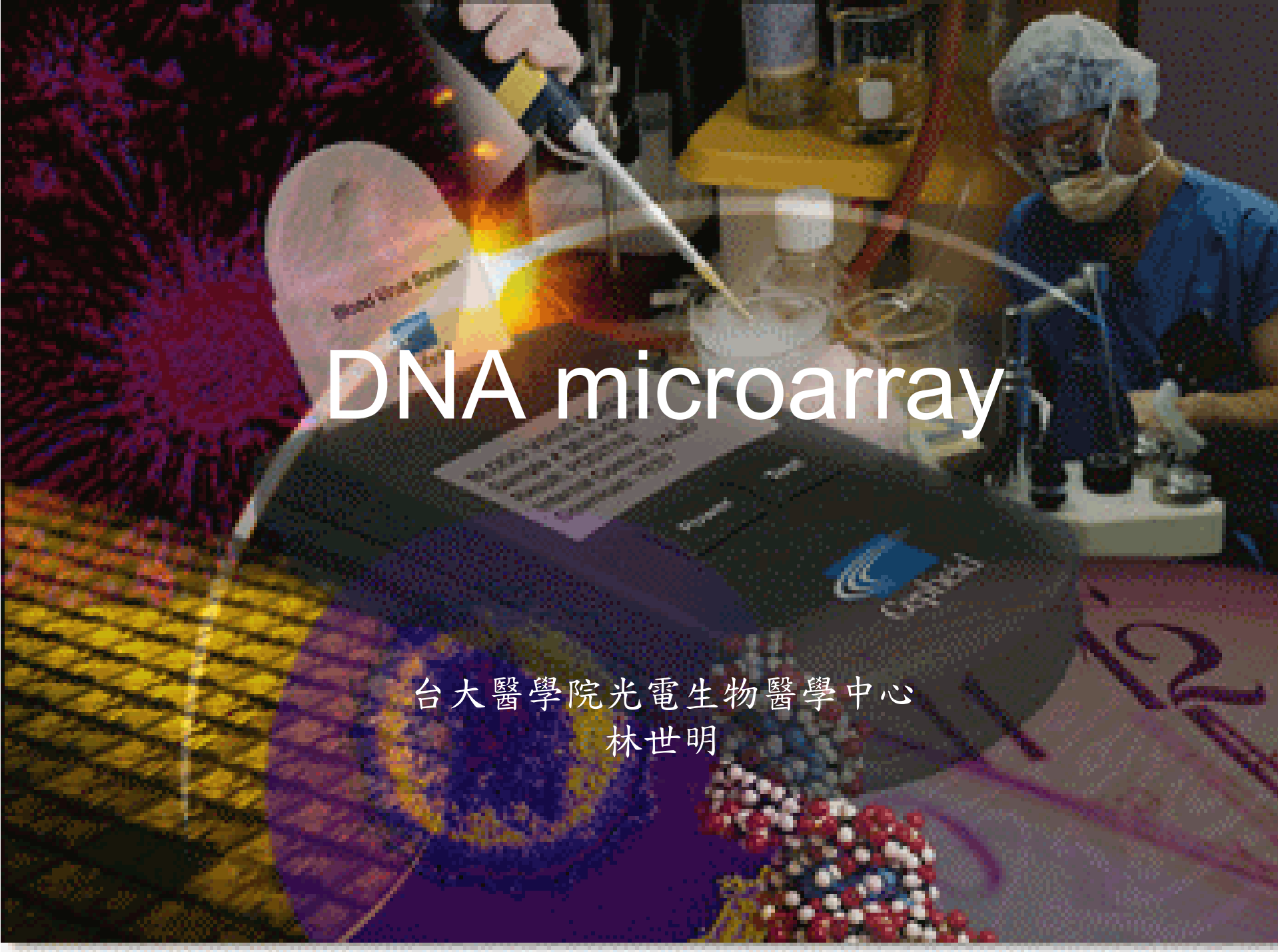
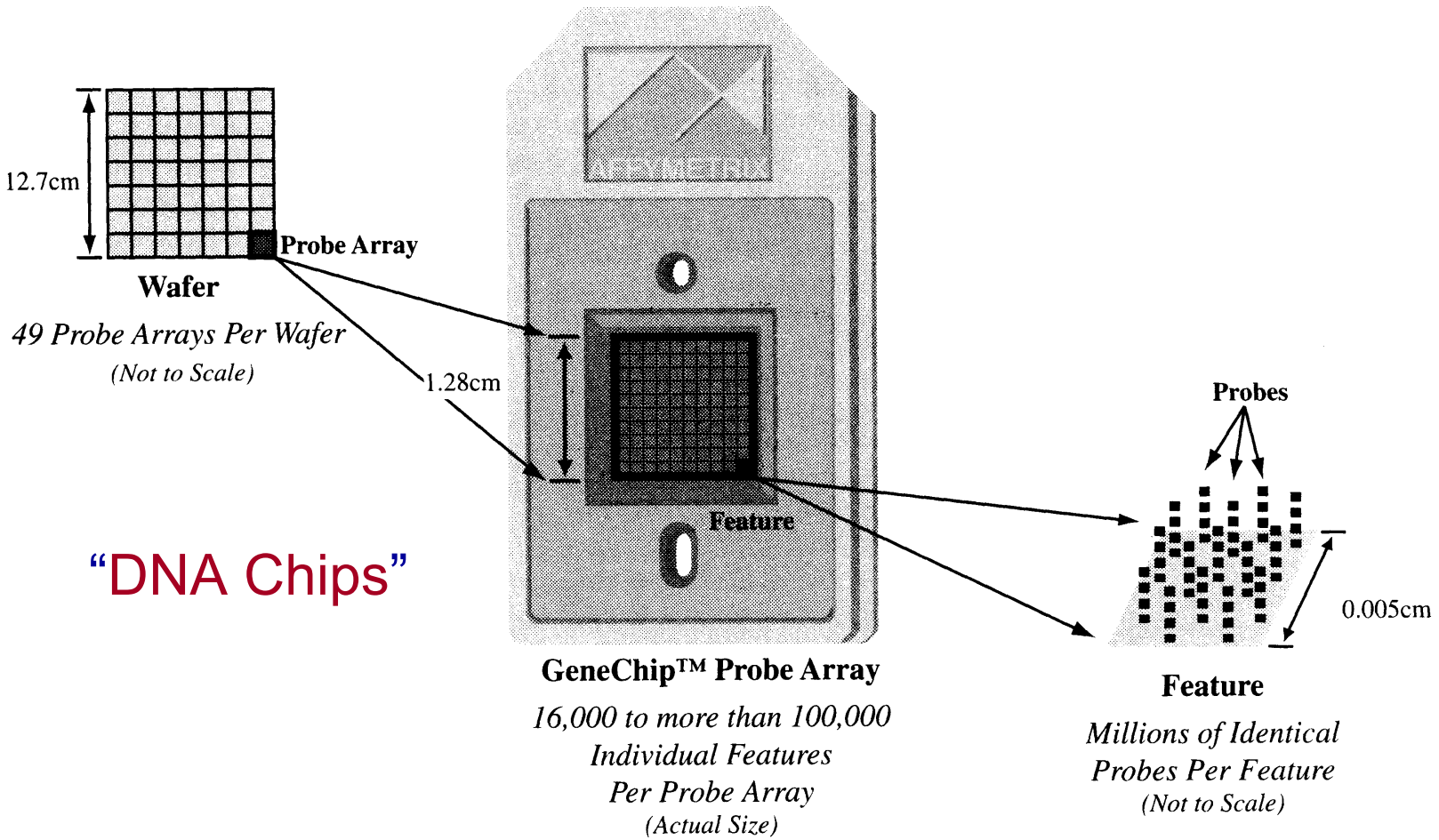


DNA microarray

台大醫學院光電生物醫學中心
林世明

Microarrays are constructed using

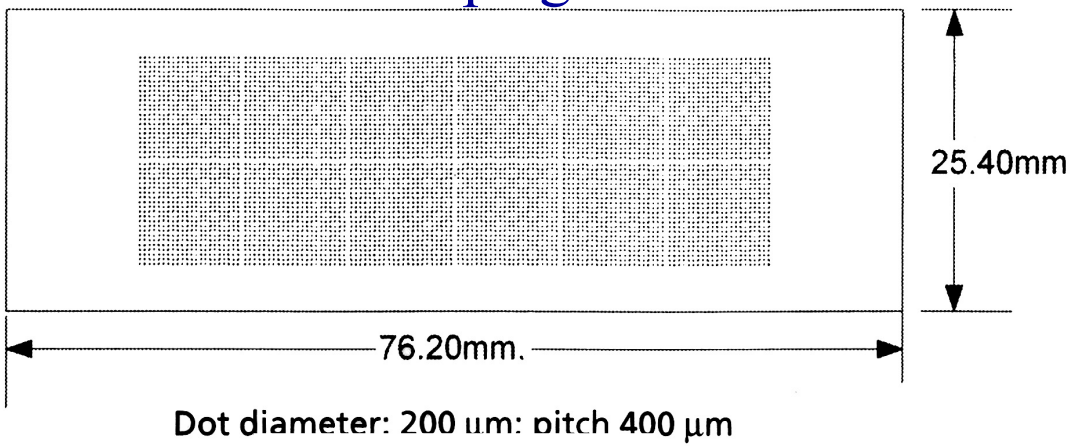
(*1*) oligonucleotides synthesized in silico ("DNA Chips")
(*2*) cDNAs (cDNA arrays) and genomic sequences

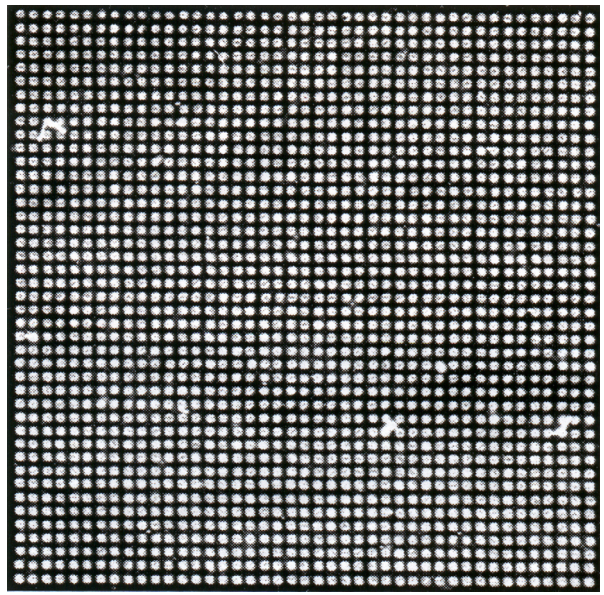


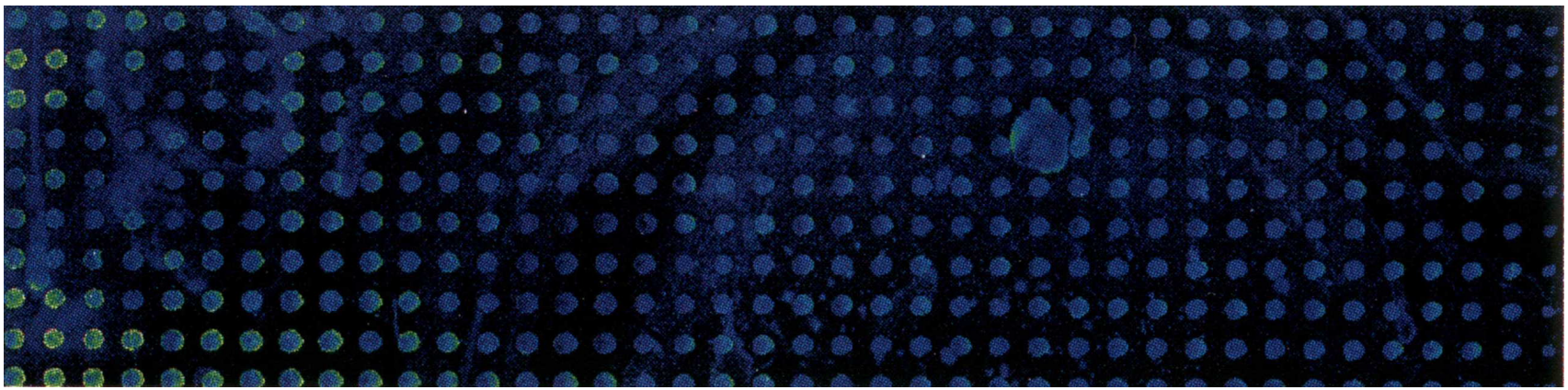
"DNA Chips"

“cDNA arrays” :

1“ X 3” microscope glass slide







Nomenclature :

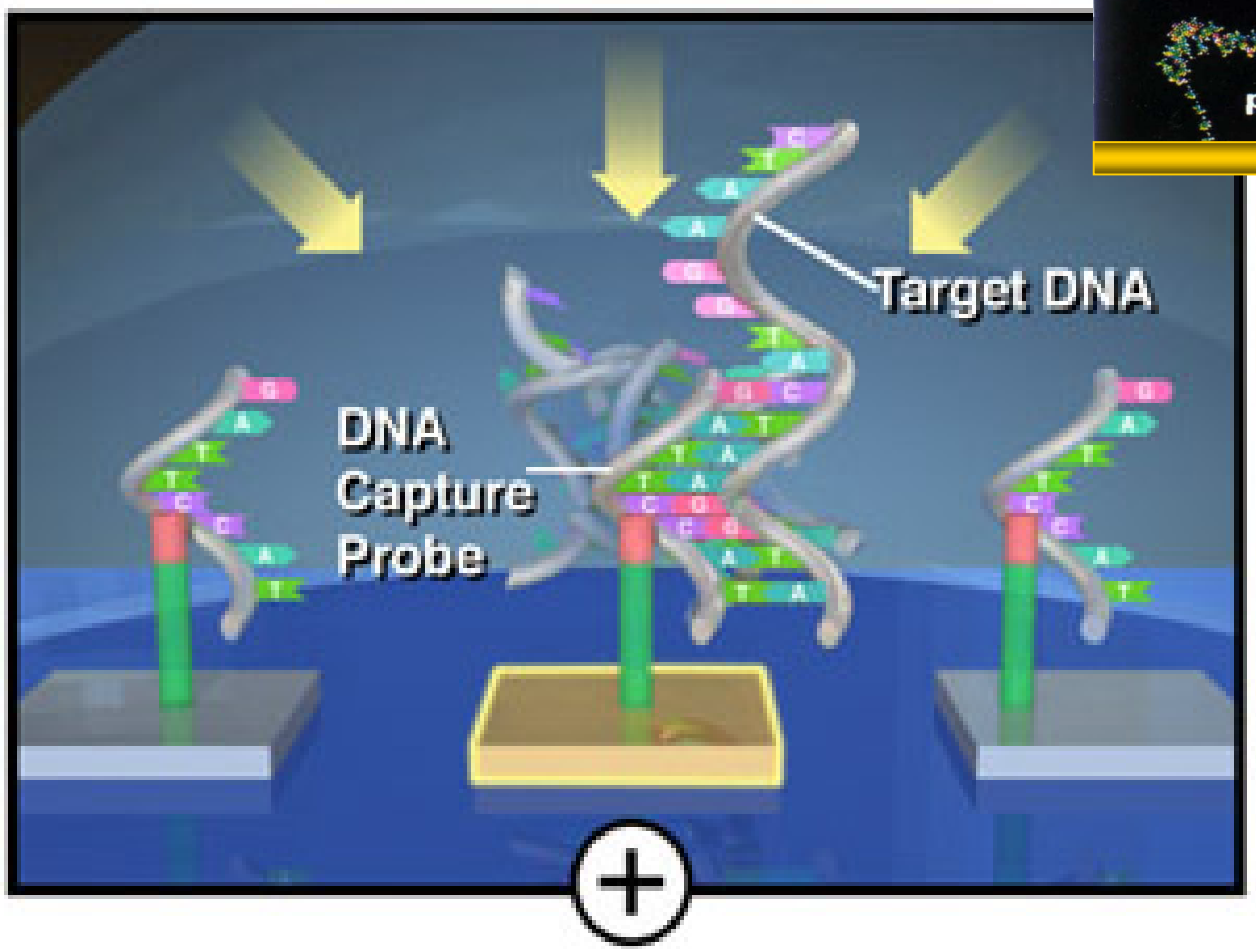
"DNA probe " – the tethered nucleic acid

