Research and Implementation Center for Business

50. Zakopane School of Physics
May 18th – 23rd, 2015

Biological evaluation of nanoparticles

BioNanoPark laboratories
of Lodz Regional Park of Science and Technology

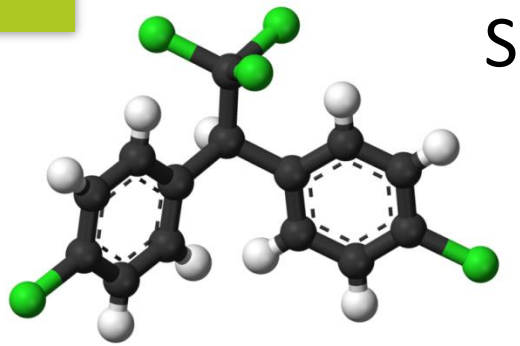
Bogdan Walkowiak
- Scientific Leader

of BioNanoPark Laboratories , Technopark of Lodz, Poland
- Head of the Department of Biophysics

Institute of Materials Science, Lodz University of Technology, Poland
- Member of Nanomaterials Working Group

European Chemical Agency, Helsinki, Finland

www.technopark.lodz.pl
e-mail: b.walkowiak@technopark.lodz.pl



Short story of DDT

In 1874 Othmar Zeidler, under the supervision of Adolf von Baeyer, has synthetised DDT. It is a colorless, crystalline, tasteless and almost odorless organochloride.

DDT's insecticidal properties were discovered in 1939 by the Swiss scientist Paul Müller, who was awarded the 1948 Nobel Prize in Physiology and Medicine for his efforts.
DDT was used extensively during World War II by the Allies to control the insect vectors of typhus and malaria.

- DDT and its metabolites are durable organic pollutants with half-life reaching 30 years.
- The International Agency for Research on Cancer classifies it as a "possible" human carcinogen.
- Human data indicate possible disruption in semen quality, menstruation, and duration of lactation.
- US Environmental Protection Agency states that DDT exposure damages the reproductive system and reduces reproductive success.

http://en.wikipedia.org/wiki/DDT

Risk resulting from presence in our environment of products of innovative technologies and the need to control this hazard was the main reason for the creation of the Laboratory Molecular and Nanostructural Biophysics in Lodz Regional Park of Science and Technology

http://en.wikipedia.org/wiki/DDT

BioNanoPark

Research and Implementation Center for Business

BioNanoPark is a project implemented by Technopark Lodz, aimed in science and industry cooperation, helping to transform ideas into business

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The structure of **BioNanoPark** laboratories

Laboratory of Industrial Biotechnology	Laboratory of Molecular and Nanostructural Biophysics
Microbiology Unit	Cell Biology Unit
Molecular Biotechnology Unit	Biochemistry Unit
Biocatalysis and Biotransformation Unit	Transcriptomics and Proteomics Unit
Biomolecular Analysis Unit	Molecular and Nanostructural Biophysics Unit
Biosynthesis and Bioproducts Separation Unit	Personalized Medical Implant Unit

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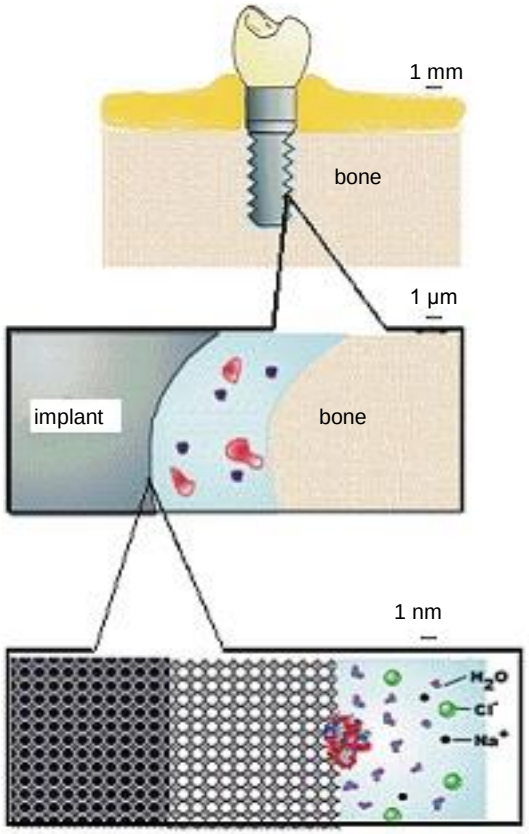
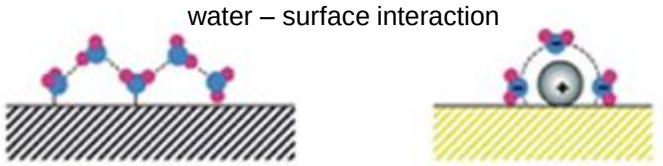
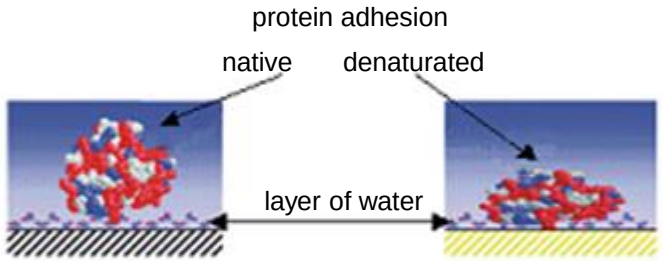
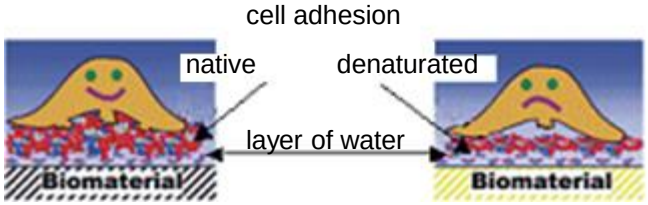
Different levels of understanding









Bengt K. Biological surface science. Current Opinion in Solid State and Materials Science. 1998, 3(5):451-459.

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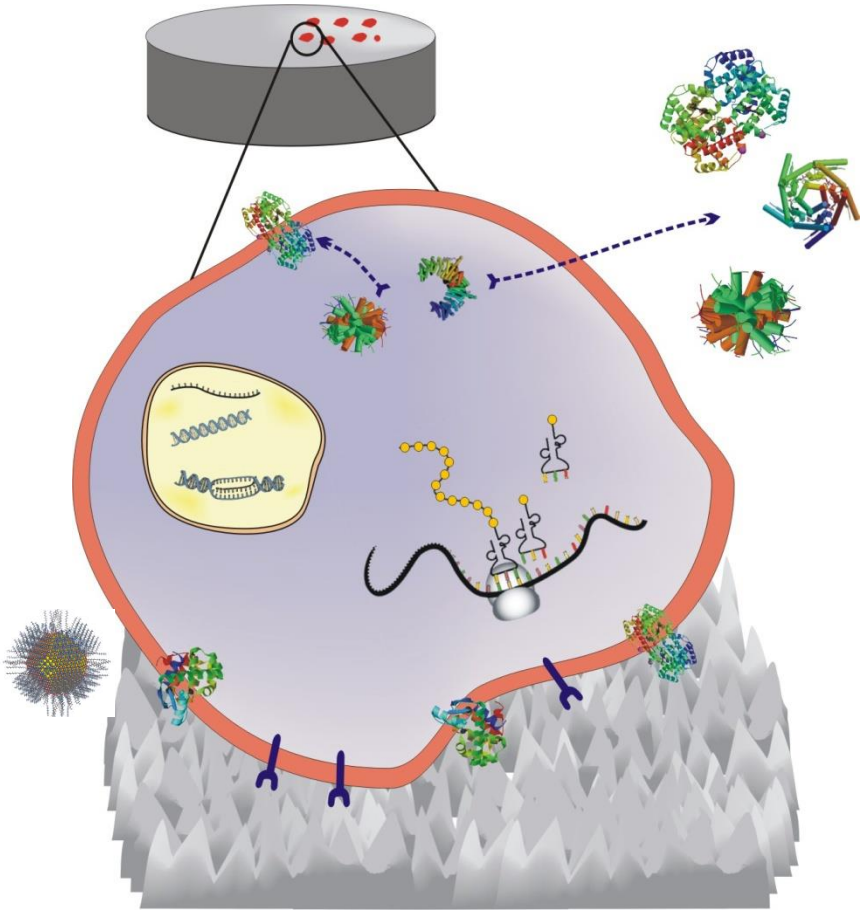
Different levels of understanding



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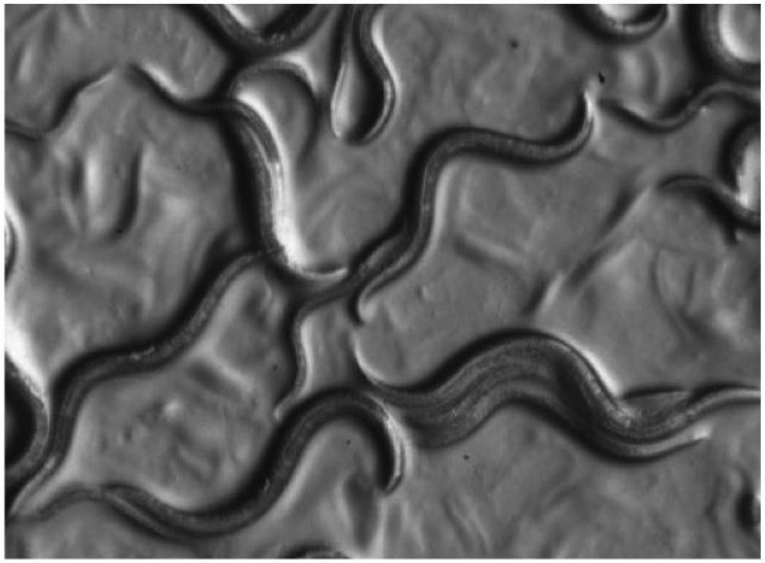
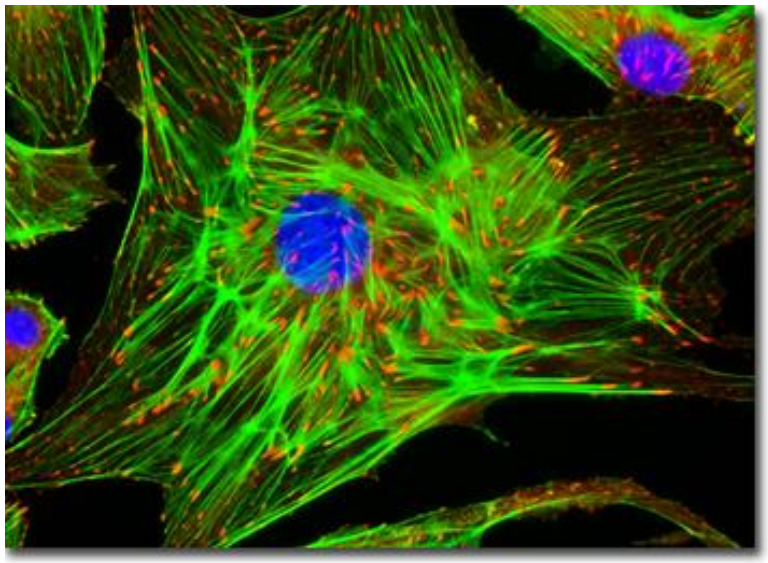
Cell Biology Unit

