FEL Radiation Use for Large Biomacromolecules Ablation

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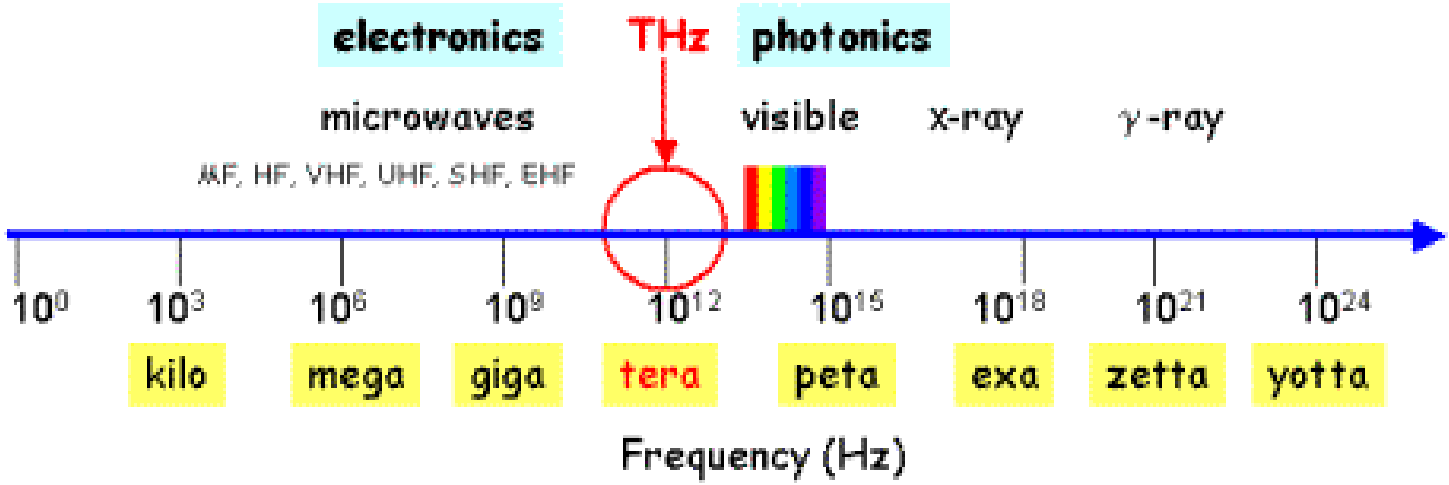
BINP


ICG


ICKC


11 building

Electromagnetic spectrum

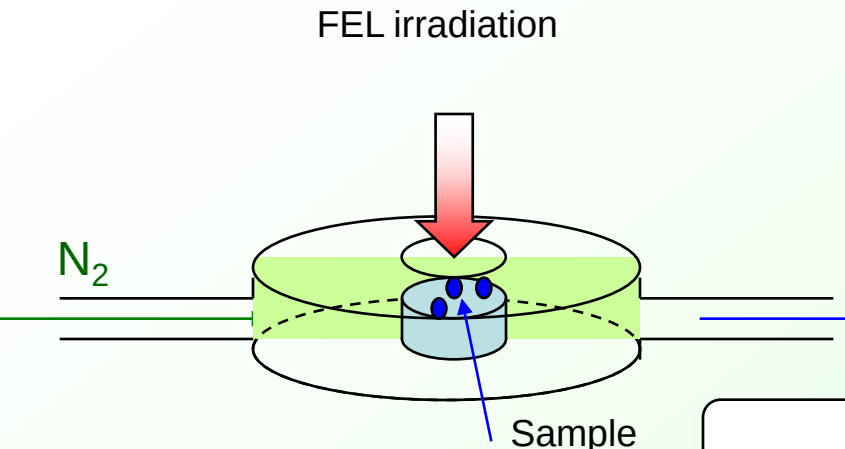


Wavenumber	Wavelength	Frequency	Photon Energy	Blackbody Temp.
1 cm^{-1}	10 mm	30 GHz	120 μeV	1.4 K
10 cm^{-1}	1 mm	300 GHz	1.2 meV	14 K
33 cm^{-1}	300 μm	1 THz	4.1 meV	48 K
100 cm^{-1}	100 μm	3 THz	12 meV	140 K
200 cm^{-1}	50 μm	6 THz	25 meV	290 K
670 cm^{-1}	15 μm	20 THz	83 meV	960 K

THE MAIN GOAL OF THIS WORK

- An investigation of a possibility of using terahertz radiation for transfer biomacromolecules and nanoparticles from a solid surface into the aerosol phase.
- We have show that this process is nondestructive – the ablated molecules conserve primary structure.
- We applied this technique to standardization of the biochip production and express analysis of nanoparticle's size.
- We named the process of biomacromolecule and nanoparticle transfer into the aerosol phase the soft nondestructive ablation.

Ablation under FEL Thz emission

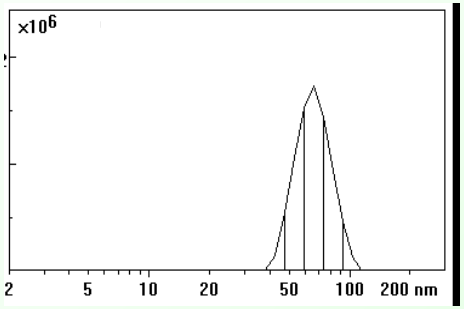


Sample in aerosol

Collection on filter

Ablation

Ablation registration



Single mode distribution

Nondistractive character of ablation proves

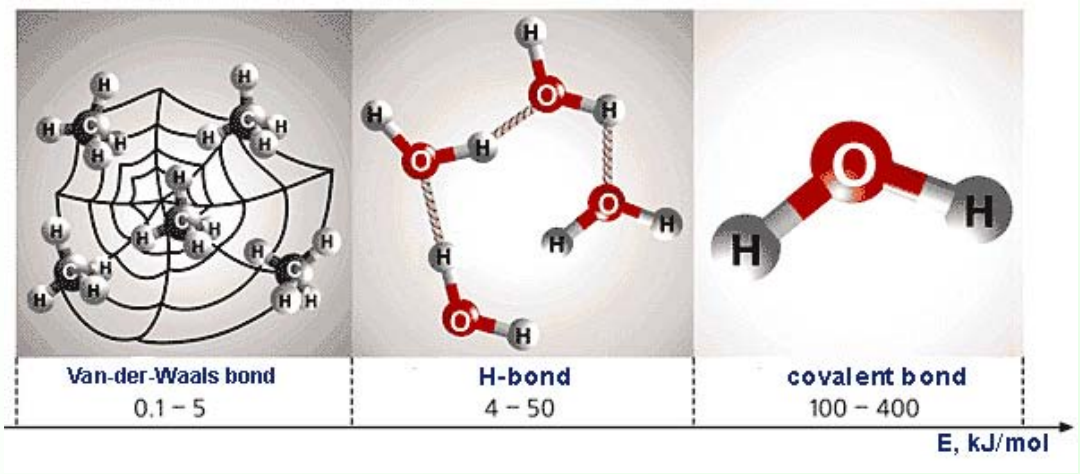
DNA

Protein

Persist ability for E.coli cell transformation
Conserve restriction site structure,
Nucleotide sequence

Persist enzyme activity,
Electrophoretic mobility,
Conserve protein structure (by mass-spec.)

Molecular bond energy



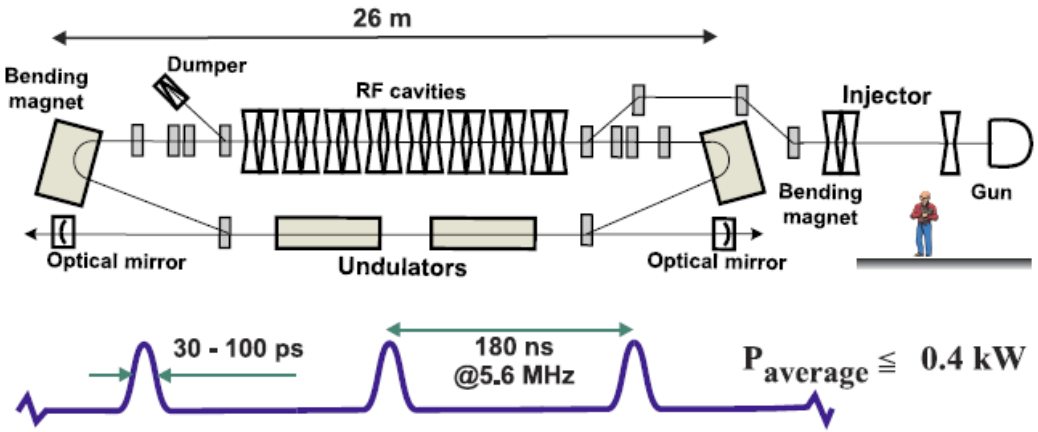
1 eV = 1,60217653(14)×10^{-19} J

- The THz quant of energy is about 0.01 eV.
- This approximately corresponds to the energies of hydrogen and Van der Waals bonds.
- So, for that reason the ablated molecules conserve their native structure.
- On this base were developed a method of the protein and nucleic acid ablation and nanoparclies

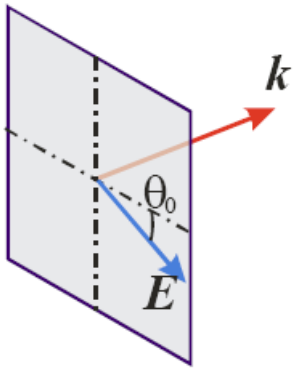
Novosibirsk Free Electron Laser

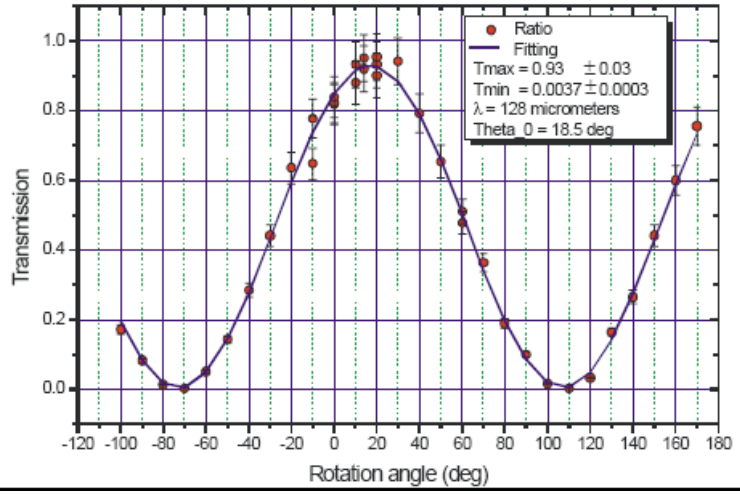
Wave length -
110-240 μm,
Average power -
400 W,
Peak power -
1 MW.

