FEL Irradiation Use for the Biochip Production Standardization

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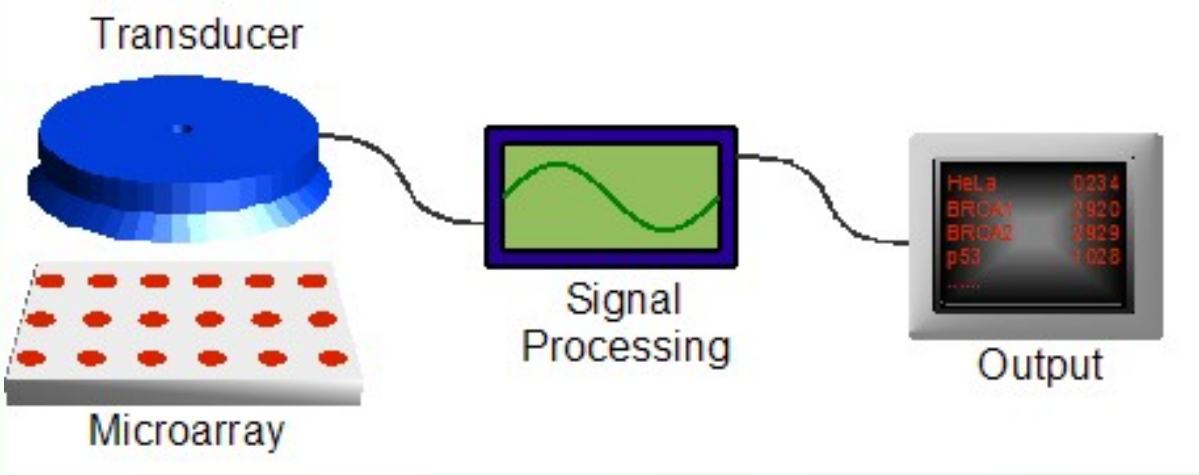
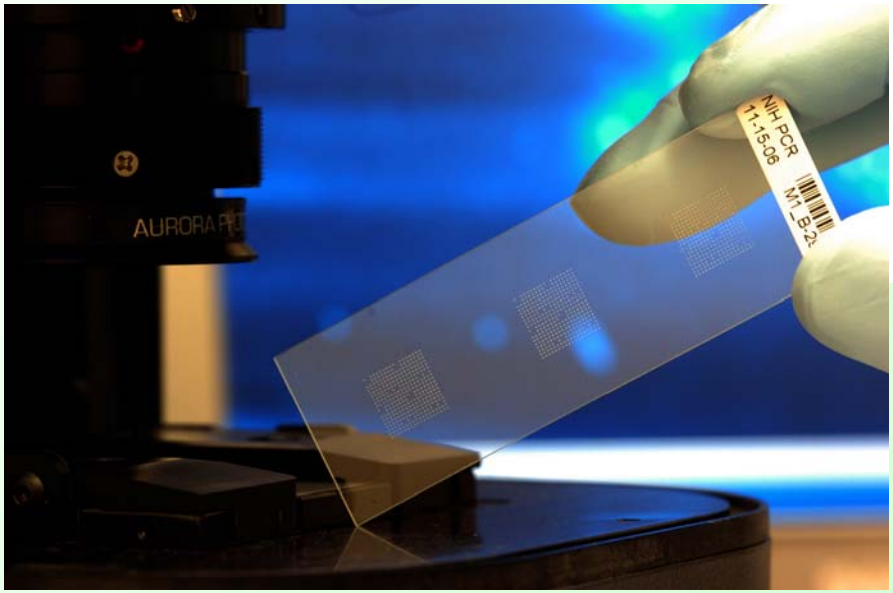
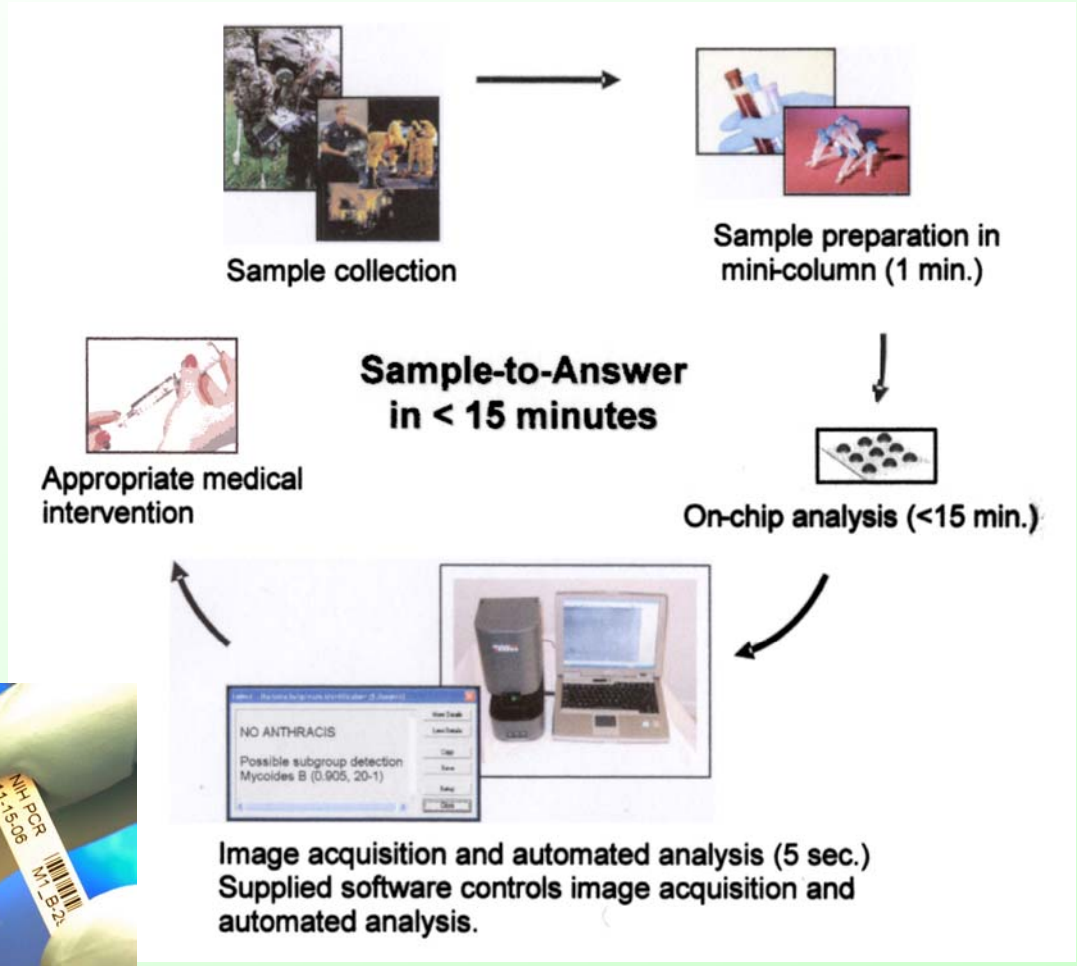
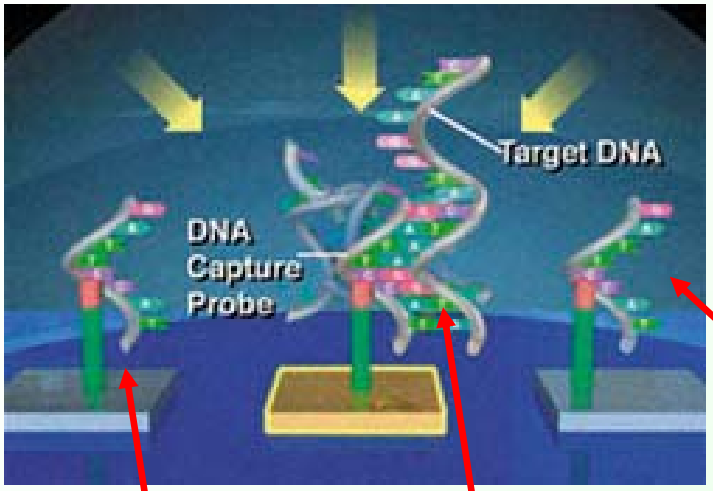


A **DNA microarray** (also commonly known as *DNA chip*, or *gene array*) is a collection of microscopic DNA spots, commonly representing single genes, arrayed on a solid surface by covalent attachment to chemically suitable matrices. DNA microarrays utilize the selective nature of DNA-DNA hybridization and fluorochrome-based detection. DNA arrays are commonly used for monitoring expression levels of thousands of genes (gene expression profiling) or for comparative genomic analysis.