BIOLOGICAL OBJECTS

Caenorhabditis elegans
C. elegans



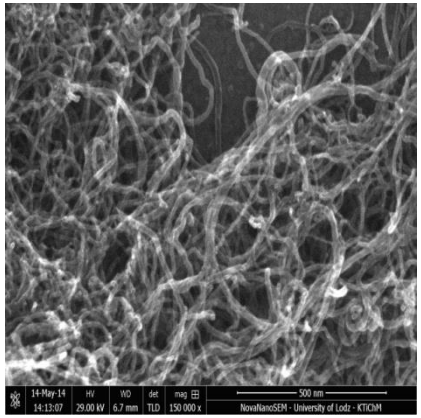
Endothelial cells
EA.hy926



Molecular and Nanostructural Biophysics Unit

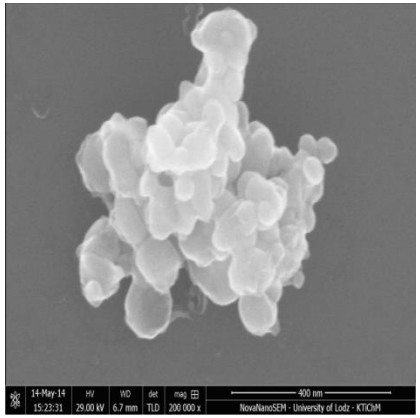
NANOPARTICLES

Multiwalled carbon Nanotubes (MWCNT)



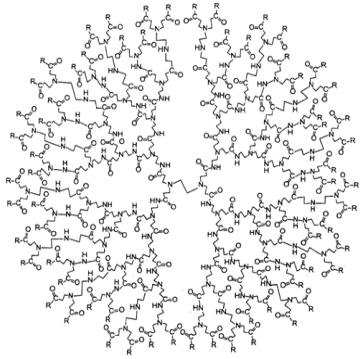
SouthWest Nanotechnologies Inc

Silver nanoparticles (SNP)



Sigma - Aldrich

Denrimers (PAMAM)



Dendritech Inc

https://data.epo.org/publication-server/rest/v1.0/publication-dates/20070321/patents/EP1382385NWB1/document.html

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METHODS USED IN RESEARCH

- *C. elegans* and endothelial cells culture in Cell Biology Unit
- *C. elegans* population observation
  (live, size and reproductive potential estimation)
- metal-oxide sensors system e-nose (nanoparticles detection)
- optical, fluorescence and scanning electron microscope imaging
- EDS spectrometry (X-ray spectrometry for elementary analysis)
- flow cytofluorymetry analysis (cell granularity, size and live/dead estimation)
- cyto- and genotoxicity tests (XTT and micronuclear tests)
- microarray gene expression analysis (transcriptome analysis)
- 2D-DIGE gel electrophoresis analysis (proteome analysis)
- MALDI-TOF/TOF and LC-ESI-MS/MS mass spectrometry analysis
  (peptide identification)

… and many other

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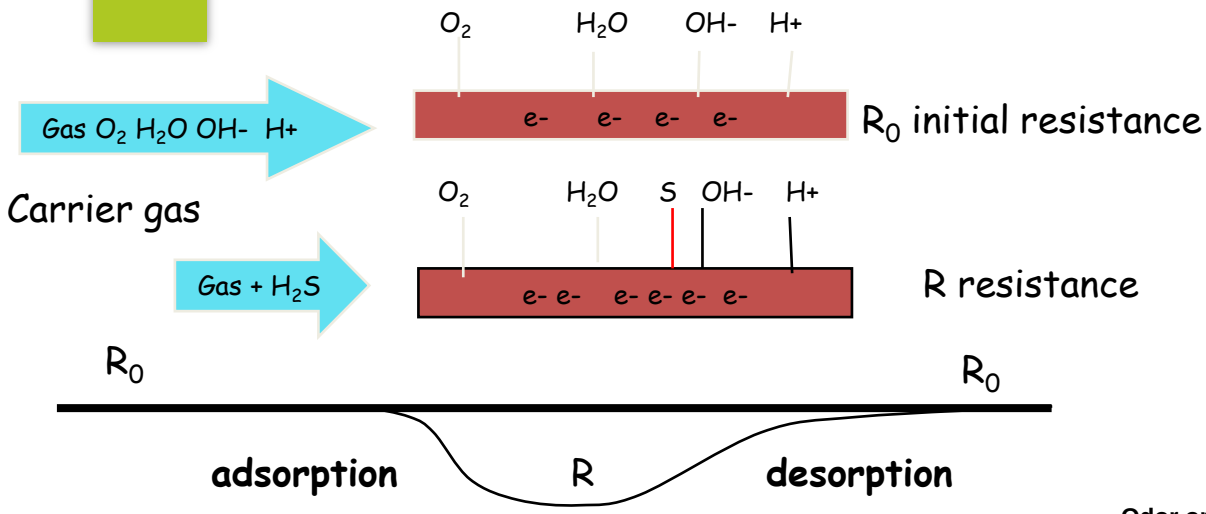


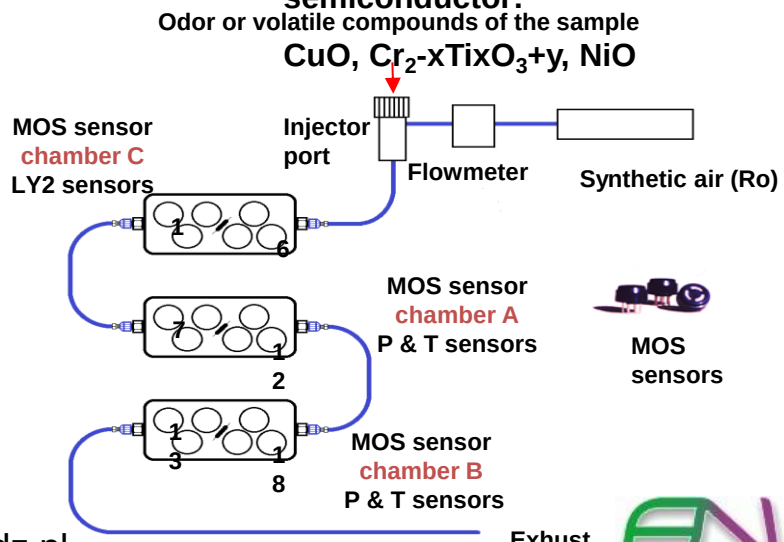
Sensors:

-n-type metal oxide semiconductor:

SnO_2, ZnO, WO_3, TiO_2, In_2O_3

-p-type metal oxide semiconductor:

CuO, Cr_2-xTiO_3+y, NiO



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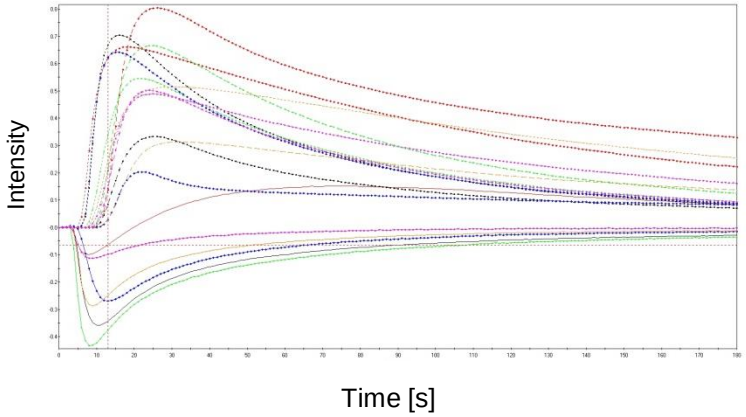
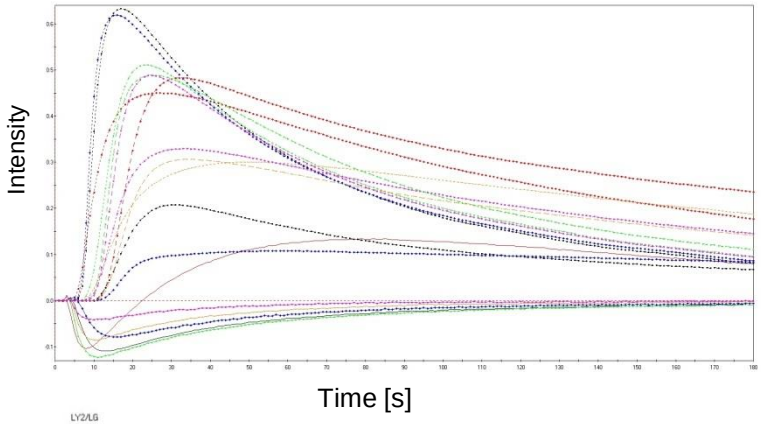
Comparison of resistance curves

Cell culture medium



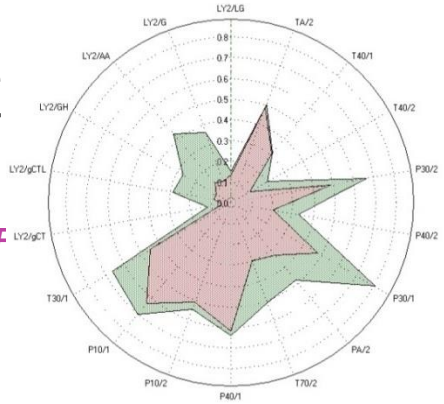
Cell culture medium without FBS



Comparison of resistance extreme values on radar graph for all sensors

Medium

Medium without F



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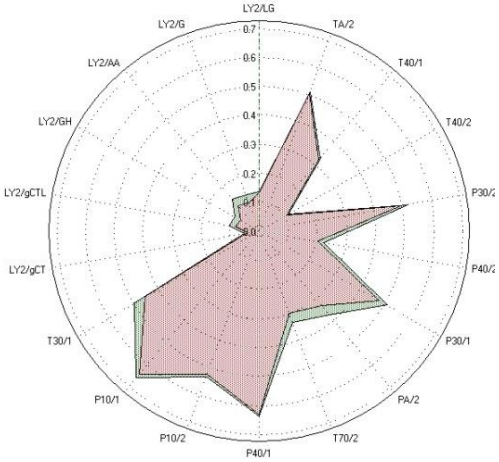
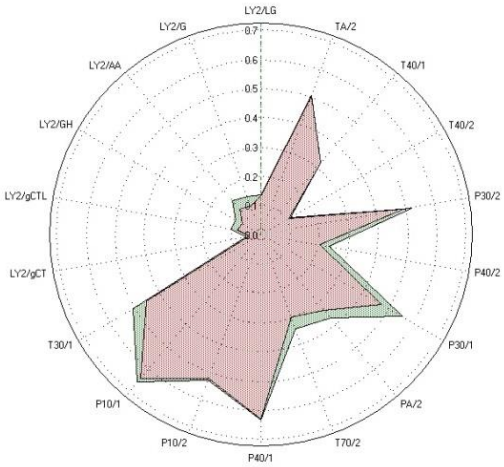
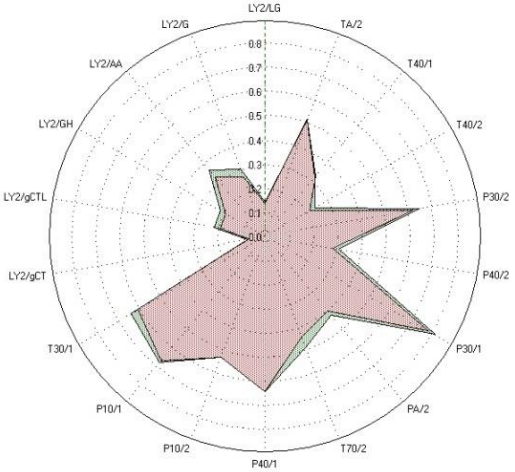
Nanoparticles detection using gas sensor array system



