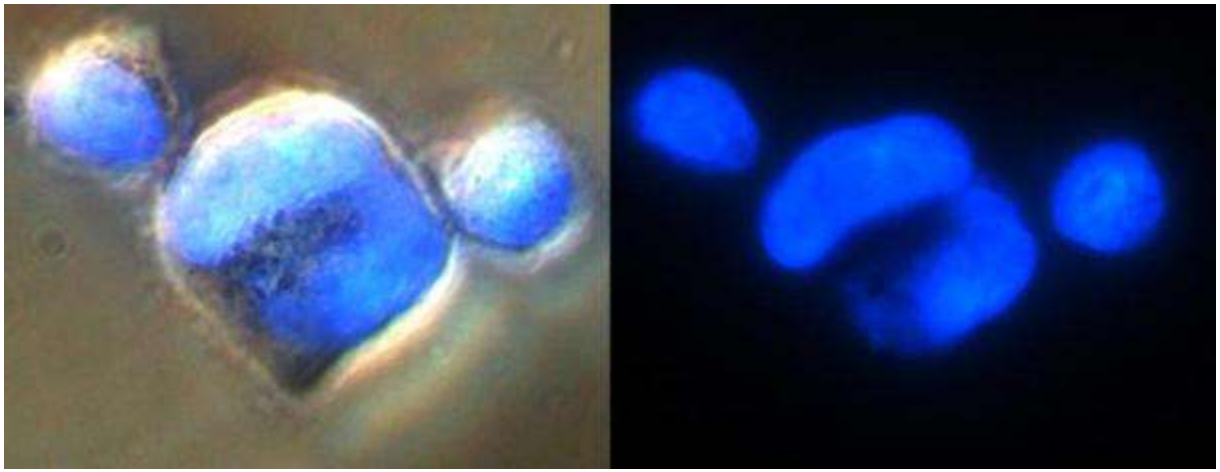
Plasmon-Resonant Gold Nanoparticles as Drug Carriers and Optical Labels for Cytological Investigations

O. Bibikova, S. Staroverov, A. Prilepskiy, V. Bogatyrev



Nanobiotechnology Laboratory, IBPPM RAS
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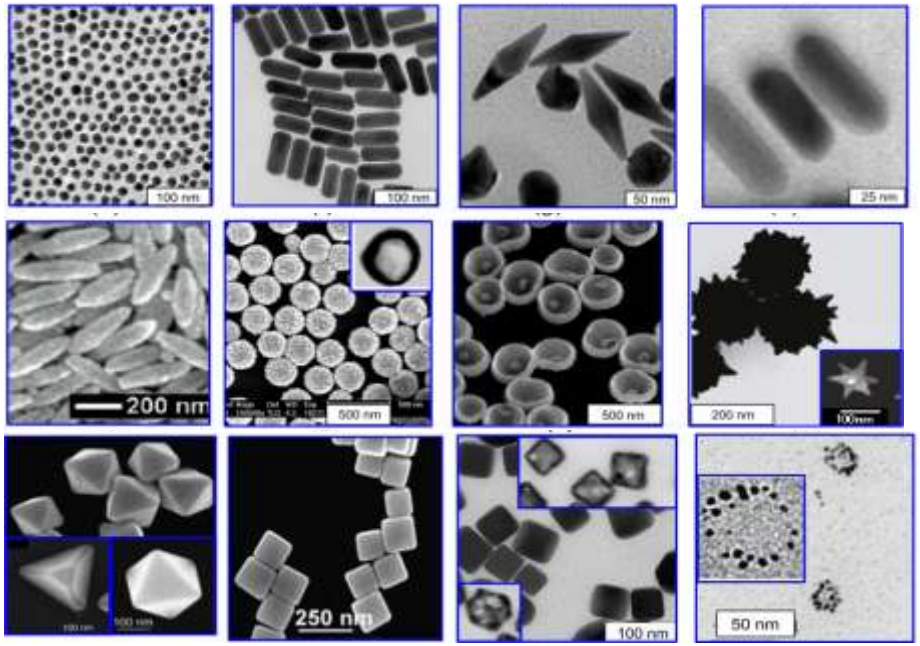
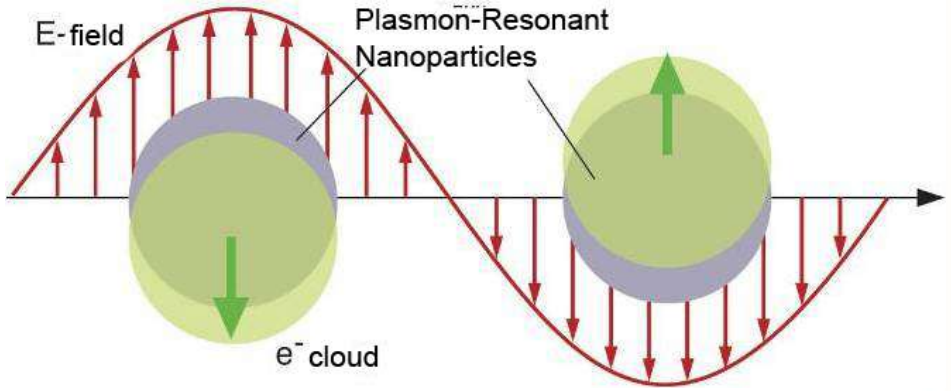
Plasmon-Resonant Nanoparticles