"target DNA" – the free nucleic acid



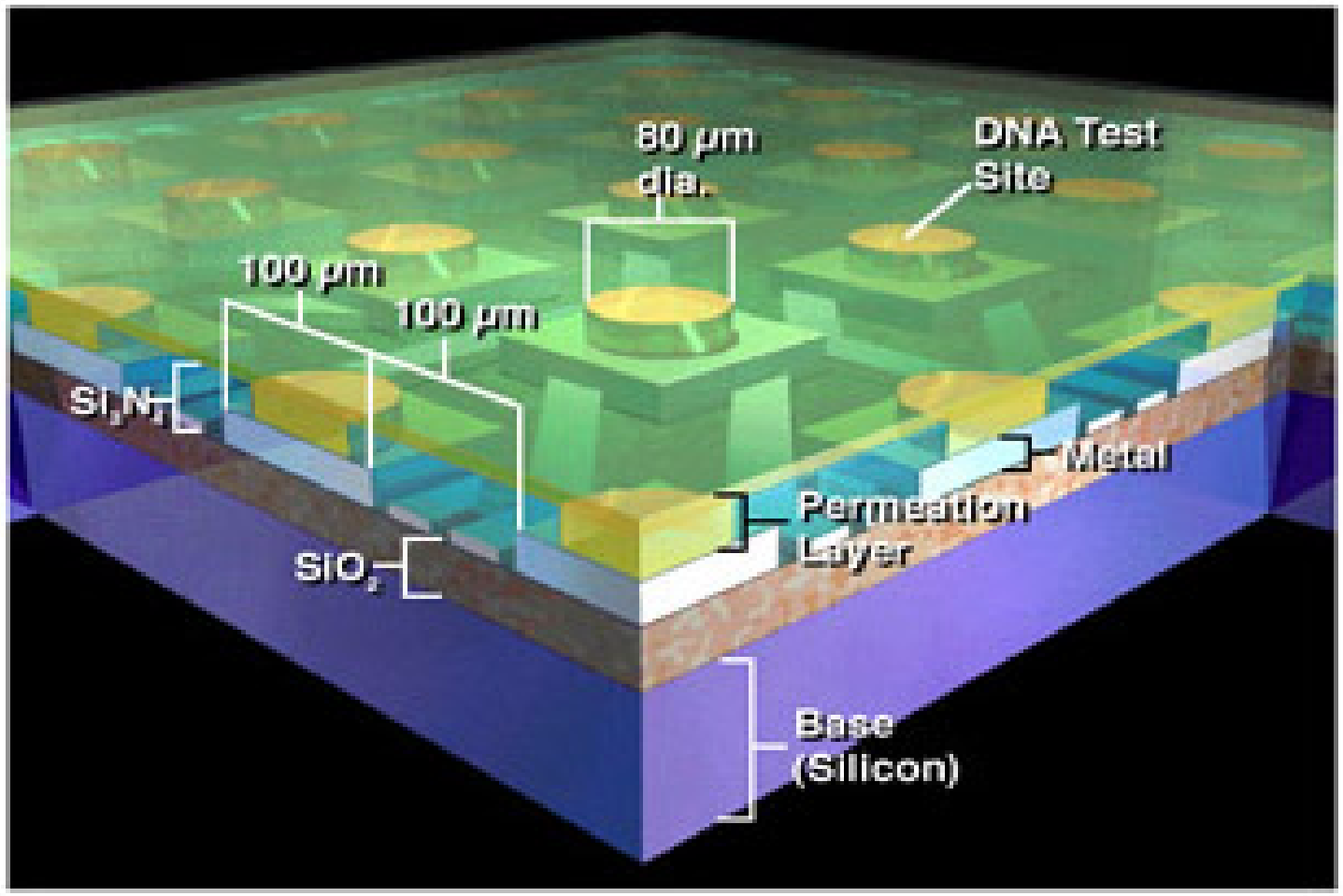
"immobile phase" DNA

"mobile phase" DNA

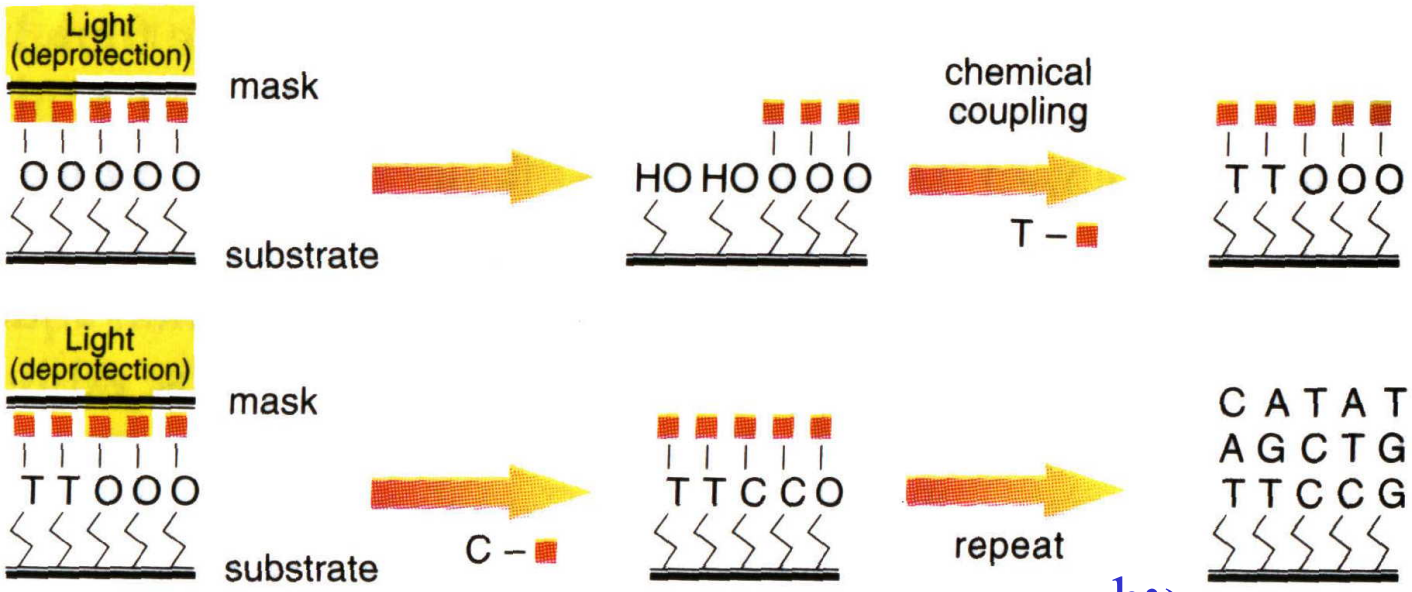


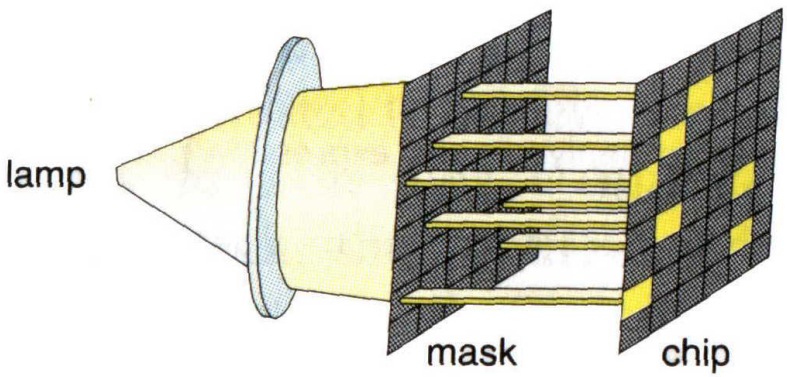
DNA chips using two techniques :

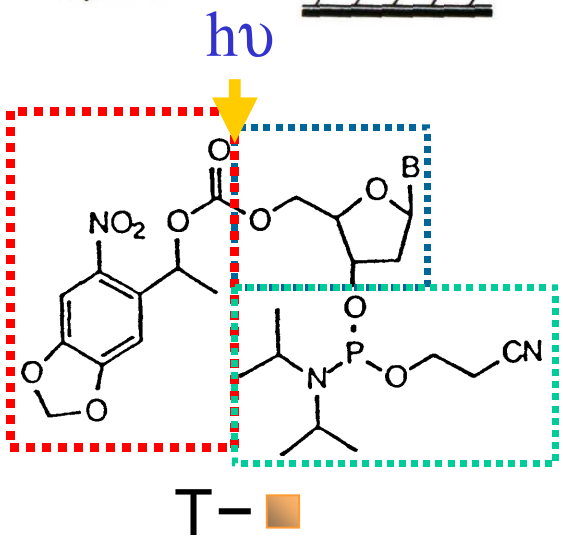
1. photolithography or micromirrors (maskless mirror)
2. solid-phase DNA synthesis

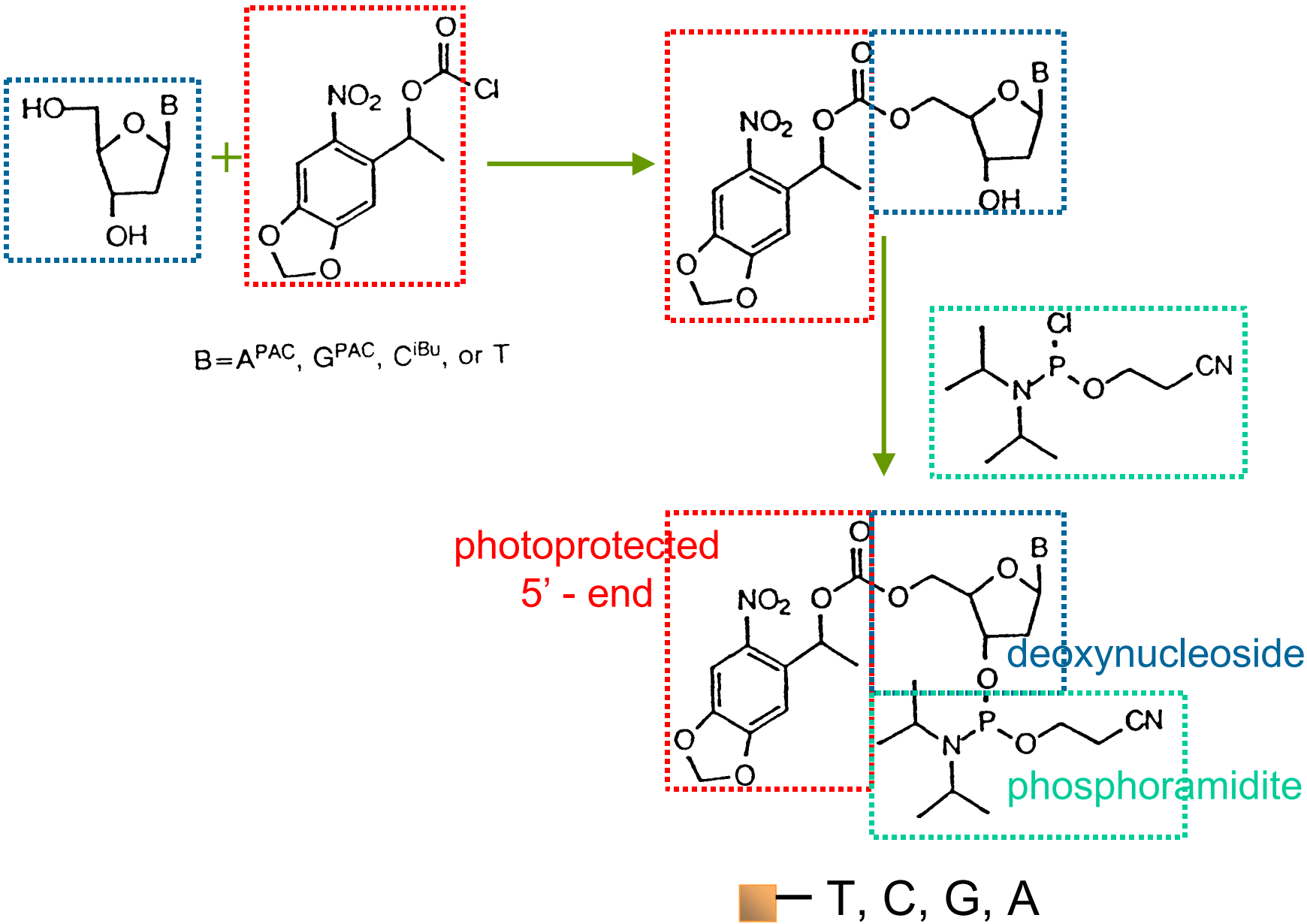


Light-directed oligonucleotide synthesis (photolithography):



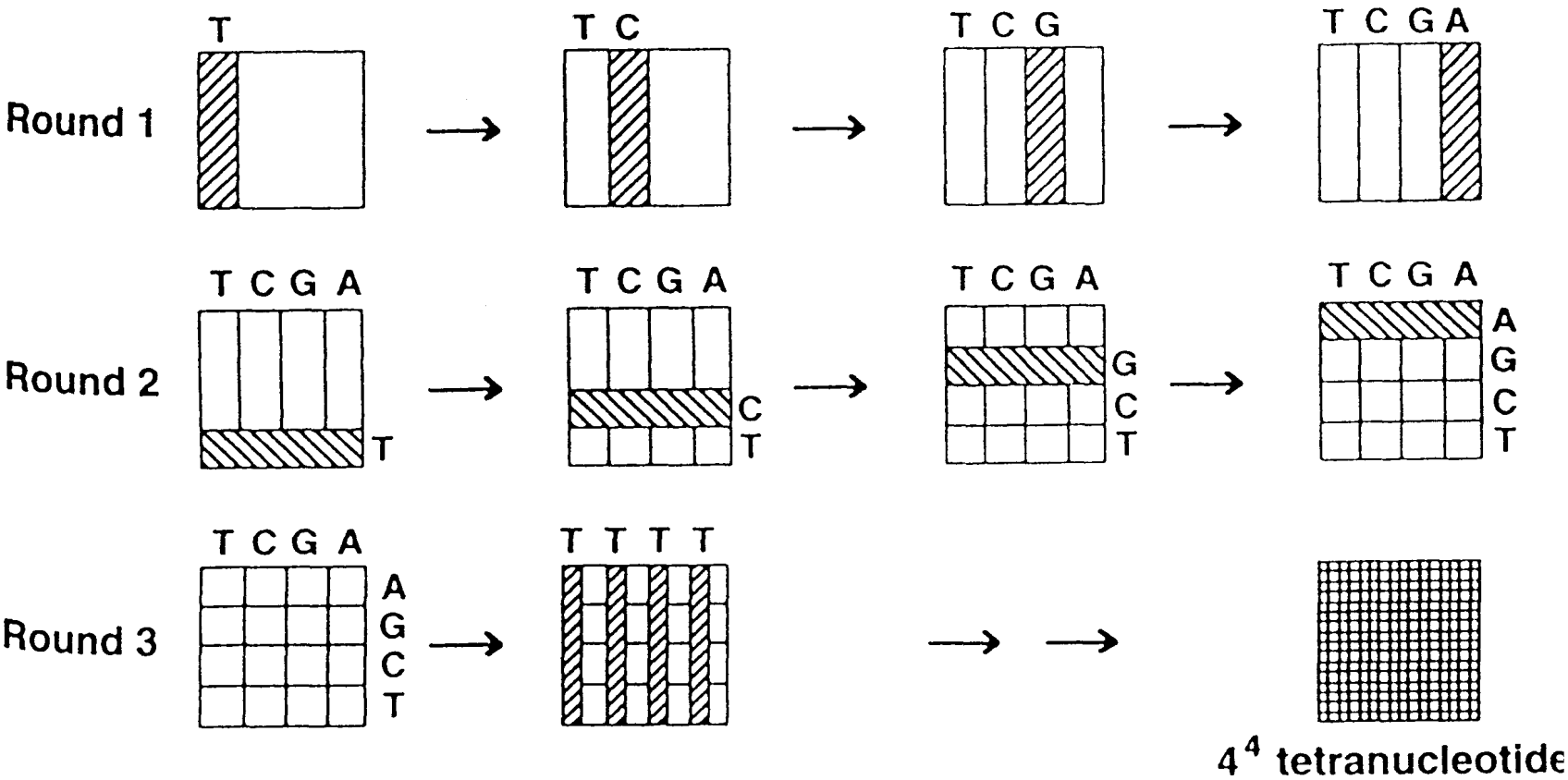




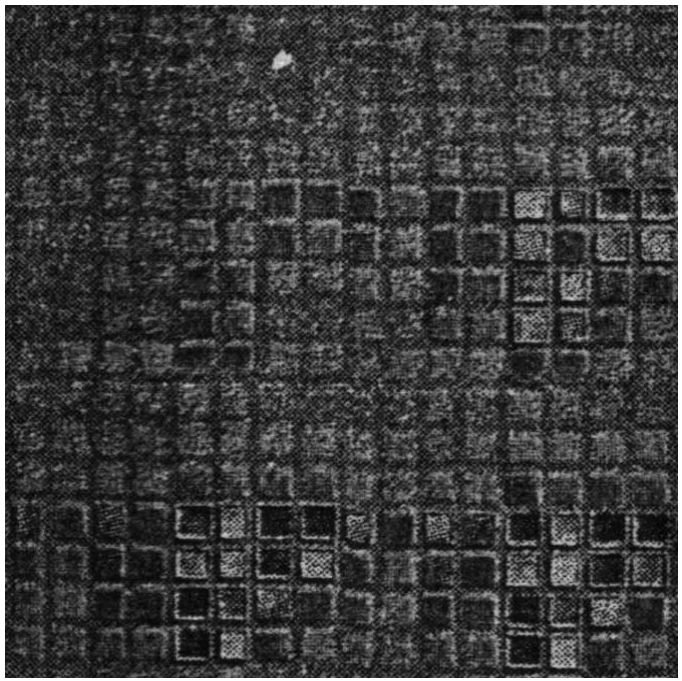

B=A^PAC, G^PAC, C^iBu, or T
photoprotected
5' - end
deoxynucleoside
phosphoramidite
T, C, G, A

Synthesis of a matrix of 4^4=256 octanucleotides and

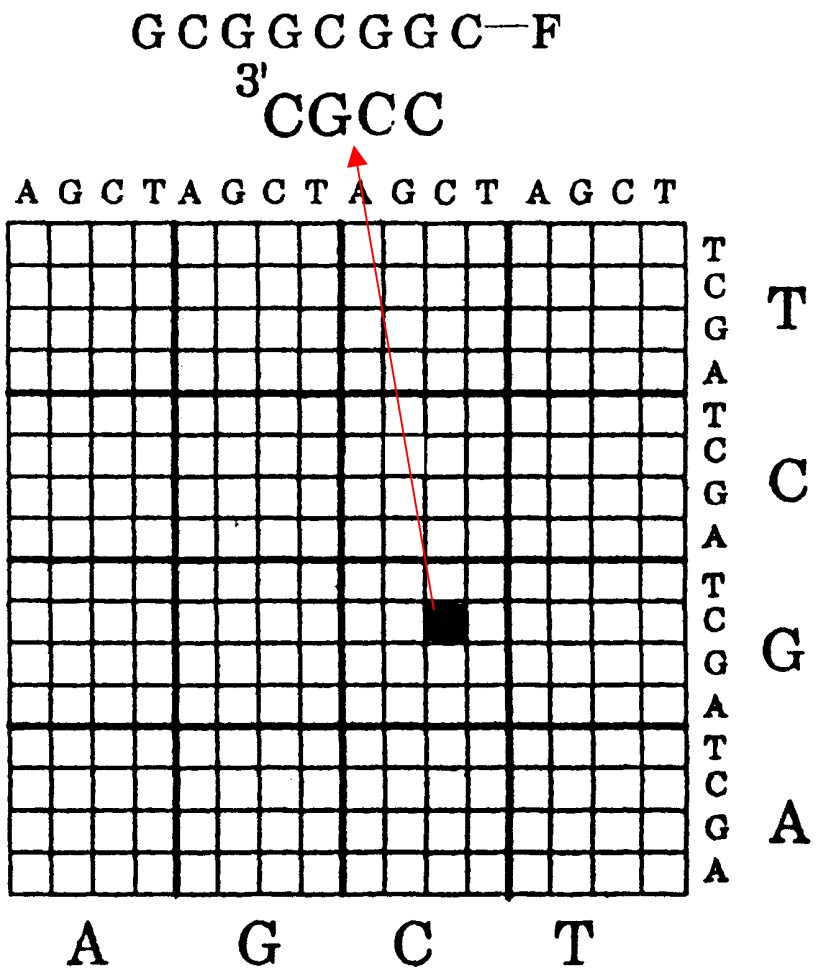
(A) Combinatorial synthesis of 4^4 tetranucleotides:



(B) Fluorescence image of a 256 tetranucleotide array following hybridisation with 5’ –GCGGCGGC-fluoresce



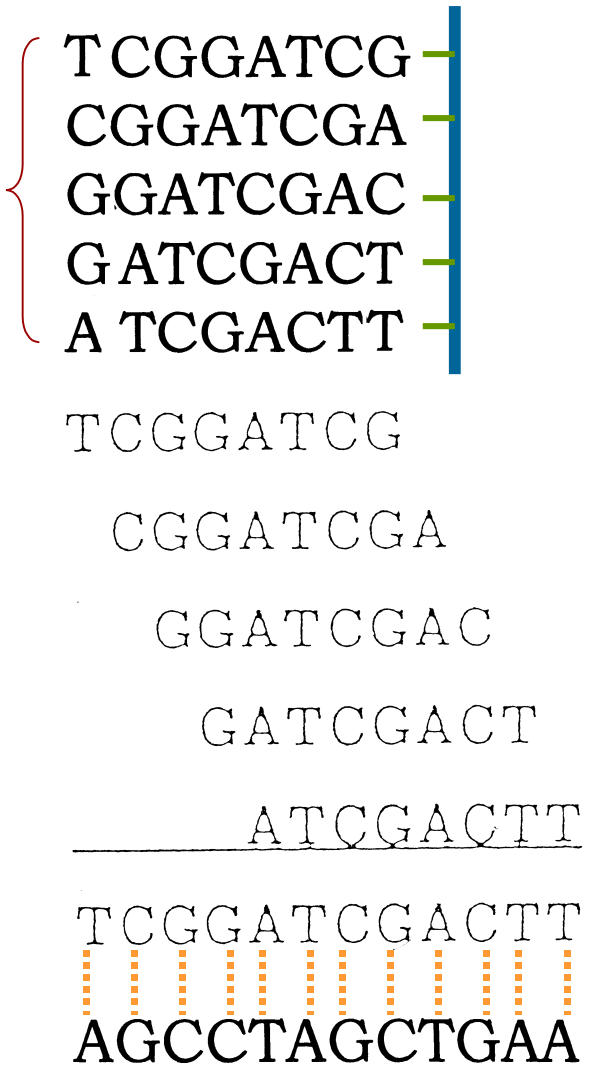
(C) Matrix decoder for the array :



Sequencing by hybridisation (SBH) :

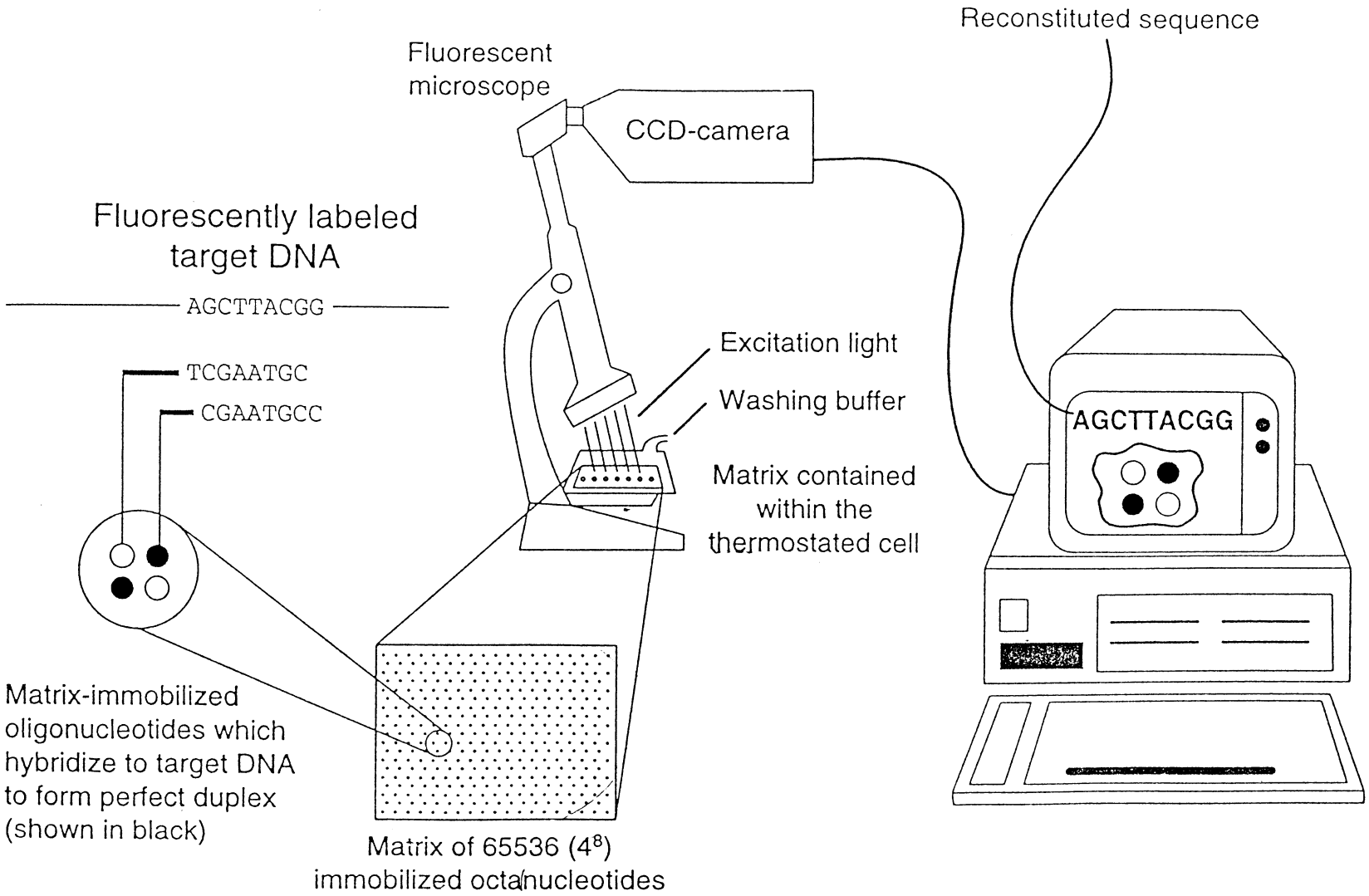
12-mer target DNA sequence : AGCCTAGCTGAA

Oligonucleotide probes :



Thus, DNA sequence is :

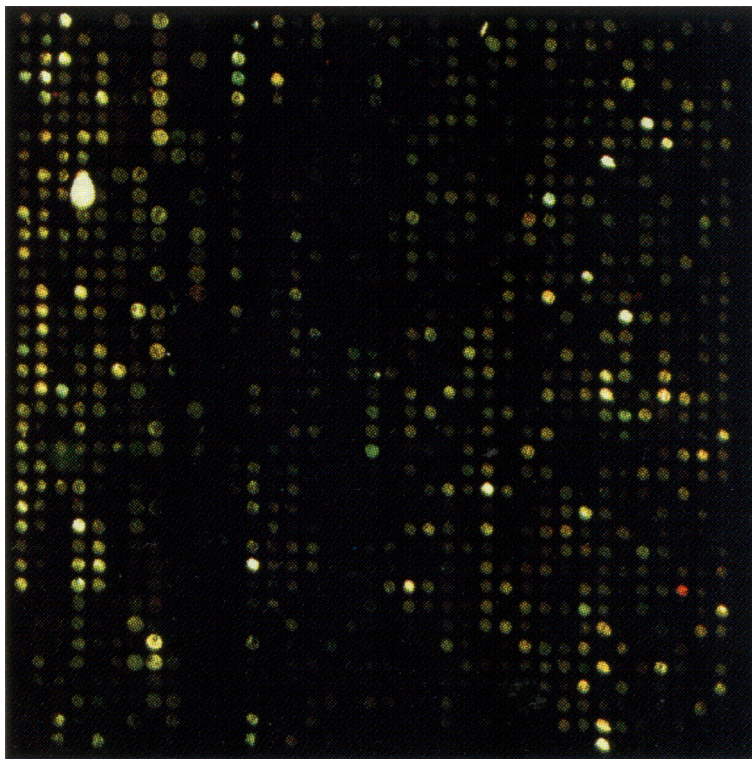
A sequenator for sequencing by hybridisation with an oligonucleotide matix (SHOM) :

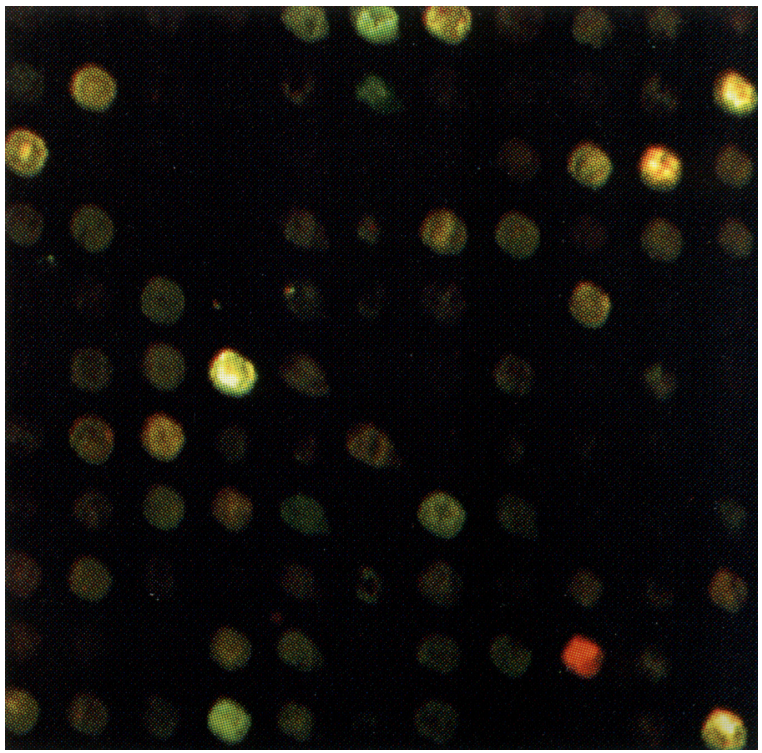


Fluorescent probes incoporated into DNA:

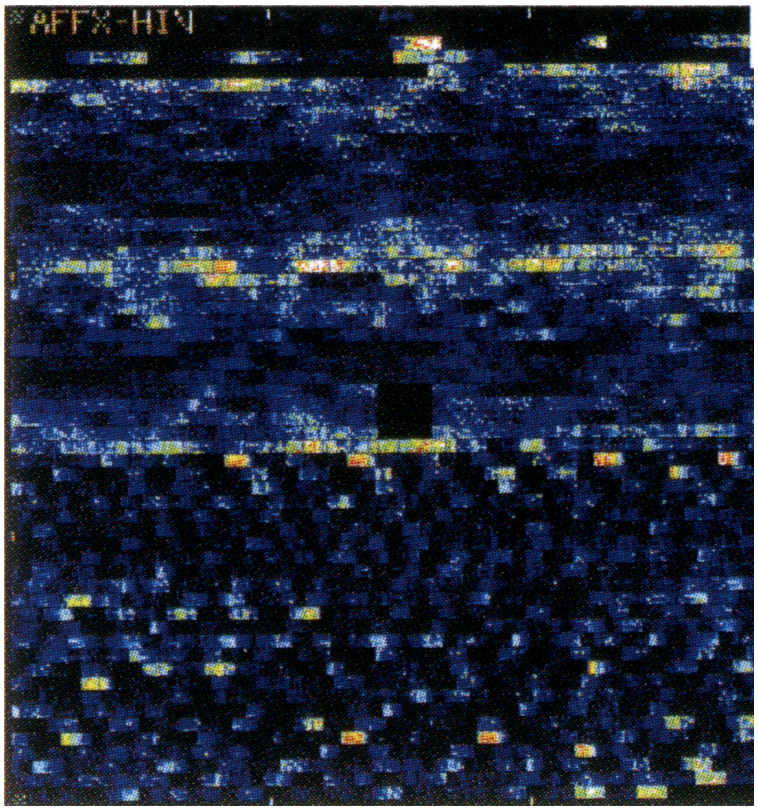
Cye 5 signal: red (633 nm)

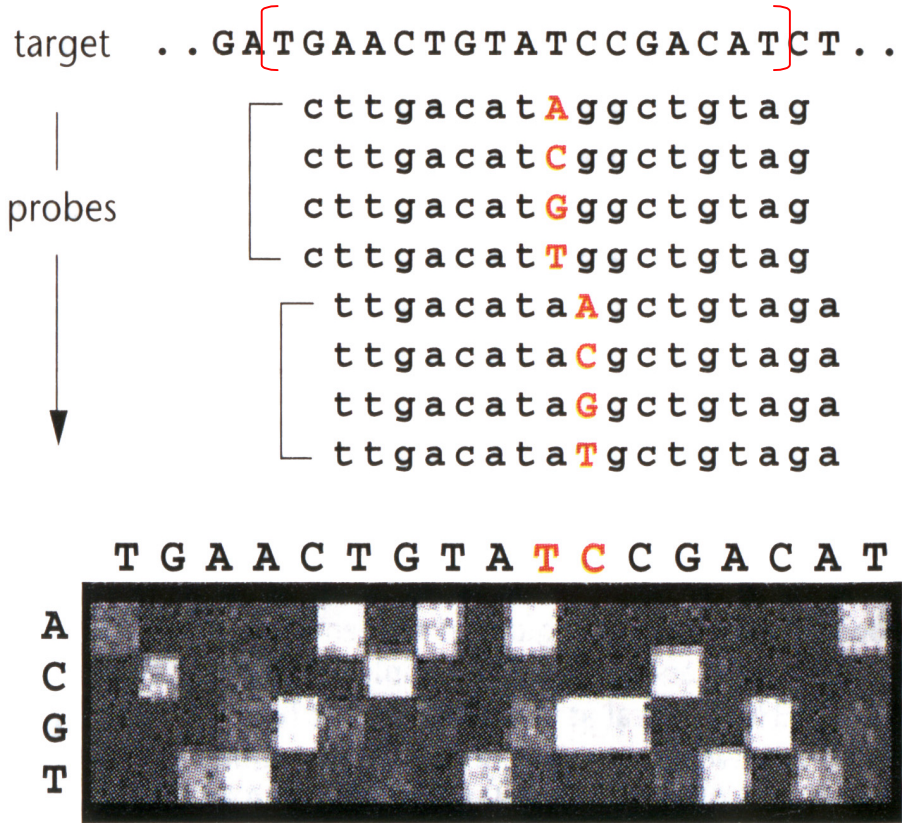
Cye 3 signal: green (532 nm)





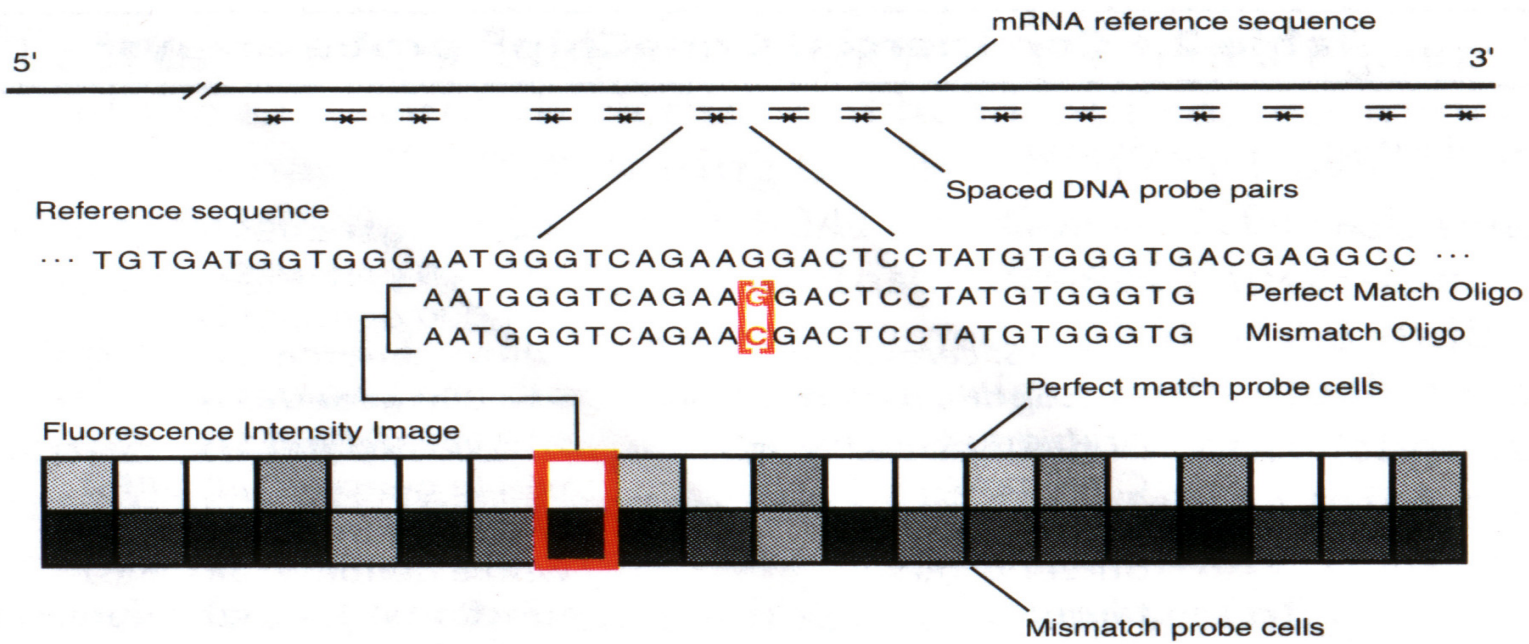
Sequence analysis array :