BIOCHIP SLIDE – Each biochip has hundreds to thousands of spots on a glass, plastic or membrane support. The biochip system **can identify infectious disease strains in less than 15 minutes** when testing protein arrays and in less than two hours when testing nucleic acid arrays.



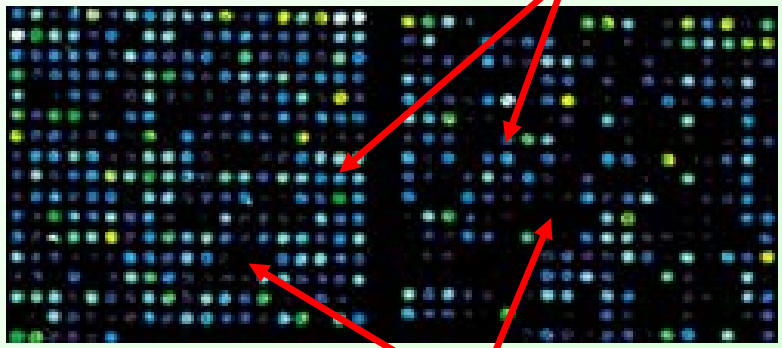




DNA-probe
(oligonucleotide)

Hybridization

None hybridization

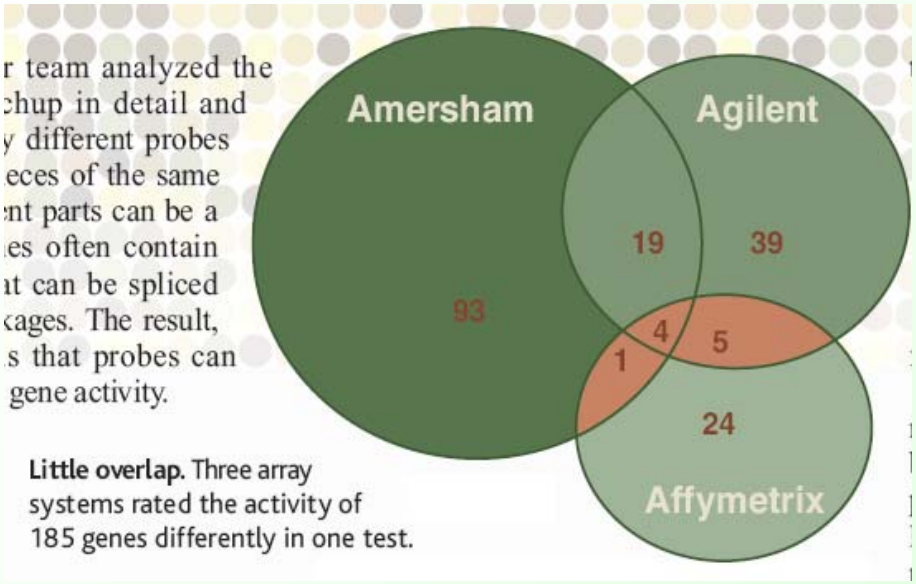


Hybridization

None hybridization

Structure of each individual spot of the chip.
Each spot contains specific DNA-probe (specific sequence).
Target DNA is labeled by fluorohrome.
After the hybridization process the chip generates signals on the spots with target DNA.
The biochip is automatically scanned and date are computered.