Different types of nanoparticles

Khlebtsov et. al. Chem Soc. Rev. 2011

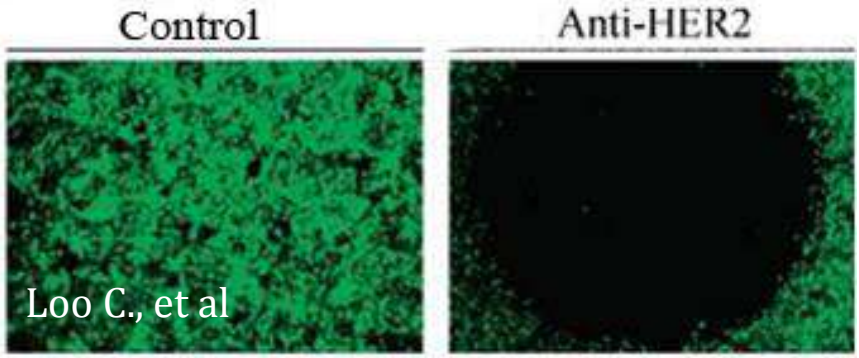


Plasmon-resonant property of nanoparticles

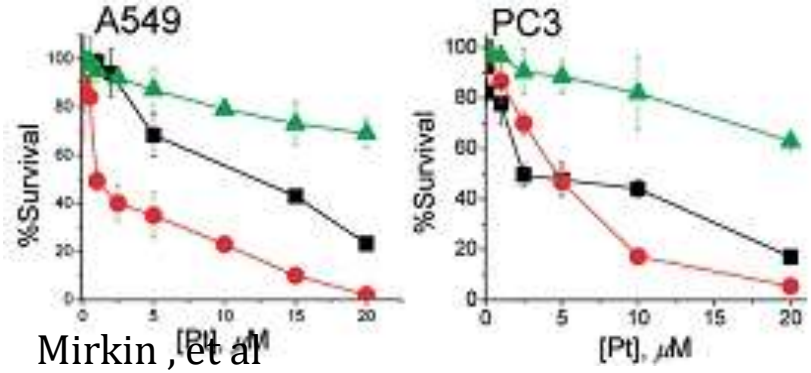
K. Fandler et. al. Adv. Mater. 1996

Gold Nanoparticles

Therapy

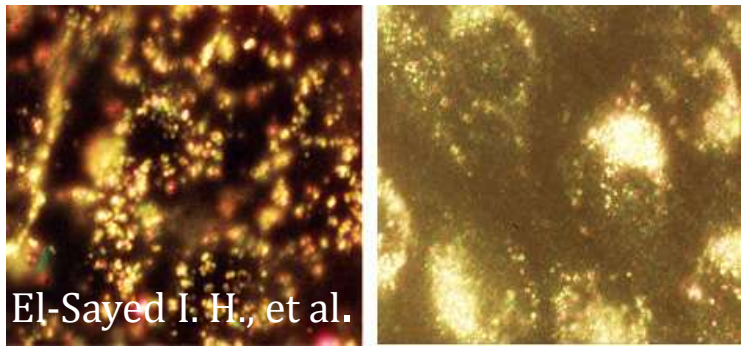


Selective photothermolysis of cancer cells with antibody-conjugated gold nanoshells

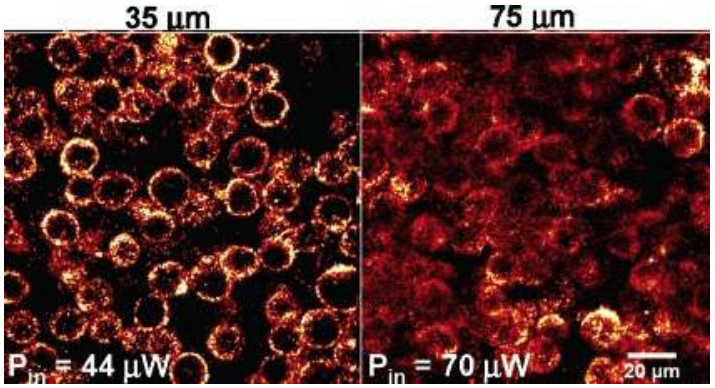


Cytotoxicity profiles of Pt-DNA-Au NP (red circles), cisplatin (black squares), and 1 (green triangles) with U2OS, A549, HeLa, and PC3 cells

Diagnostic



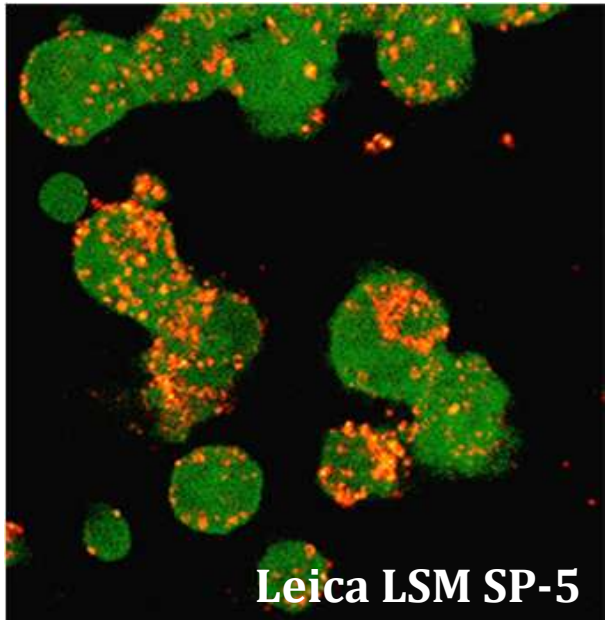
Dark-field images of cancer cells with anti-EGFR-conjugated gold nanoparticles



Cancer cells with antibody-conjugated gold nanorods

The Aim of the Research

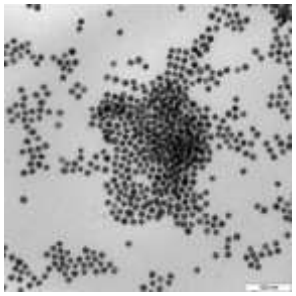
The aim is to investigate the penetration of gold nanoparticles with variable morphology and surface functionalization into animal cells by using mixed labels and to explore the influence of complex consist of gold nanoparticles (GNPs) anti-cancer drug prospidin to the physiological functions (endocytosis, respiratory activity, viability) of tumor cell lines.