MWCNT in medium without FBS
Medium without FBS



SNP in medium without FBS
Medium without FBS



PAMAM Dendrimers in PBS
PBS

Molecular and Nanostructural Biophysics Unit

SCANNING ELECTRON MICROSCOPY

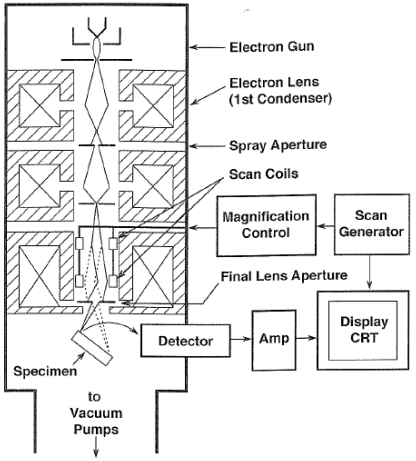
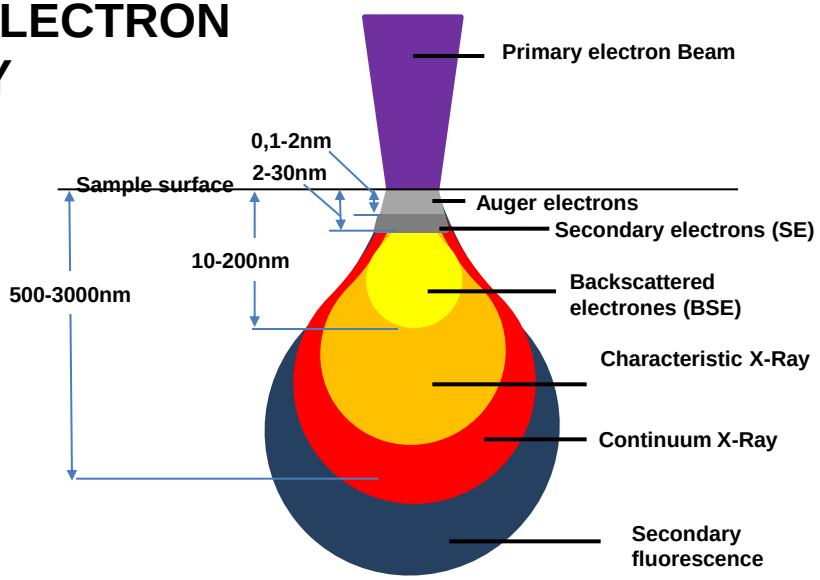
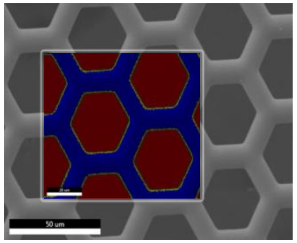
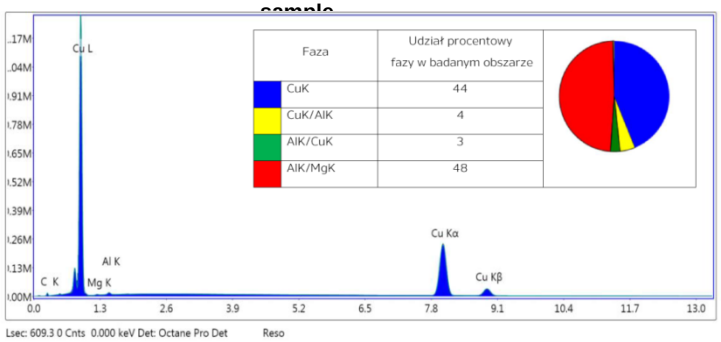


Figure 2.2. Schematic drawing of the electron column showing the electron gun, lenses, the deflection system, and the electron detector.





Range and spatial distribution of electron emission in the sample



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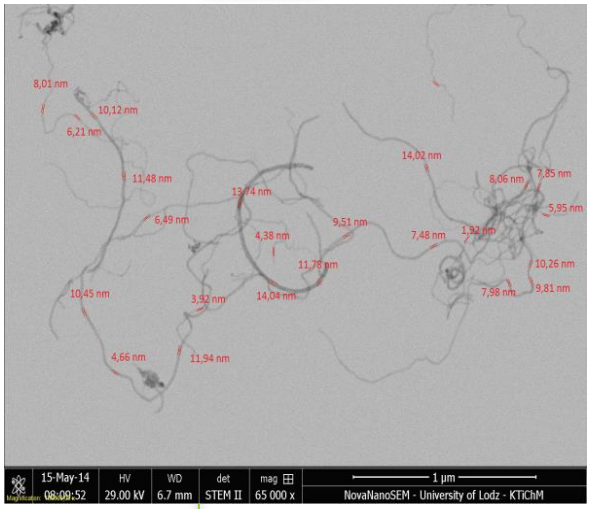
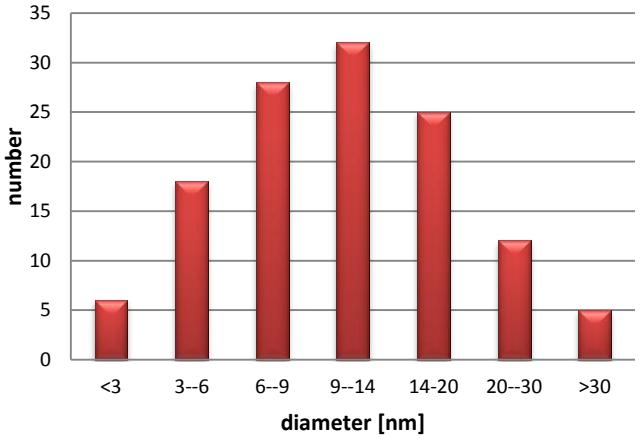
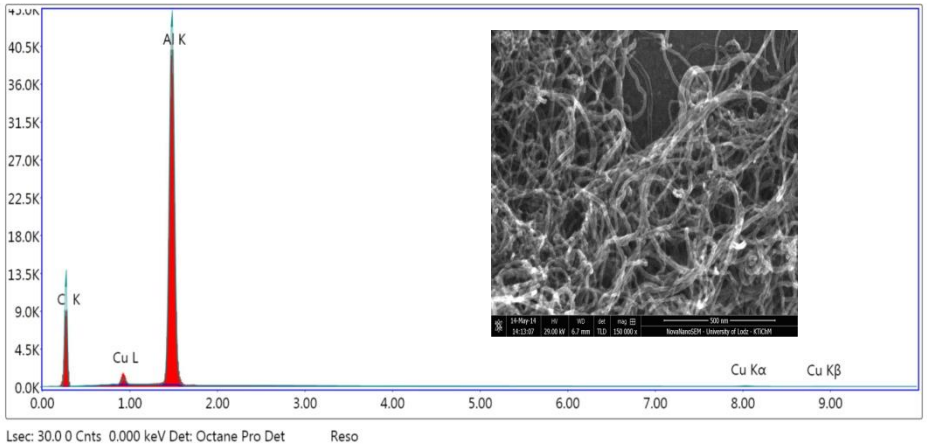
CMWNT





	SouthWest Inc.	measured
mean±SD [nm]		12.3 ± 7.7
median [nm]	6.6	10.4



element	concentration [%]	deviation [%]
C	58.9	9.6
Al	38.7	2.3
Cu	2.2	14.4

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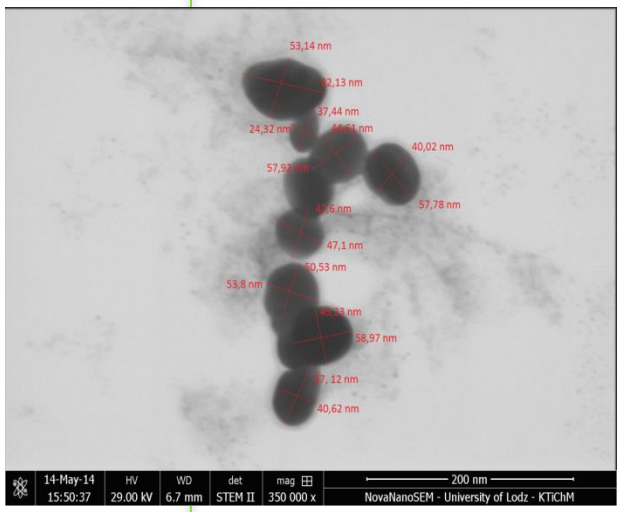
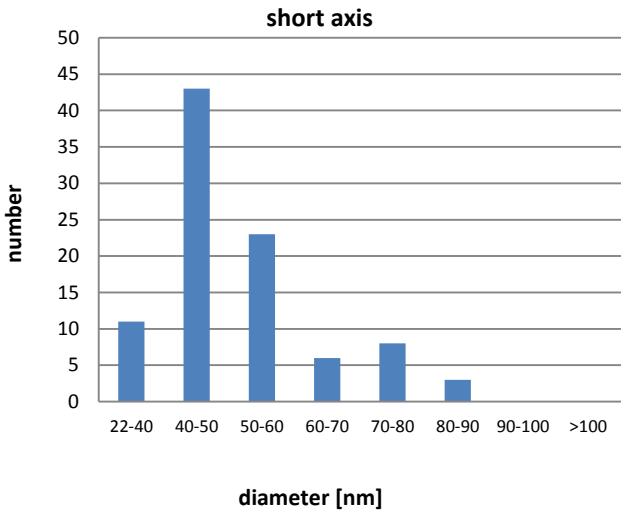
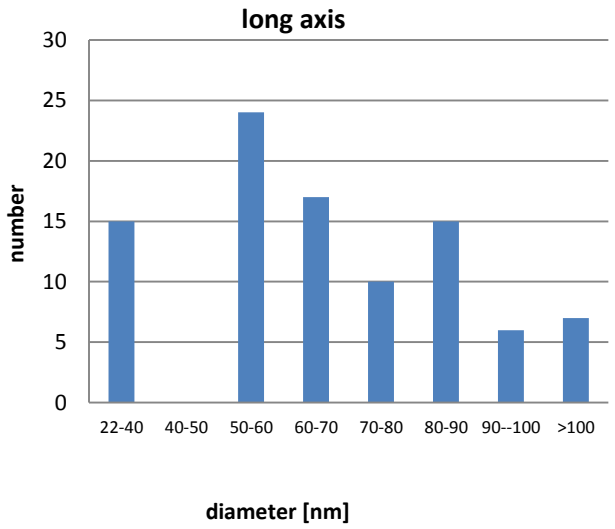
SNP

	Sigma
Diameter [nm]	<100







	short axis	long axis
mean ± SD [nm]	50.8 ± 13.0	69.3 ± 20.5
median [nm]	48.3	66

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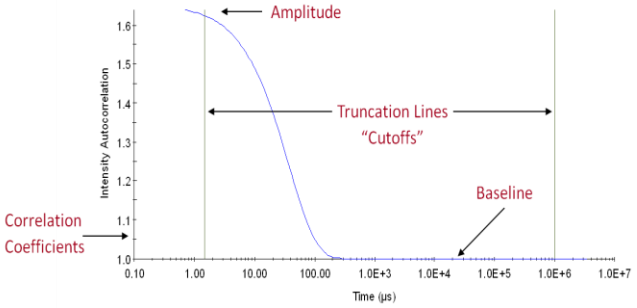
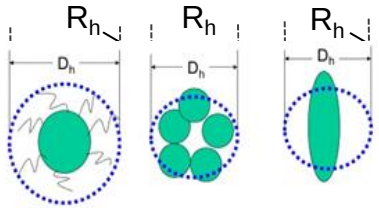
Dynamic light scattering



Stokes – Einstein relationship describes the relationship between particle size and the rate of their Brownian motion

$$R_h = \frac{k_B T}{6\pi n D_t}$$



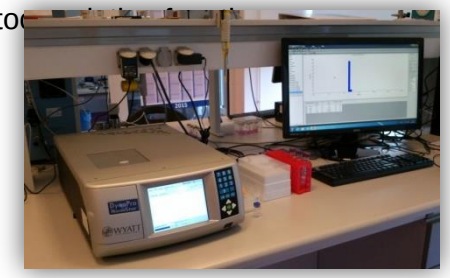
k_B – Boltzmann constant
T – temperature (Kelvin)
n – solvent viscosity
R_h – hydrodynamic radius

