Paper presentation

Size-dependent endocytosis of gold nanoparticles studied by three-dimensional mapping of plasmonic scattering images

Sheng-Hann Wang[1,2], Chia-Wei Lee[1,3], Arthur Chiou[2], Pei-Kuen Wei[1,2]

[1]Research Center for Applied Sciences, Academia Sinica, 128, section 2, Academia Road, Nankang, Taipei 11529, Taiwan. [2]Institute of Biophotonics Engineering, National Yang Ming University, Taipei, 112, Taiwan. [3]Department of Material Science and Engineering, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei, Taiwan 10617.

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By
Kamalesh
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Introduction

- Understanding the endocytosis process of AuNPs is important for the drug delivery and photodynamic therapy applications.
- The fluorescent labeling suffers from photobleaching and no long term observation is possible.
- AuNPs have large optical scatterings at 550-600 nm wavelengths due to localized surface plasmon resonances.
- Using an enhanced contrast between yellow and blue CCD images, AuNPs can be well distinguished from cellular organelles.
- Targeting can be achieved with the help of AuNPs coated with aptamers for surface mucin glycoprotein.

Identifying AuNPs in scattering images

- The scattering cross-section of a nanoparticle is usually described by the Mie scattering theory. According to which scattering depends on,

$$C_s(\lambda) = \frac{32\pi^4}{3\lambda^4} r^4 n^4 \frac{[\varepsilon_r(\lambda) - n^2]^2 + \varepsilon_i^2(\lambda)}{[\varepsilon_r(\lambda) + 2n^2]^2 + \varepsilon_i^2(\lambda)}$$

r - radius of the nanoparticle,

λ - incident wavelength,

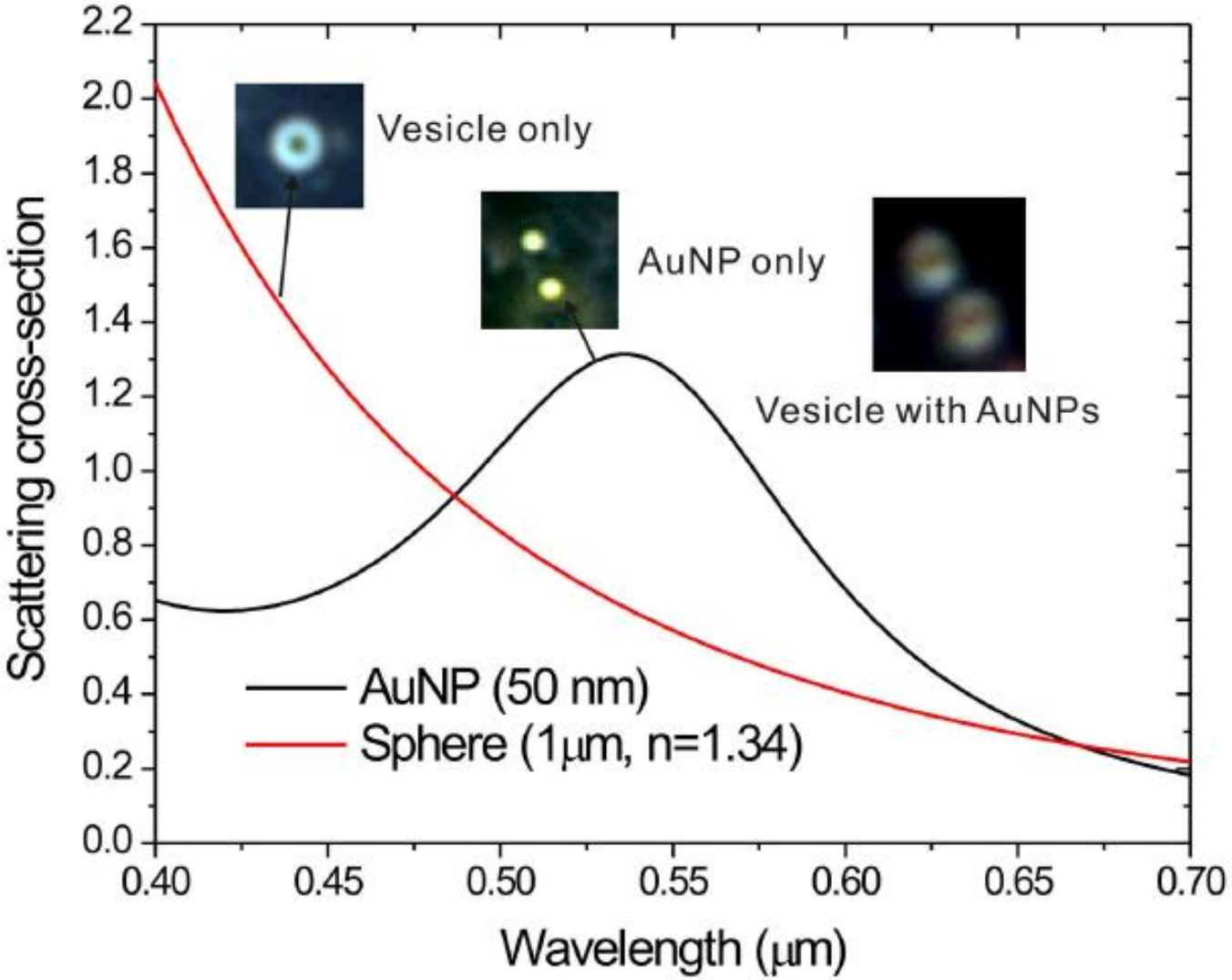
n - refractive index of environmental medium

εr and εi the real and imaginary parts of the dielectric constant of the nanoparticle, respectively.

