeadArray technology by Illumina Inc.

10.8. Bead-Array Technology

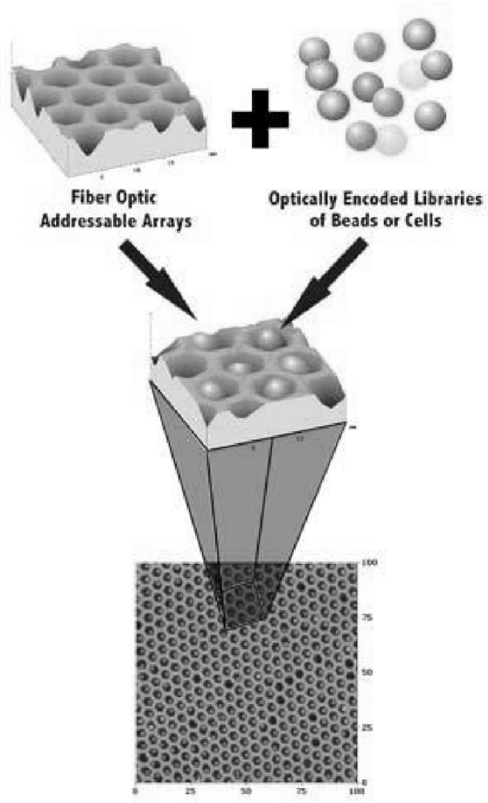
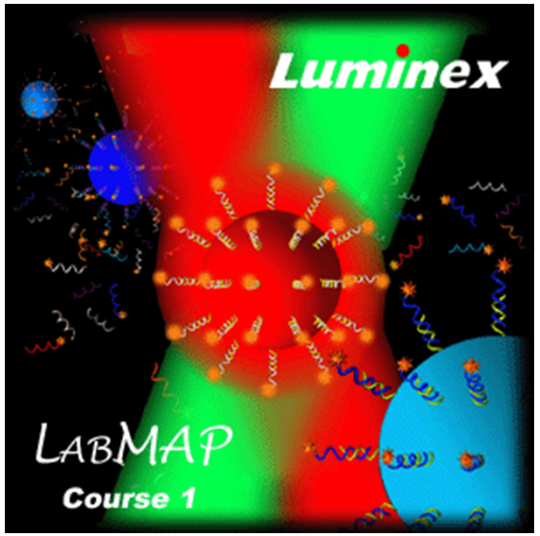


Fig. 10.38. BeadArray technology by Illumina Inc.

LabMap Technology

Luminex

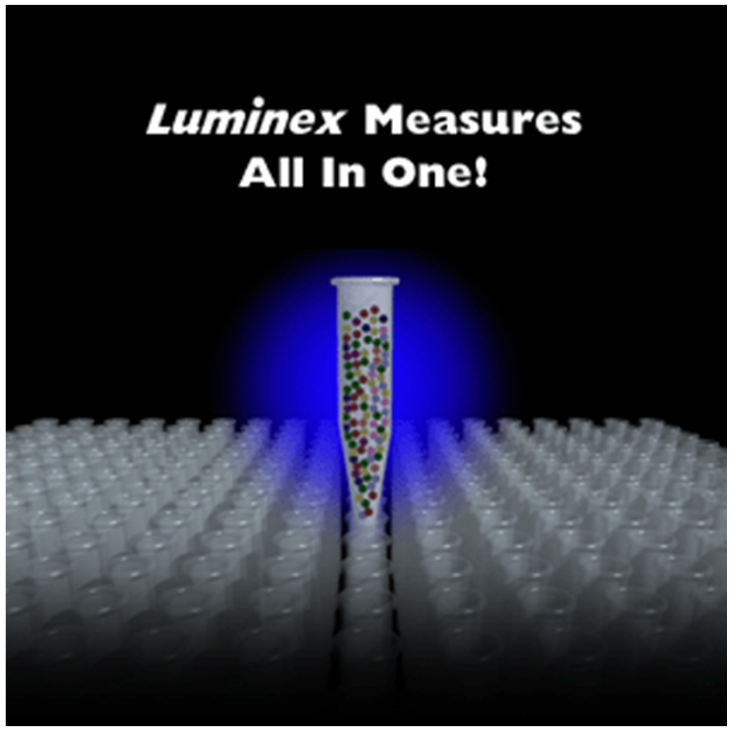
- LabMAP technology
  - Measures multiple analytes simultaneously in single reaction vessel
  - Delivers wealth of information simply and inexpensively
- Ingredients
  - Microspheres • Fluidics • Optics • Computers
- Molecular reactions on surface of microscopic beads called microspheres
  - Thousands of molecules attached to surface
  - Internally color-coded microspheres
  - Assigned color-codes identify reaction throughout test
- Magnitude of biomolecular reaction
  - Measured using second molecule called reporter
  - Signaling extent of the reaction by attaching to molecules on microspheres
  - Also color signal by reporter
  - Two sources of color