Laser radiation at the user stations

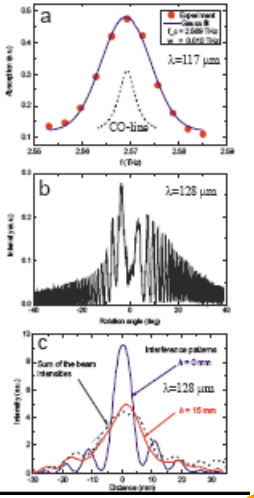


Novosibirsk FEL emerges continuous train of 70-ps terahertz pulses.
The monochromatic radiation can be tuned within range of 120 – 240 μm.
Laser beam is transmitted to user station hall through 14-m beamline.
Plane-polarized coherent laser beam diameter at user stations is equal to 80 mm





Coherence length of laser radiation is 40 – 100 ps depending on electron beam characteristics



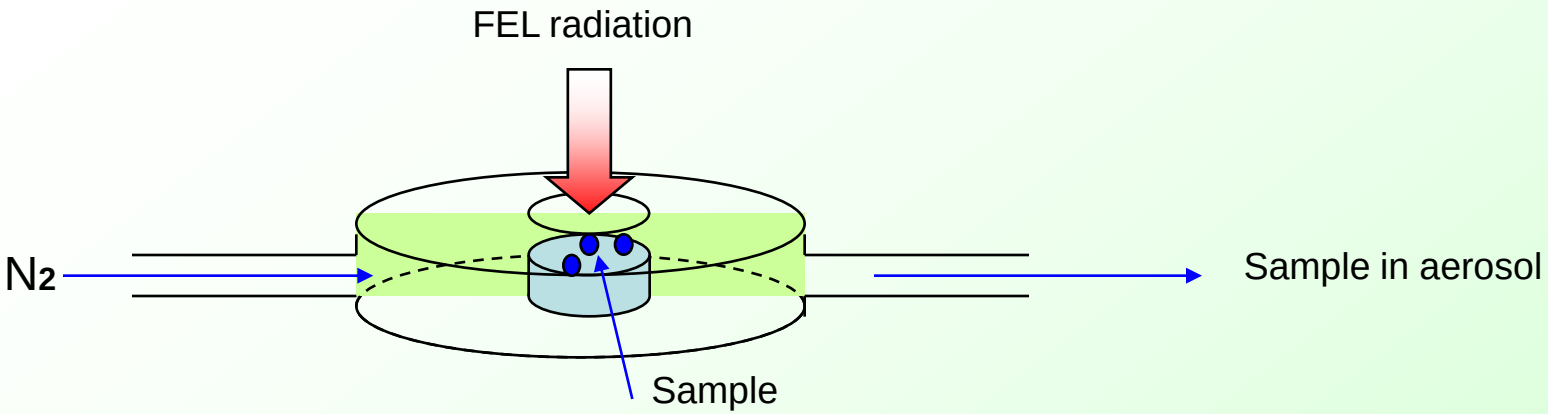
Ablation is defined as removal of a material from a surface by vaporization, chipping or other erosive processes. The term occurs in physics associated with atmospheric circulation, in glaciology, medicine and passive fire protection.

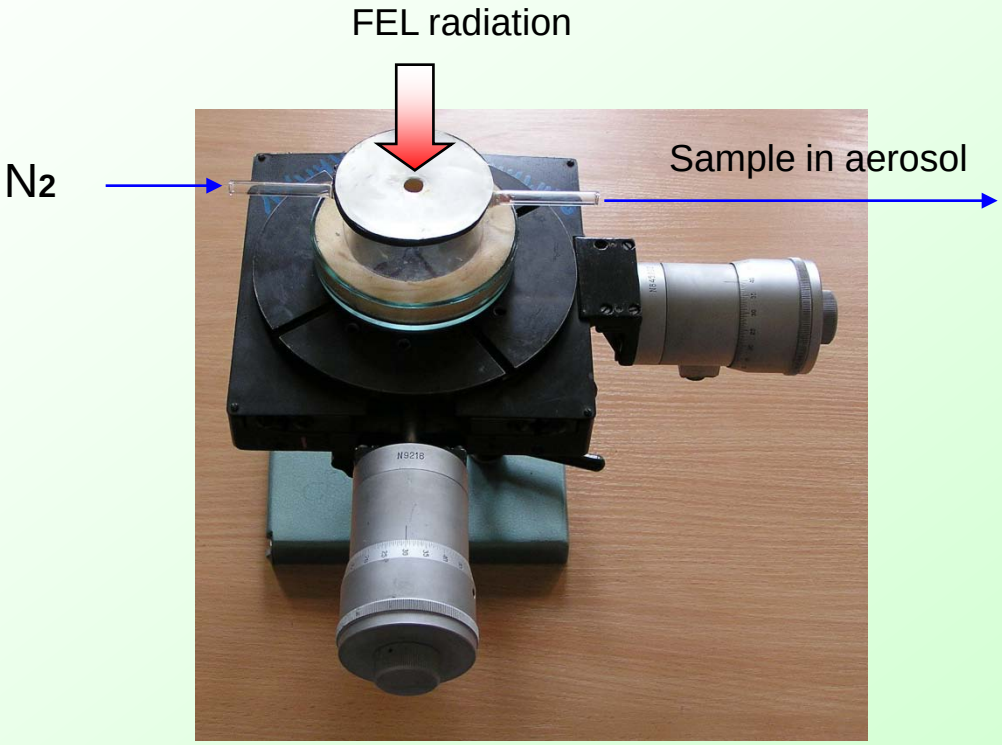
In glaciology, ablation is used to define the removal of ice or snow from the surface of a mass of ice.

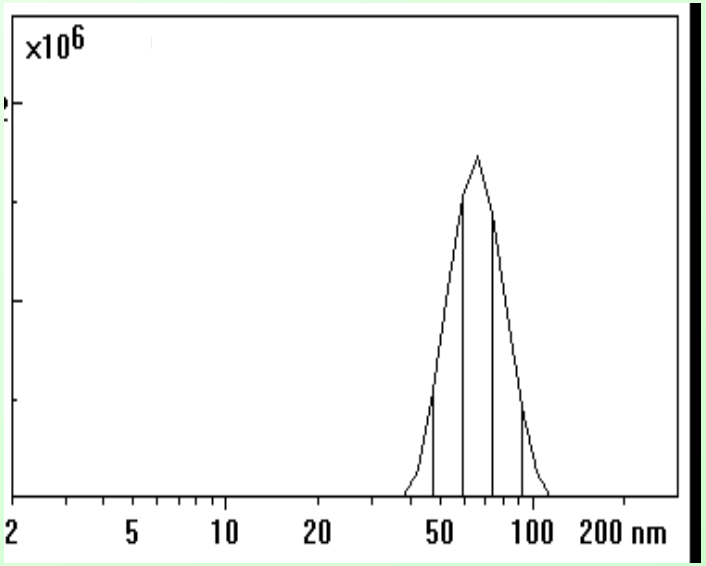
In medicine, ablation is the same as removal of a part of biological tissue, usually in surgery.