The results of a study comparing major, commercially available, microarray platforms



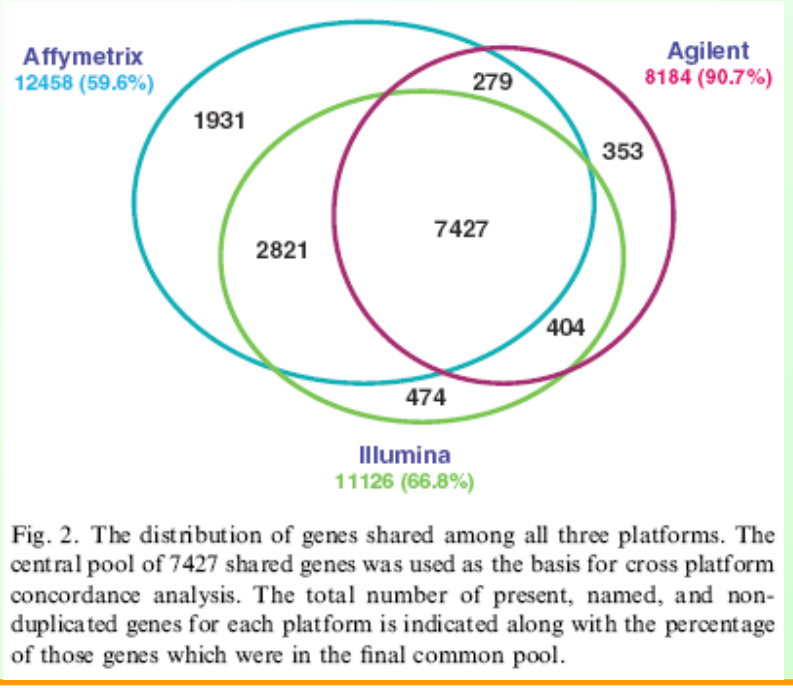


Fig. 2. The distribution of genes shared among all three platforms. The central pool of 7427 shared genes was used as the basis for cross platform concordance analysis. The total number of present, named, and non-duplicated genes for each platform is indicated along with the percentage of those genes which were in the final common pool.

An average 4 % for all platforms across all possible [according Margaret Cam]

Getting the Noise Out of Gene Arrays
Eliot Marshall
SCIENCE 2004, V. 306, 22, OCTOBER, pp.630-631

An average 22.8% for all platforms across all possible

A rapid method for microarray cross platform comparisons using gene expression signatures
Chris Cheadlea, Kevin G. Beckerb, Yoon S. Cho-Chungc, Maria Nesterovac,Tonya Watkinsa, William Wood IIIb, Vinayakumar Prabhub, Kathleen C. Barnes
Molecular and Cellular Probes 21 (2007) 35–46

The inevitable use of a variety of different platforms has compounded the difficulty of effectively comparing data between projects, laboratories, and public access databases.

The need for consistent, believable results across platforms is fundamental problem.

NEEDS:

Technology for the direct analysis of the target DNA.

Novosibirsk Free Electron Laser

Wave length -
110-240 μm,
Average power -
400 W,
Peak power -
1 MW.

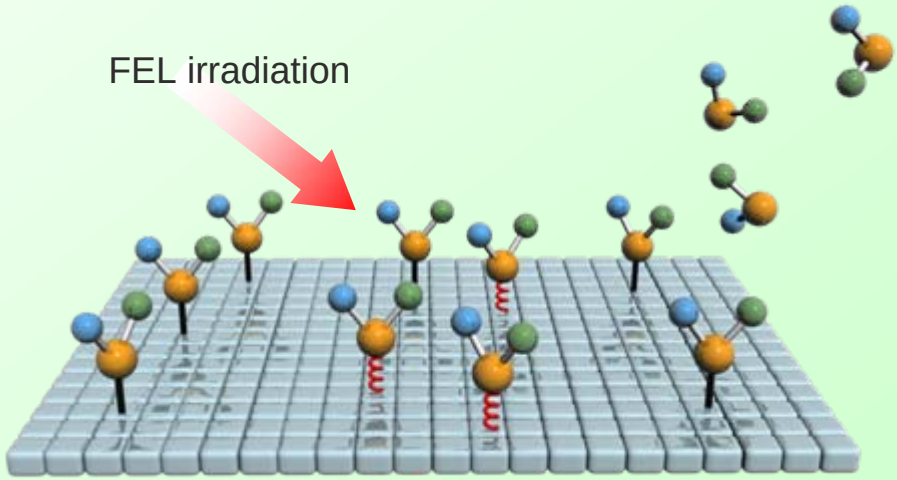
ABLATION is defined as the removal of material from the surface of an object by vaporization, chipping, or other erosive processes. The term occurs in space physics associated with atmospheric reentry, in glaciology, medicine and passive fire protection.