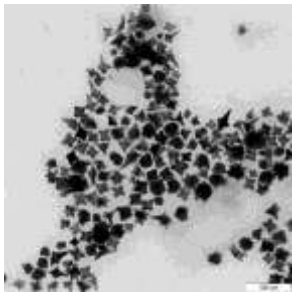
The confocal microscopy image of cells incubated with gold nanoparticles

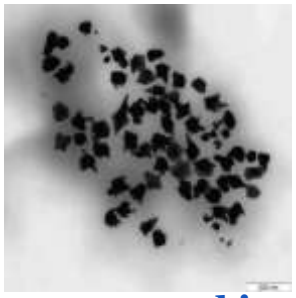
Materials and Methods

nanospheres

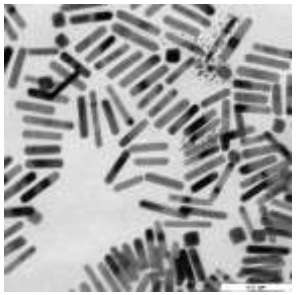


nanostars





nano-sea-urchins



nanorods

Nanoparticles (GNP):
Gold nanospheres with diameter 15 nm and 50 nm (CG 15 and CG50), CTAB-coated gold nano-sea-urchins (NSUs), HEPES-coated gold nanostars (NS), CTAB-coated gold nanorods (NR)

Fluorescent labels:
PI (3 mkg/ml), DAPI (0,25 mkg/ml) and AO (3 mkg/ml)

Anticancer drug:
Prospidin

Cells:
HeLa and SPEV-2 cancer cell lines

Confocal microscope

Leica TCS-SP5

Dark-field and fluorescent microscope

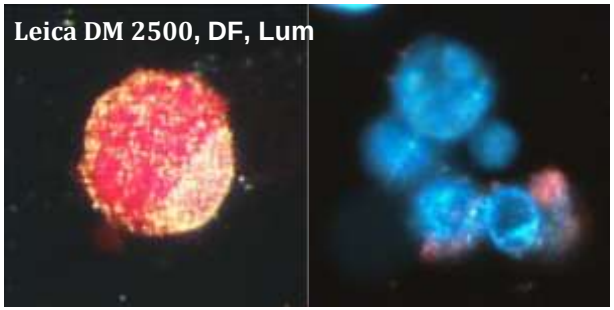
Leica DM 3000

Leica CLS 100x

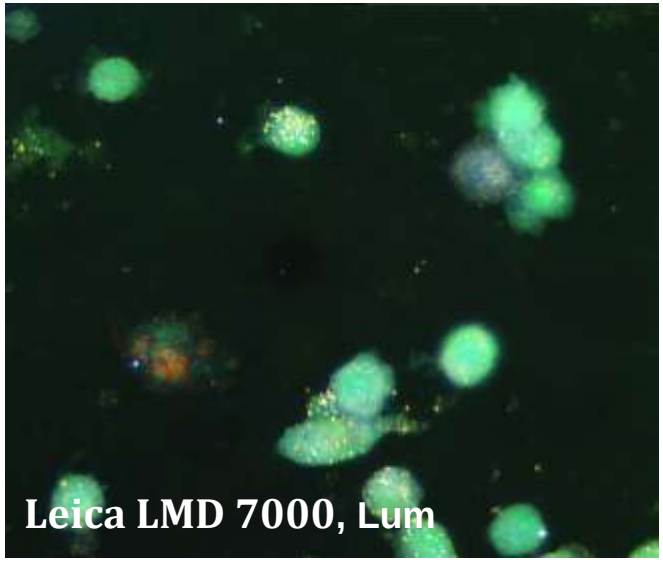
Leica DM 2500

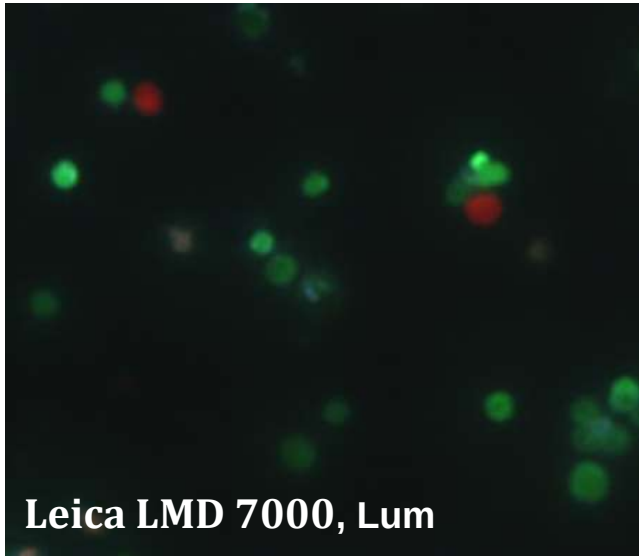
Mixed Labels and Mixed Microscopic Regime

Mixed labels consist of gold nanoparticles and fluorescent labels



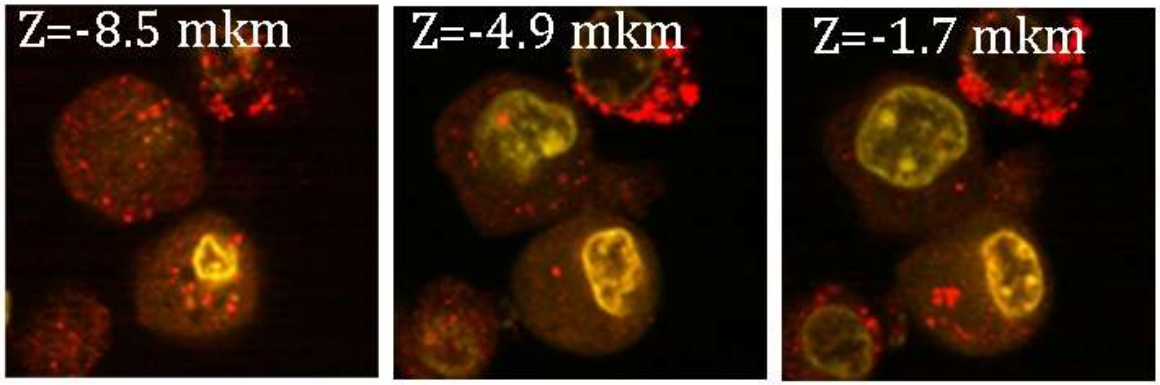
Live-Dead method (FDA, PI)