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

A scheme of our experiment experiment

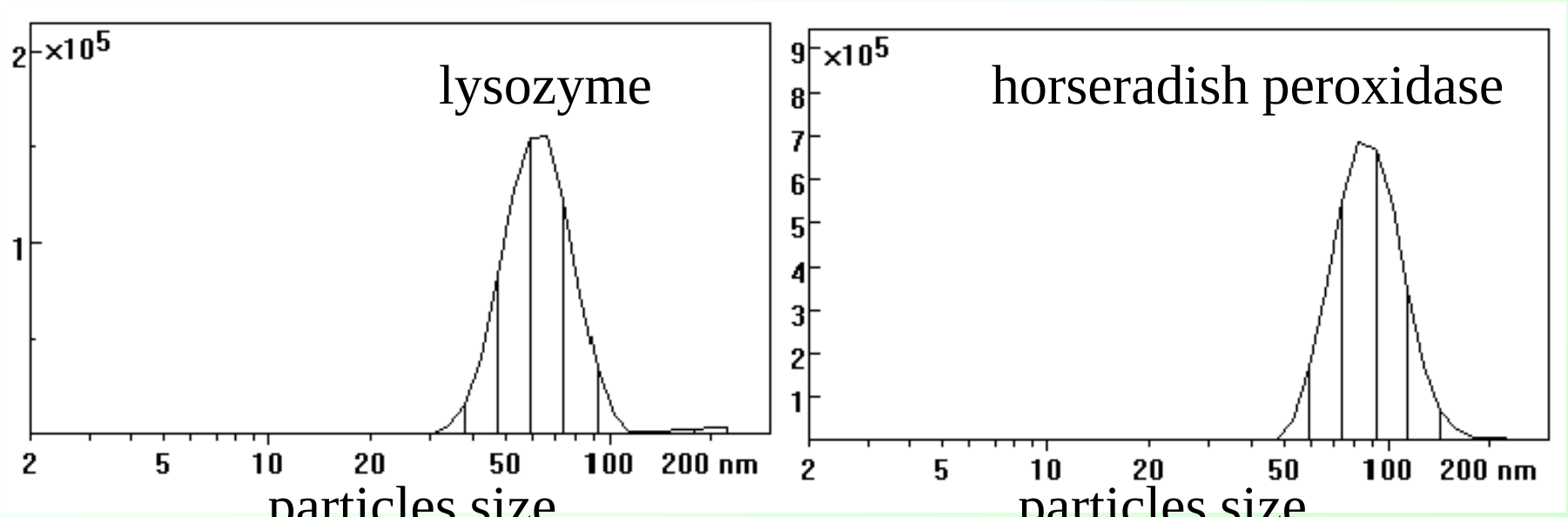






Particle size measurement by aerosol spectrometer

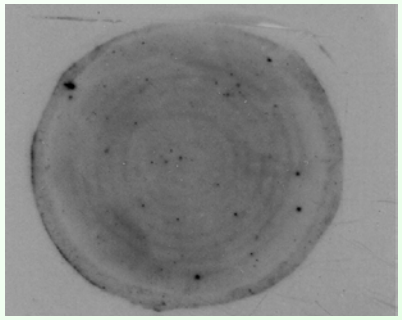
Size distribution for the aerosol particles formed as a result of soft ablation of proteins using FEL radiation



In our first experiments were ablated two commercially available enzymes: lysozyme and horseradish peroxidase. Both protein samples under irradiation generated aerosol particles with single distribution mode. We observed the particle sizes of 60 nm for lysozyme and 100 nm for horseradish peroxidase. They are correlated with their respective molecular weights.

Nondestructive character of protein soft ablation

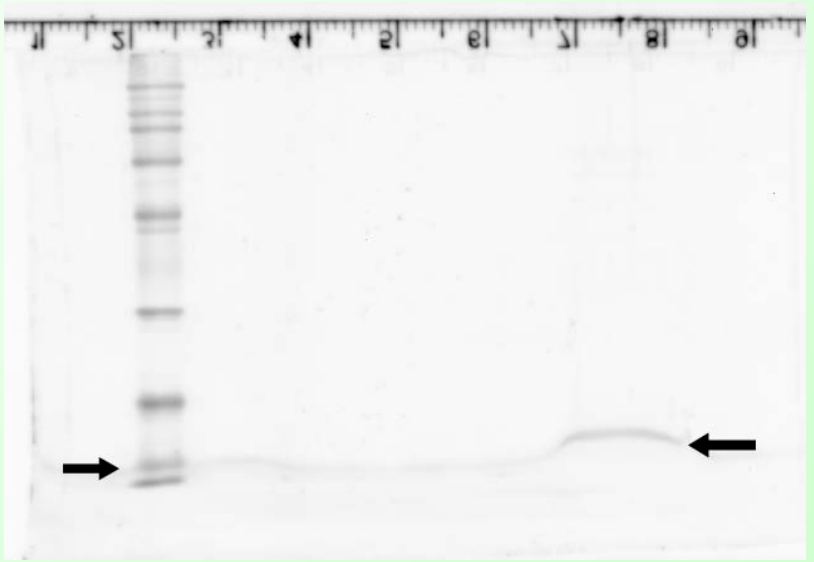
Control Protein after ablation



Sample on filter
Hystochemical staining technique

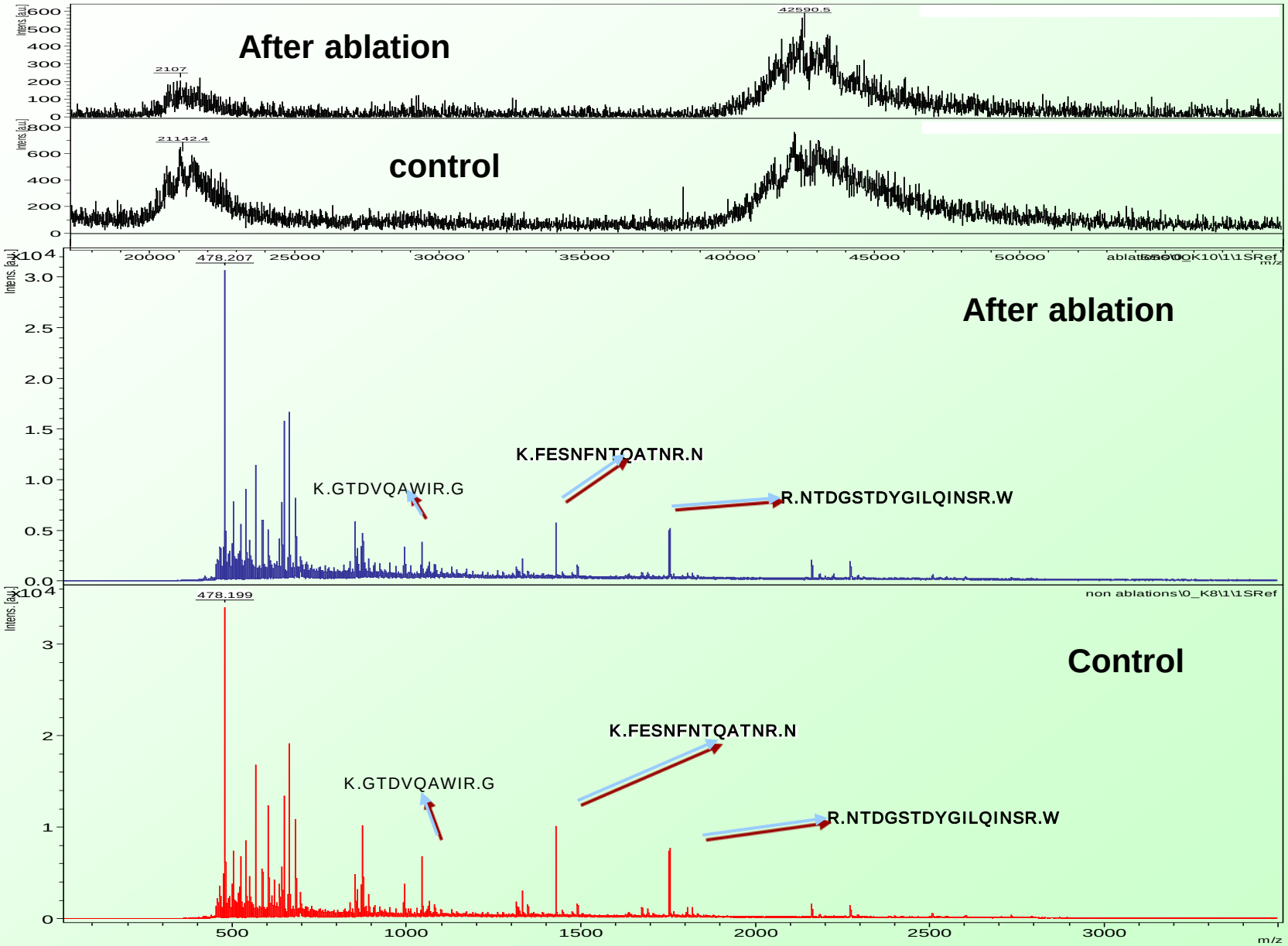
PAAG electrophoregramm (native)

Control Protein after ablation

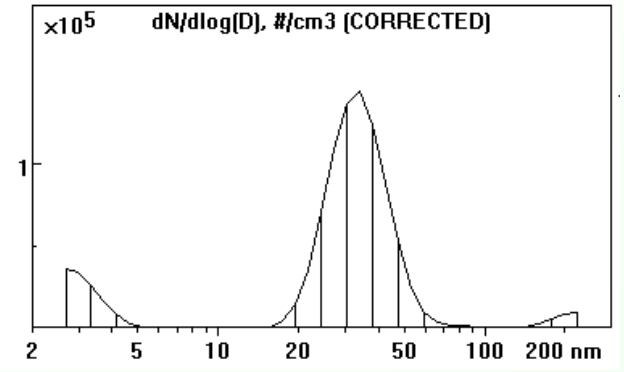


Lisozyme,SDS - electrophoregram, Flamingo staining
Control – SDS - PAGE standards broad range (BioRad).