In glaciology, ablation is used to define the removal of ice or snow from the surface of a mass of ice.

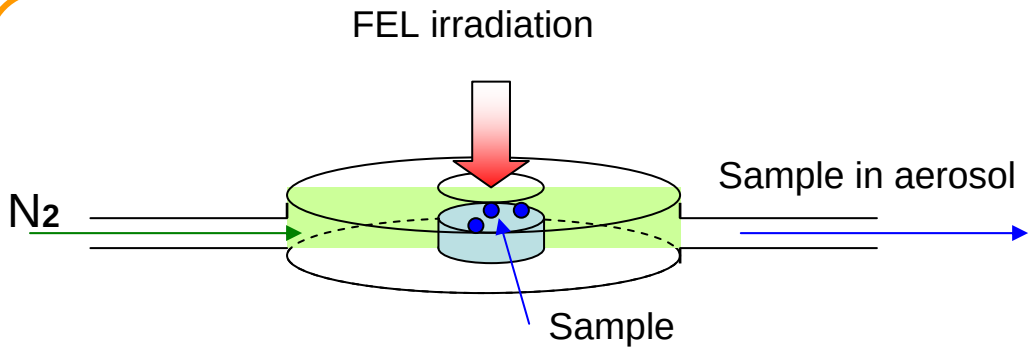
In medicine, ablation is the same as removal of a part of biological tissue, usually in surgery.

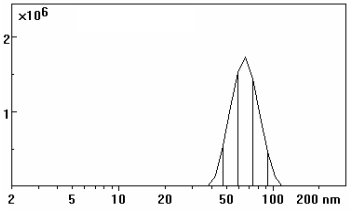
In our case ablation is transfer of biomacromolecules from solid surface into aerosol phase under FEL THz irradiation.

•Binding energy of biomacromolecules and surface corresponds with THz energy quantum.
•Low energy quantum (~0.01 eV) retains the covalent bonds in molecules intact.
•For that reason the ablated molecules conserve native structure.
•On this base were developed methods of protein and nucleic acid ablation for biomacromolecules transfer from solid surface into aerosol phase.



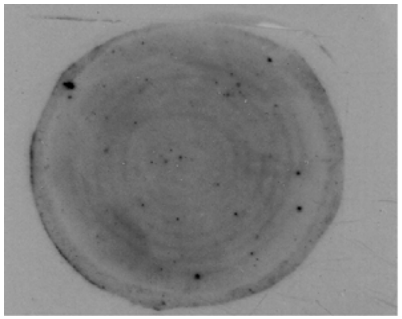
Ablation of Horseradish peroxidase





Detection by aerosol spectrometer

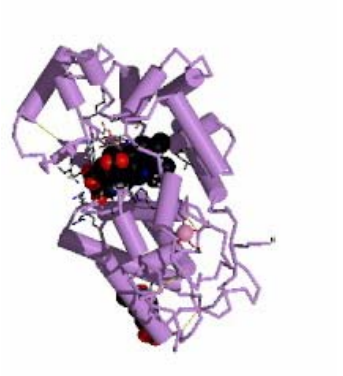
Collected on the filter



Histochemical staining
(biological activity test)