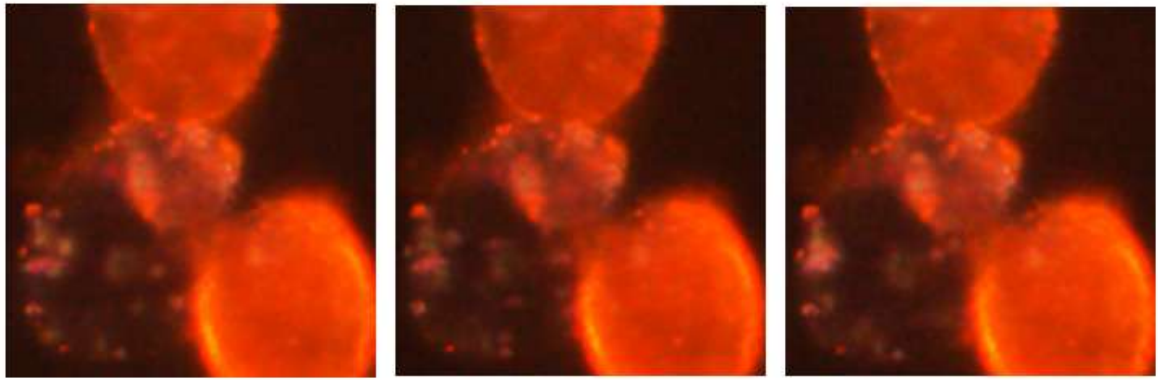
The Localization of Gold Nanoparticles

CG (10^10 particles/mL) , overnight, DAPI



Leica LSM SP-5

Medial optical slices off cells SPEV-2

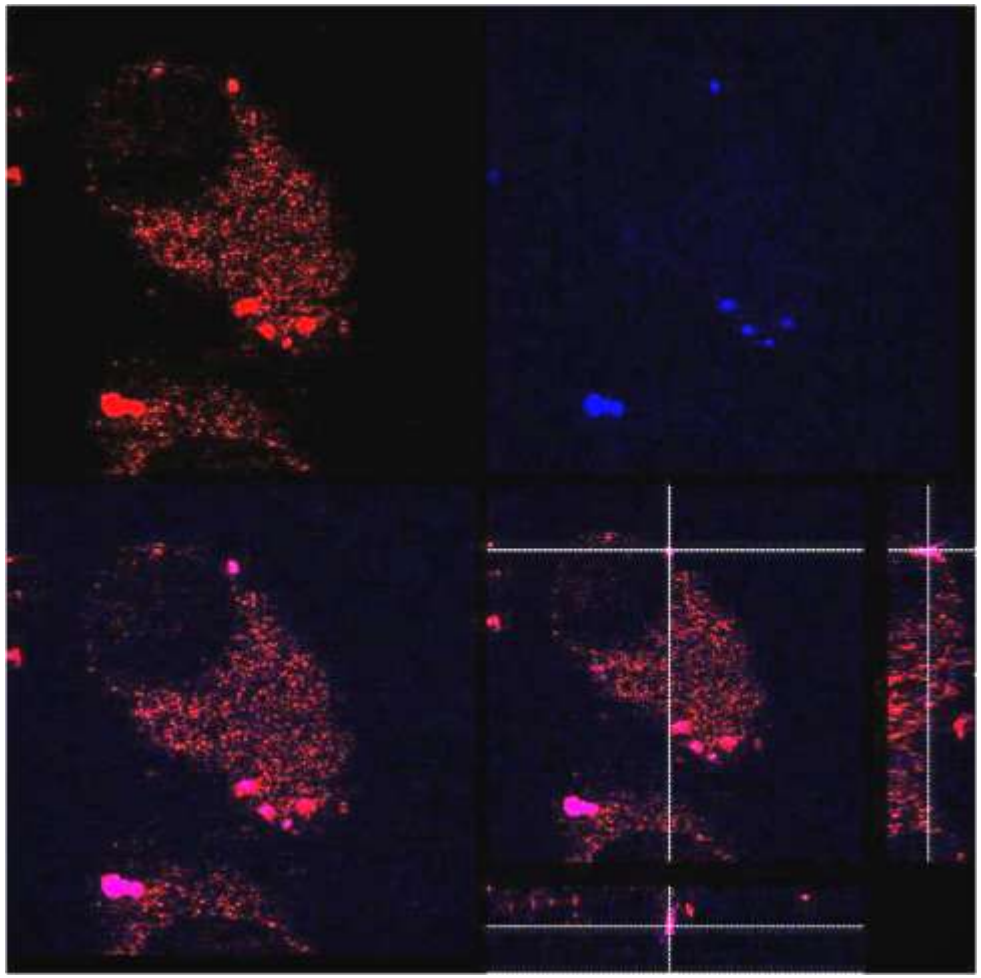


Leica DM2500

Movement of gold nanospheres within cells

The Localization of Gold Nanoparticles

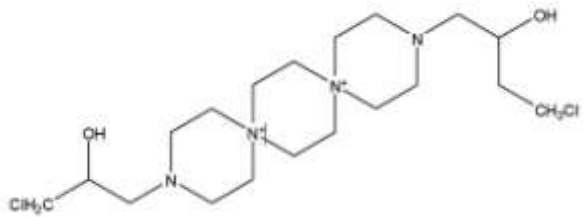
NSUs (10^10 particles/mL) , overnight, DAPI