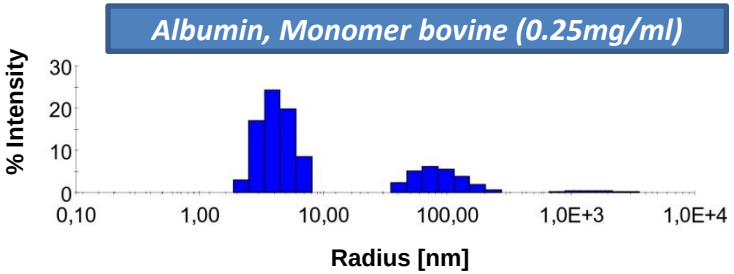
Autocorrelation function:

$$G^2(\tau) = \langle I \rangle^2 \left(1 + \alpha \exp\left(-2D_t q^2 \tau\right)\right)$$

$$q = \frac{4\pi n_0}{\lambda_0} \sin(\theta/2)$$

***D_t* – diffusion coefficient ,** calculated from the fitted autoc





Peak	Radius [nm]	%Intensity	%Mass
1	4.28	72.4	99.9
2	93.27	25.7	0.1
3	1594.89	1.9	0.1

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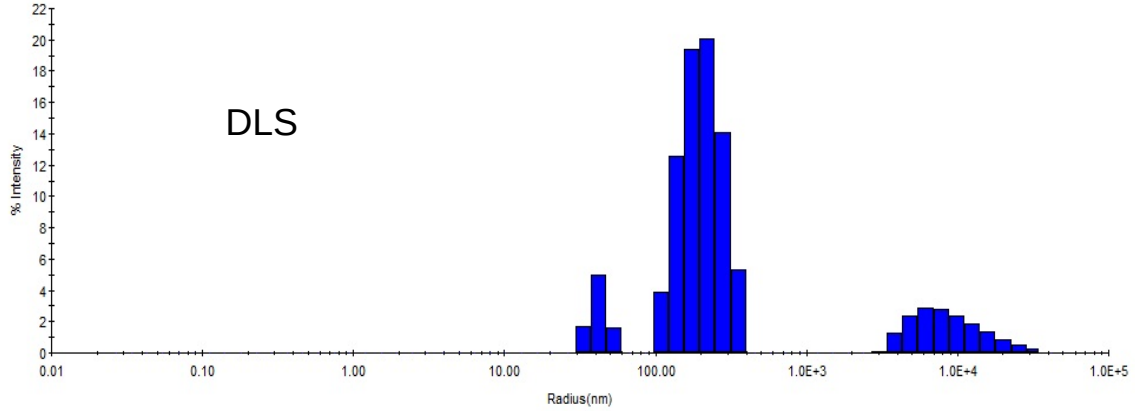
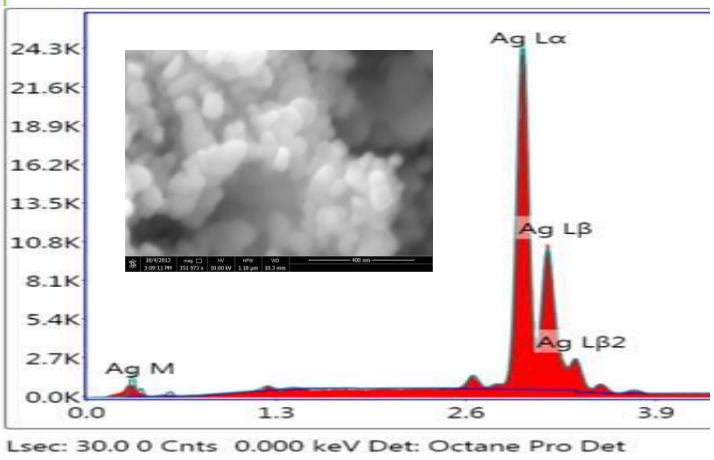
DLS





element	concentration [%]	deviation [%]
Ag	100	1.2

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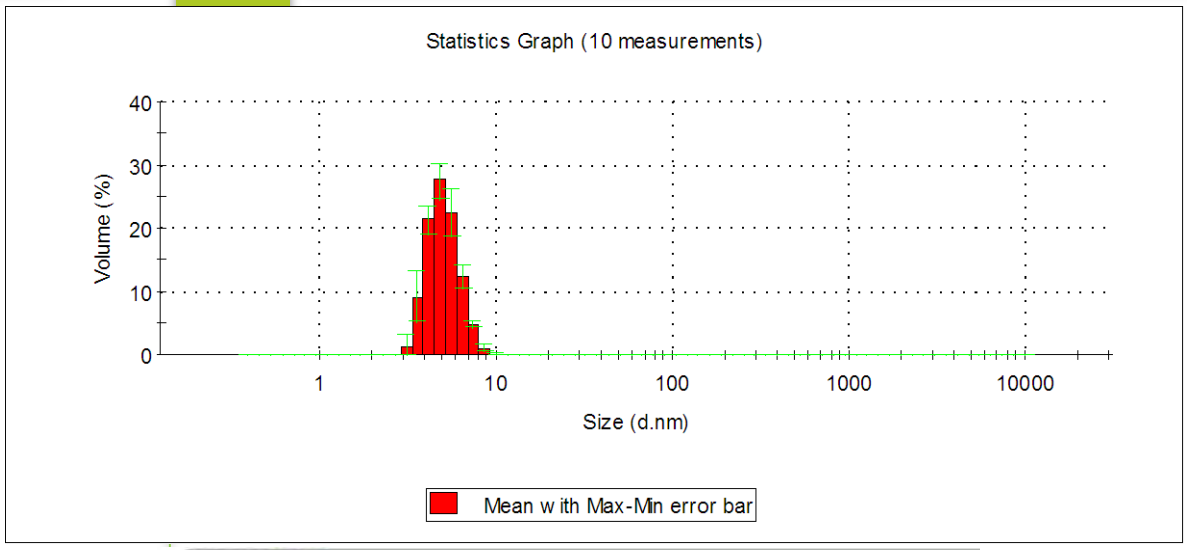
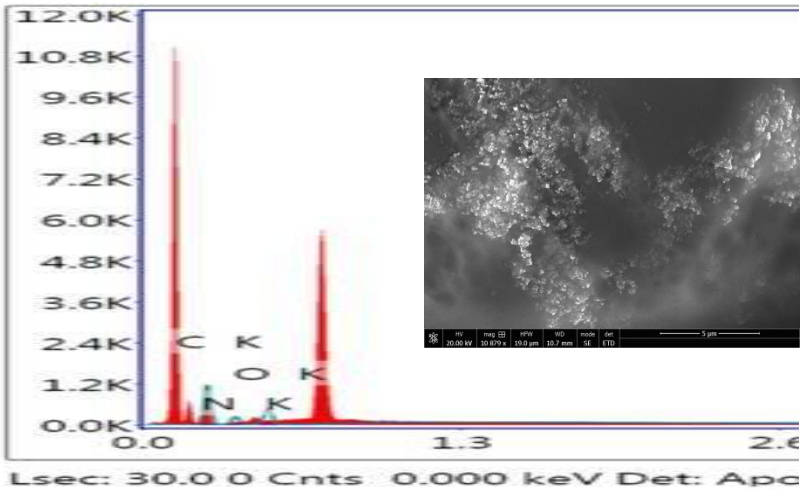


PAMAM

	Dendritech Inc	measured
diameter [nm]	4.5	4.5



element	concentration [%]	deviation [%]
C	36.1	6.2
N	28.4	13.1
O	35.6	11.8

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Cell Biology Unit

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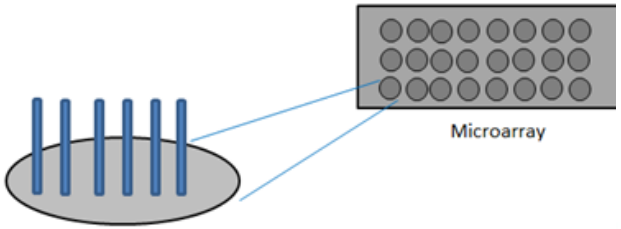
C. Elegans – population observations

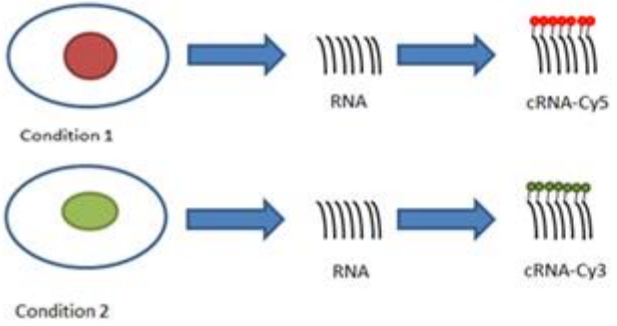
	MWCNT 10^{13}/ml			SNP 10^{13}/ml			PAMAM 10^{13}/ml		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Δ live	-	+	-	+	--	--	-	-	--
Δ size	---	---	----	---	---	----	---	----	----
Δ eggs			+			+++			

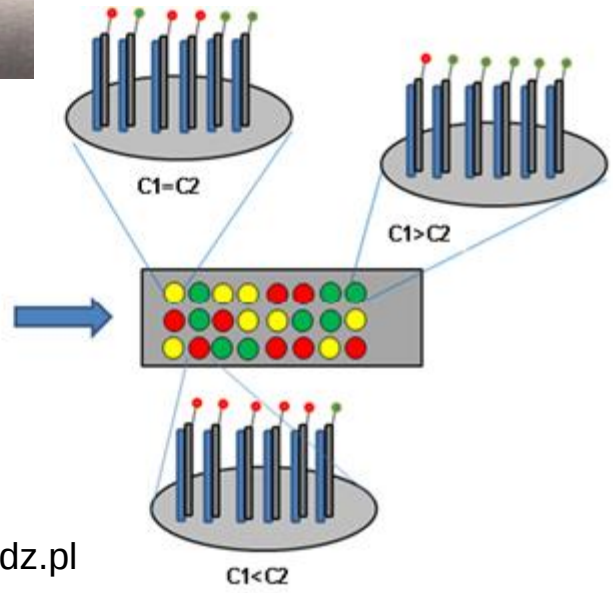
Δ number	increase	decrease
0-2	+	-
2-10	++	--
10-50	+++	---
>50	++++	----

Transcriptome and Proteome Unit

RNA MICROARRAY TECH







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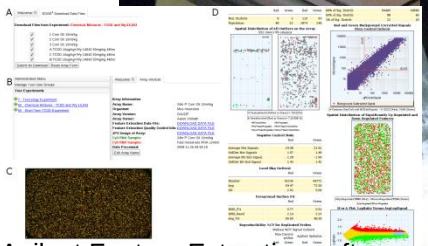
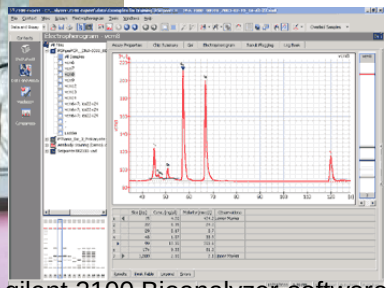
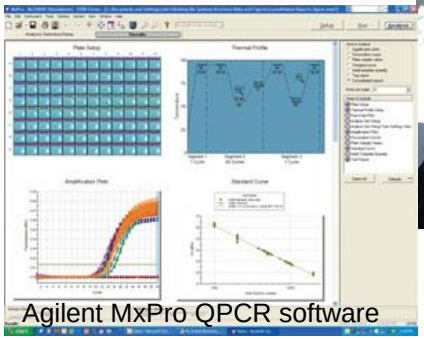
Transcriptomics Unit equipment and software



Agilent Feature Extraction software



Agilent 2100 Bioanalyzer software



Agilent MxPro QPCR software

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Transcriptomics and Proteomics Unit