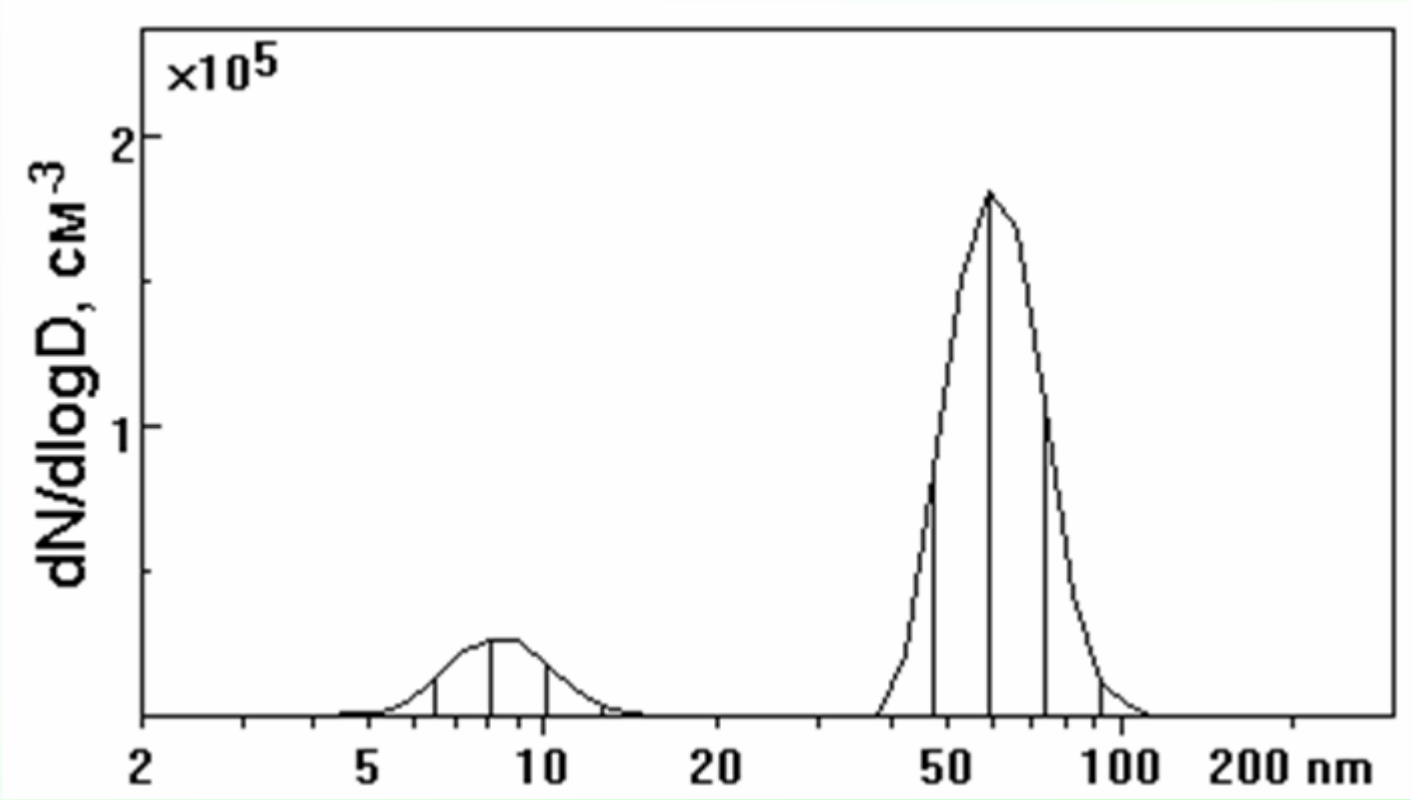
Control ablated protein

Electrophoregram
(molecular mass test)