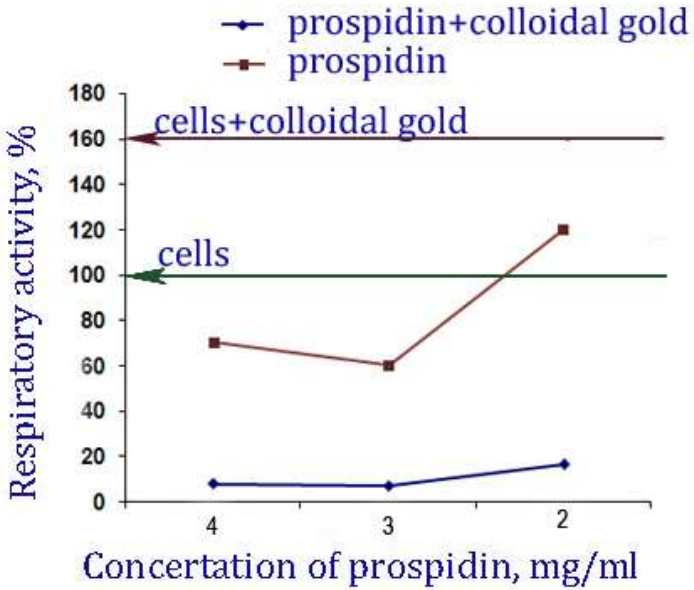
1. The signal reception in narrow band (543 nm), Rayleigh scattering of cells
2. The signal reception in 643 nm, NSUs shining,
3. The mixed image.
4. The medial projections on all axis show the localization of NSUs within cells.

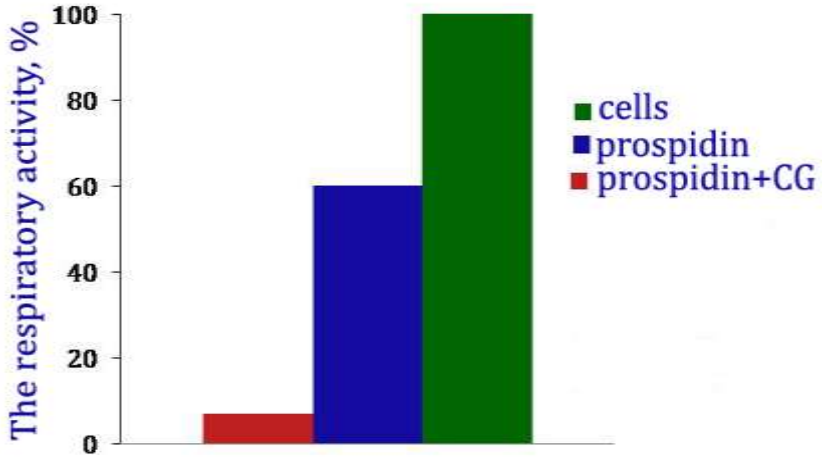
Leica LSM SP-5

Influence of Prospidin – GNP Complex on Cancer Cells

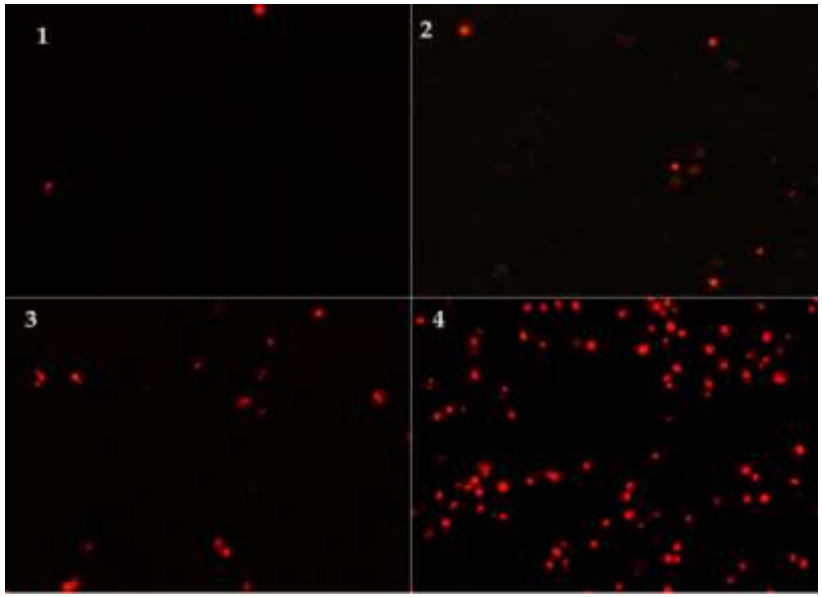


Prospidinum (prospidin)





Influence of Prospidin – GNP Complex on Cancer Cells



1. Control;
2. Gold nanospheres;
3. Prospidin;
4. Gold nanospheres+prospidin.

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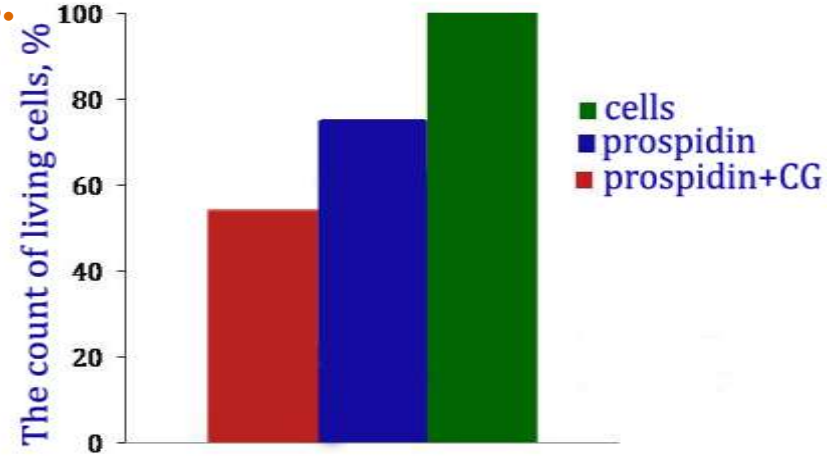
Quantity adjustment of dead cells.
ImageJ is used for counting cells.