Gene expression monitoring with oligonucleotide arrays :





Molecular interactions on microarrays (1/3) :

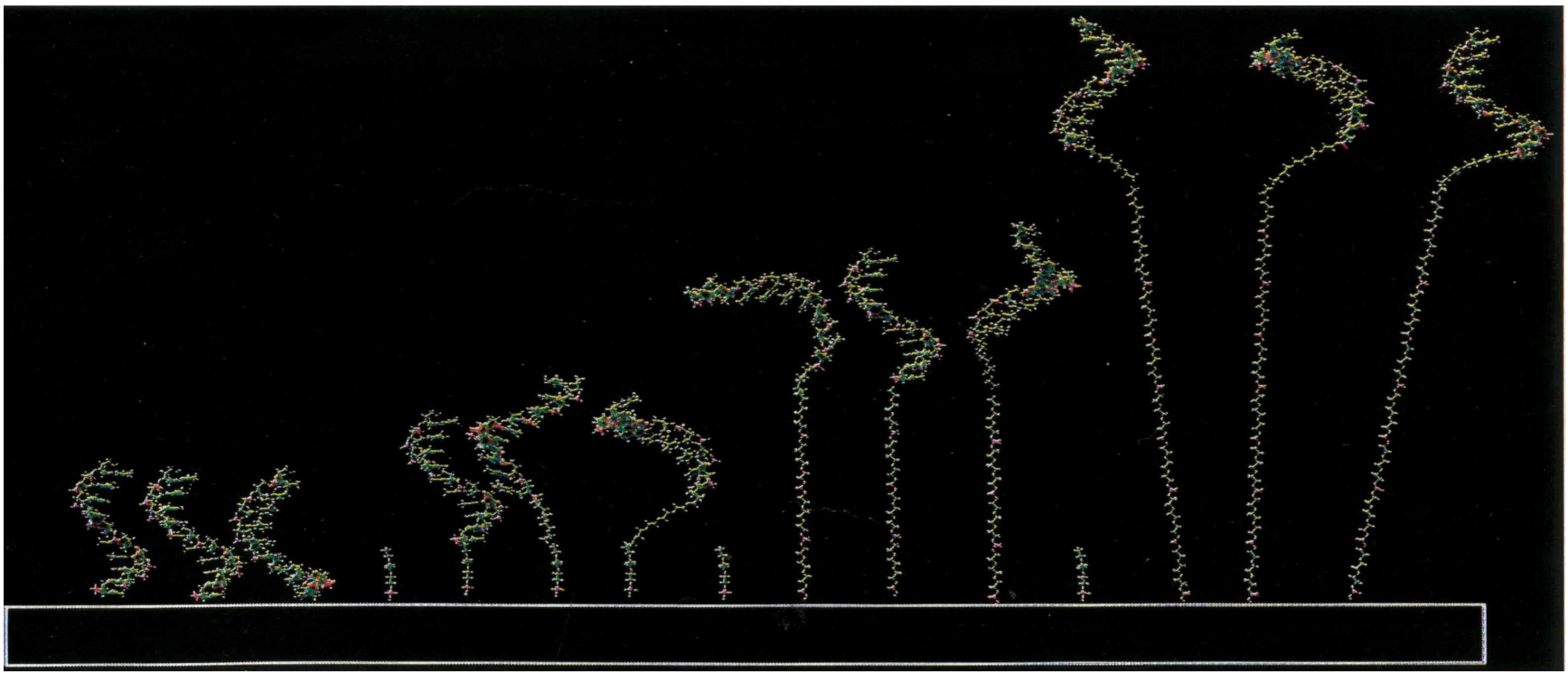
1. The density of oligonucleotides on the surface is :

~ 10 pmol per mm^2 on aminated polypropylene,

~ 0.1 pmol per mm^2 on aminated glass after ammonia deprotection

- equivalent to one molecule per 39 square angstroms

2. Spacers help to overcome steric interference

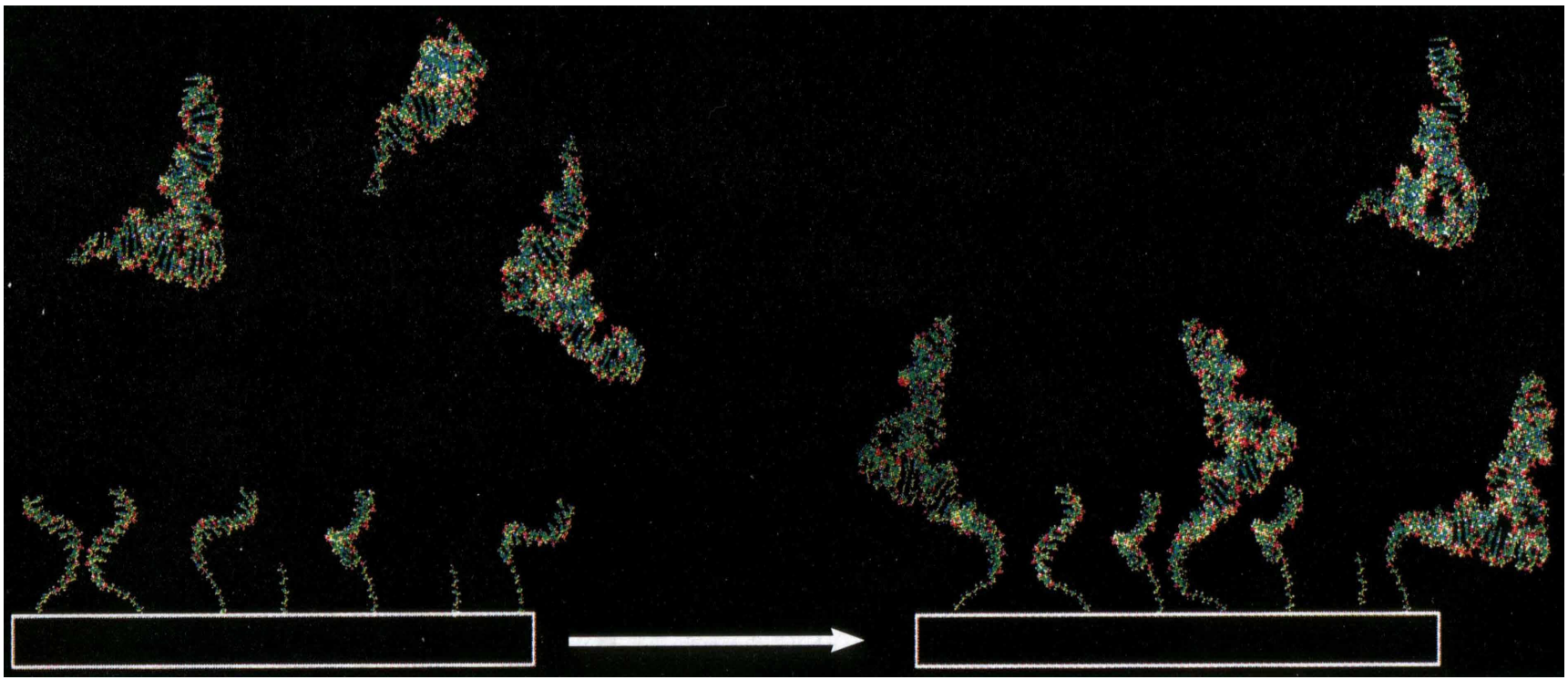


The advantages of glass slides :

1. DNA samples can be covalently attached onto a treated glass surface.
2. glass is a durable material that sustains high temperatures and washes of high ionic strength.
3. it is non-porous so the hybridization volume can be kept to a minimum, thus enhancing the kinetics of annealing probes to targets.
4. as a consequence of its low fluorescence, it does not significantly contribute to background'noise'.

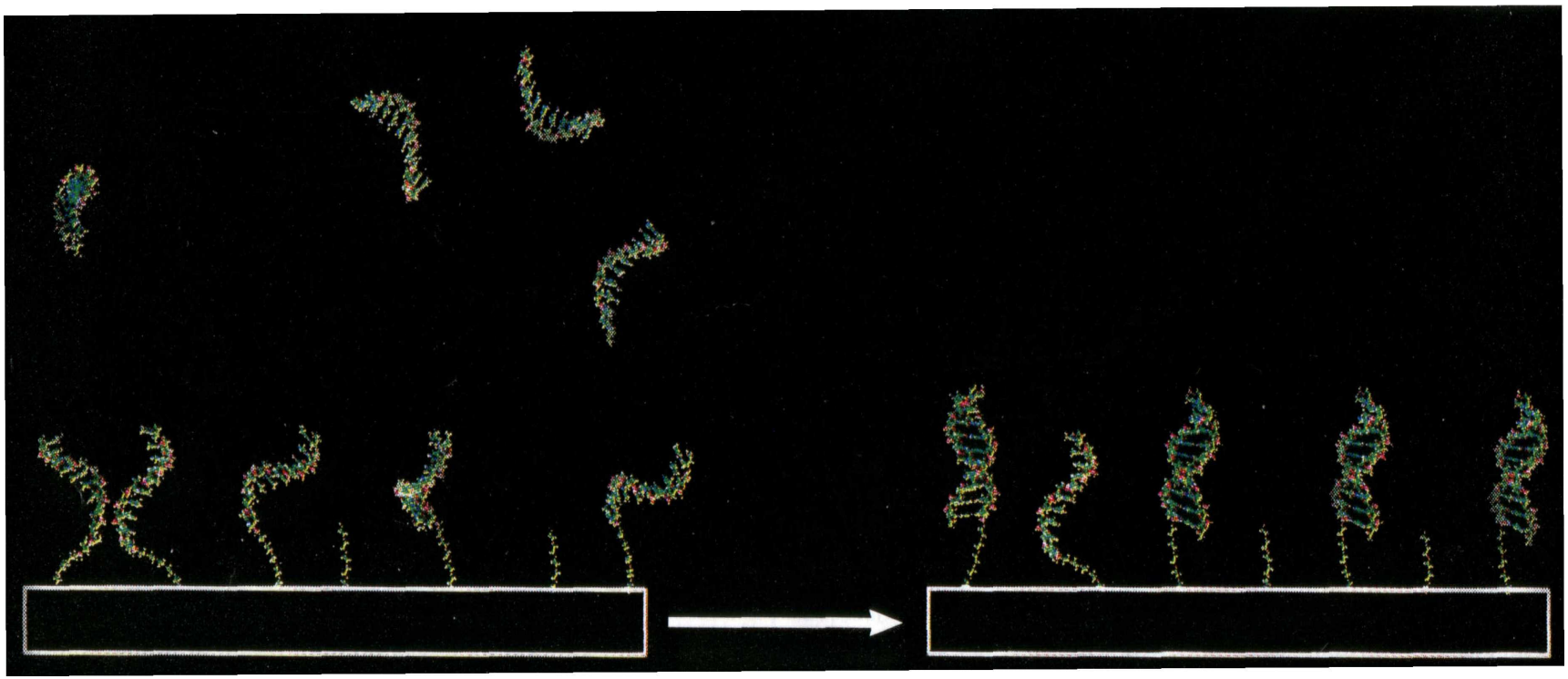
Molecular interactions on microarrays (2/3) :

1. long target are likely to fold in on themselves as a result of intramolecular Watson-Crick base pairing
2. the structure of long target hides parts of the target from the oligonucleotide probes
3. large targets are also likely to be inhibited by their bulk from approachimg the surface



Molecular interactions on microarrays (3/3) :

1. short targets are better able than large targets to interact with tethered oligonucletides
2. they are less likely to have bases hidden from duplex formation by intramolecular base pairing
3. as they are less bulkly, they will more readily penetrate the closely packed lawn of oligonucleotides

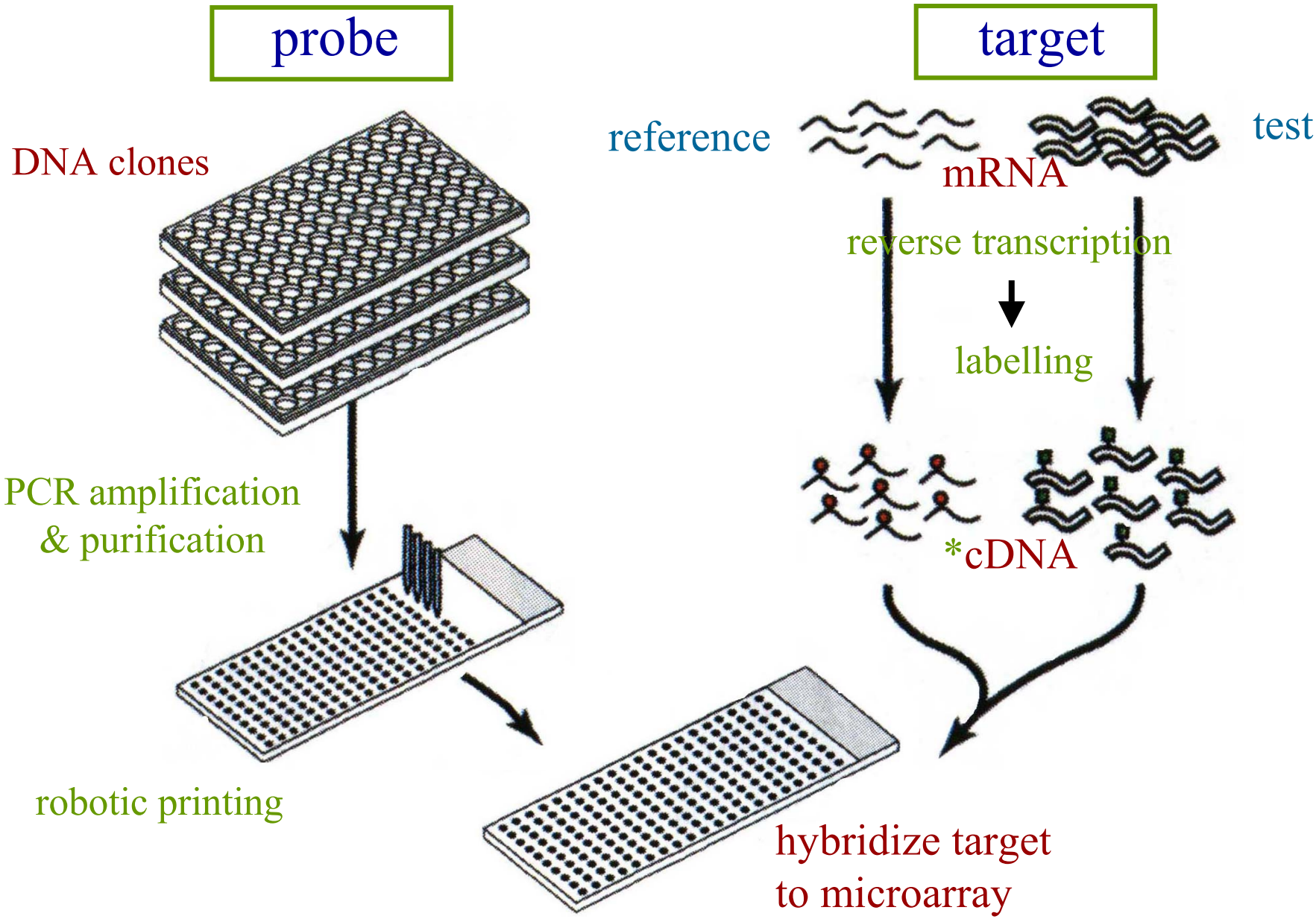


Atomic force microscopy of DNA on a microarray :

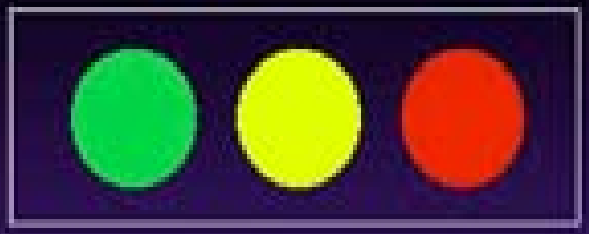


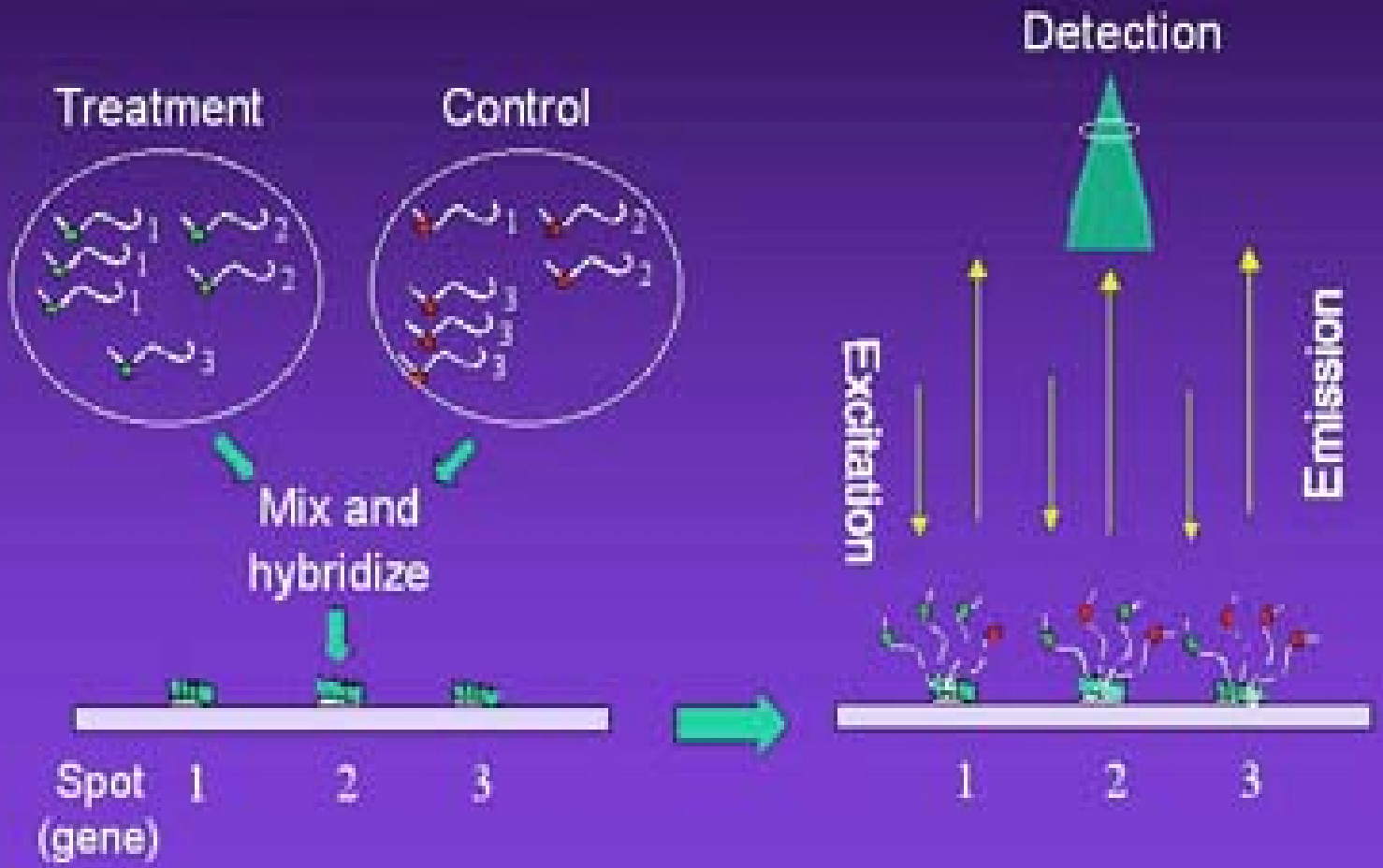
* the yeast DNA is clearly deposited at a sufficient density to allow many kinds of strand-to-strand interactions

cDNA microarray schema :