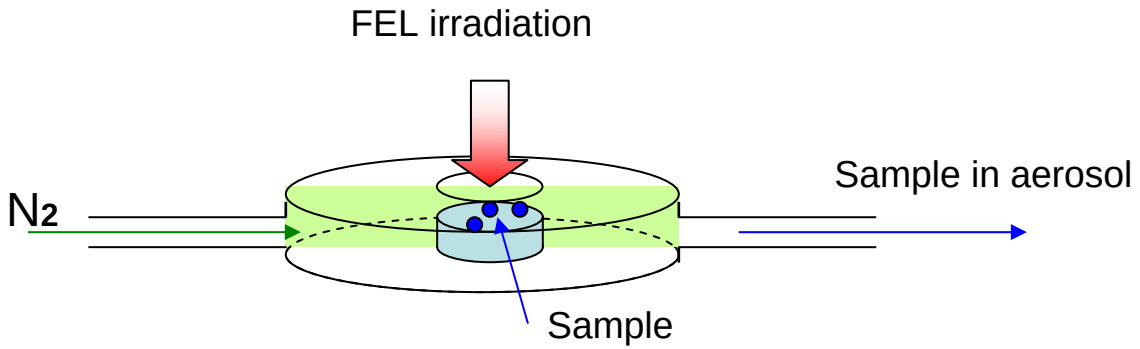


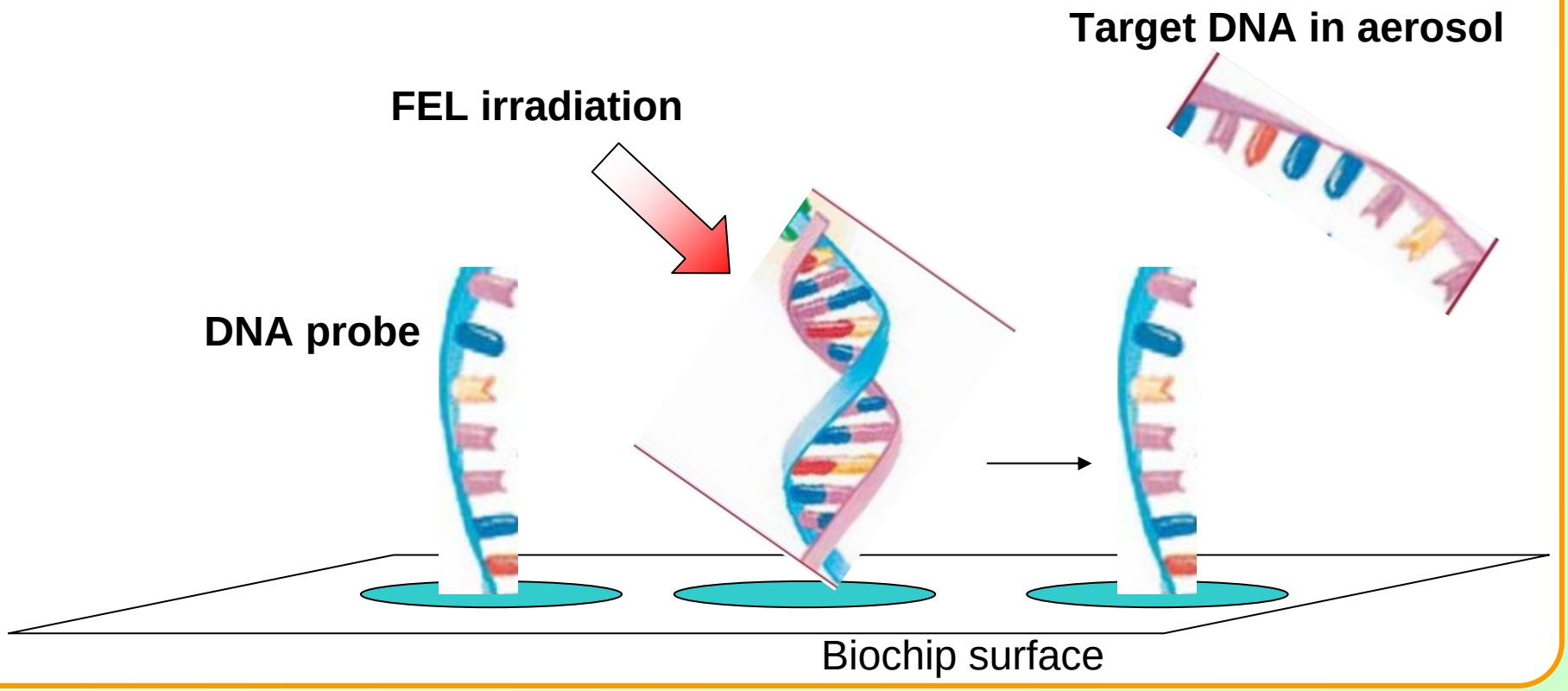
3D structure

Size distribution for the aerosol particles formed as a result of mild nondestructive ablation of lambda phage DNA in a mixture with BlueScript plasmid DNA using FEL radiation



Principle of experiment with biochip





DNA probe: 3' – TGTGACTCTAGACTATA - 5'