Dependence between Dye Luminosity Enhancement and Type of Nanoparticles

CG15 , CG50 and NSUs (10^9 particles/mL) , 12 h, PI, DAPI and AO

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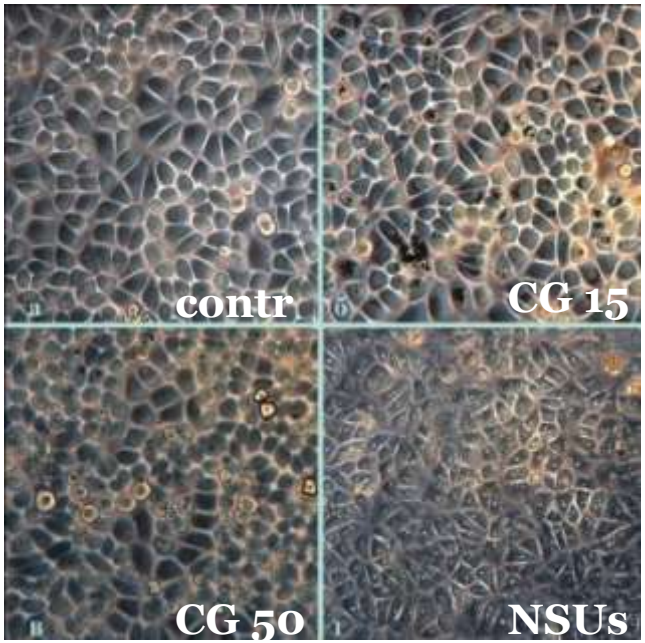
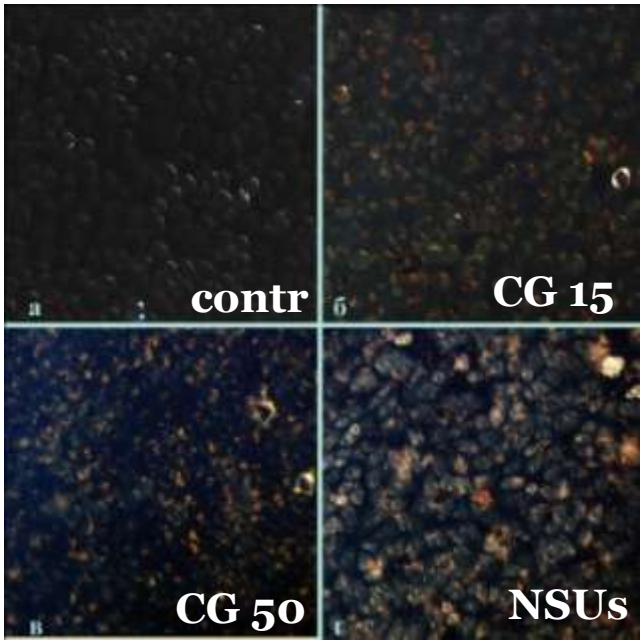


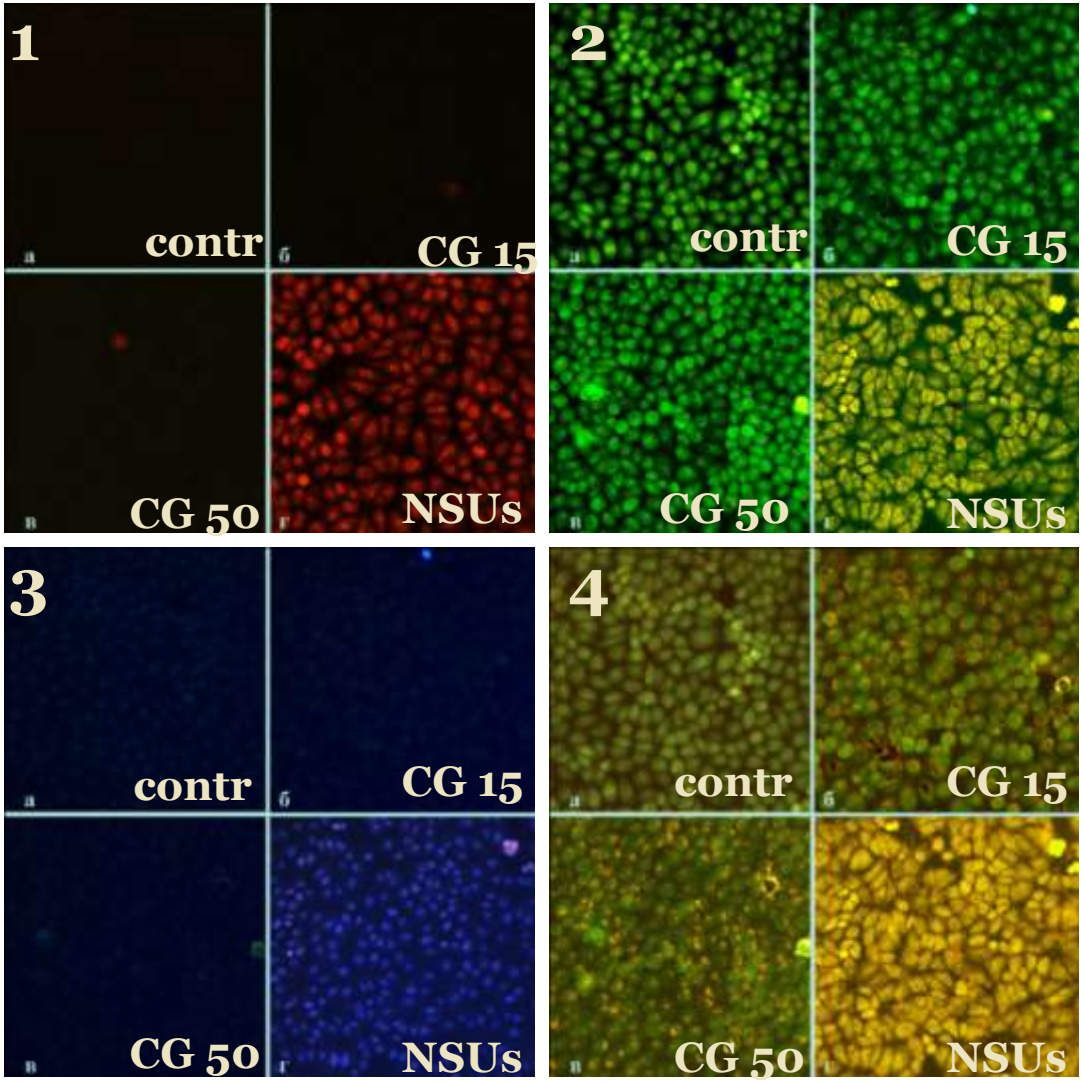
The phase contrast image of cells incubated with different type of nanoparticles