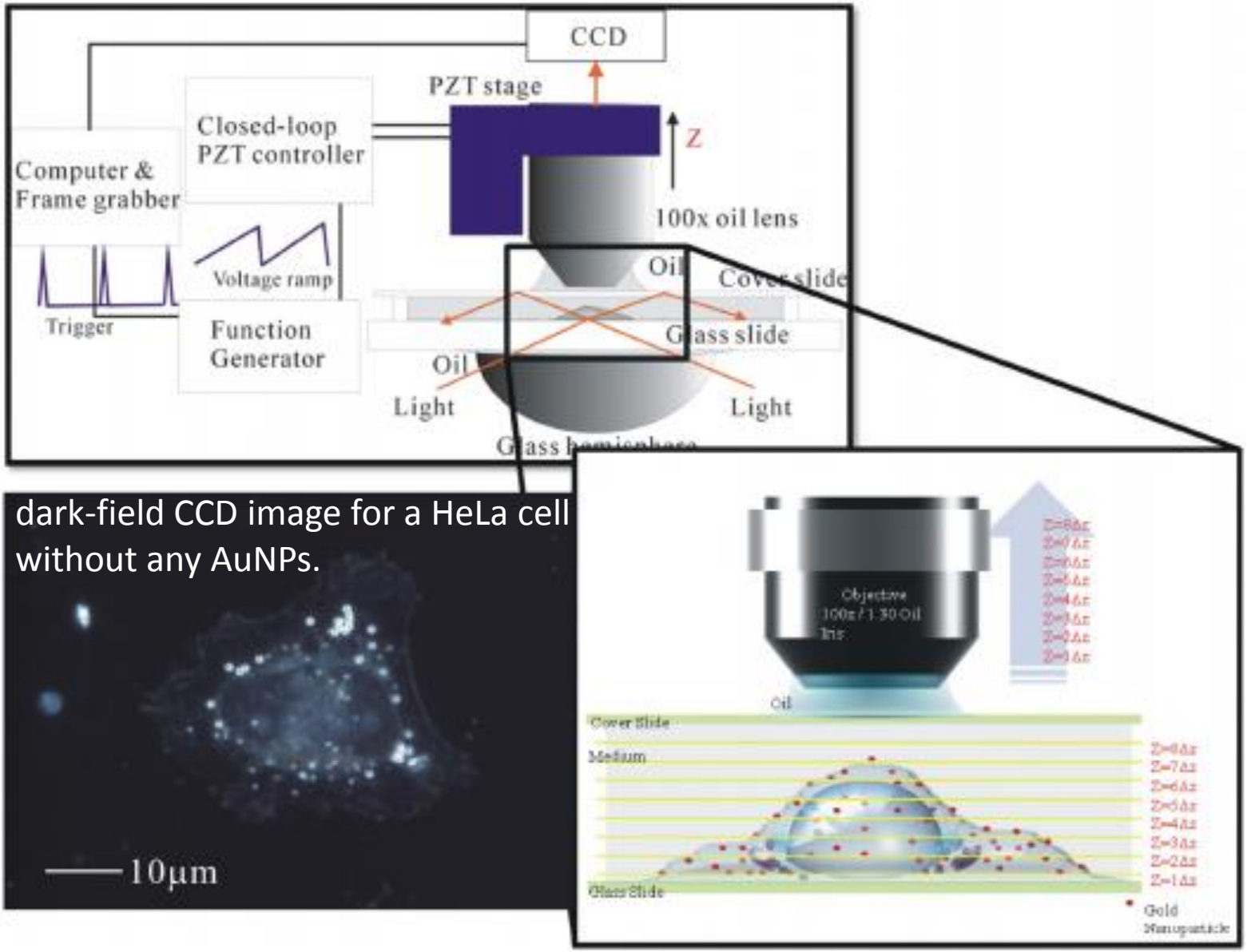
Identifying AuNPs in scattering images

- The AuNP has a negative dielectric constant.
- On the other hand, the dielectric constant of cellular organelles is positive.
- Single 50 nm AuNP shows as yellow and the dielectric sphere shows as blue.
- For AuNP inside vesicle scattering image is visualised as an orange centre with a blue periphery.