Biochip surface - $MMTNHCH_2CH_2CH_2CH_2CH_2CH_2$

$$\begin{array}{l} \\ | \\ O \\ | \\ [\text{probe DNA}]\text{-}\ N_2\text{-}P\text{-}OCH_2CH_2CN \end{array}$$

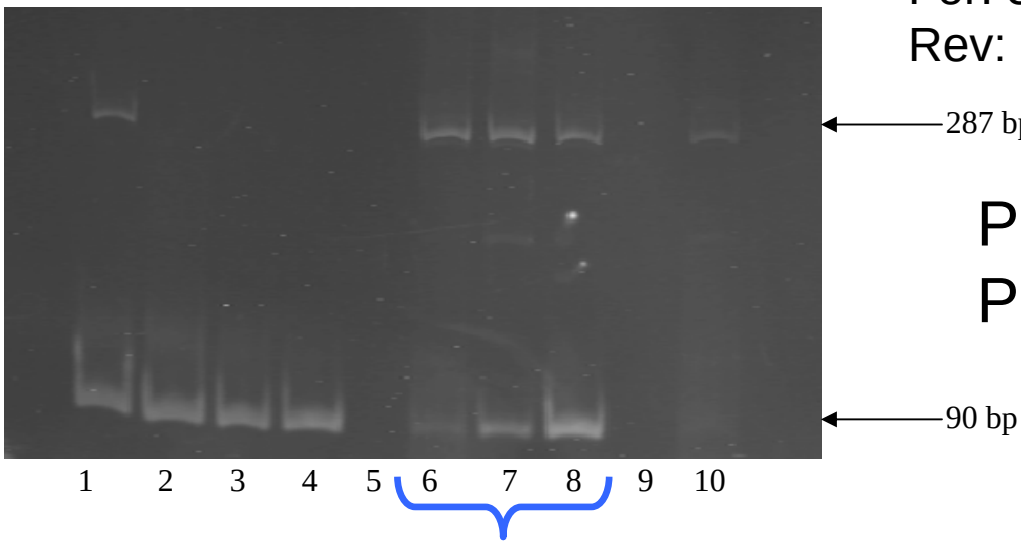
6-(4monomethoxytritylamino)hexyl-(2cyanoetyl)-(N,N-diissopropyl)- phosphoramide

Probe DNA is covalently binded to biochip surface

TARGET DNA ABLATED AMPLIFICATION PRODUCT IDENTIFICATION BY ELECTROPHORETIC ANALYSIS

PCR primers

For: 5’-TTCCCTCCTGAGTTCCCC-3’
Rev: 5’-ATATCAGATCGCAGTGTGCC-3’



PCR -
Polymerase Chain Reaction

Target DNA ablated amplification product

1,2,3,4.10 – different controls of PCR system; 5 – empty;
6 – ablation (wave length 133.69 μm);
7 – ablation (wave length 130.33 μm);
8 – ablation (wave length 128.5 μm);
9 – empty.

target DNA 5' - CCCTCCTGAGTTCCCCTACACACACAACCAC

ACACAACCACACACAACCACACACAACCACACA

CAACGGCACACTGCGATCTGATAT - 3'

DNA probe 3' – TGTGACTCTAGACTATA - 5'

Hybridization

Ablation,
Amplification

Sequencing of the target DNA after ablation:

target DNA 5'TTCCCTCCTGAGTTCCCCTACACACACAACCACACACAACCACAC

ablated DNA 5'TTCCCTCCTGAGTTCCCCTACACACACAACCACACACAACCACAC

ACAACCACACACAACCACACACAACGGCACACTGCGATCTGATAT-3'

ACAACCACACACAACCACACACAACGGCACACTGCGATCTGATAT-3'

Real ablation of DNA molecules from biochip surface under THz-emission was shown!

Target DNA was kept intact and is suitable for Polymerase Chain Reaction (PCR) and sequence analysis. ,

It should be noted: The method of nondestructive ablation allows to transfer large proteins and DNA molecules into aerosol. This allows to analyze biochip hybridization products from single spot by its direct analysis.

We plane to use this technique for standardization of biochip production.

THANK YOU FOR YOUR ATTENTION