The dark field image of cells incubated with different type of nanoparticles

We have explored penetration nanoparticles with variable morphology and surface functionalization into animal cells .The particles are non-toxic. NSUs enhance Rayleigh scattering of cells.

Dependence Between Dye Luminosity Enhancement and Type of Nanoparticles



1. The fluorescent image of cells(filter N21 515-560 nm)
2. The fluorescent image of cells(filter I3, , 450–490 nm)
3. The fluorescent image of cells(filter A, 340-380 nm)
4. The image of cells made in mixed (dark field and filter I3) regime

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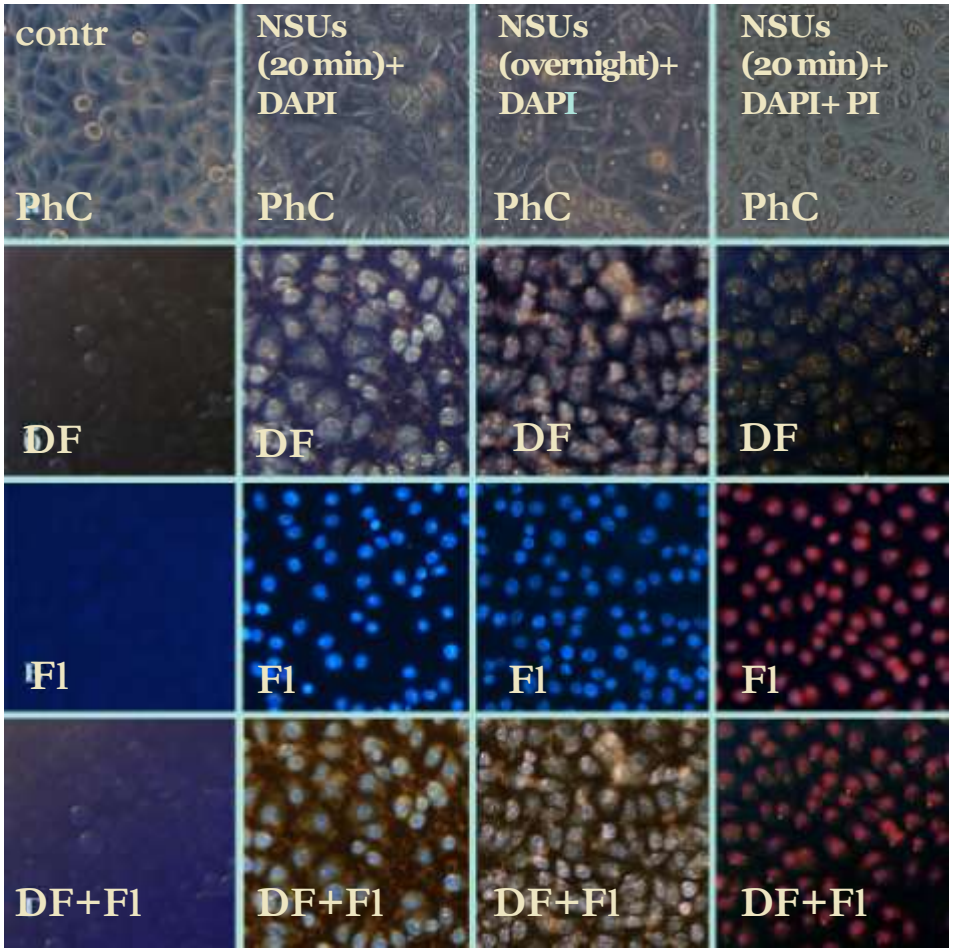
Dependence Between Dye Luminosity Enhancement and Type of Nanoparticles