Pathway name	Number of active genes	MWCNT	SNP	PAMAM
		Number of affected genes	Number of affected genes	Number of affected genes
Aging	3	3	-	-
Cytoplasmic ribosomal proteins	63	6	-	19
Insulin sygnaling pathway	8	2	2	-
Dauer formation	23	3	-	-
Immune responses in the intenstine	25	3	4	-
Metapathway signal transduction	107	6	9	-
Mitochondrial unfolded-Prorein response	18	2	-	-
II division – first embryonic mitosis	124	-	15	-
Fatty acid biosynthesis	17	-	4	-
Fatty acid Beta Oxidation	34	-	8	-
Vulval development	18	-	3	-
Glycogen Metaobolism	21	-	3	-
Glycolysis	25	-	3	4
DNA replication	25	-	-	8
LIN-12-Notch Lateral signaling	15	-	-	5
Sex determination	17	-	-	5
Translation factors	31	-	-	7
Total number:	564	25	51	48

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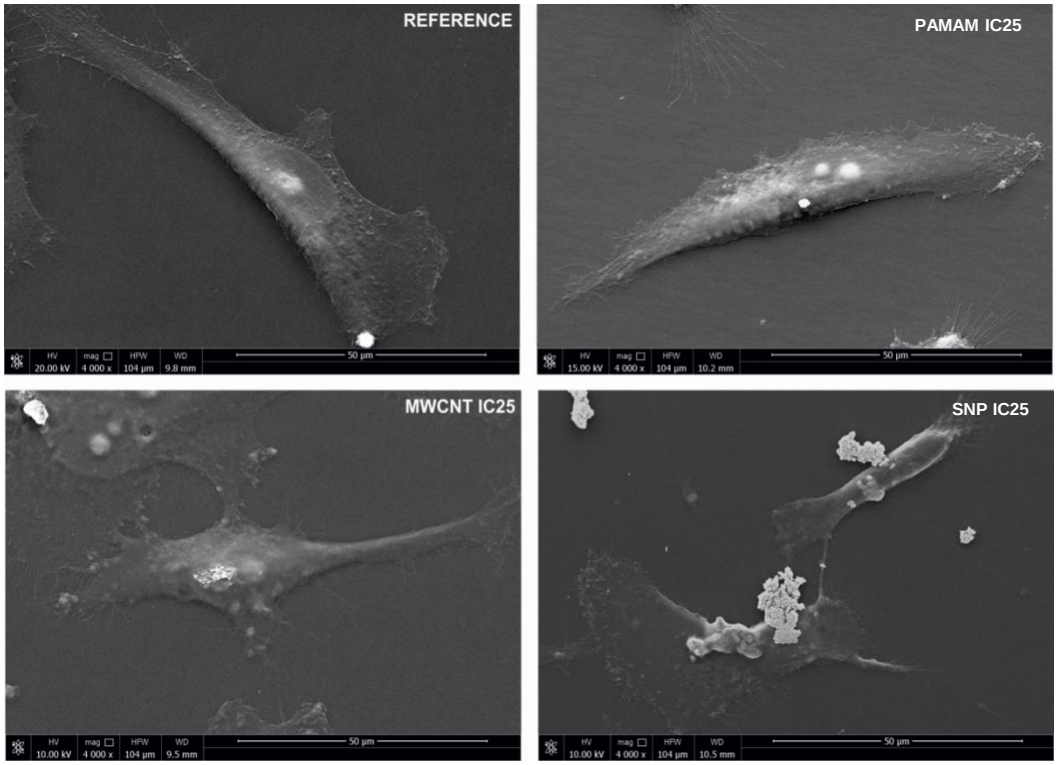
Molecular and Nanostructural Biophysics Unit



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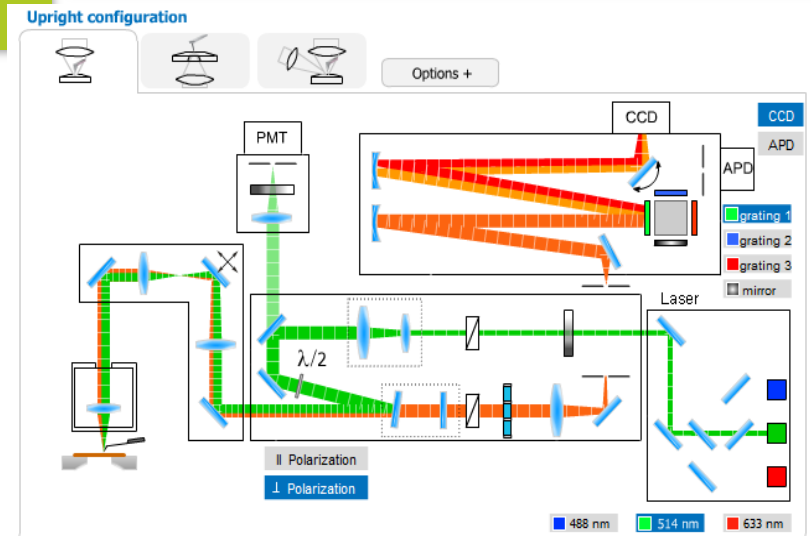
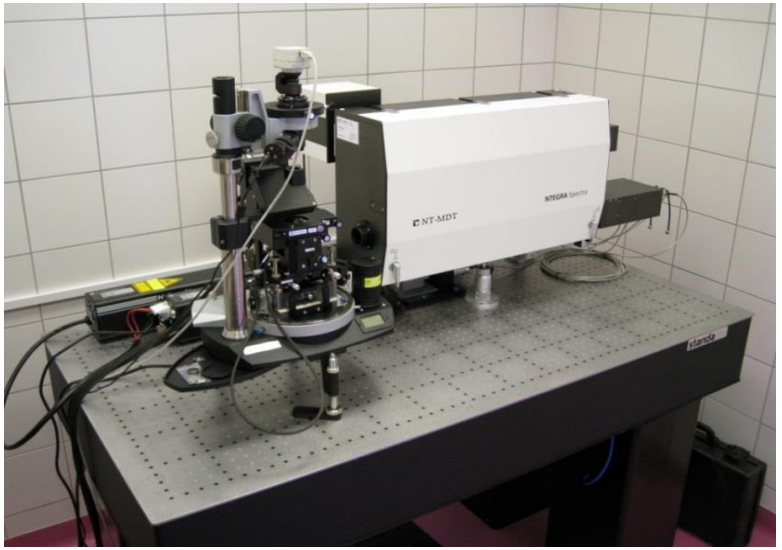
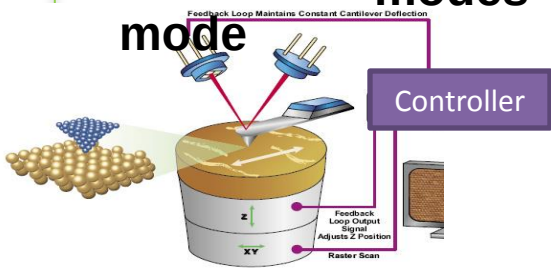
Molecular and Nanostructural Biophysics Unit



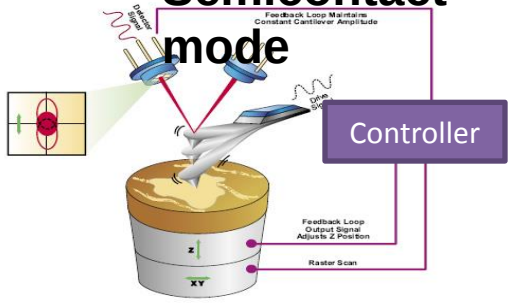


Basic AFM modes

Contact mode



Semicontact mode



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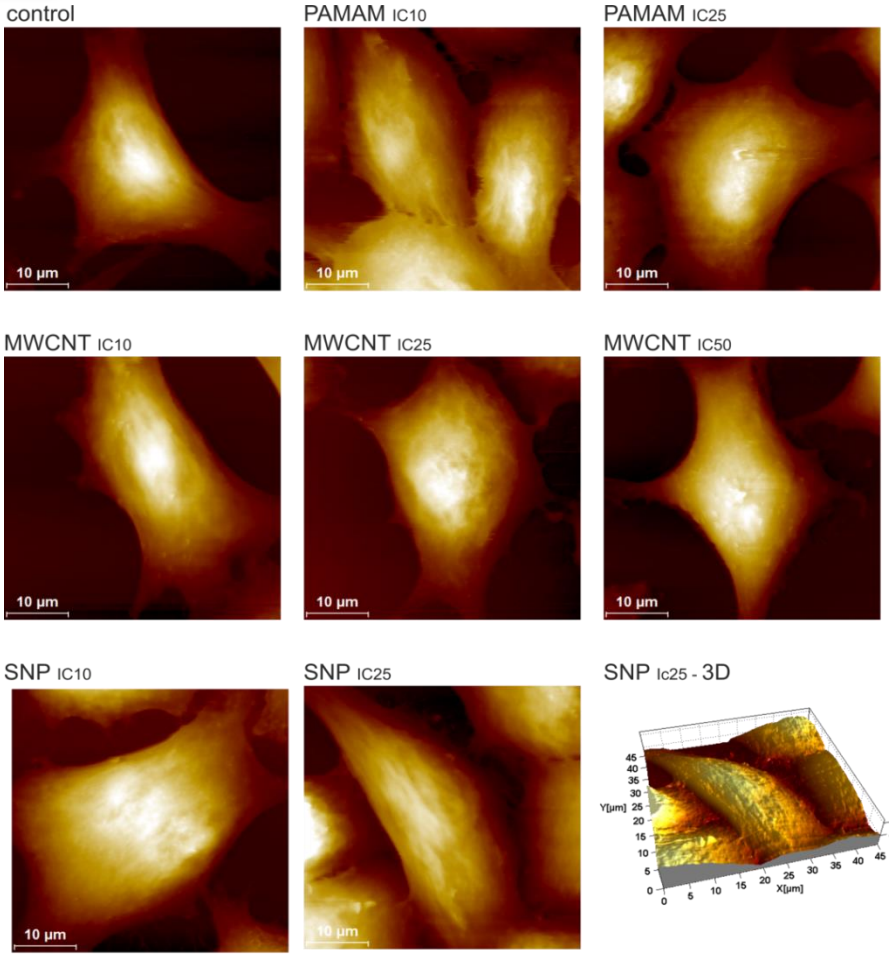
Molecular and Nanostructural Biophysics Unit



SAMPLE	DS [1/µm²]
Reference	155 ± 56
MWCNT IC25	401 ± 69 (**)
SNP IC25	294 ± 99
PAMAM IC25	239 ± 60

Average values of density of summits calculated for AFM images (2µmx2µm) for reference cells and incubated with nanostructures at IC25 concentration.
ANOVA, **P < 0.01

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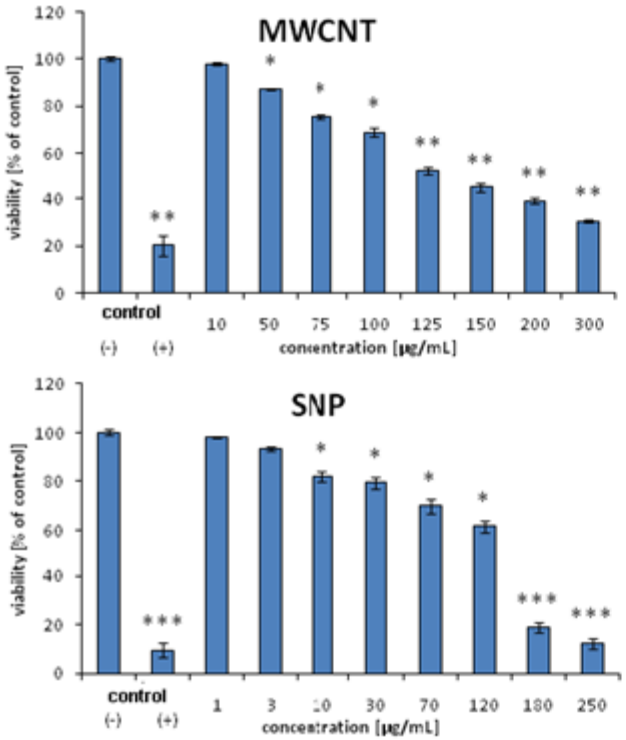
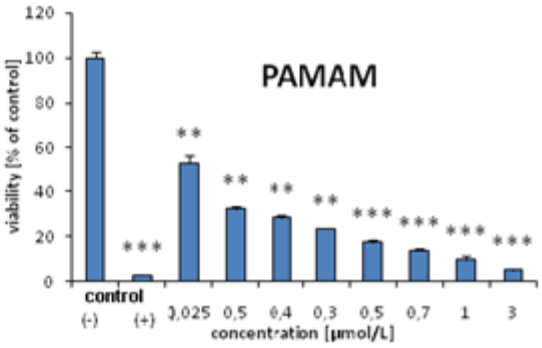
Cell Biology Unit