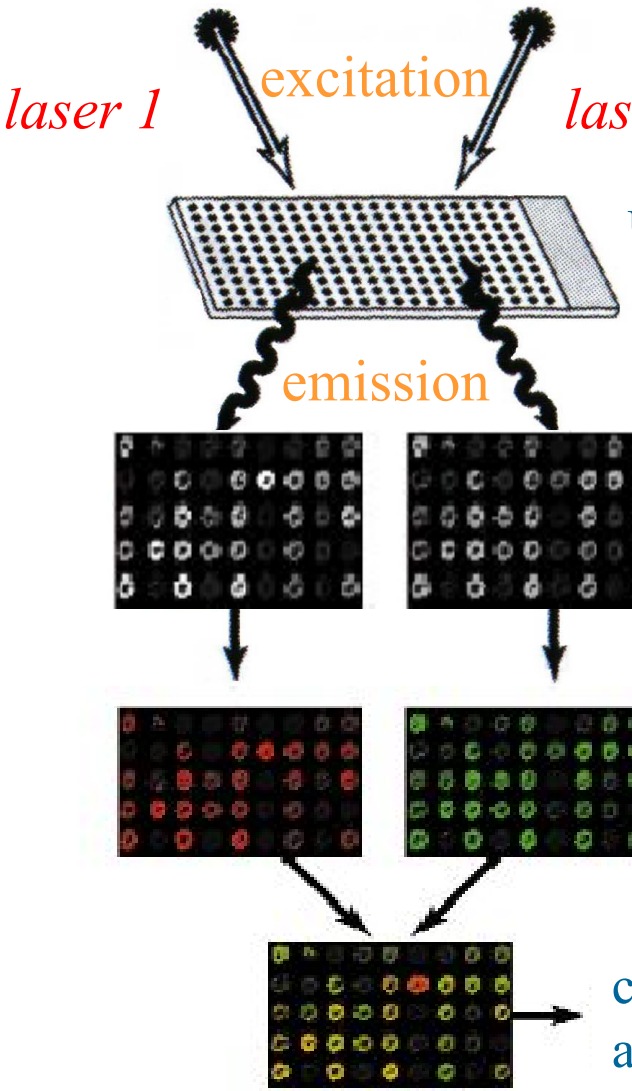


Relative Abundance Detection





cye 5-cDNA cye 5-cDNA



using a scanning confocal laser microscope.

monochrome images from the scanner are imported into software



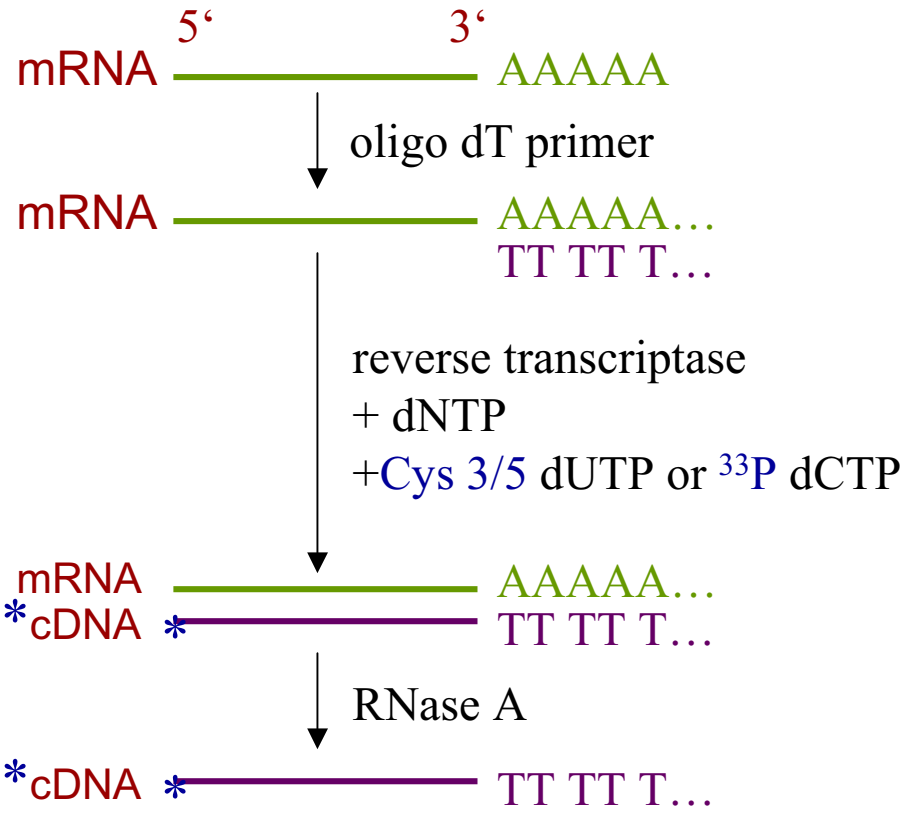
the images are pseudo-coloured and merged

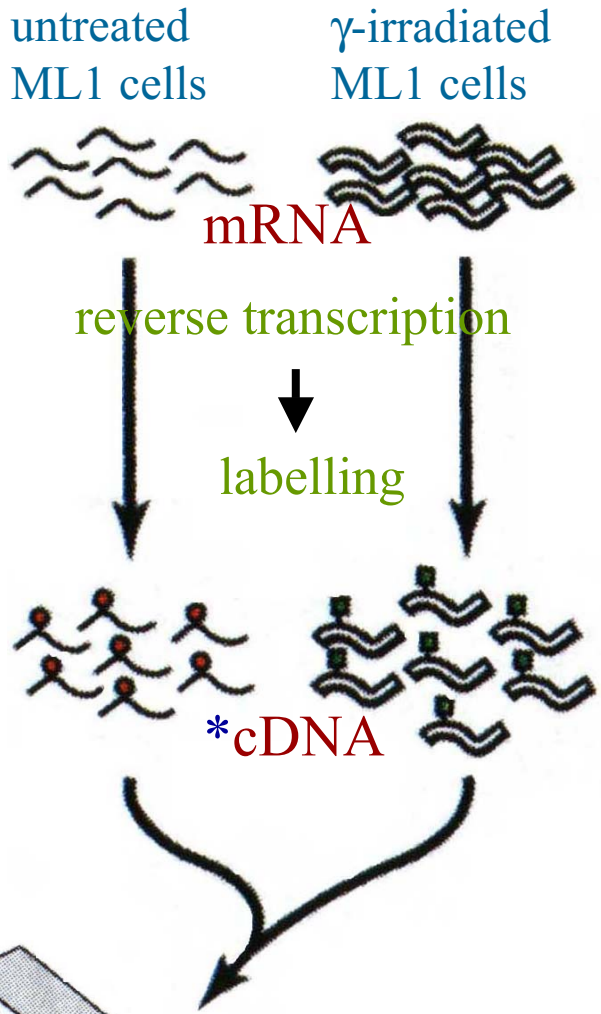


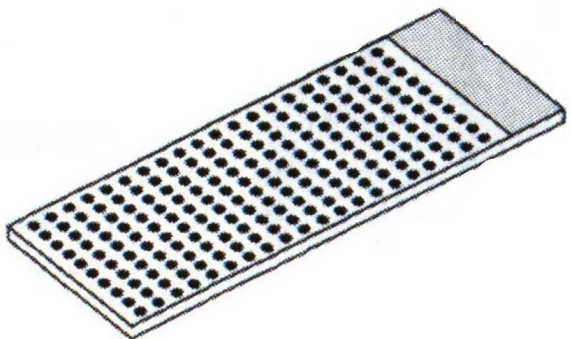
computer analysis

- gene name
- clone identifier
- intensity value
- intensity ratio
- …..

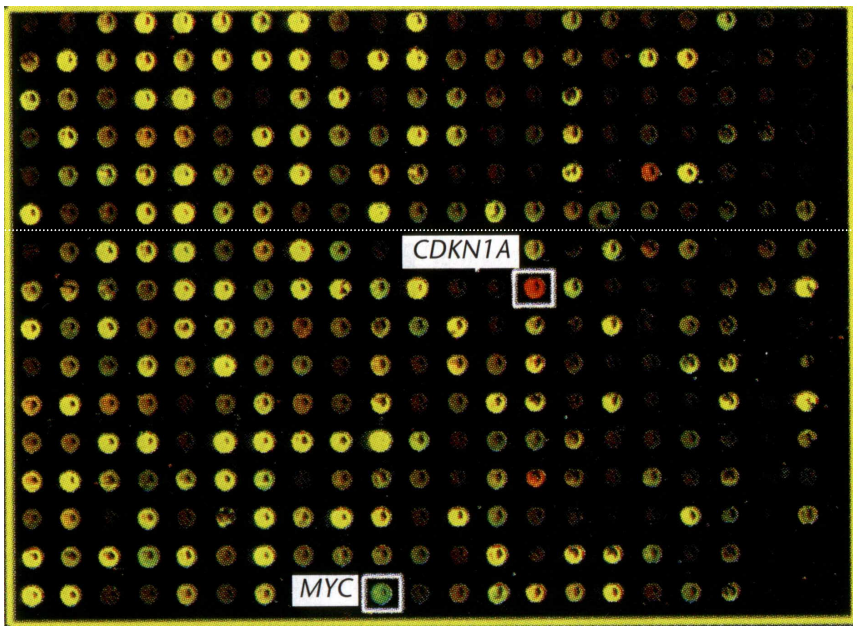
cDNA microarray 例子：

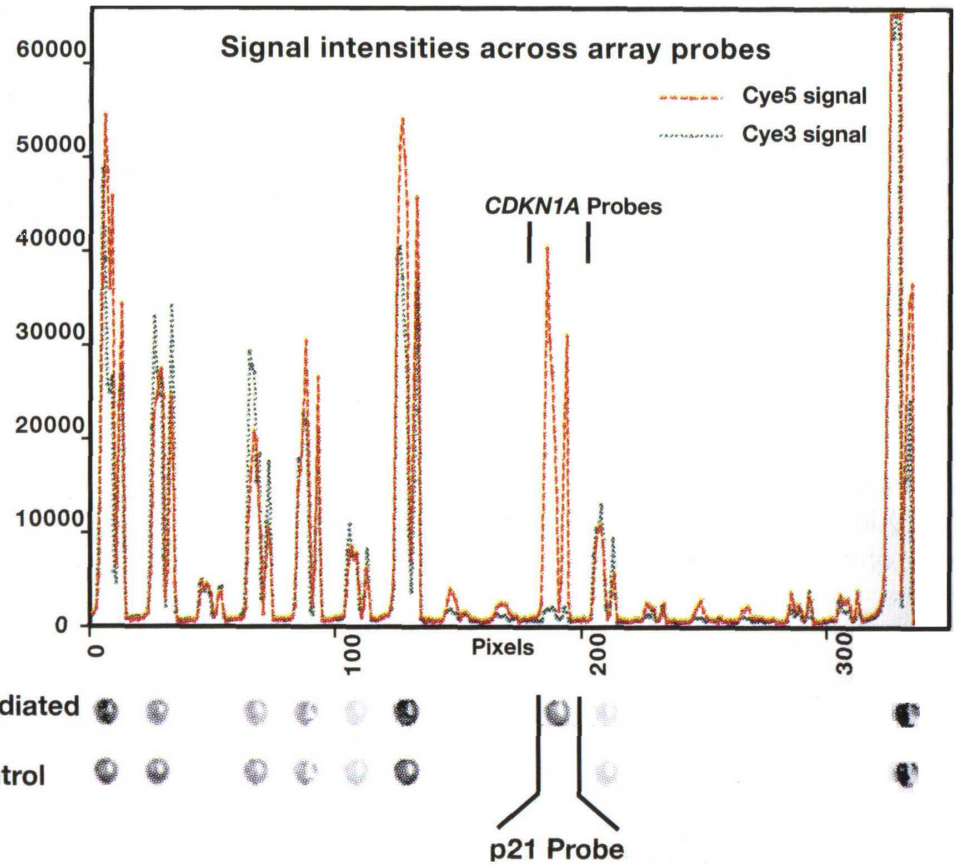




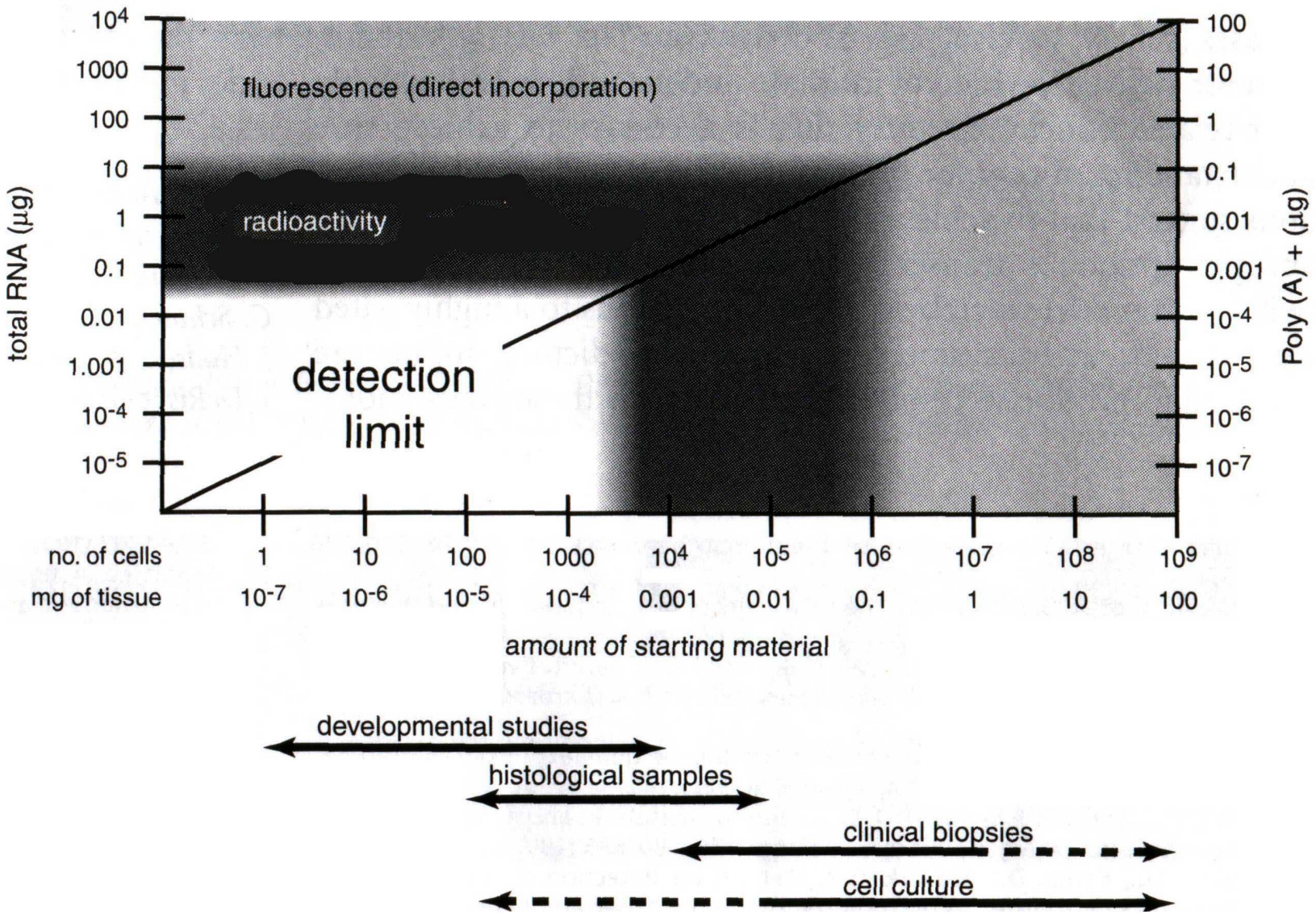


Flurorescent detection for cDNA probes (Cye 5- & Cye 3- cDNA) :