NSUs (10^9 particles/mL) , 12 h or 20 min, DAPI or PI+DAPI

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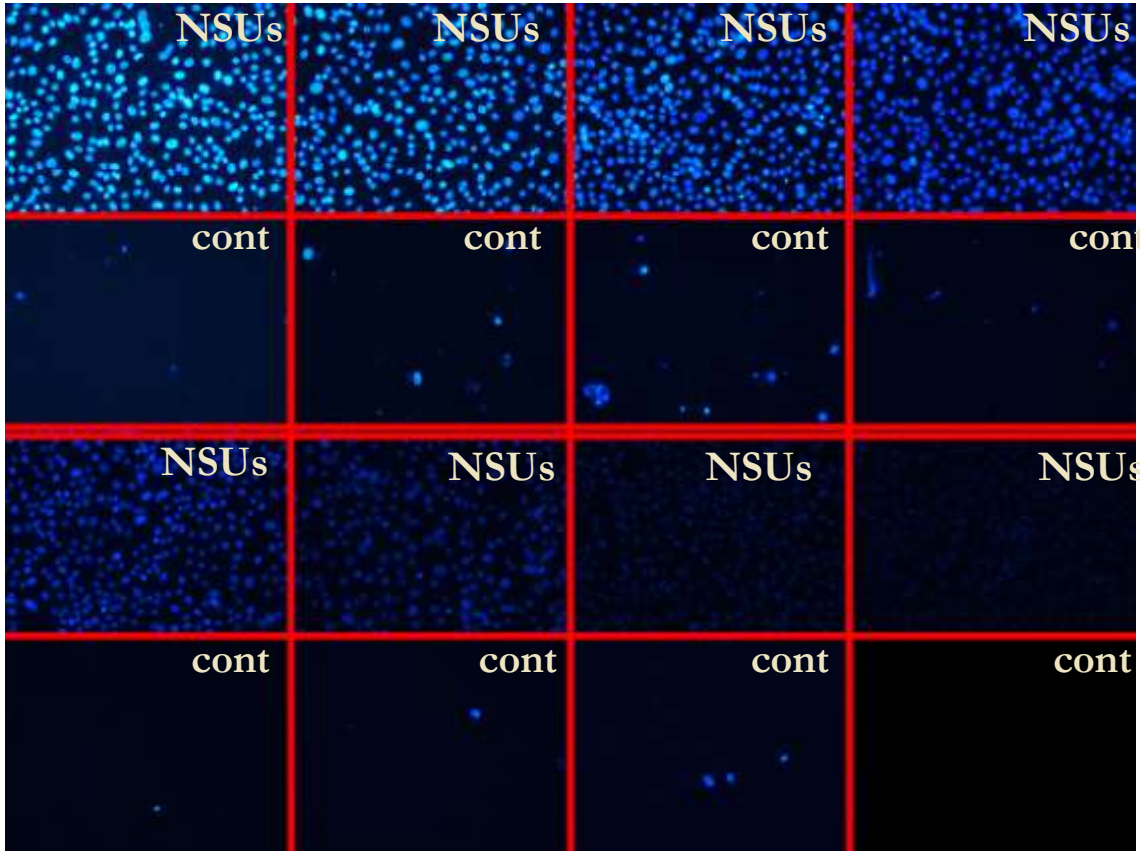
Enhancement is independent of incubation time.

Dependence Between Dye Luminosity Enhancement and Type of Nanoparticles

NSUs (10^9 particles/mL) , 20 min, DAPI (double serial dilutions method from 0, 5 mkg/ml to 4 ng/ml) .



The fluorescent image of cells stained by DAPI and incubated with NSUs (on the top) and control.

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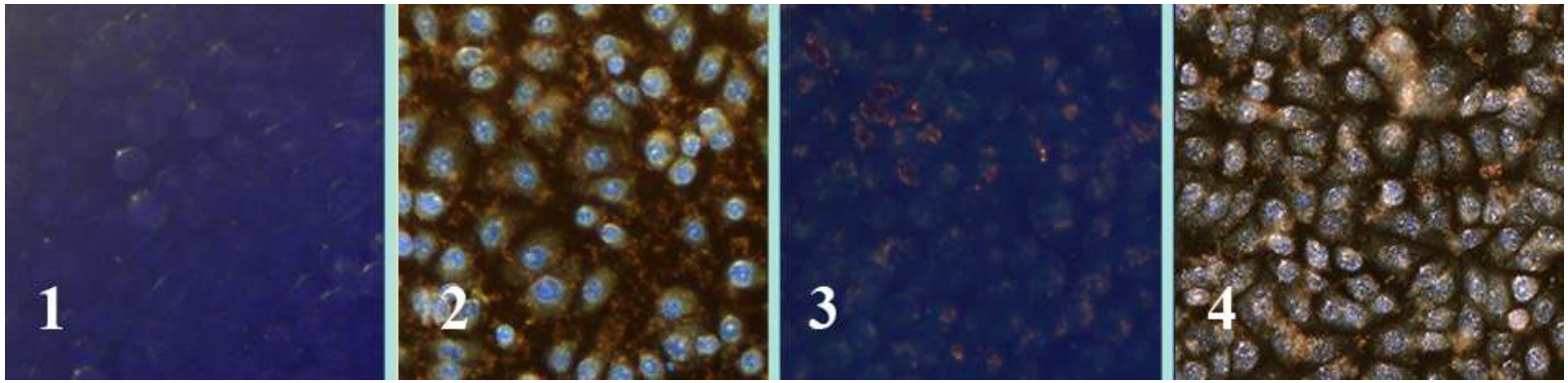
Decrease of dye luminosity in cells with NSUs. Some luminous (dead) cells in control.

Dependence Between Dye Luminosity Enhancement and Type of Nanoparticles

NSUs , NS, NR (10^9 particles/mL) , 20 min, DAPI



Dye luminosity enhancement depends of surface property. In contrast to CTAB-coated gold nanoparticles, HEPES-coated gold nanostars did not show high contrast and fluorescence enhancement. The mechanism of the dye luminosity enhancement is assisted with endocytosis or nonfatal cell membrane damage.

Conclusions

1. The use of combined microscopic facility (fluorescence and dark field) allows evaluation of the cell population heterogeneity by the morphophysiological parameters.

2. The intracellular localization of gold nanoparticles has been shown for nanospheres and nano-sea-urchins by confocal standard microscopy.

3. The prospidin – gold nanospheres complex inhibits the viability of the cells. The decrease of the respiratory activity and the changing the ratio of the live and dead cells are investigated.

4.Dye luminosity enhancement depends of surface property. In contrast to CTAB-coated gold nanoparticles, HEPES-coated gold nanostars did not show high contrast and fluorescence enhancement. The mechanism of the dye luminosity enhancement is assisted with endocytosis or nonfatal cell membrane damage.

Thanks for your attention!

Acknowledgments:
This work was supported in part by grants from the Russian Foundation for Basic Research № 07-04-00301a, № 07-04-00302a and № 12-04-00629a. President and the Ministry of Education and Science of the Russian Federation (nos. MK-1057.2011.2, 2.1.1/2950, 14.740.11.260, and 02.740.11.0484)
We thank the program "U.M.N.I.K" (Russia) and CIMO (Finland) for supporting the research.