Cell viability XTT test





IC parameter	MWCNT [µg/mL]	SNP [µg/mL]	PAMAM [µM]
IC10 ± SD	51.7 ± 0.3	2.8 ± 0.1	0.005 ± 0.0001
IC25 ± SD	74.1 ± 2.1	74.8 ± 4.5	0.012 ± 0.001
IC50 ± SD	166.6 ± 11.7	139.6 ± 7.1	0.024 ± 0.003

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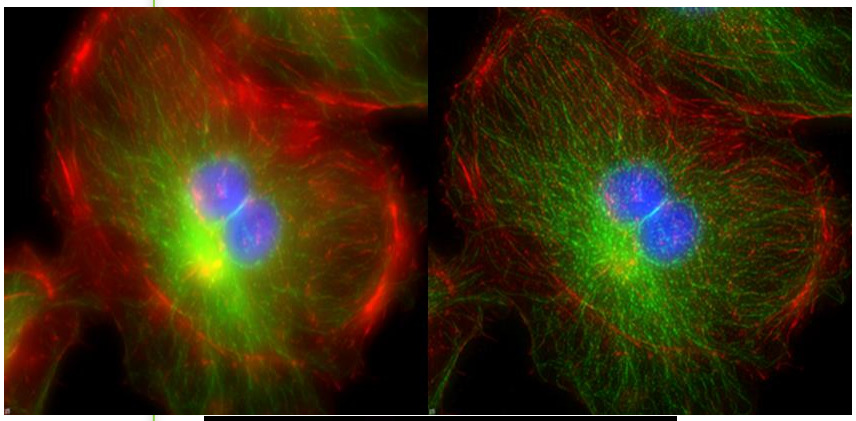
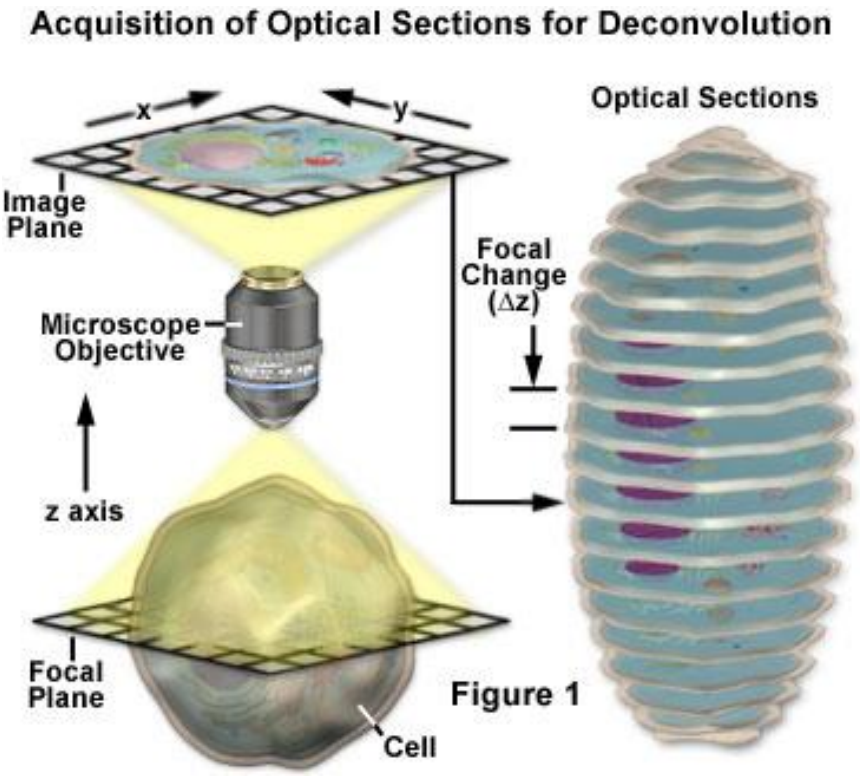
Cell Biology Unit

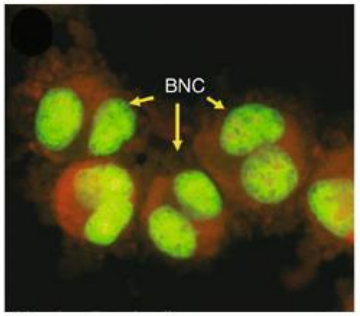
InCell Analyzer 2000
Non-confocal high content cell analysis system with advanced function of deconvolution



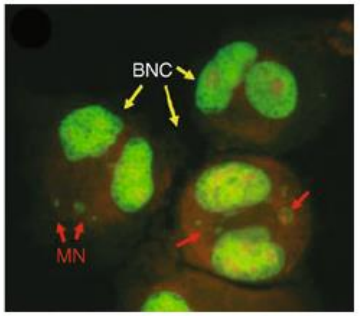


Cell Biology Unit

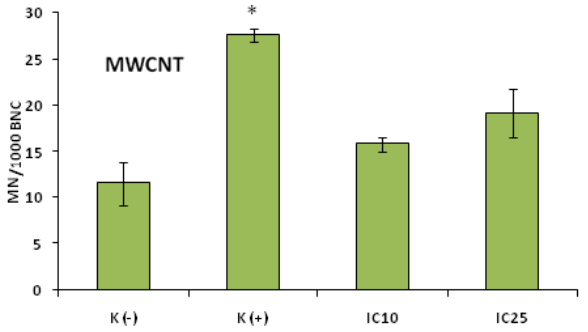
Cell genotoxicity micronucleus test

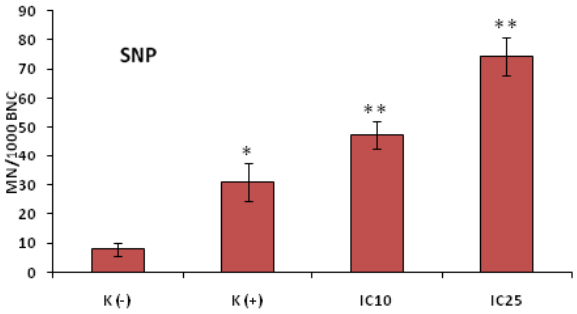


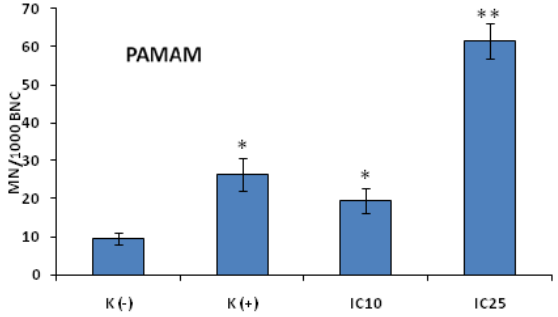
CONTROL



TRATED



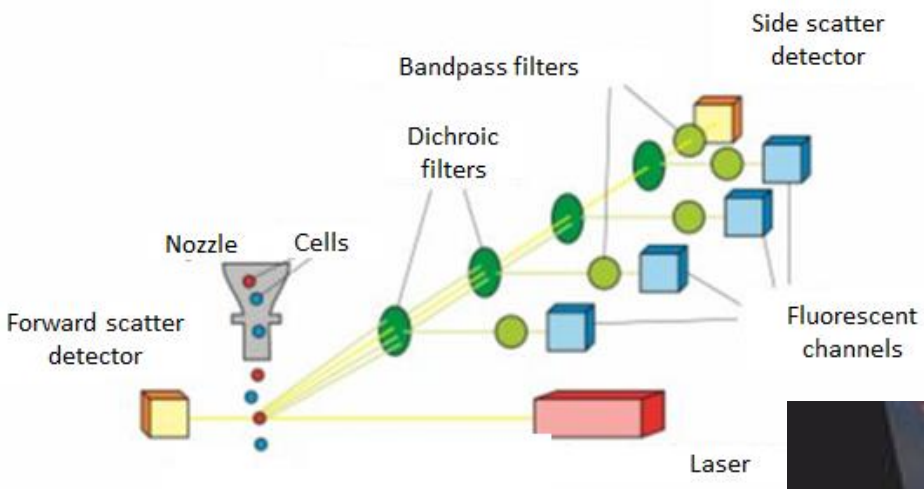


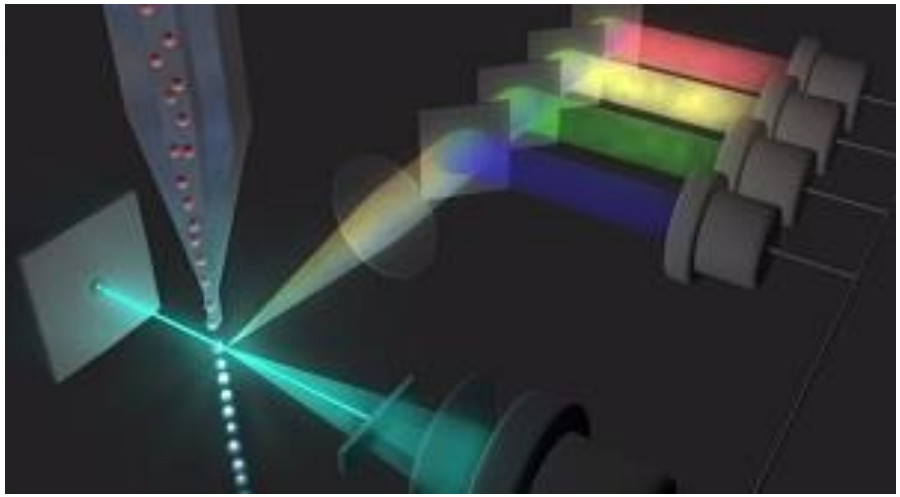


Parametr IC	MWCNT [µM]	SNP [µM]	PAMAM [µM]
IC50	9.9×10^{-11}	1.4×10^{-11}	2.4×10^{-2}

Cell Biology Unit

Flow cytometer





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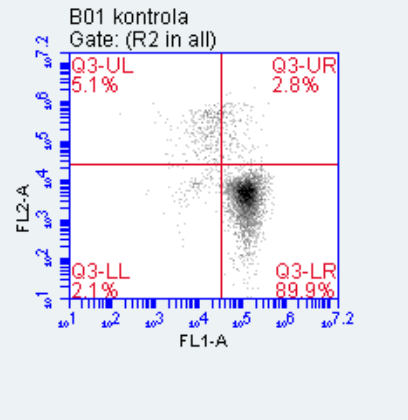
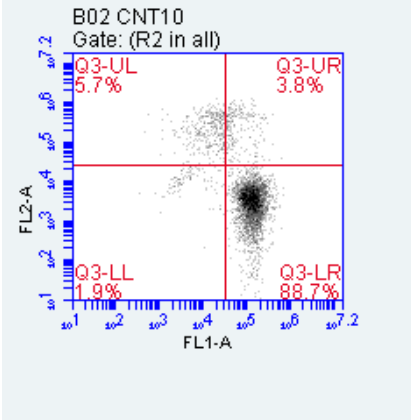
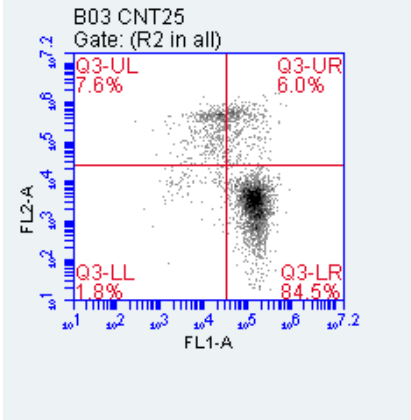
Cell Biology Unit