Identifying AuNPs in scattering images



Dark-field optical sectioning microscopy



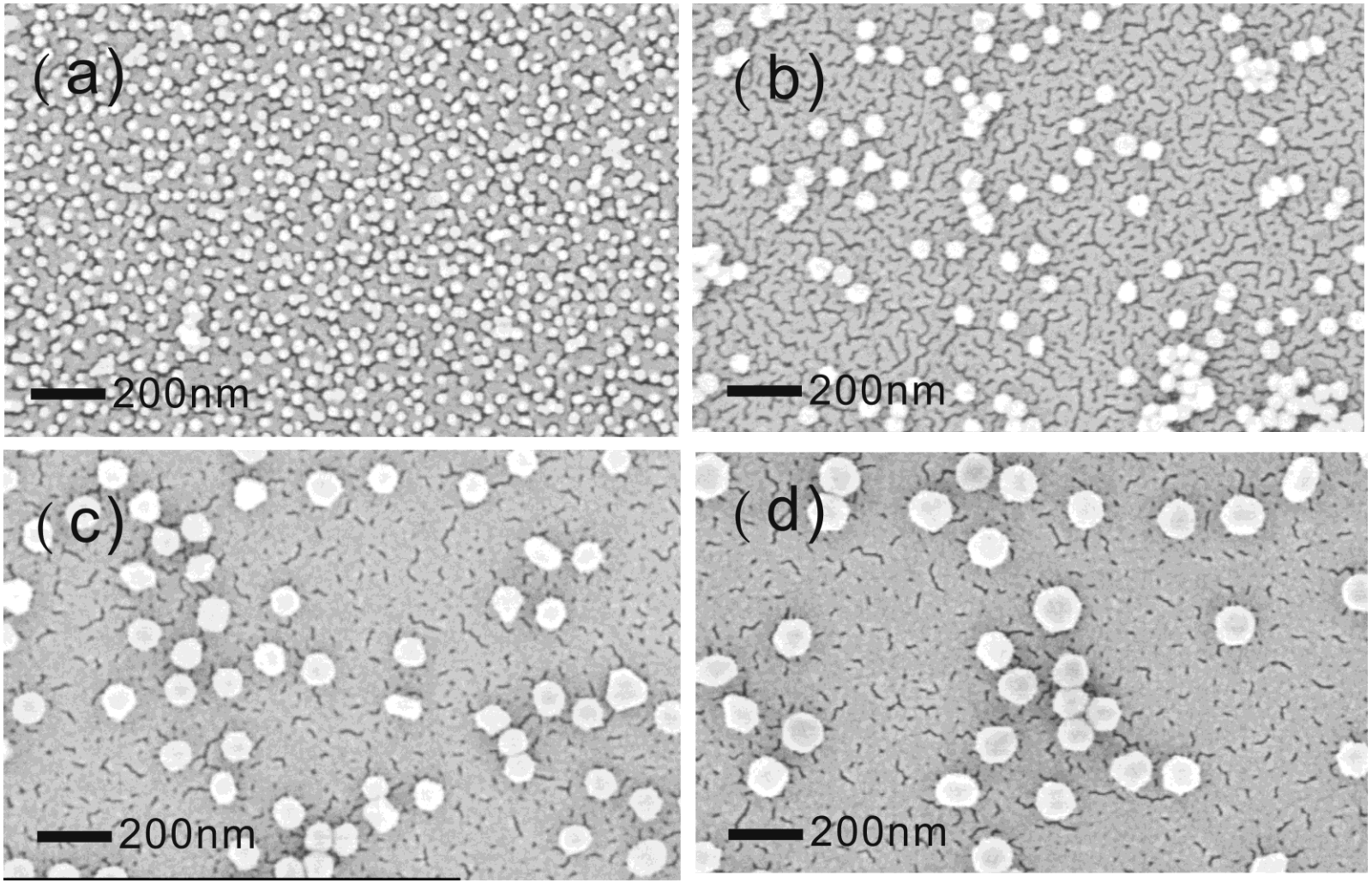
dark-field CCD image for a HeLa cell without any AuNPs.

Experimental setup

- Experiment was done in a chamber maintained at 37°C humidified atmosphere.
- The light source was a 60 W metal halide lamp.
- In dark-field sectioning microscopy, it made a 16 μm movement from the focal position.
- Interaction of AuNPs (Citrate reduced) was studied with two kinds of cancer cells, non-small lung cancer cells (CL1-0) and HeLa cells.
- AuNPs were modified with aptamer for cellular surface mucin glycoprotein (MUC1) which is over-expressed in the extracellular matrix of cancer cells.

SH-(CH2)10-GCAGTTGATCCTTTGGATACCCTGG

SEM images of AuNPs



SEM images for 13 nm, 45 nm, 70 nm and 110 nm AuNPs on glass substrates.