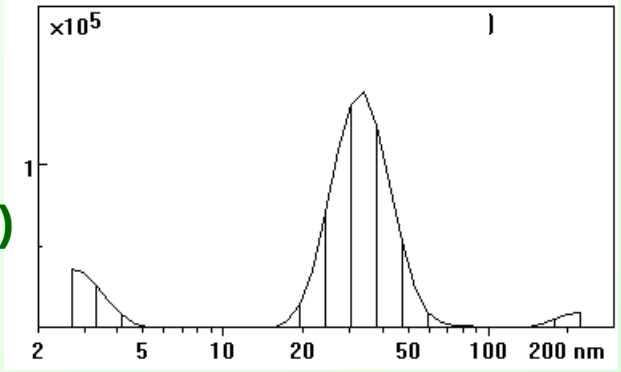
Experiments proving the nondestructive character of soft ablation by MALDI-TOF technique



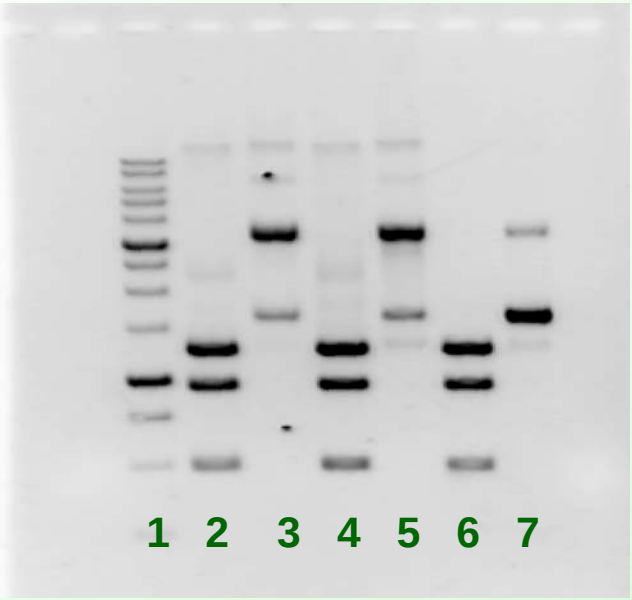
pUC18 plasmid ablation



Ablation (left) –
Collection –
Secondary ablation (right)
Identical distributions

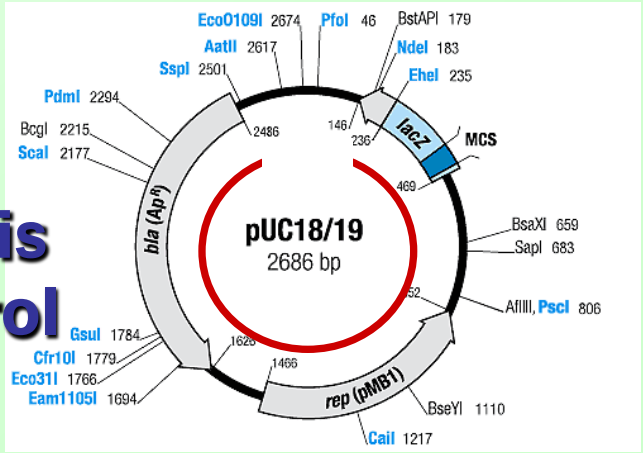


Electrophoregram of control and ablated plasmides Alw 441 hydrolyzates



1 Kb DNA marker (SibEnzyme);
2,4 Alw441 hydrolyzate of ablated plasmide;
3,5 ablated plasmide;
6 Alw441 hydrolyzate of control plasmide;
7 control plasmide.

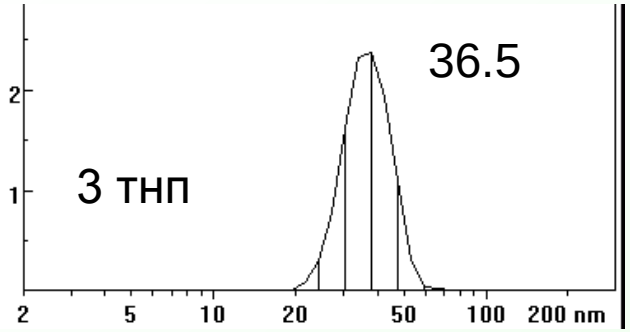
Sequence of ablated plasmid is identical to control



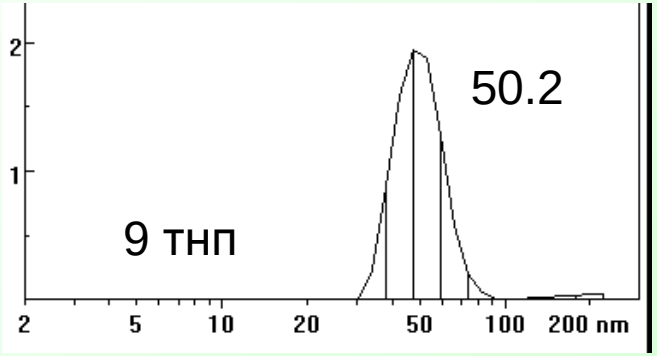
Methodes based on the soft nondistructive ablation

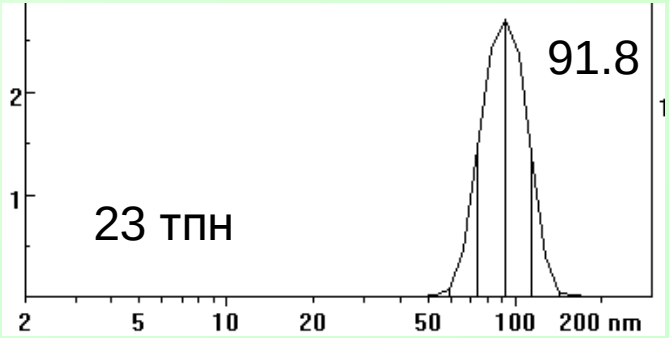
Nanoparticles size measurement

Phage λ (Hind III) DNA fragments ablation

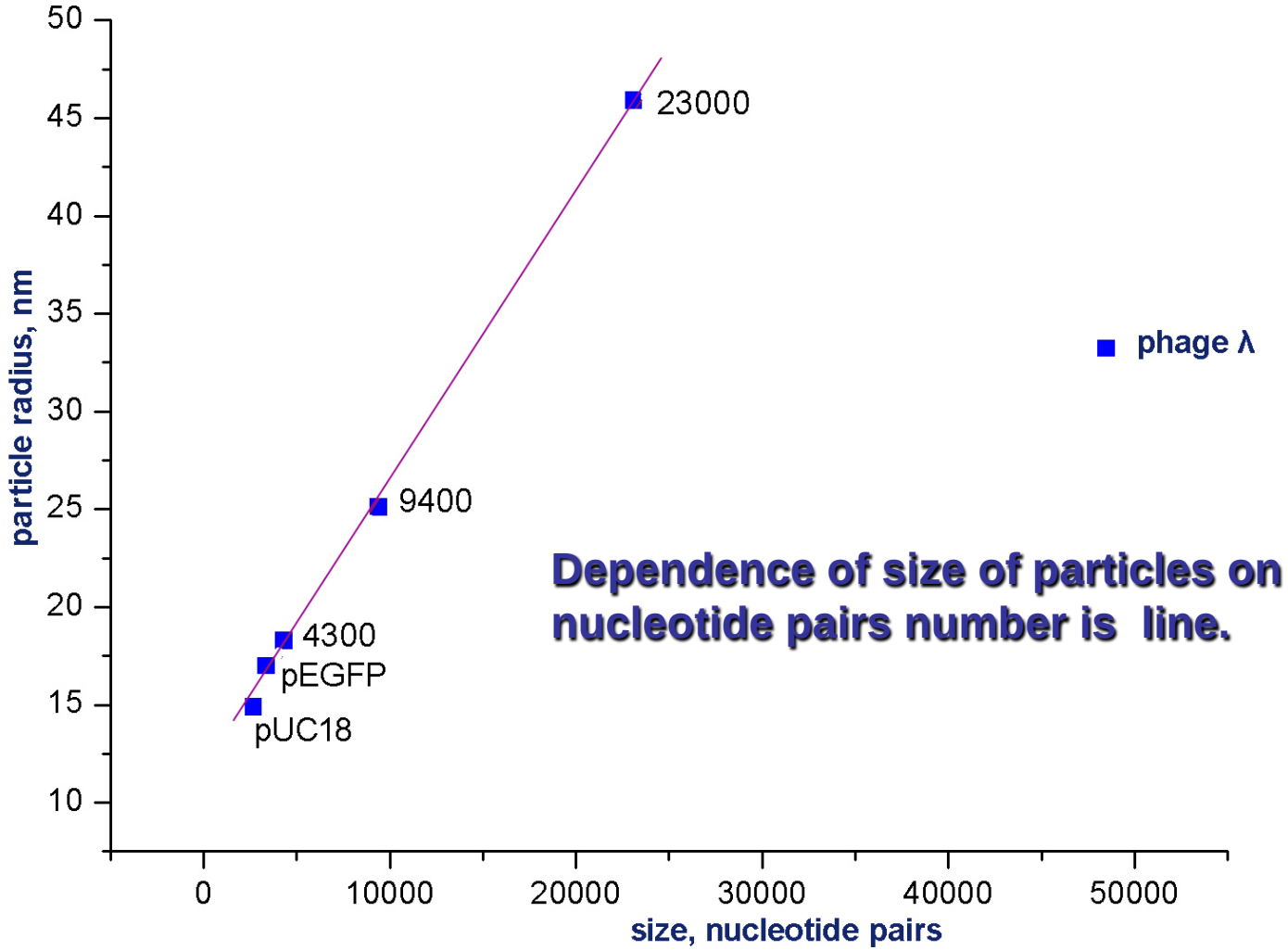


Each DNA fragment produced single size mode distribution
Its proves nondistructive character of DNA ablation