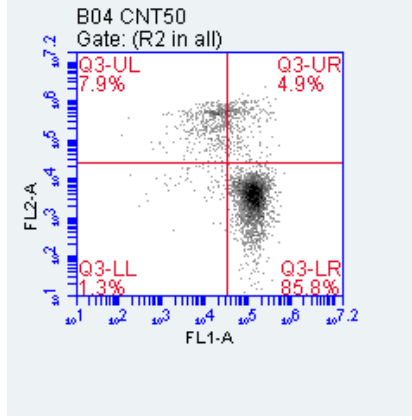
MWCNT

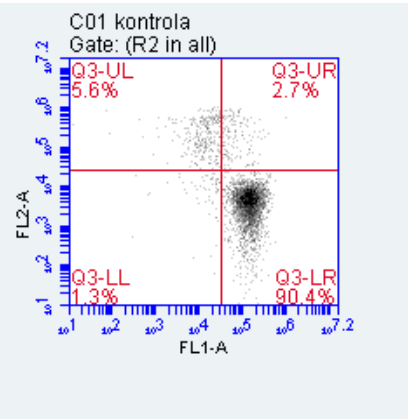
SNP

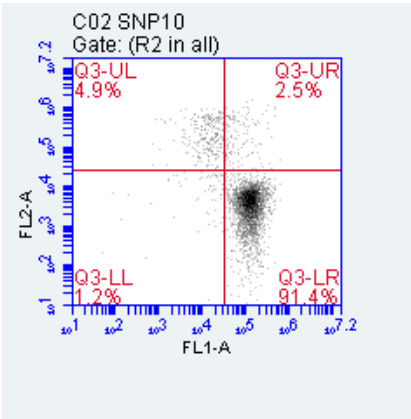


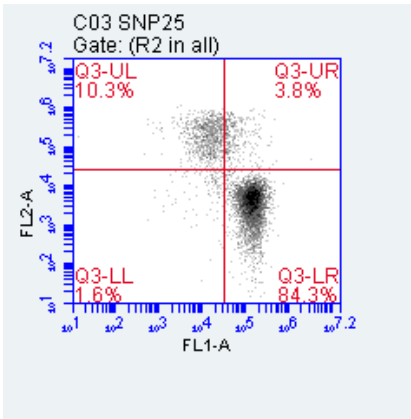


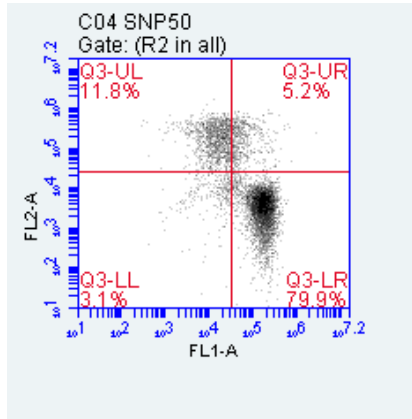












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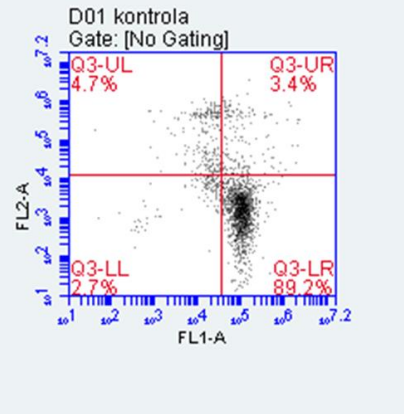
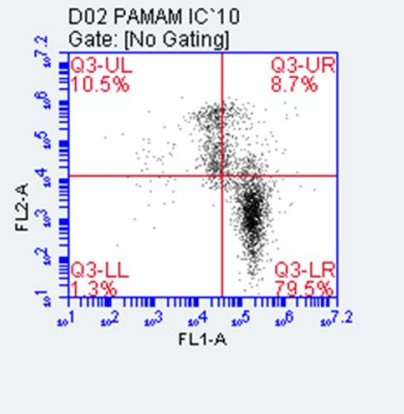
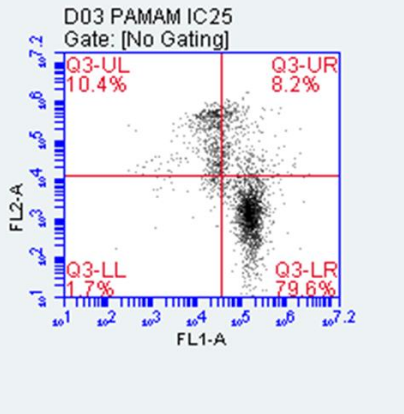
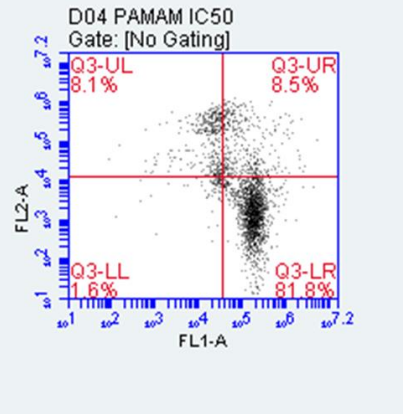
Cell Biology Unit

PAMAM









	LIVE % OF TOTAL
CONTROL	91,5
MWCNT IC10	79,3
MWCNT IC25	78,3
MWCNT IC50	75,7
SNP IC10	92,3
SNP IC25	84,1
SNO IC50	72,4
PAMAM IC10	87,1
PAMAM IC25	87,4
PAMAM IC50	81,2

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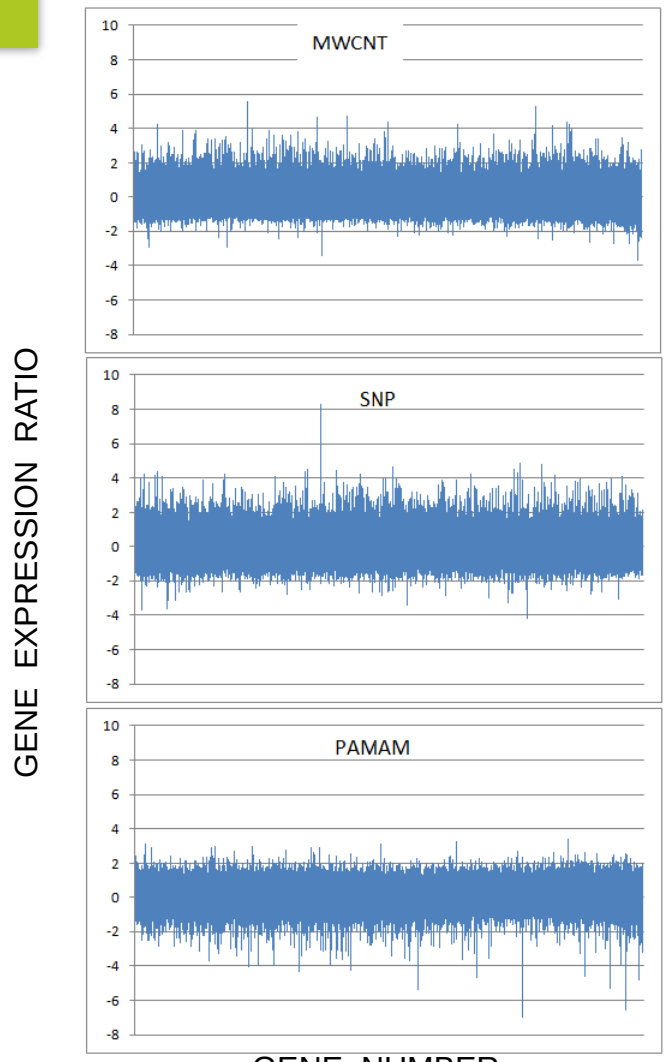
Transcriptomics and Proteomics Unit



Identified:
13 signalling pathways with changed level of gene expression

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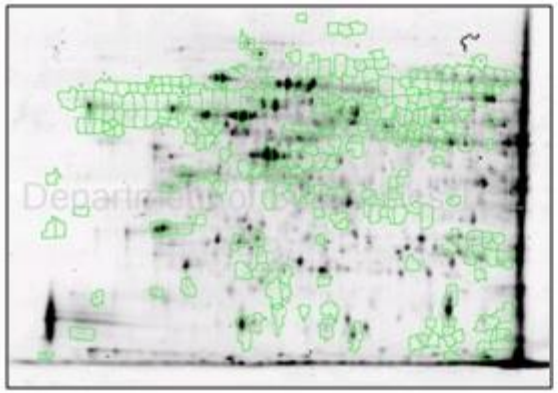
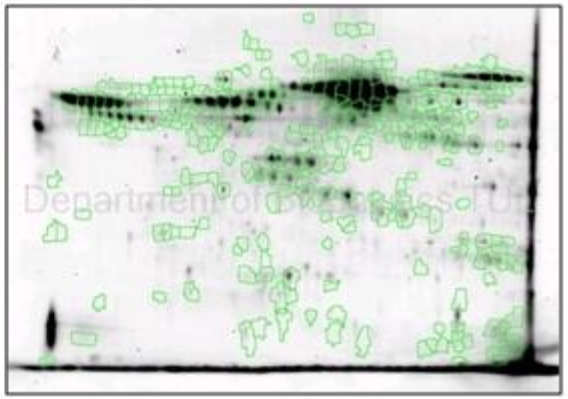
Transcriptomics and Proteomics Unit