Zeta potential analysis to confirm aptamer immobilization on AuNP surface

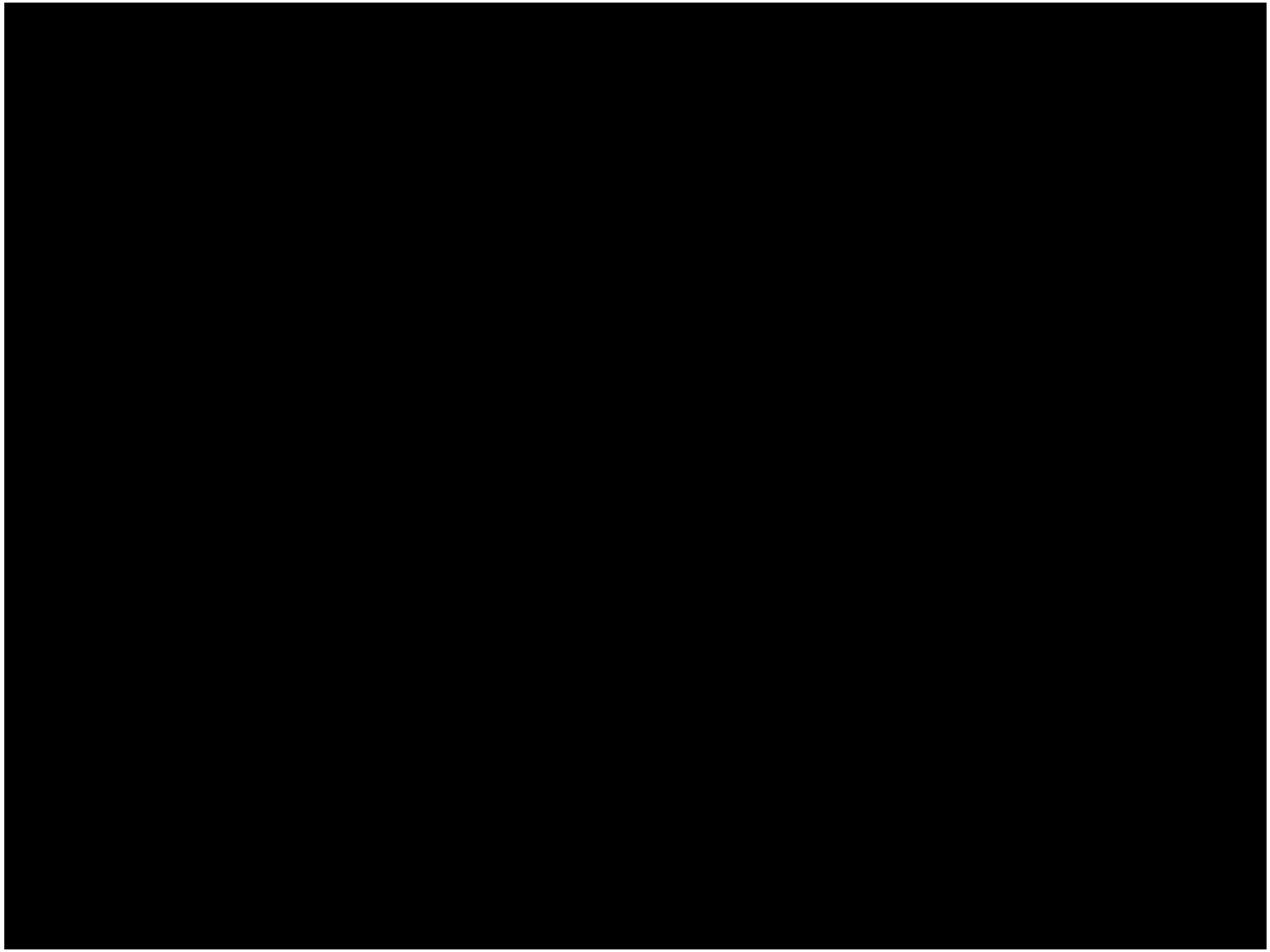
Size of gold nanoparticles (nm)	Zeta potential before ssDNA conjugated (mV)	Zeta potential after ssDNA conjugated (mV)
13 ± 2.6	-13.99 ± 1.75	-27.27 ± 1.03
45 ± 3.1	-17.83 ± 1.31	-28.69 ± 1.07
70 ± 4.9	-19.14 ± 1.48	-24.66 ± 1.88
110 ± 5.1	-10.25 ± 0.80	-19.48 ± 0.97