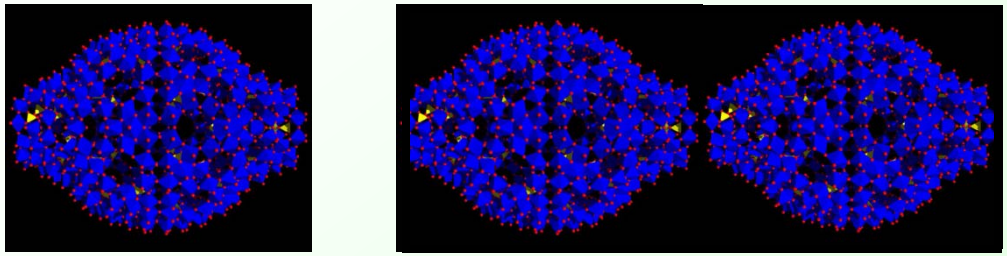


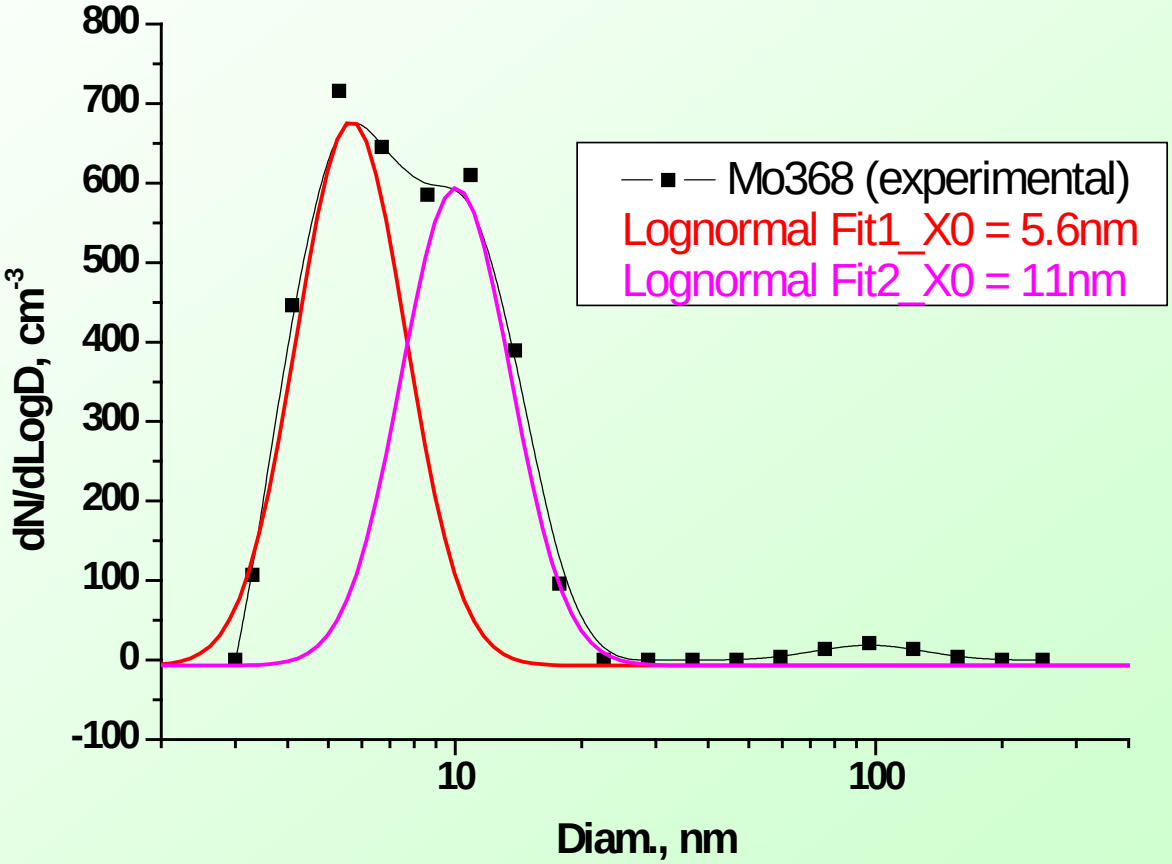
Size of particles in dependence of nucleotide pairs number



Dependence of size of particles on nucleotide pairs number is line.