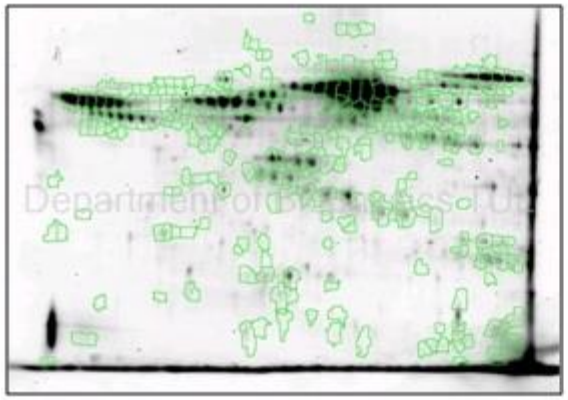
2D-DIGE

CONTROL

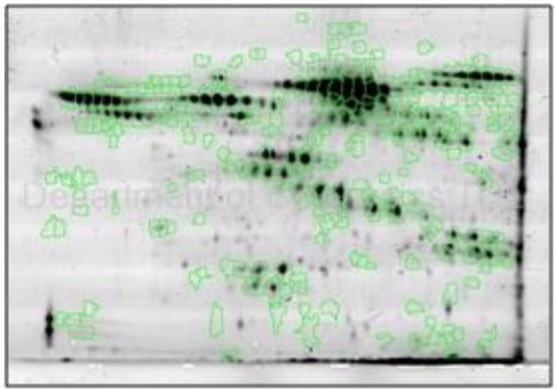


MWCNT





SNP



PAMAM

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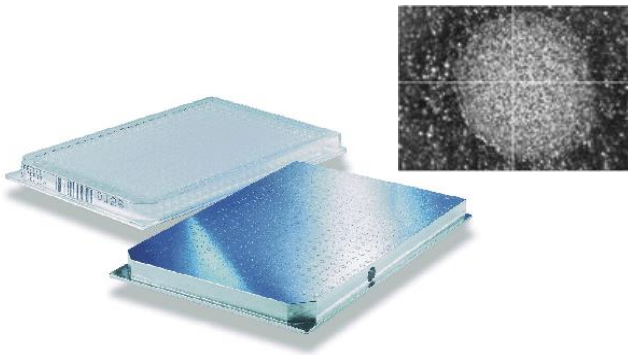
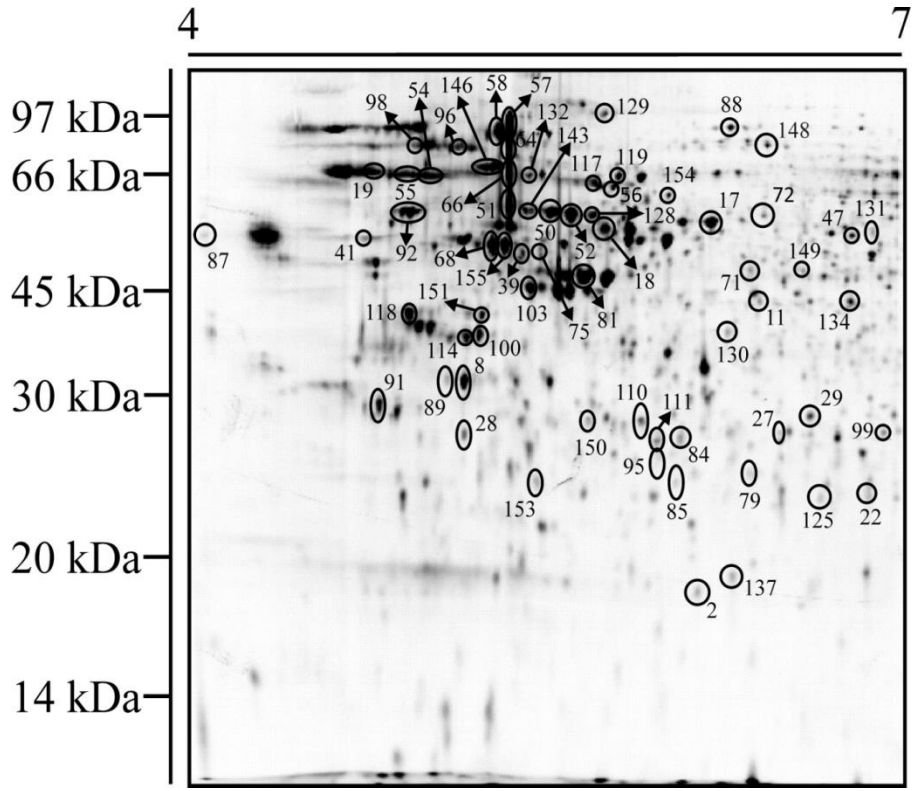
Transcriptomics and Proteomics Unit

Identified:
57 proteins with changed level of expression



steel target for sample application



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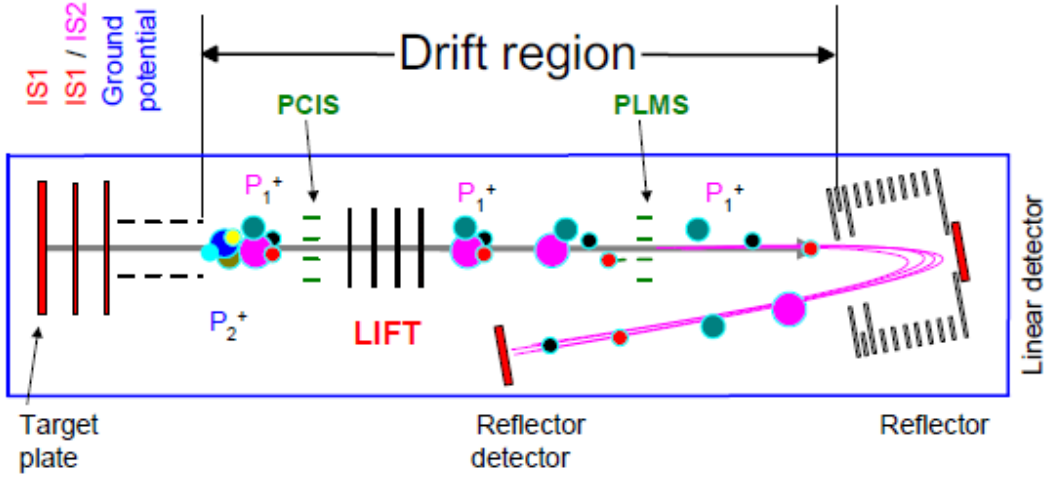
Transcriptomics and Proteomics Unit



Kinetic energy of ion leaving the source with mass *m* and total charge *q=ze*

$$qVs = \frac{mv^2}{2} = Ek$$

Ion will run distance *d* with uniform motion in time *t*

$$t = \frac{d}{v}$$

Measurement t^2 allows calculation mass to charge ratio *m/z* lub *m/q*, assuming a constant value of the expression in parentheses

$$t^2 = \frac{m}{q}\left(\frac{d^2}{2Vs}\right) = \frac{m}{z}\left(\frac{ed^2}{2Vs}\right)$$

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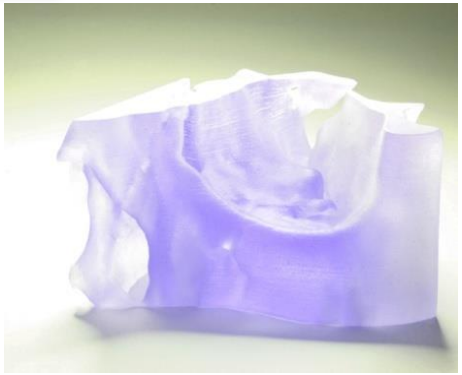
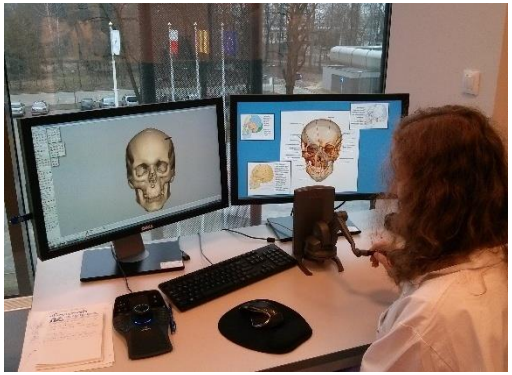
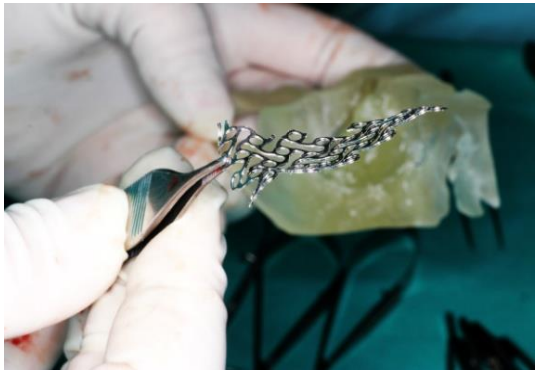
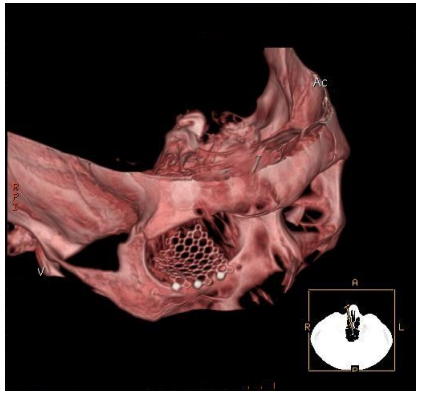
Personalized Medical Implant Unit

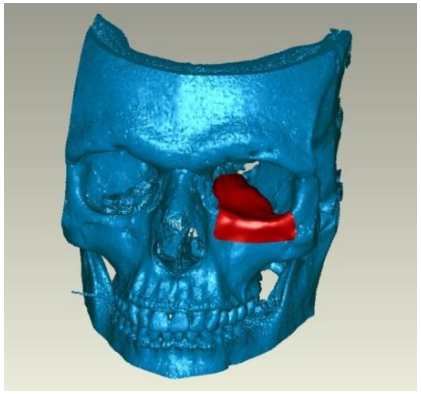








Sensable Phantom Desktop -
Haptic device,
EDEN 350 - 3D Printer,
Software:
Reverse engineering software,
CAD - Solidworks 2013,
Dedicated software for conversion
of CT data to 3D models.



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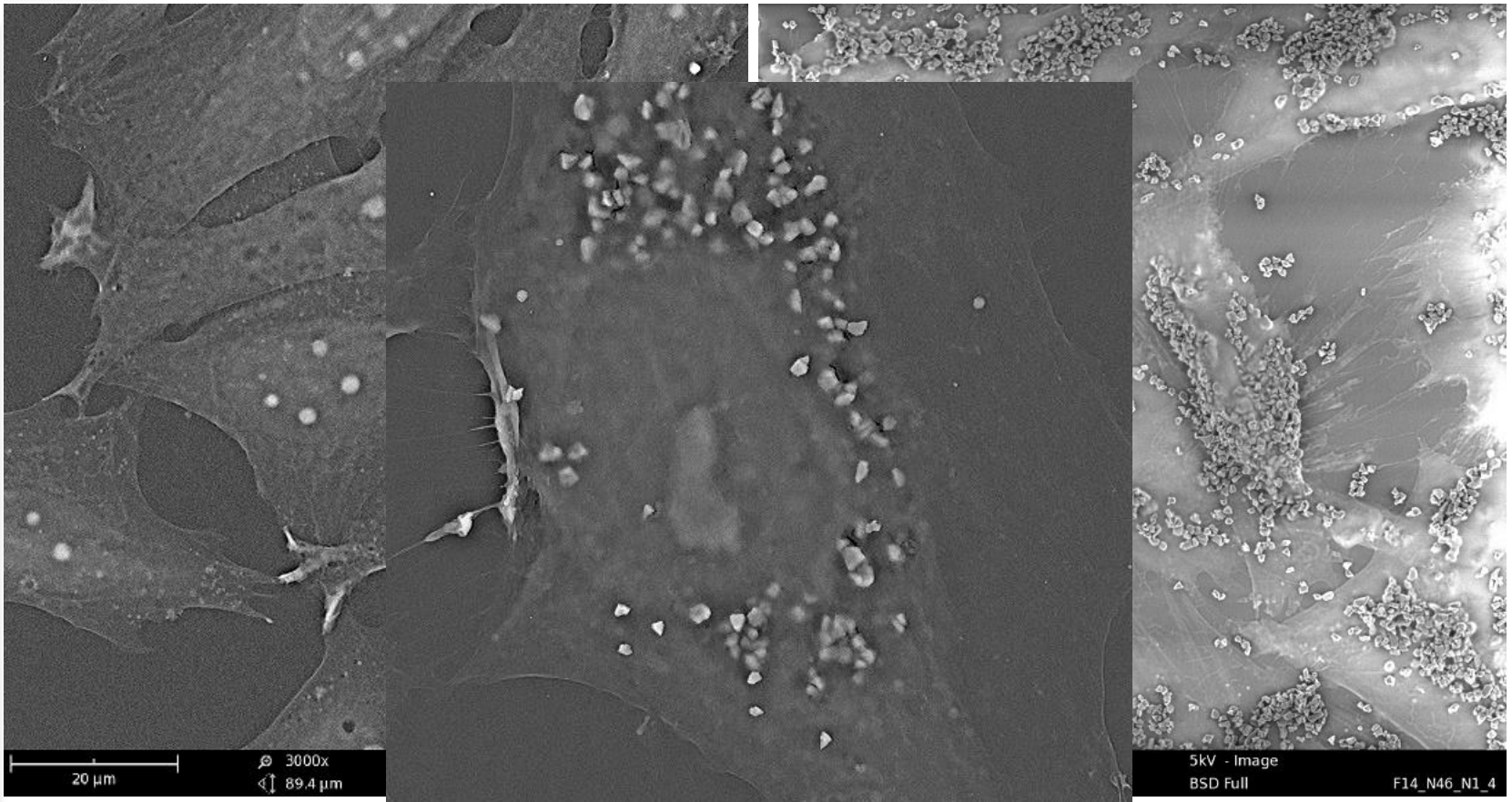
Microdiamonds and osteoblast cells



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