Cell-nanoparticle interactions

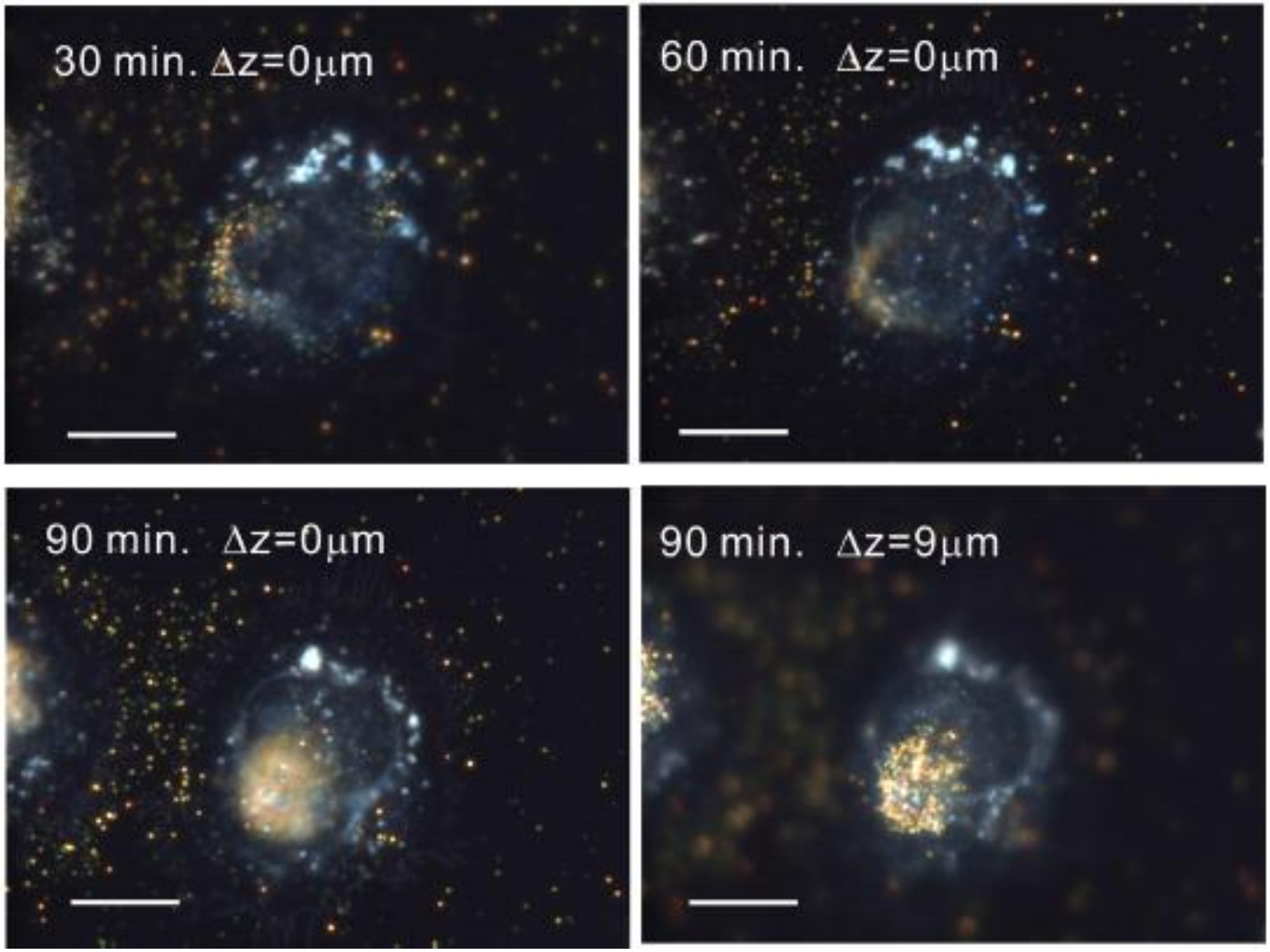
- Lung cancer cells were incubated for 10 mins with AuNPs and then washed.

- Images were recorded with 5 min interval and 100 ms exposure time for 1.5 hours