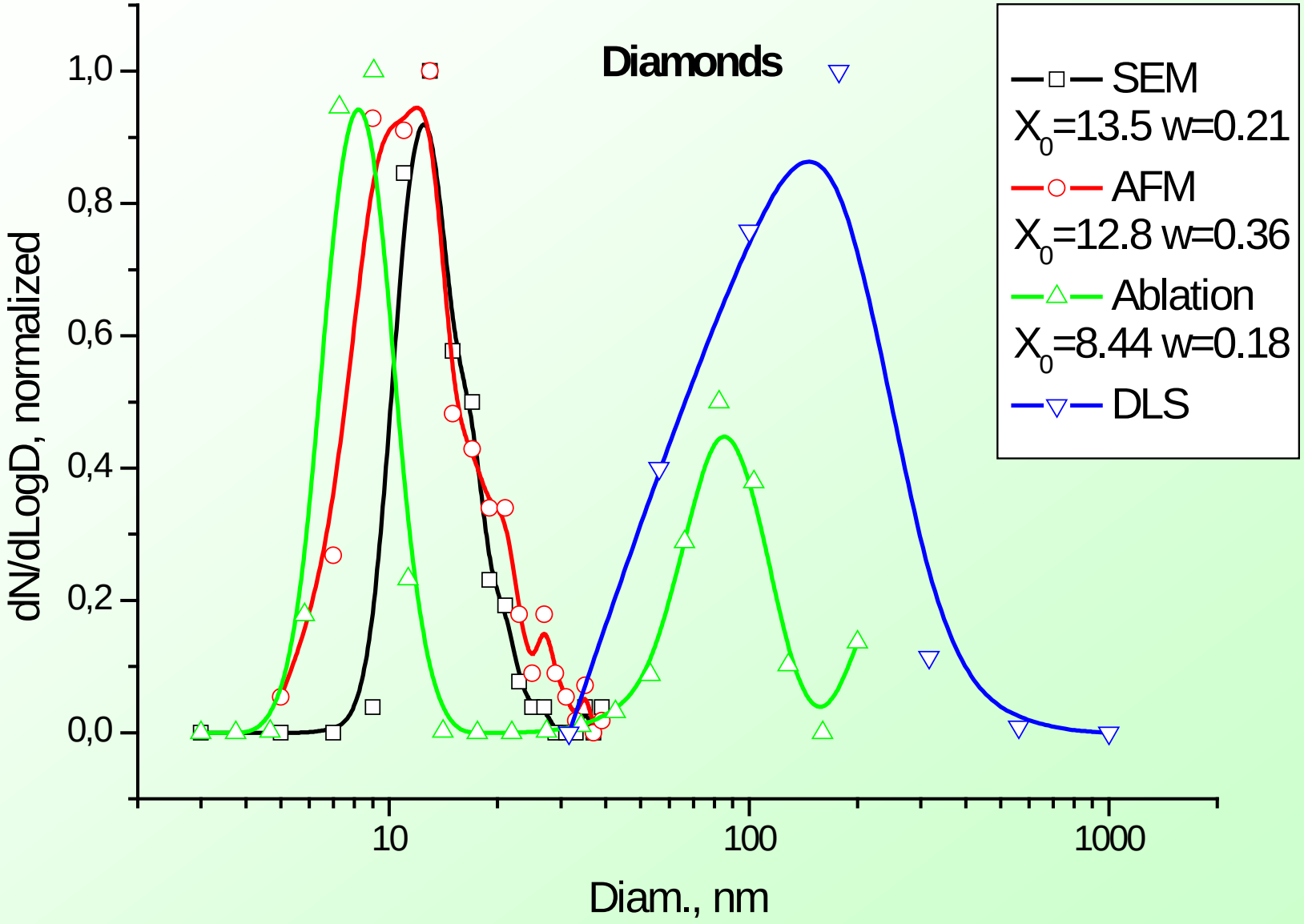
Fullerene like structure



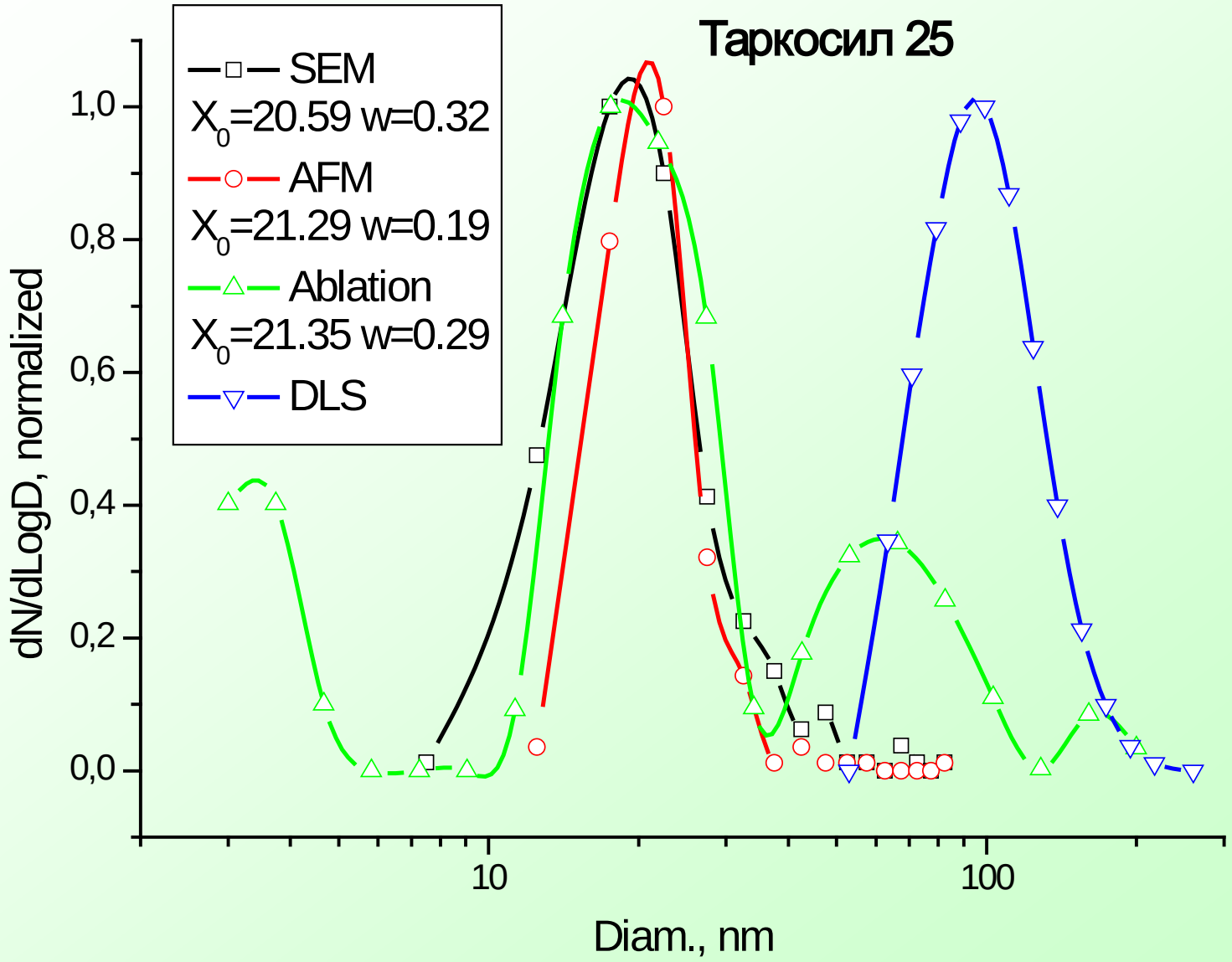


Mo_{368} – 5,6 нм; $\{Mo_{368}\}_2$ – 11,2 нм

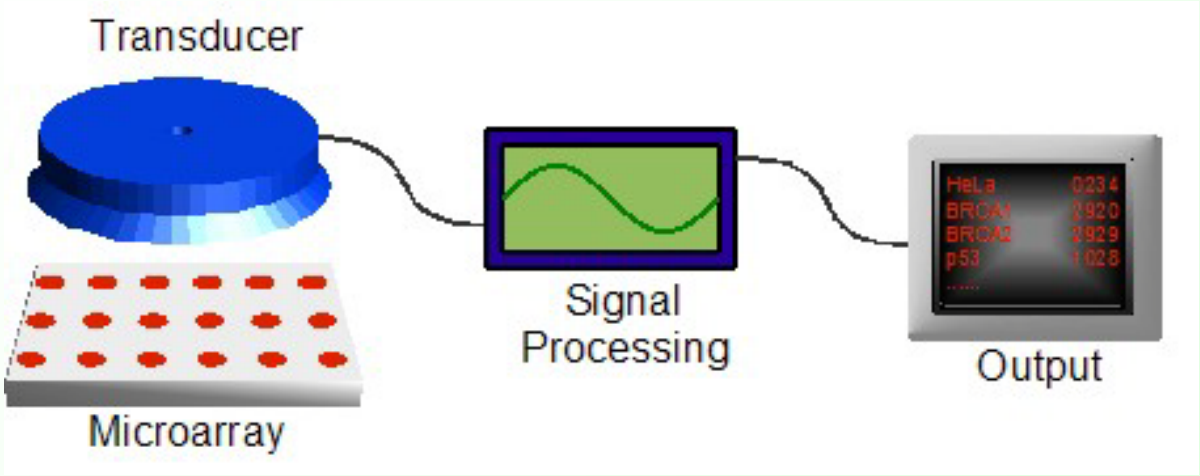
Diamonds Ablation (comparison)



SiO_2 Ablation (comparison)

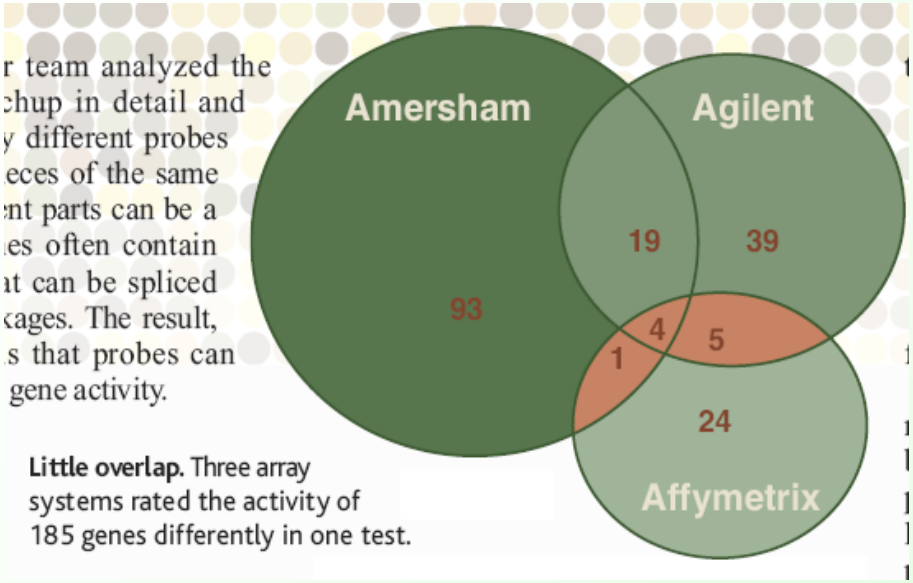


Biochip investigation



A **DNA microarray** (also known as the DNA chip, or gene array) is a collection of microscopic DNA probe spots, generally representing single genes, arrayed on a solid surface by a covalent attachment to the 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.

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



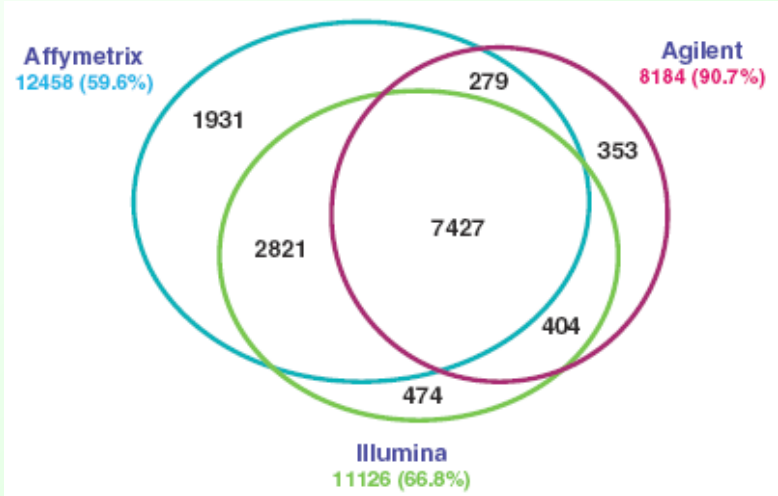


Fig. 2. The distribution of genes shared among all three platforms. The central pool of 7427 shared genes was used as the basis for cross platform concordance analysis. The total number of present, named, and non-duplicated genes for each platform is indicated along with the percentage of those genes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

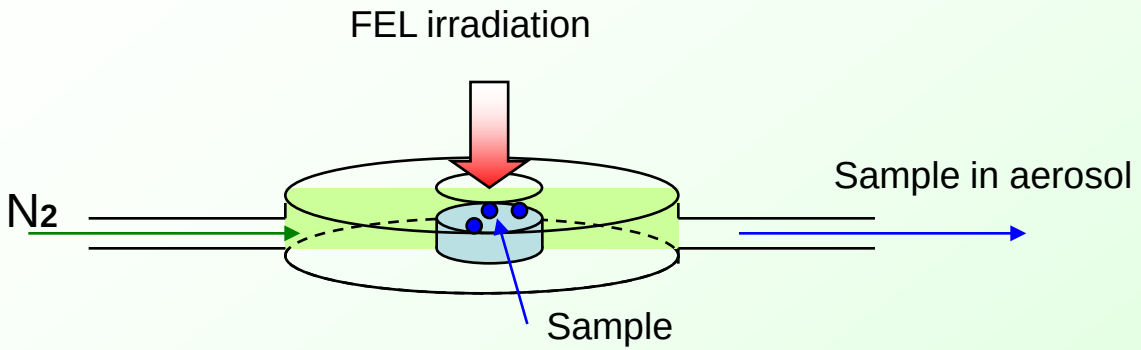
The inevitable use of a variety of different platforms has compounded the difficulty of effectively comparing data between projects, laboratories, and public access databases.