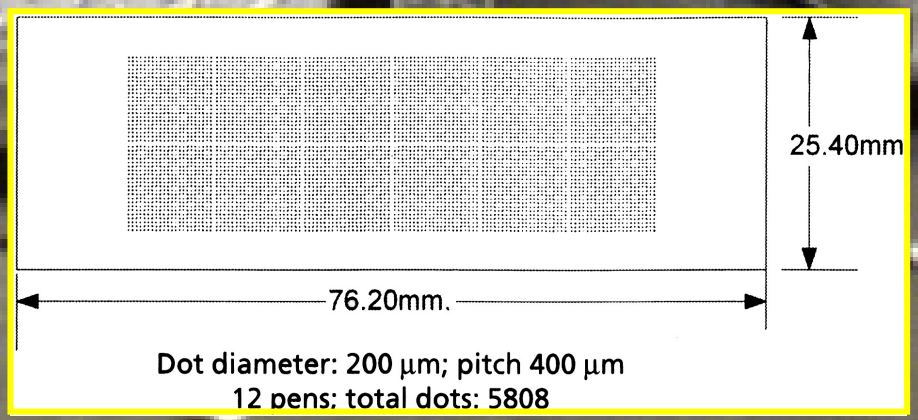


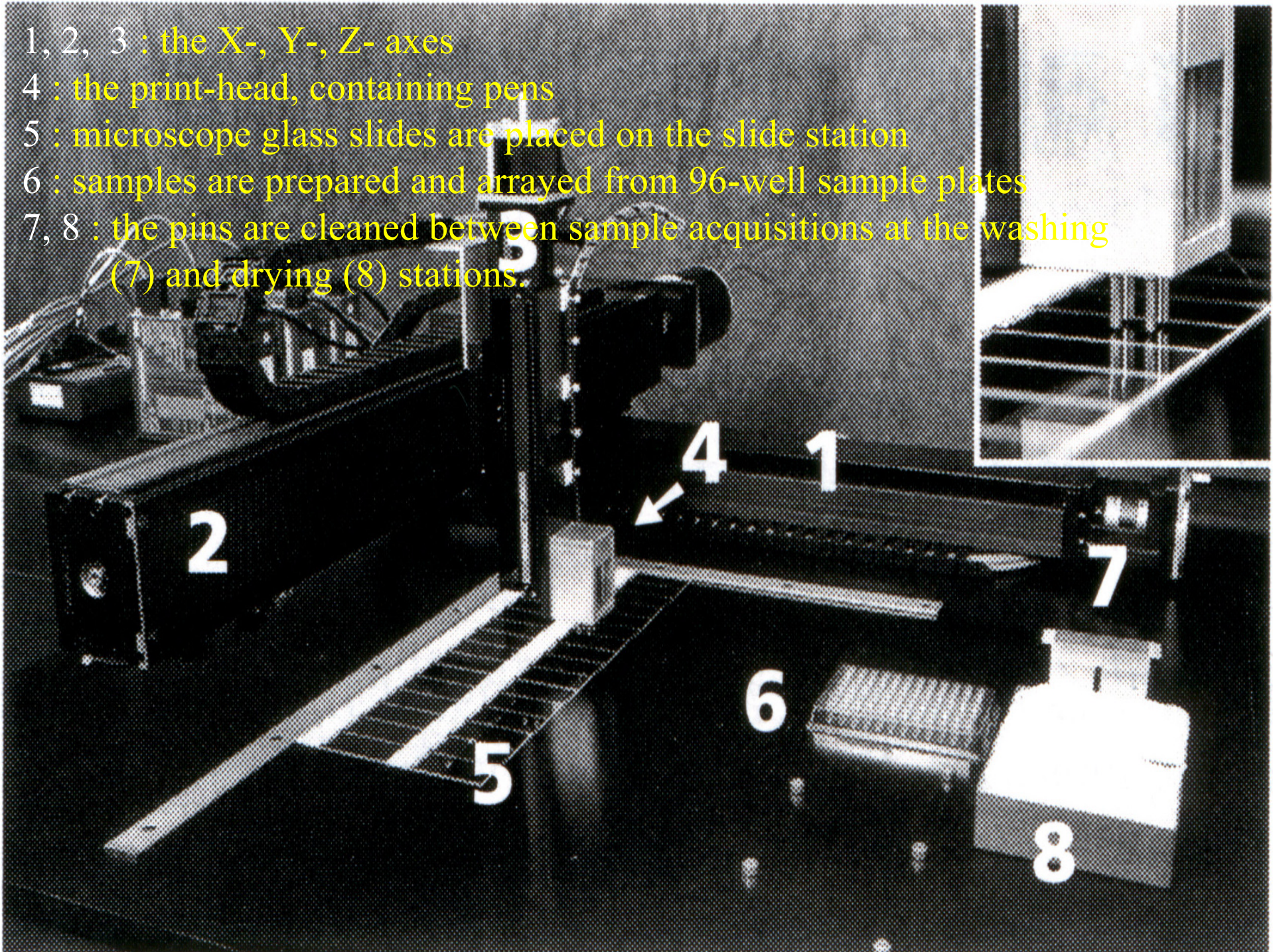


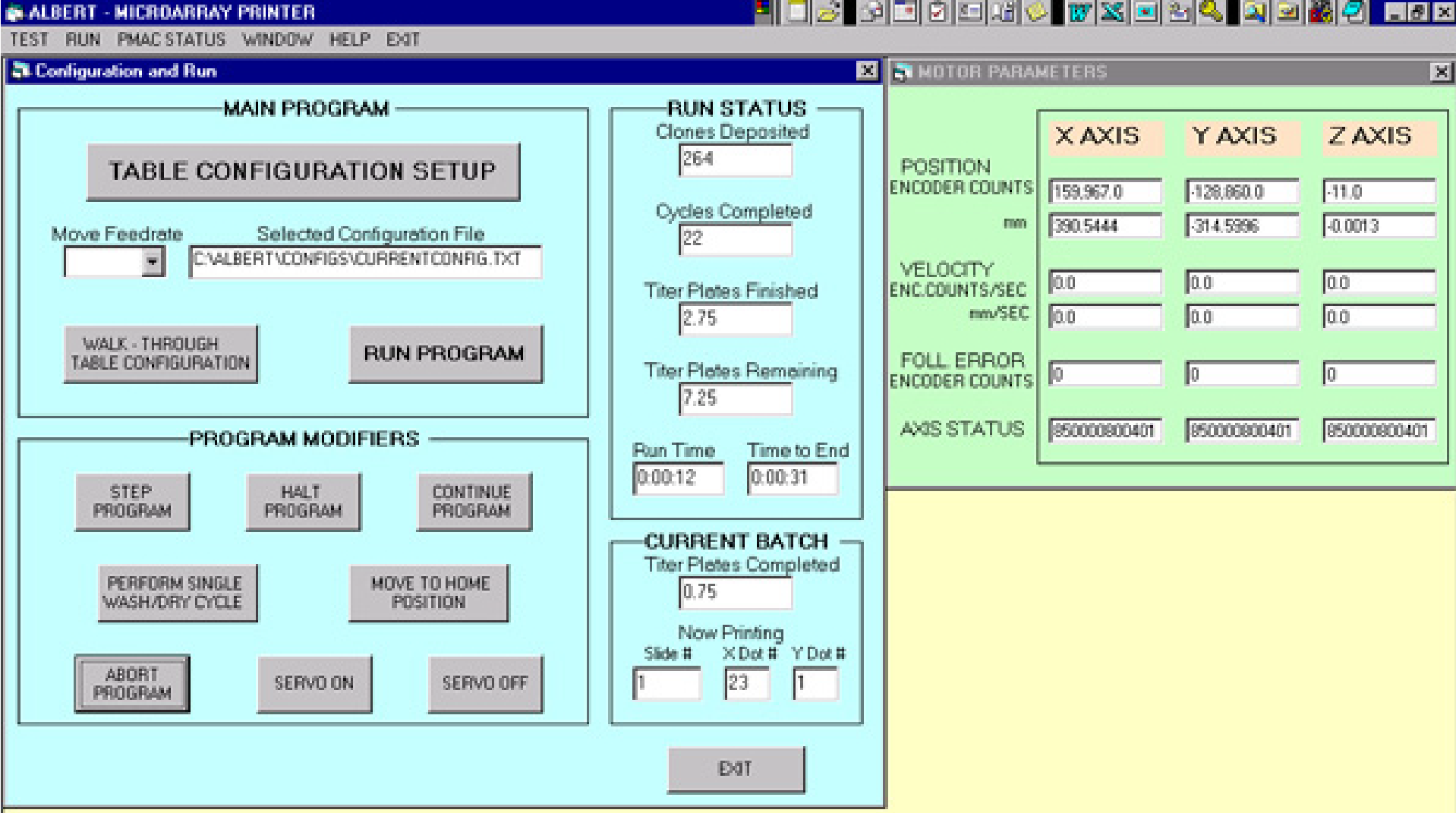
Detection schemes and applications of cDNA microarrays :



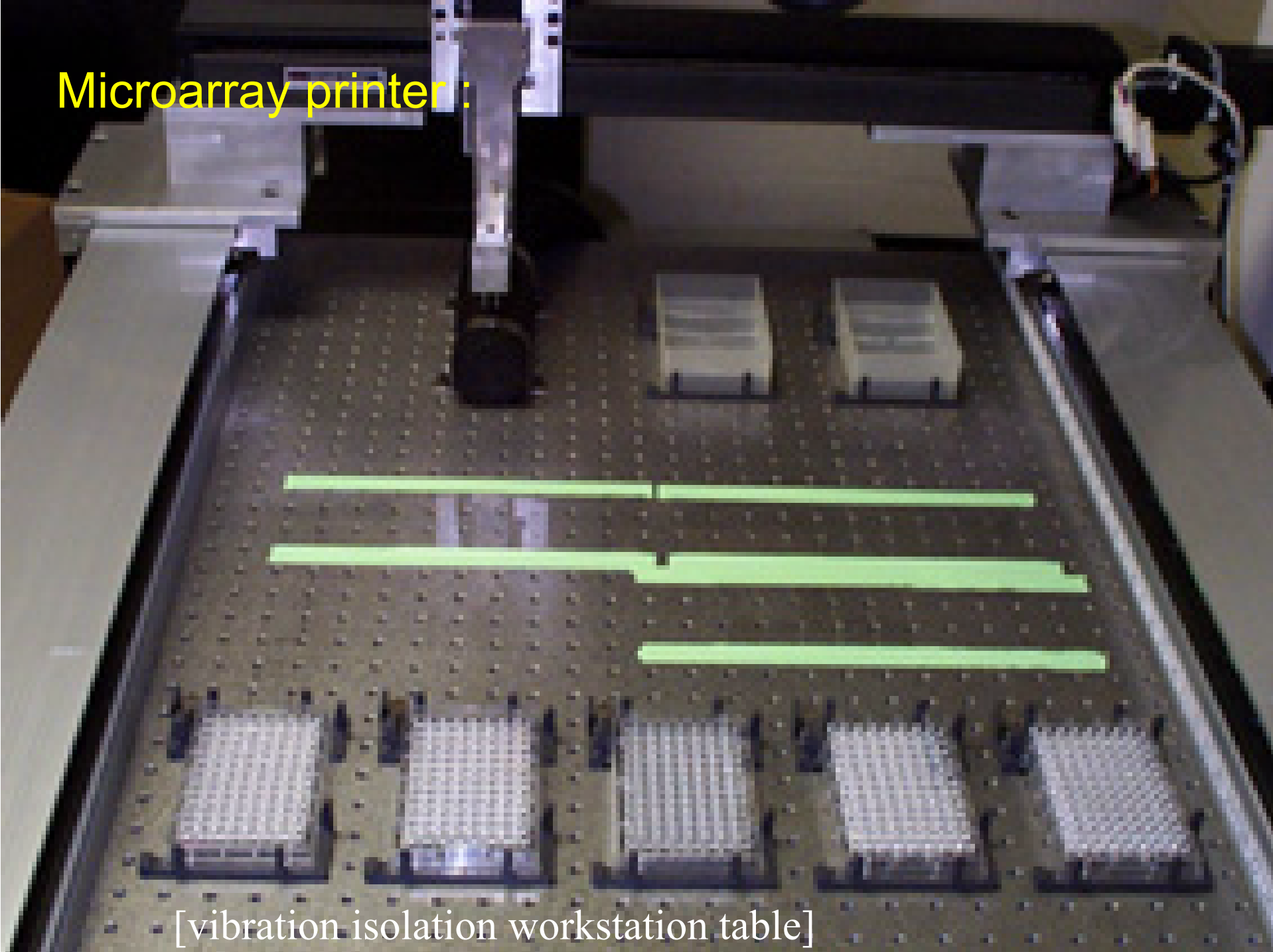
Making and reading DNA microarrays

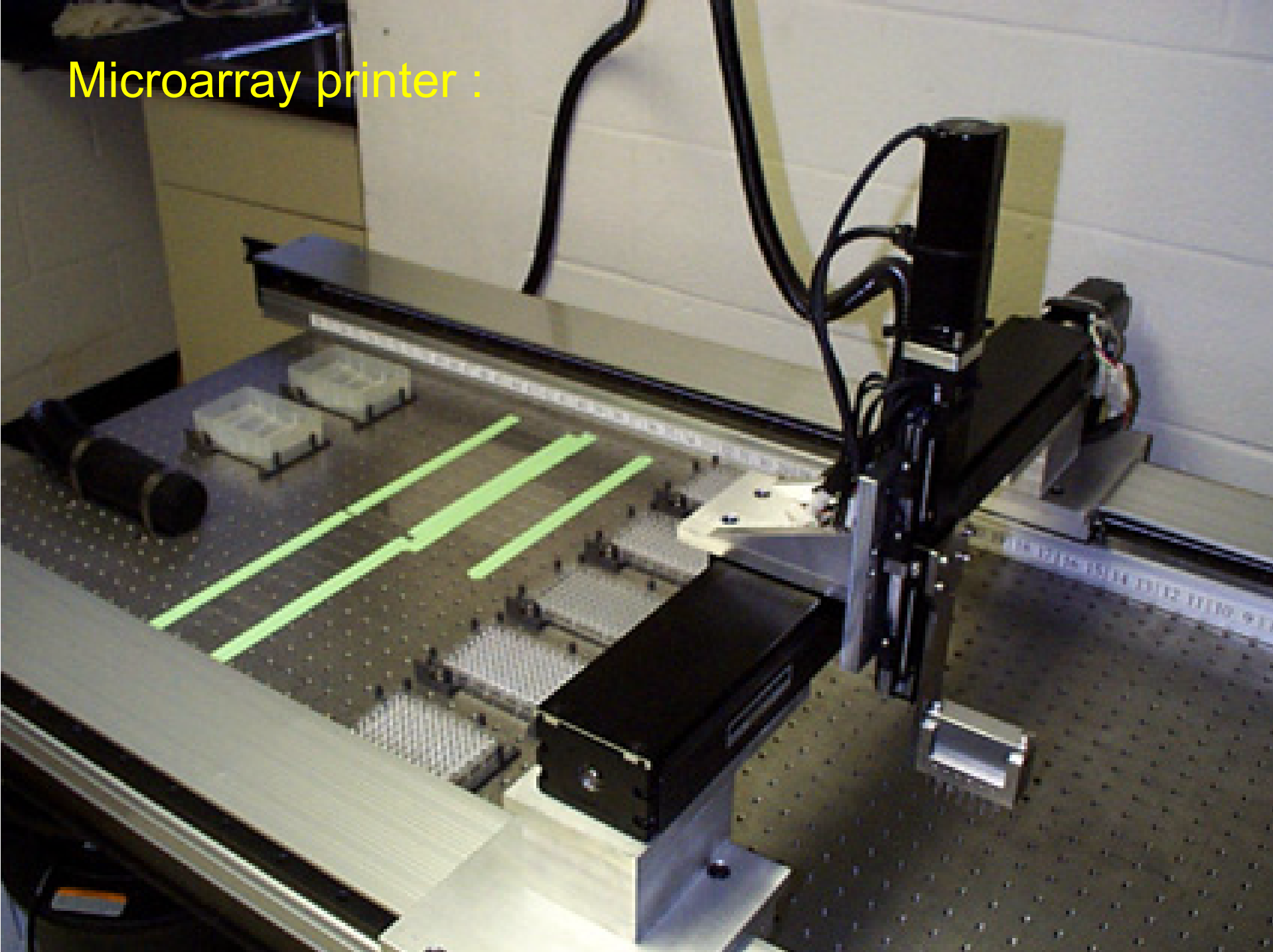


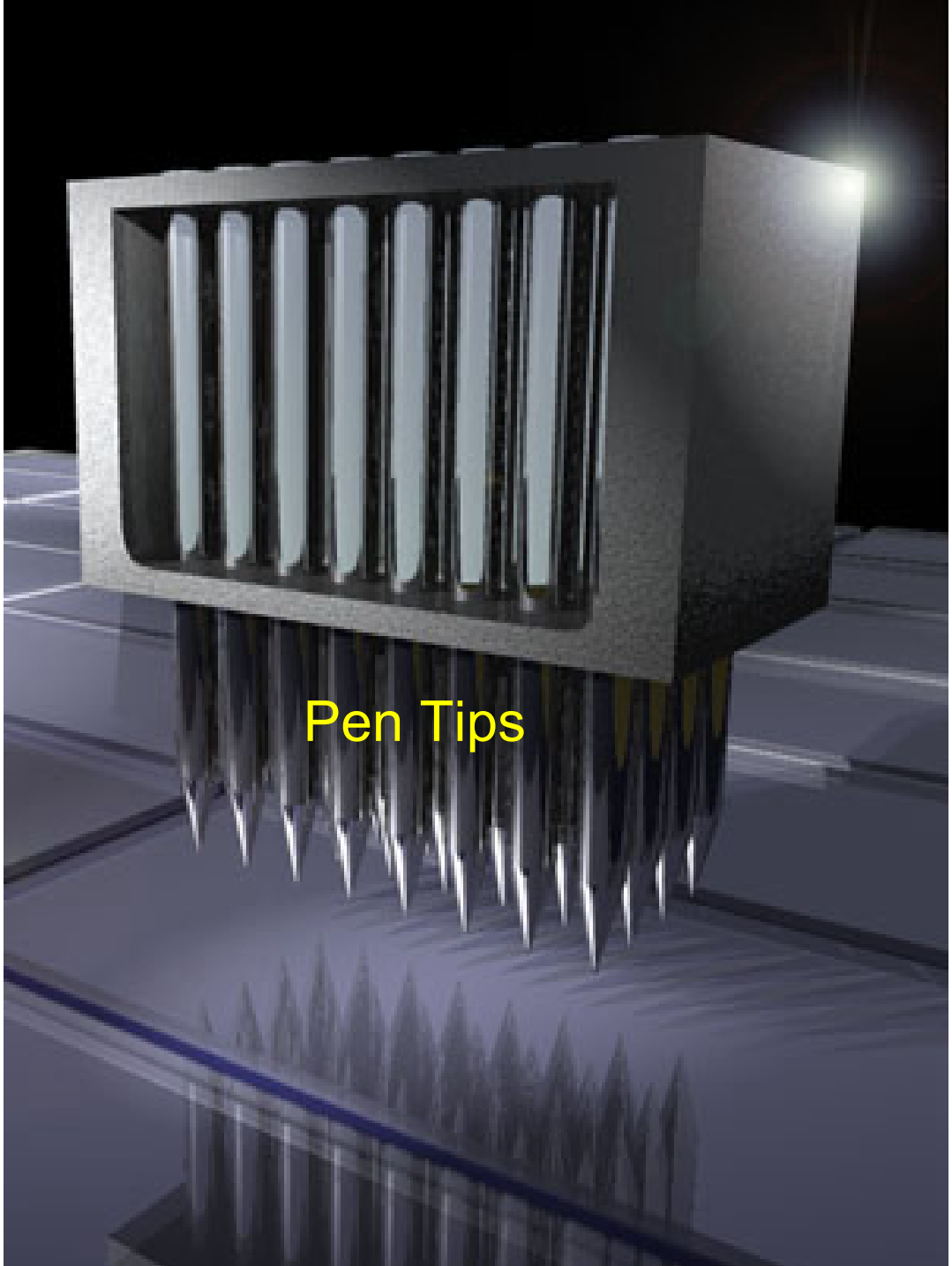

1, 2, 3 : the X-, Y-, Z- axes
4 : the print-head, containing pens
5 : microscope glass slides are placed on the slide station
6 : samples are prepared and arrayed from 96-well sample plates
7, 8 : the pins are cleaned between sample acquisitions at the washing (7) and drying (8) stations.
1
2
3
4
5
6
7
8