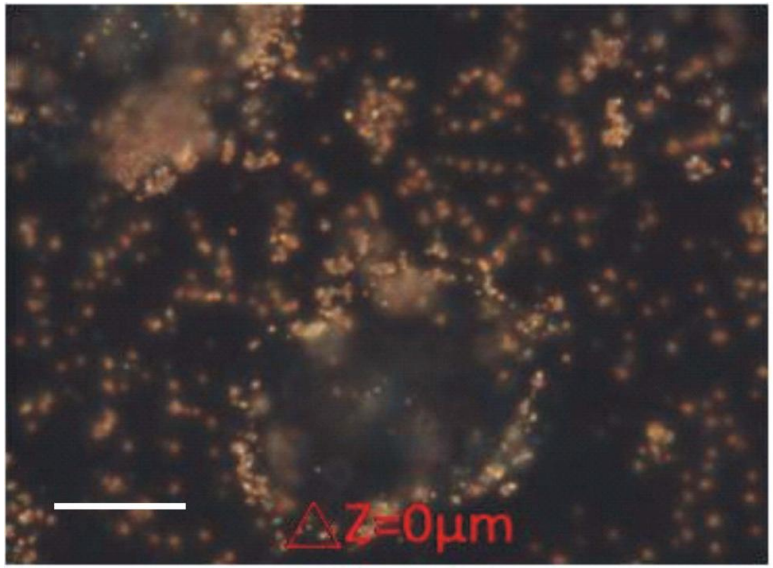
Cell-nanoparticle interactions

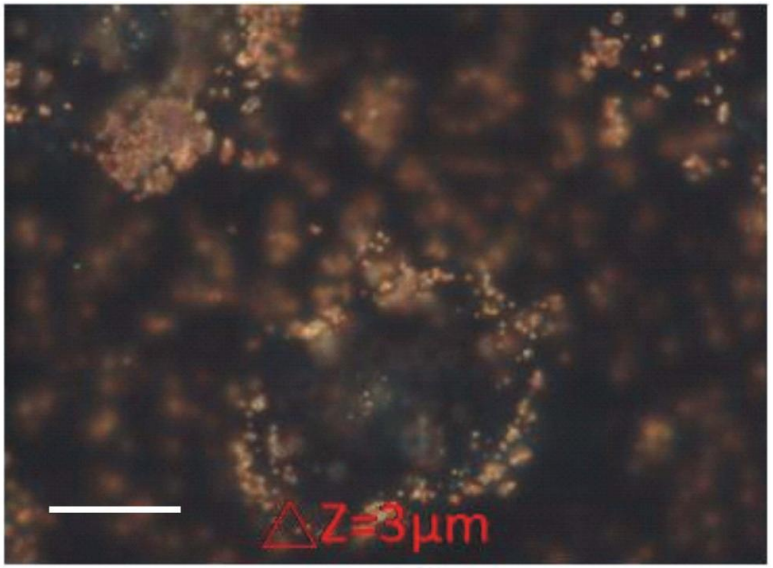


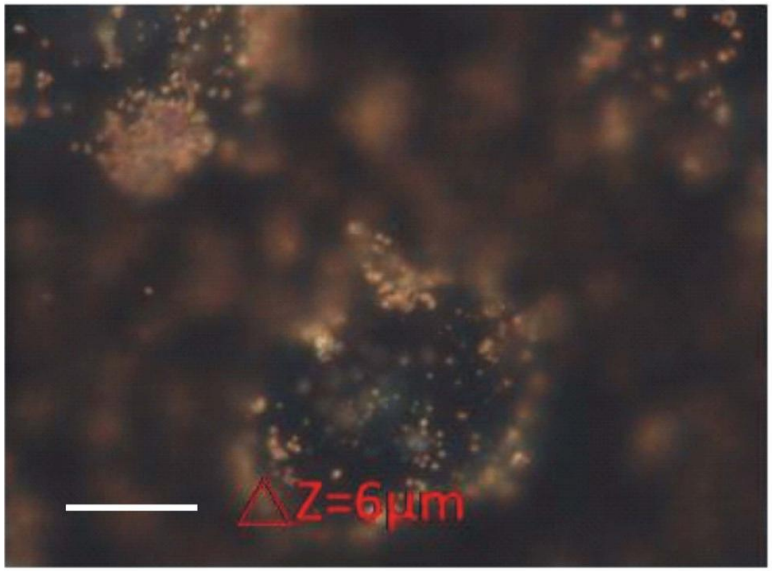
Interaction of 70 nm AuNPs