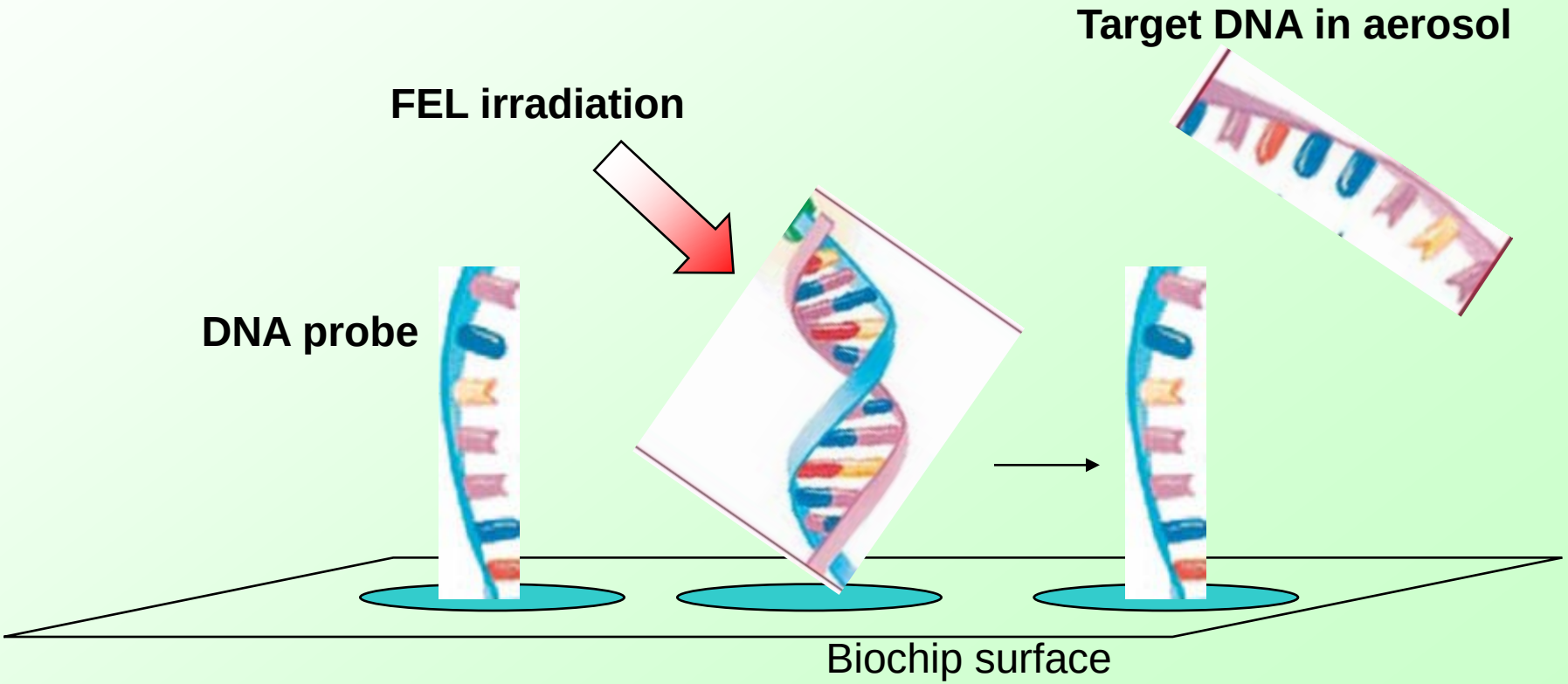
The need for consistent, believable results across platforms is fundamental problem.

NEEDS:

Technology for the direct analysis of the target DNA.

Principle of experiment with biochip

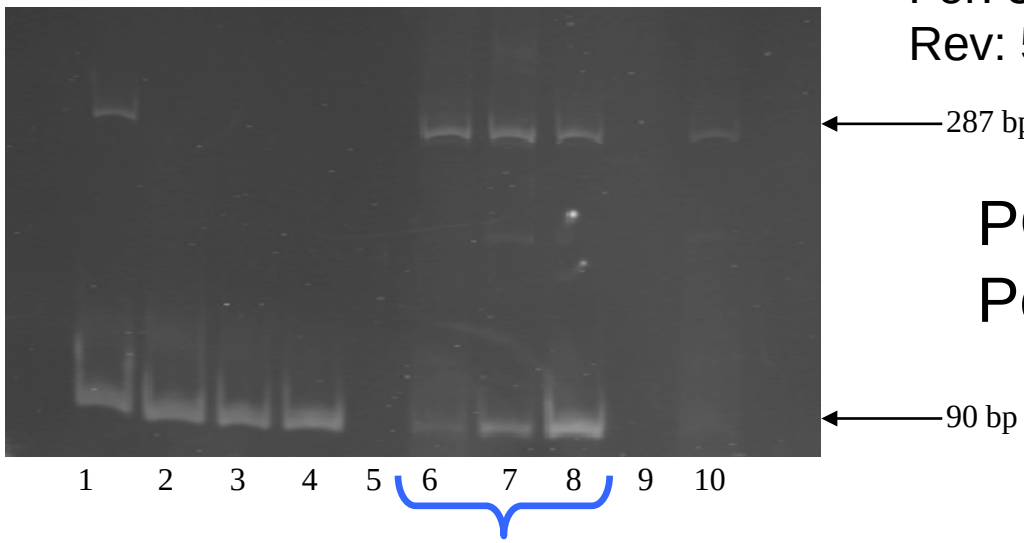




TARGET DNA ABLATED AMPLIFICATION PRODUCT IDENTIFICATION BY ELECTROPHORETIC ANALYSIS

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'

Conclusion

1. We developed the new non-destructive method of soft ablation of bio-micromolecules and nano-particles.
2. This method is suitable for non-destructive transfer of proteins into the aerosol phase.
3. We also show this method is suitable for non-destructive transfer of DNAs into the aerosol phase.
4. Using this method we developed the technique for the direct analysis of target DNA of biochips.
5. Using this method we developed the technique for nanoparticles size measurements.

Future plans

To develop method for standardization of the biochip manufacture.
To study a possibility of applicability of non-destructive ablation for mass-spectrometry.
To study a possibility of non-destructive ablation for nanoparticle mass measurements.
Use Thz irradiation for Identification of biomacromolecules.

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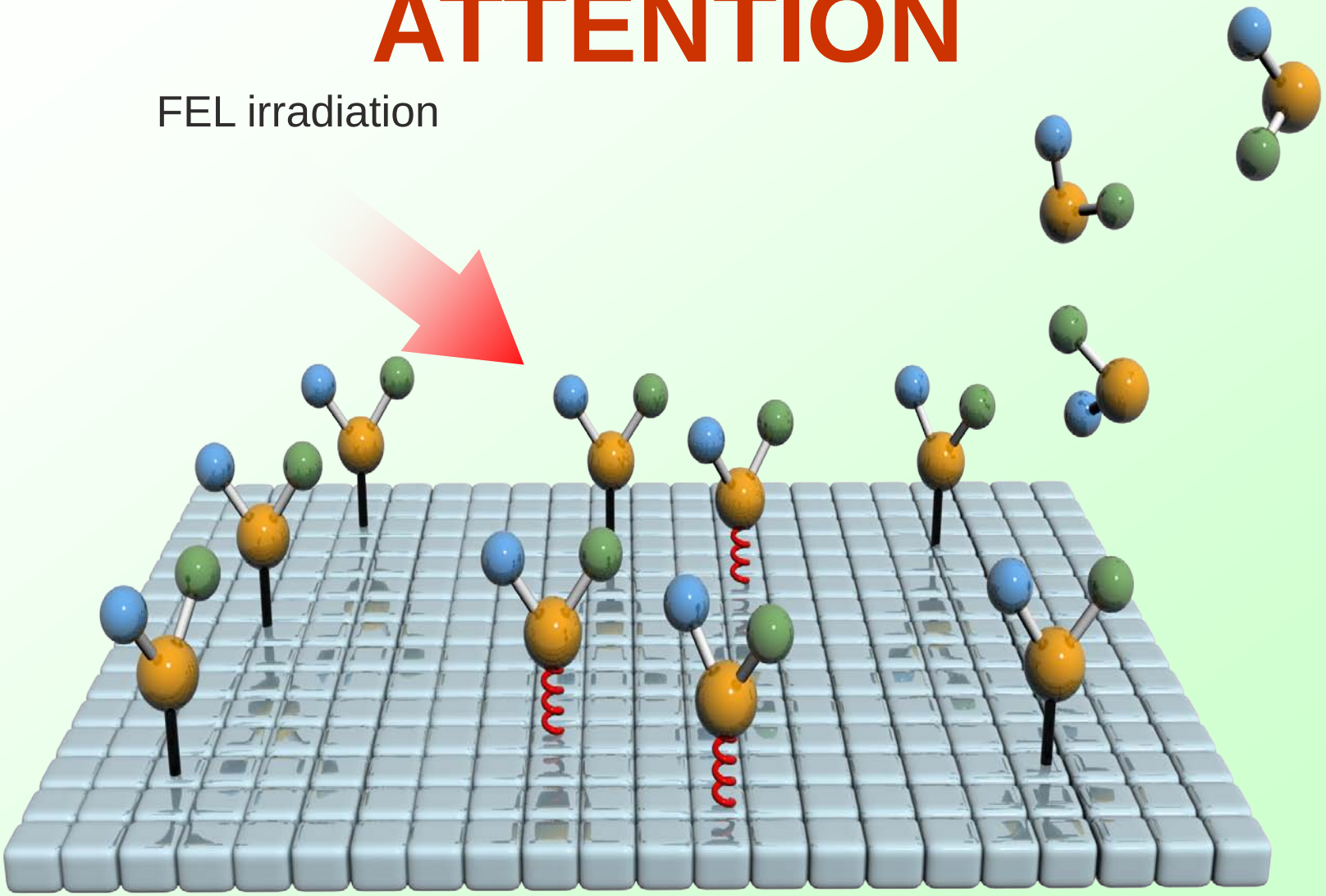
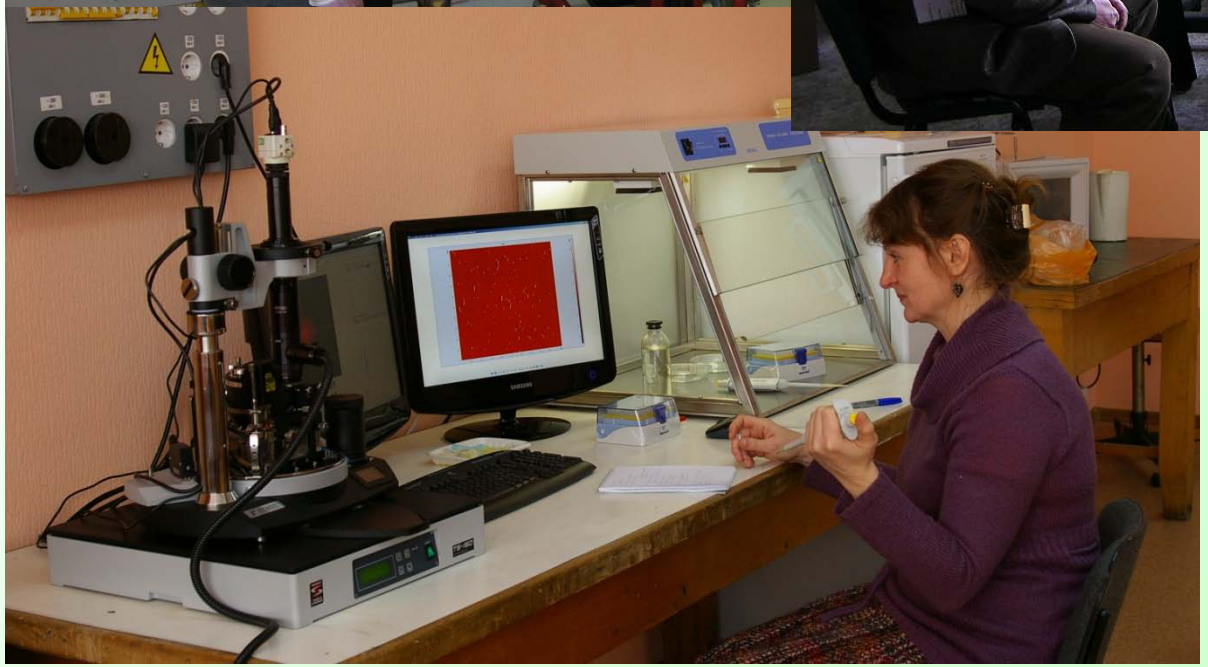
Alexander S. Kozlov, Sergey B. Malyshkin, Alexander K. Petrov (ICKC SB RAS, Novosibirsk)