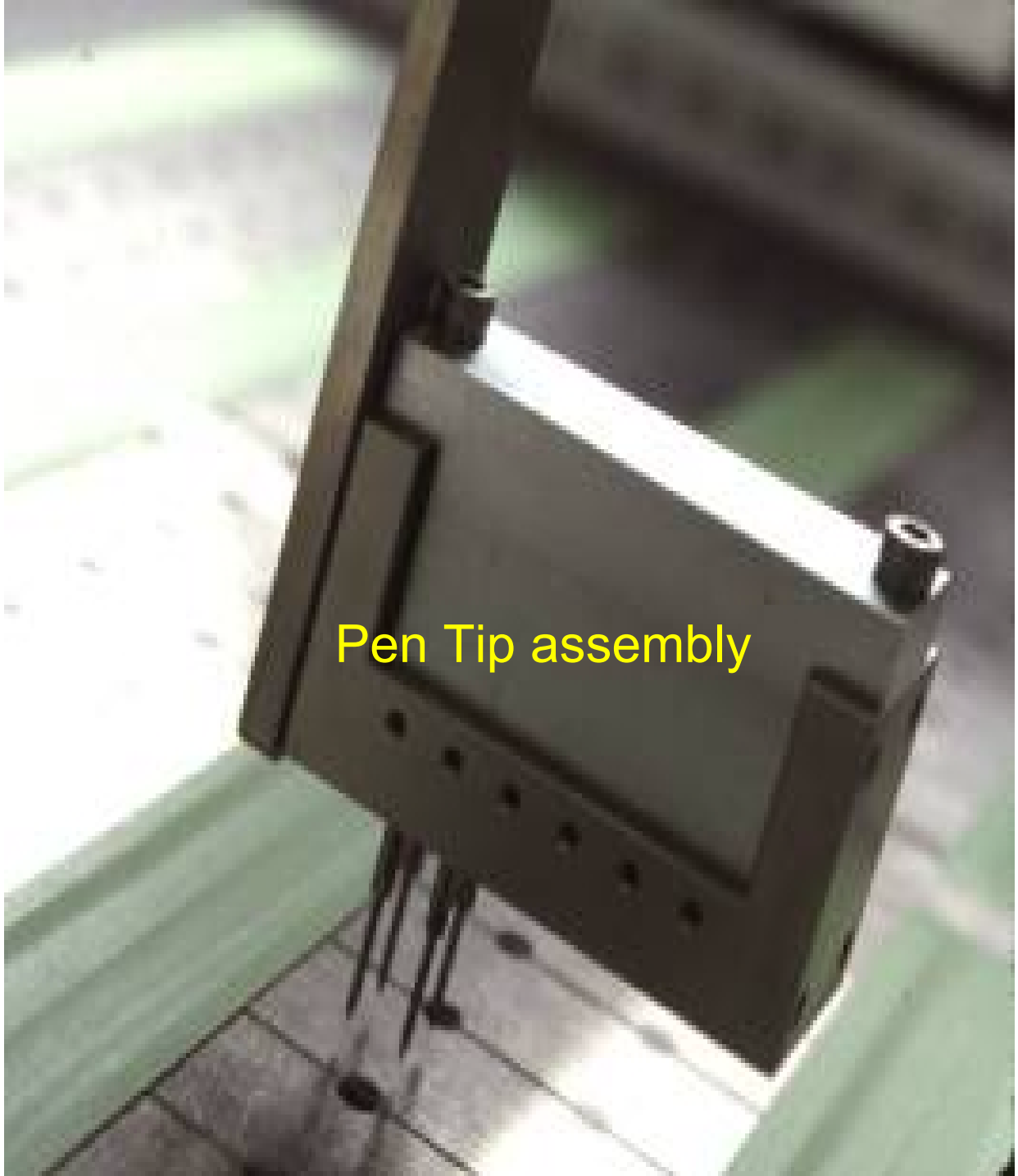


Program used to set up the various card parameters and to tune the servos for the exact combination of hardware components and performance requirements for this system.


Microarray printer :
[vibration isolation workstation table]


Microarray printer :


Pen Tips

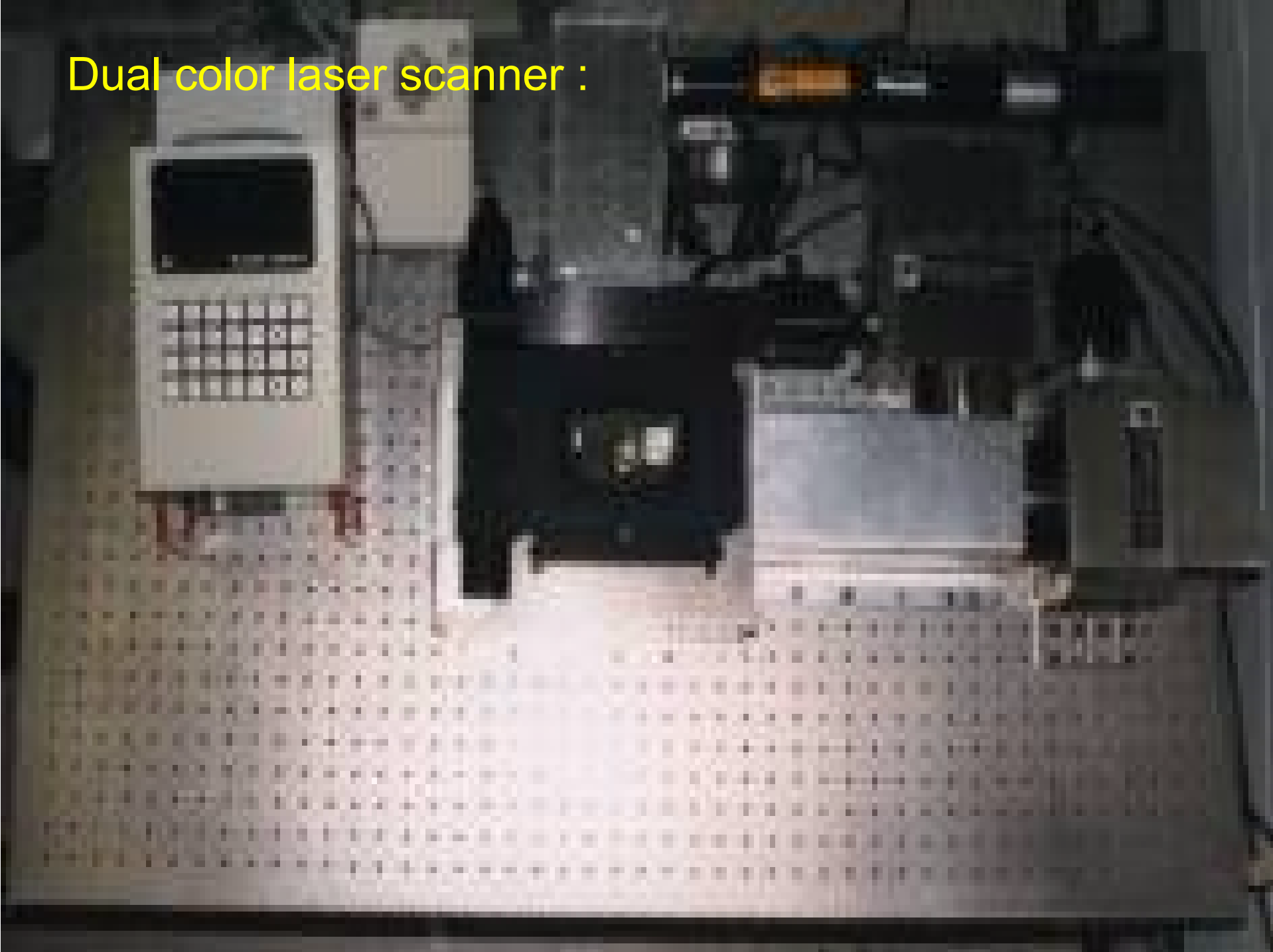

Pen Tip assembly

DNA quantity and spot size :

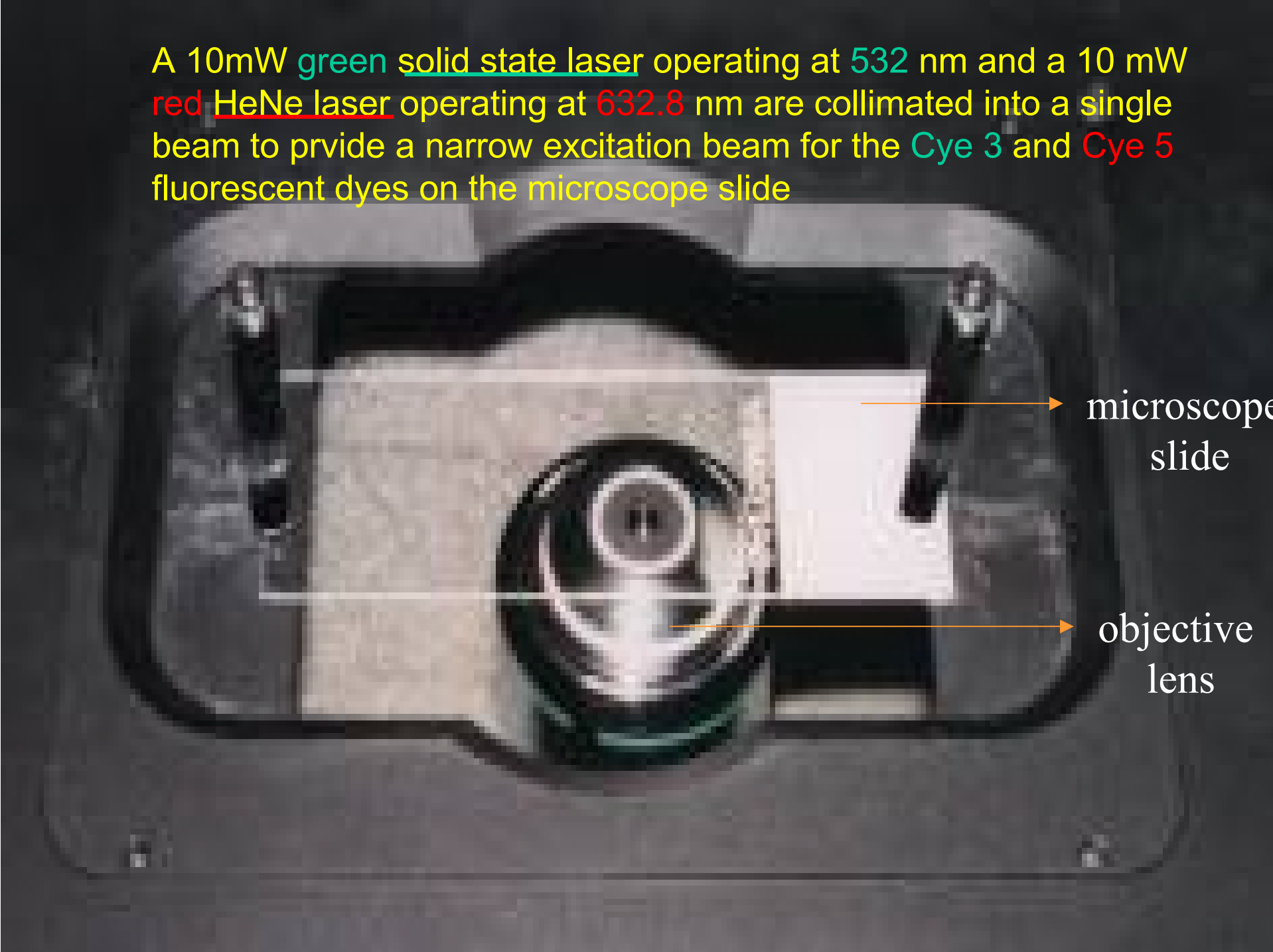
Radius of spot (μm)	Volume of spot (nl)	Amount of DNA in spot (ng)
250	32.71	16.35
200	16.75	8.37
150	7.07	3.53
100	2.09	1.05
50	0.26	0.13

The DNA content of each spot is calculated assuming a sample concentration of 500 ng/μl

Dual color laser scanner :



A 10mW green solid state laser operating at 532 nm and a 10 mW red HeNe laser operating at 632.8 nm are collimated into a single beam to prvide a narrow excitation beam for the Cye 3 and Cye 5 fluorescent dyes on the microscope slide

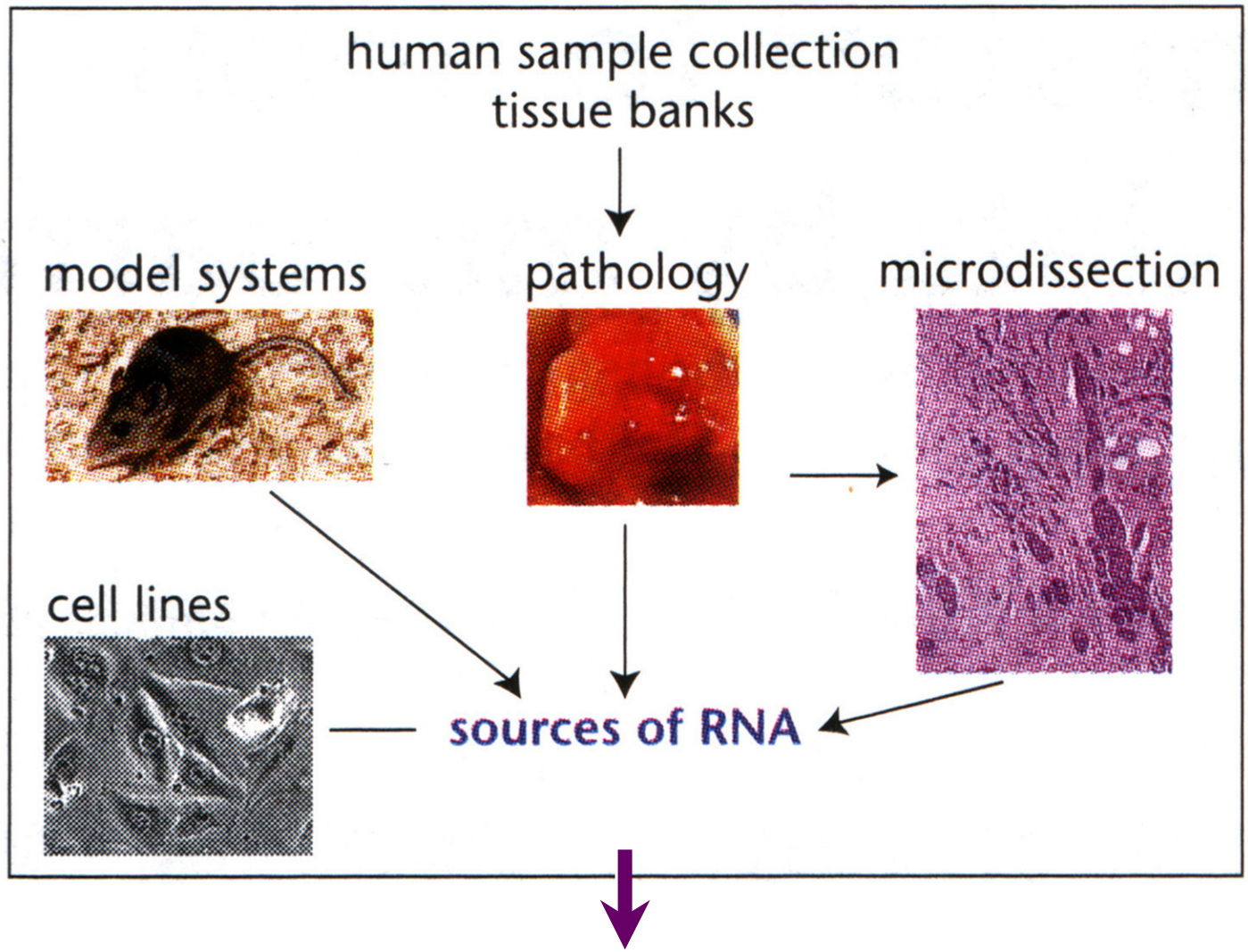


Components for microarray printer and scanner :