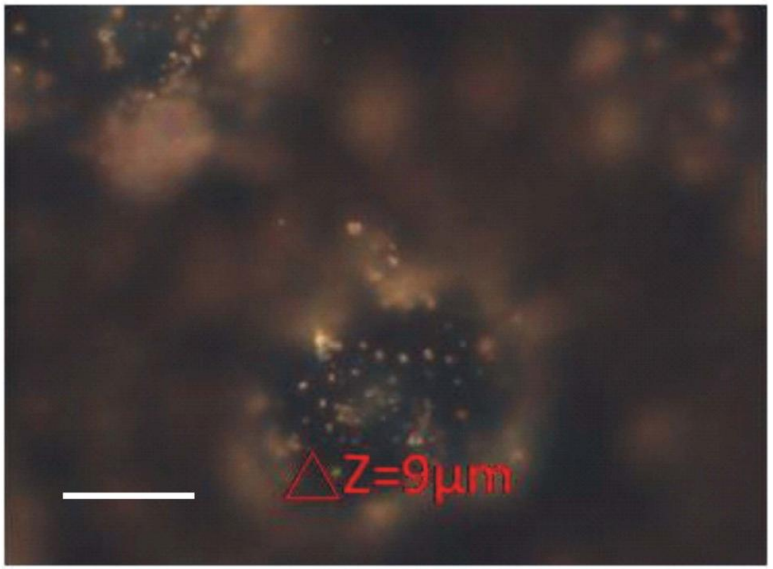


Interaction of 110 nm AuNPs



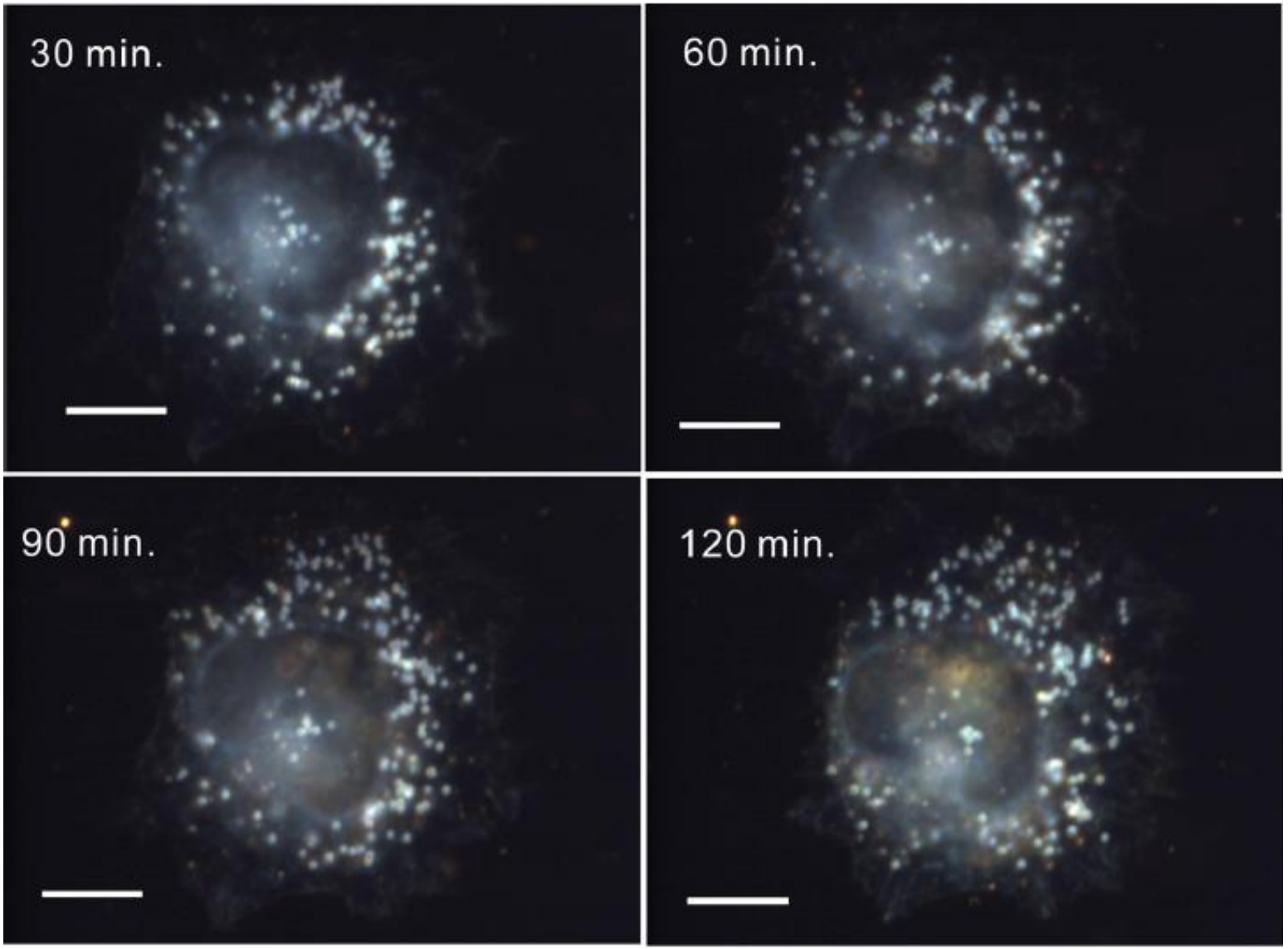






120 mins

Interaction of 45 nm AuNPs



Δz = 0 µm