LabMap Technology

Luminex

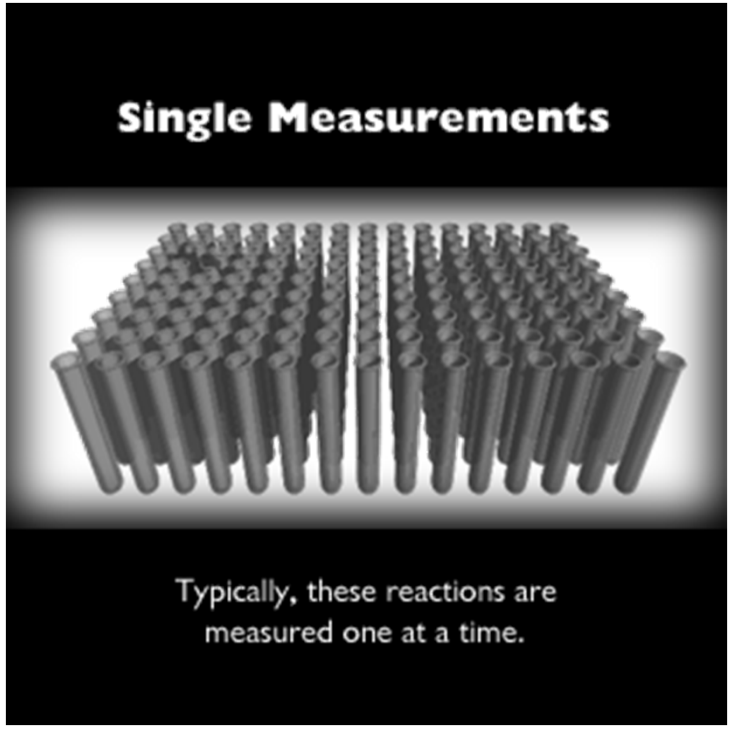
- Performance of a test

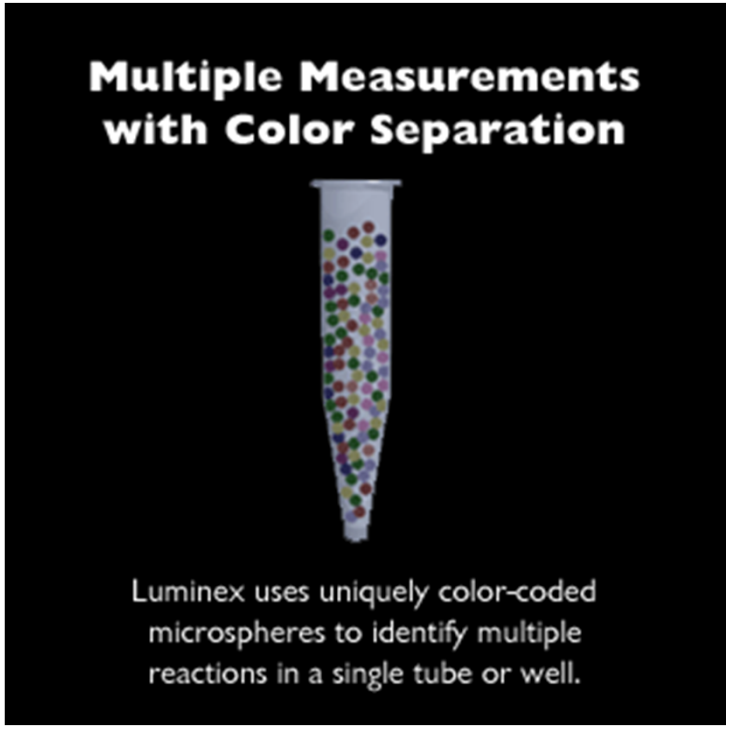


signals into real-time, quantitative data for each reaction

LabMap Technology

Luminex





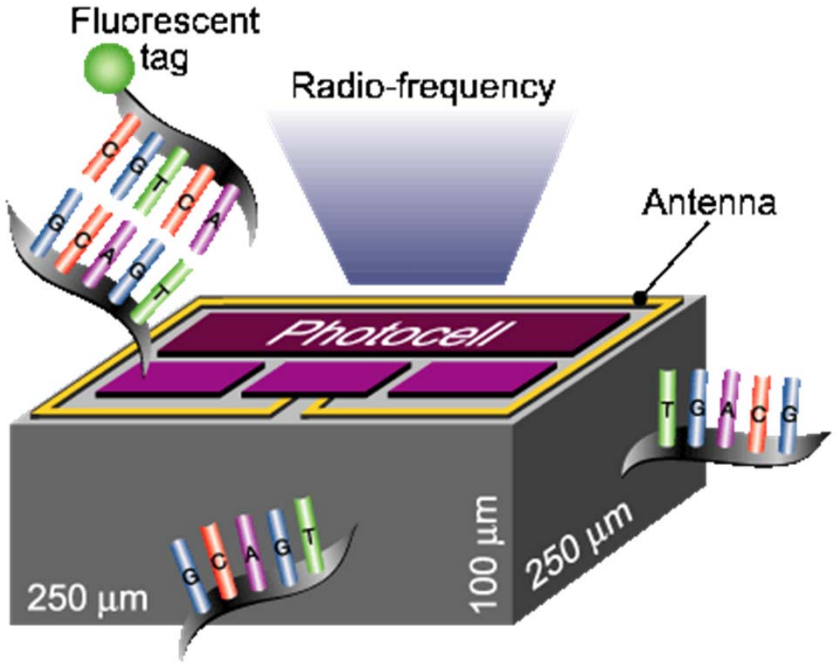
Alternative & Complementary Technologies

1. Microarrays
    1. Electrically Controlled Microarrays
    2. “Encoded Particle” Microarrays
        1. Color-Encoded Beads
        2. **Microtransponders**
2. Lab-on-a-Chip
    1. Electrokinetic Systems
    2. Centrifugal Systems
    3. DNA Amplification & Detection
    4. Diffusion-Controlled Systems
3. Microreactors
4. Drug Delivery Systems
    1. Microdisplacement Pumps
    2. Pressure Reservoir
    3. Intelligent Pills

Microtransponders

- Light-powered microtransponders and nanotransponders
  - Identification of probes
  - Analogous to *xy*-position in microarray
  - Recently developed nanotransponder
    - 250 x 250 x 100 microns
  - Performing nucleic acid-based assays