THANK YOU FOR YOUR ATTENTION

FEL irradiation



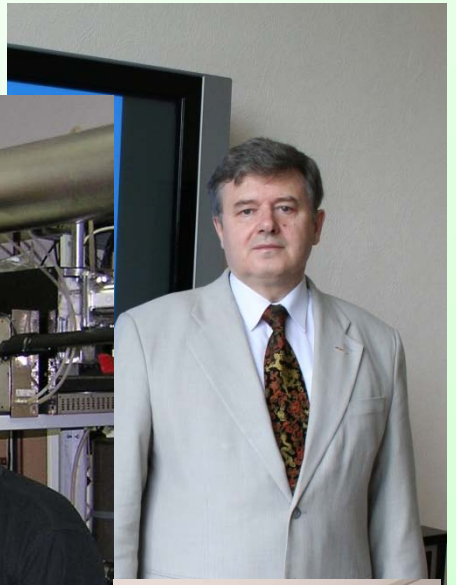
Работа коллектива по разработке метода мягкой неразрушающей абляции высоко оценена Жоресом Алферовым



Центр синхротронного и терагерцового излучения оборудован атомно-силовым микроскопом

Анатолий Чубайс оценивает перспективы использовании метода мягкой неразрушающей абляции в нанотехнологиях

Зарубежные коллеги обсуждают результаты P.Weightman, Великобритания, J.Neil, США



Работа коллектива по разработке метода мягкой неразрушающей абляции признана достижением РАН за 2006 г.

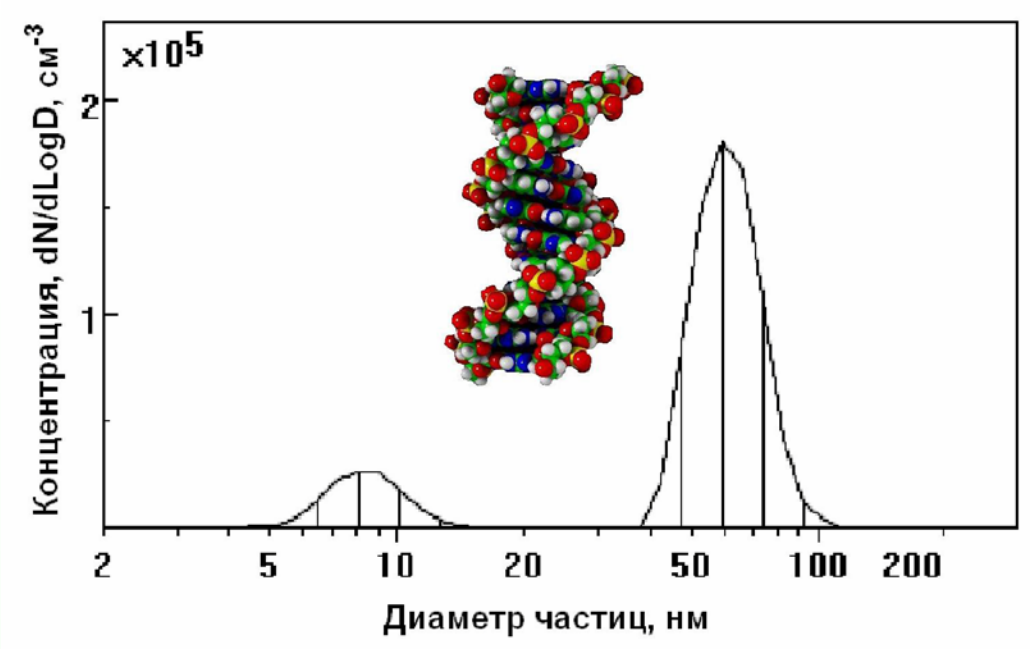
DNA probe: 3' – TGTGACTCTAGACTATA - 5'

Biochip surface - $MMTNHCH_2CH_2CH_2CH_2CH_2CH_2$

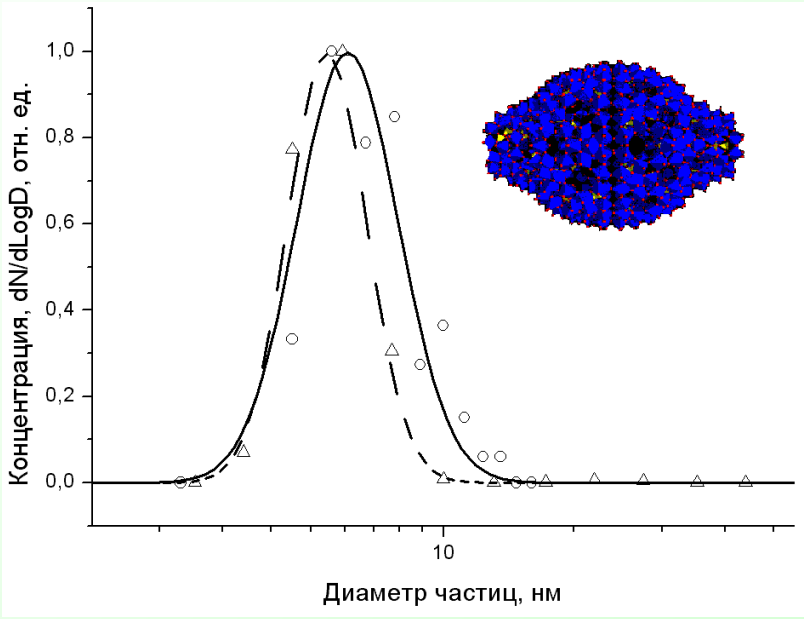
$$\begin{array}{l} \\ | \\ O \\ | \\ [\text{probe DNA}]\text{-}\ N_2\text{-}P\text{-}OCH_2CH_2CN \end{array}$$

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

Probe DNA is covalently binded to biochip surface



При абляции чистых материалов образуются наночастицы одной фракции. Так при абляции только ДНК фага образуются частицы средним размером 70нм, а ДНК плазмиды дает – 7нм.



Фуллереноподобные комплексы на основе молибдена (Mo368), синтезированные в ИНХ СО РАН (В.П. Федин), имеют характерный размер ~5.6нм. Сопоставление размерных распределений аэрозольных частиц полученных при помощи ДСА и на электронном микроскопе дает хорошие результаты.