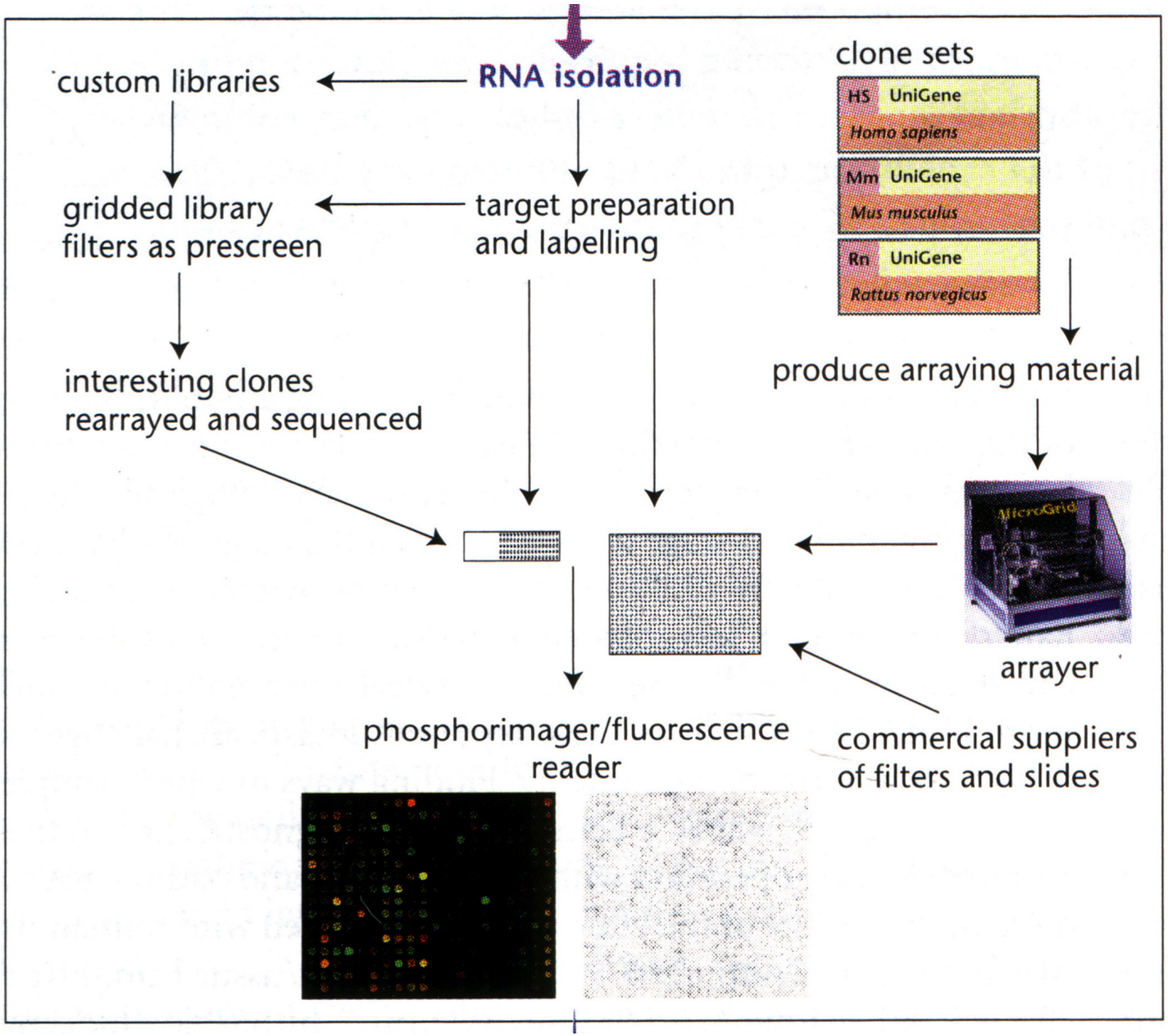
- Green (532 nm) and Red (633 nm) lasers for simultaneous two colour dye excitation
- Ludl motorized X,Y,Z microscope stage and electronics with sub-micron resolution
- Dual PMTs and housings (with cooling capability) for two colour detection and preamplifiers
- Nikon 20×infinity focus objective, filter cube and Z axis slide
- Dual beam splitter filter, laser clean-up filters, beam combiner, and bandpass filters
- A PC based A/D converter and anti alias filters
- Digital trigger circuit for conversion of stage encoder signals to A/D converter triggers
- 300 MHz Pentium PC running software developed in HPVEE graphical language
- Laser table, various fabricated metal parts, optical mounts and mirrors

– from start to finish –
for obtaining expression data by microarray :

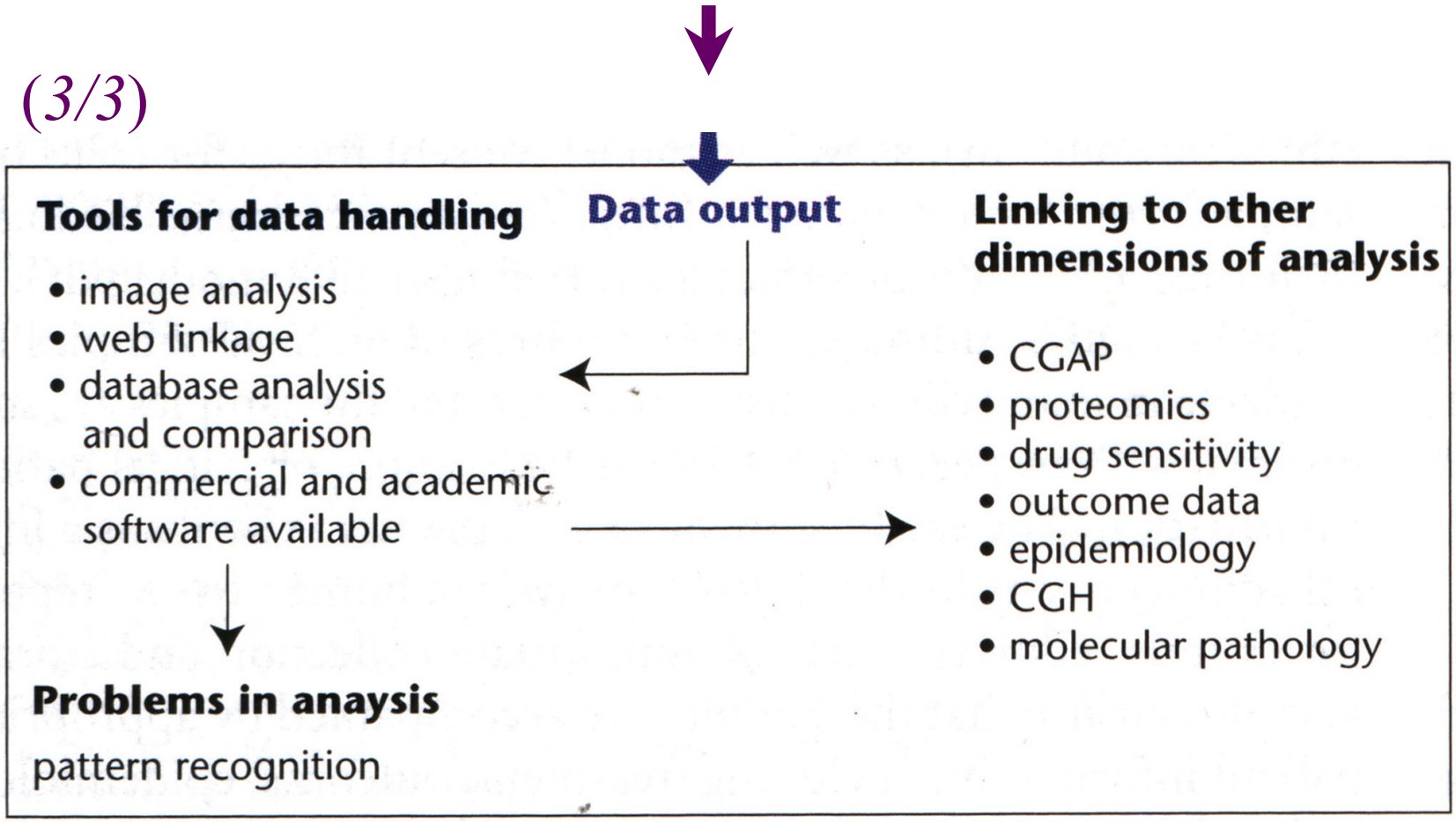
(*1/3*)

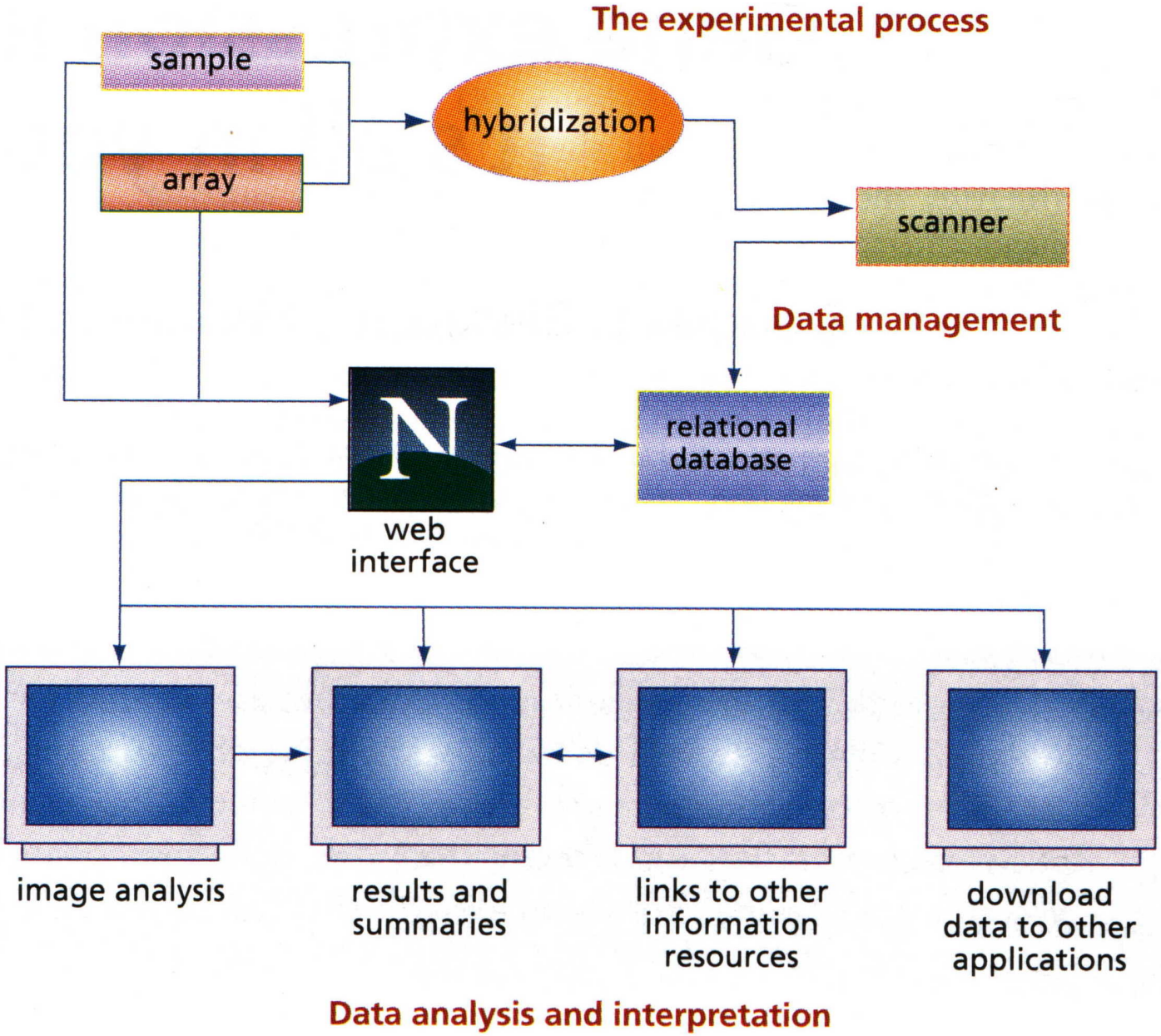


(2/3)


RNA isolation
custom libraries
gridded library
filters as prescreen
target preparation
and labelling
interesting clones
rearrayed and sequenced
clone sets
HS UniGene
Homo sapiens
Mm UniGene
Mus musculus
Rn UniGene
Rattus norvegicus
produce arraying material
MicroGrid
arrayer
phosphorimager/fluorescence
reader
commercial suppliers
of filters and slides

(3/3)


Tools for data handling
• image analysis
• web linkage
• database analysis and comparison
• commercial and academic software available
Problems in anaysis
pattern recognition
Data output
Linking to other dimensions of analysis
• CGAP
• proteomics
• drug sensitivity
• outcome data
• epidemiology
• CGH
• molecular pathology


The experimental process
sample
array
hybridization
scanner
Data management
relational database
web interface
image analysis
results and summaries
links to other information resources
download data to other applications
Data analysis and interpretation

Resources :

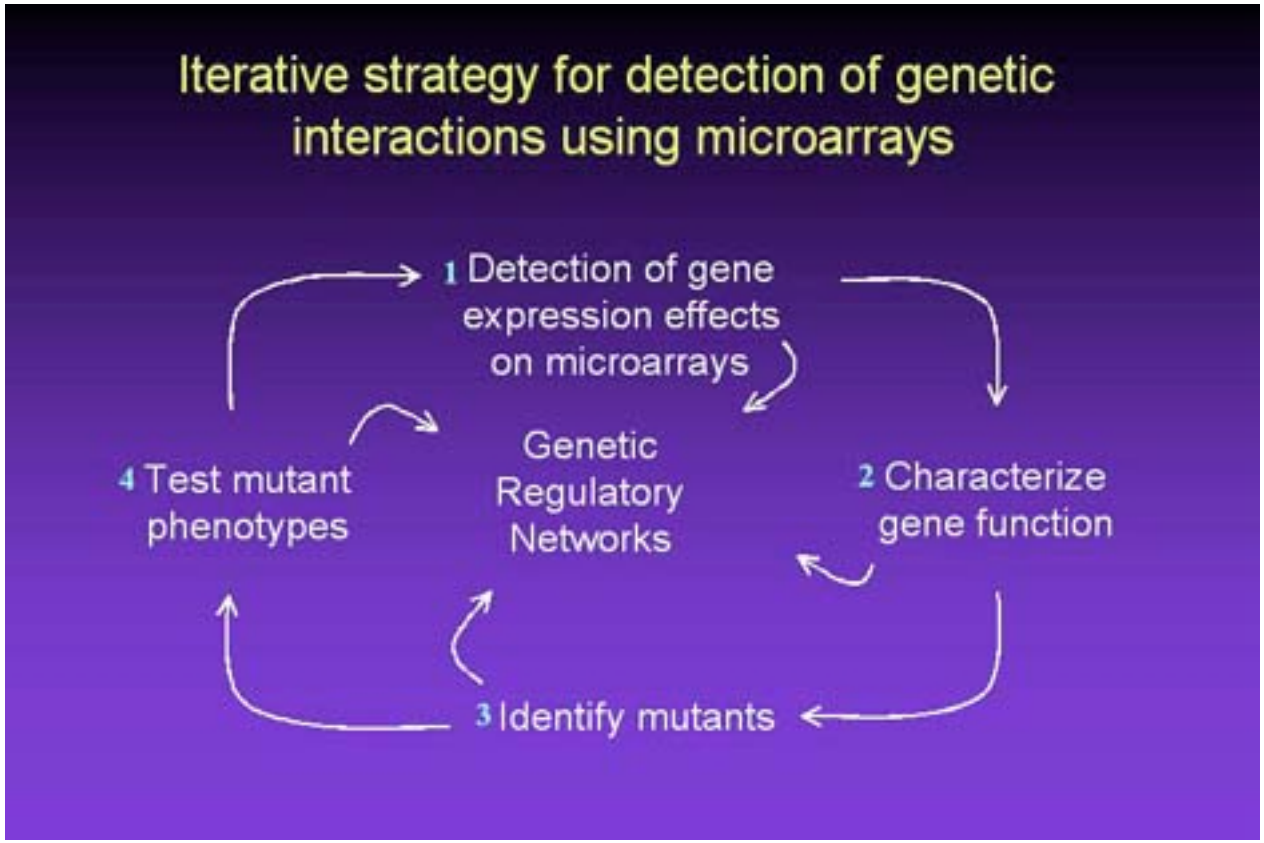
Image Analysis		
BioDiscovery	http://www.biodiscovery.com/software.html	BioDiscovery's ImaGene Image Analysis Software
ScanAlyze	http://bronzino.stanford.edu/ScanAlyze	Brown Lab's Image Analysis software
Microarray Data Warehousing & Analysis		
Affymetrix	http://www.affymetrix.com/products/lims/lims.html	GeneChip LIMS data warehouse
Brown Lab, Stanford University	http://cmgm.stanford.edu/pbrown/explore/	Searchable database of published yeast microarray data
MicroArray Project, NIH	http://www.nhgri.nih.gov/DIR/LCG/15K/HTML/dbase.html	Database schema and software tools for analysis of high-throughput gene expression data
Rosetta Inpharmatics	http://www.rosetta.org/	Resolver data warehouse & analysis software
Silicon Genetics	http://www.sigenetics.com/GeneSpring/Overview.htm	GeneSpring data warehouse & analysis software

Table 3 • Hybridization solutions

Solution A (used at 42 °C)	50% formamide 6×SSC 0.5% SDS 5×Denhardt's reagent (0.5 g Ficoll, 0.5 g polyvinylpyrrolidone, 0.5 g bovine serum albumin)
Solution B (used at 65 °C)	6×SSC 0.5% SDS 5×Denhardt's reagent
Solution C (used at 65 °C)	10% SDS 7% PEG-8000

Table 4 • Protocol for hybridization

- moisten array prior to UV-cross linking
- cross-link at 0.25 J/cm^2 to 0.35 J/cm^2
- moisten array again and heat snap for 3 s
- wash in 0.1% SDS for 30 s
- rinse in water for 1 min
- denature by placing array in 95 °C water for 3 min
- immediately transfer to ice-cold ethanol
- air dry
- prepare targets and reconstitute in 15 µl hybridization solution.
- prehybridize array with hybridization solution containing blocking agent for 30 min
- incubate probes with the array for 8–24 h
- wash the array in 0.1% SDS, 0.2×SSC for 5 min at RT, then in 0.2×SSC for 5 mins at RT
- dry by centrifugation at 2000 rpm for 2 min


Iterative strategy for detection of genetic interactions using microarrays
1 Detection of gene expression effects on microarrays
2 Characterize gene function
3 Identify mutants
4 Test mutant phenotypes
Genetic Regulatory Networks

Genotyping arrays :