- Present strategy
  - Application of microtransponder technology to
    - DNA probe diagnostics
    - Single nucleotide polymorphism (SNP) detection
    - Proteomics
- Future plans
  - Other aspects of genomics
  - Pharmaceutical drug discovery
  - Combinatorial chemistry
  - Radio frequency identification (RFID)
  - ...

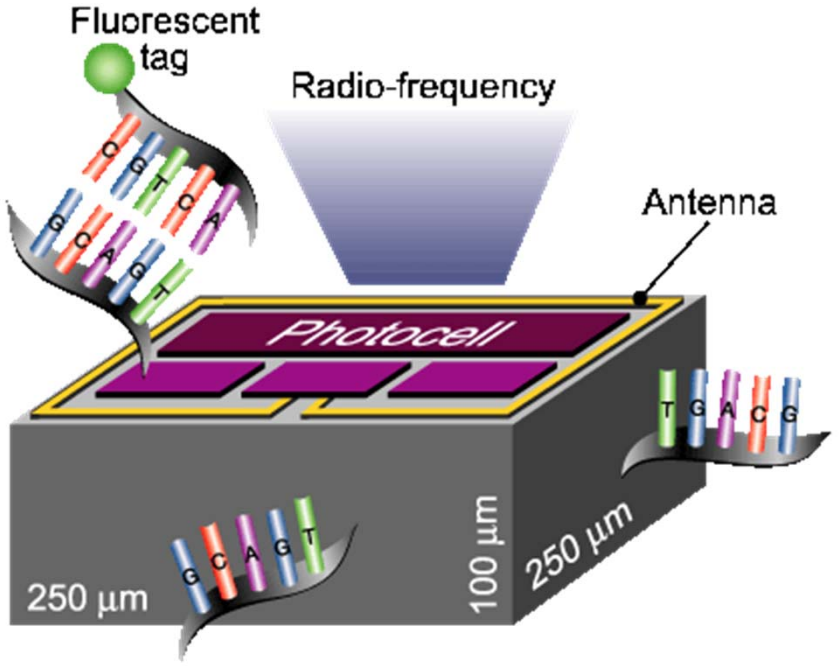
PHARMASEQ



Microtransponders

PHARMASEQ

- DNA probes covalently linked to the microchip surface
  - Recognition of specific target DNA sequence tagged with fluorescent dye
- Microchip's surface
  - Loop antenna
  - Photocell, logic
  - ROM
  - Clock circuitry
- Identification
  - Laser light activates microtransponder
  - Transmits RF identification signal
- Target detection
  - Simultaenously
  - Light induces fluorescence of dye molecules on surface of microtransponder (positive result).
  - In contrast, microchips not coated with labeled target DNA will only transmit identification signal (negative result).



Microtransponders

PHARMASEQ