Interaction of 45 nm AuNPs after 120 mins

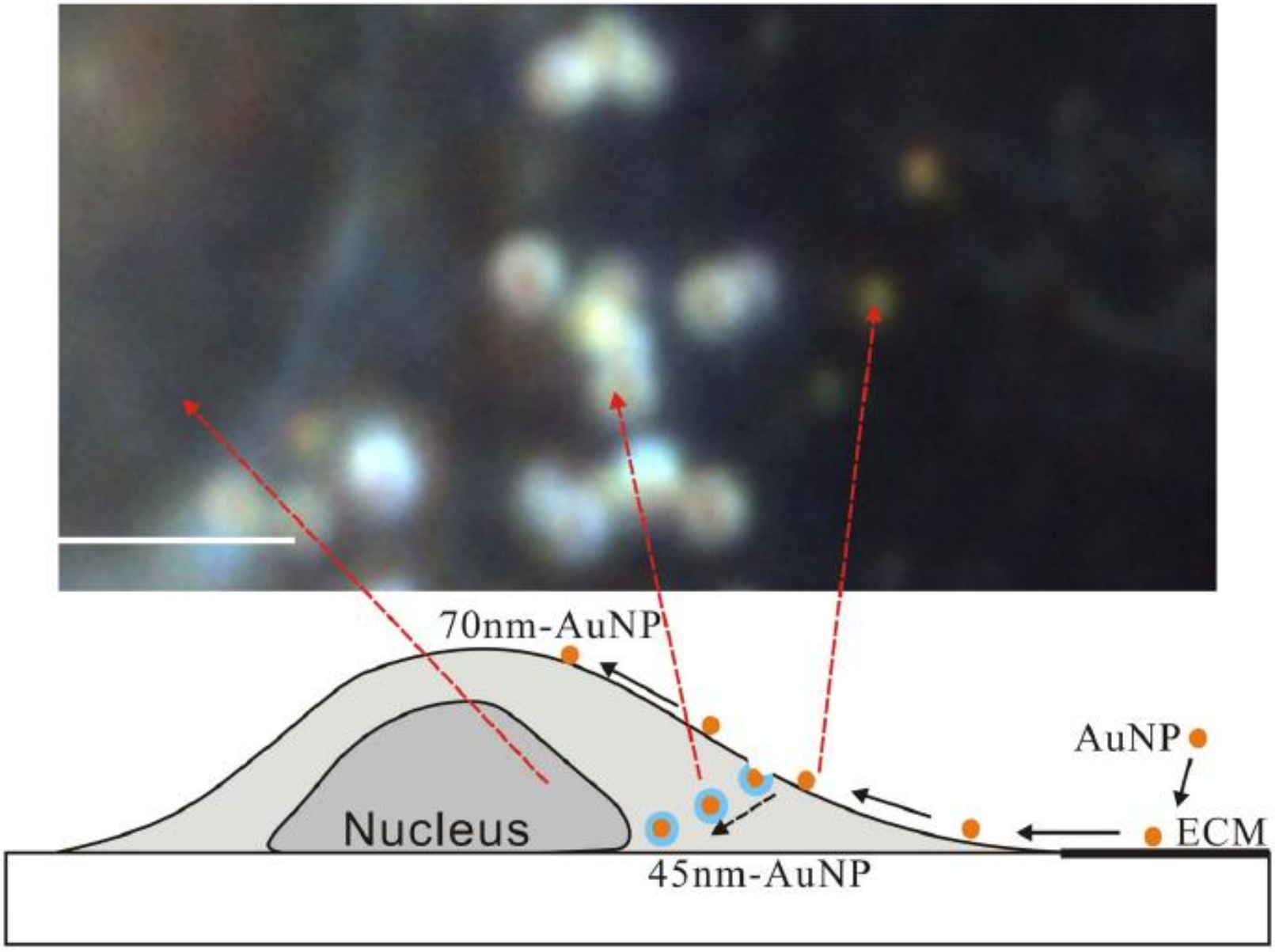
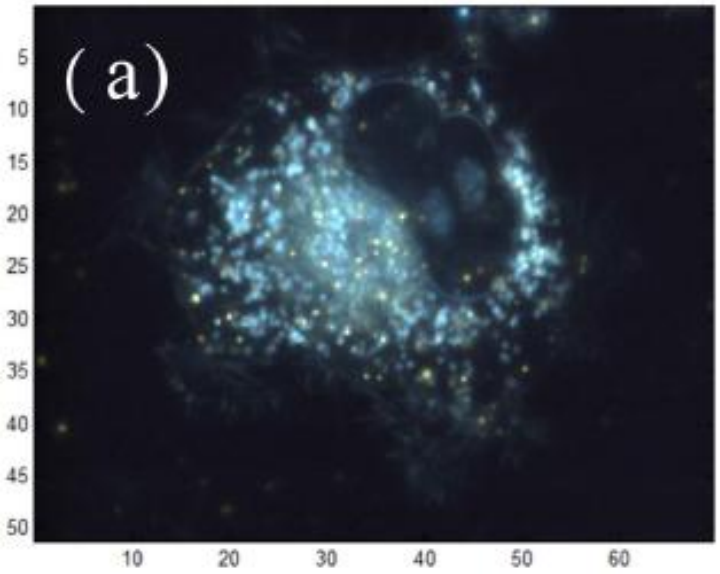
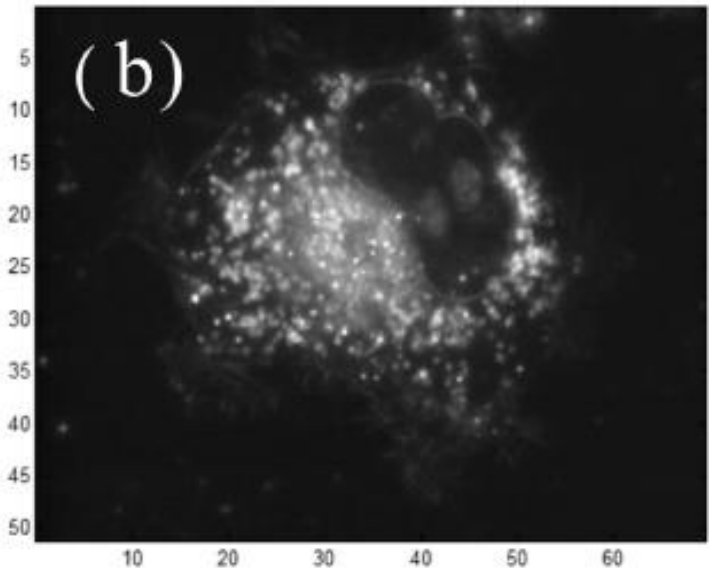


Image processing

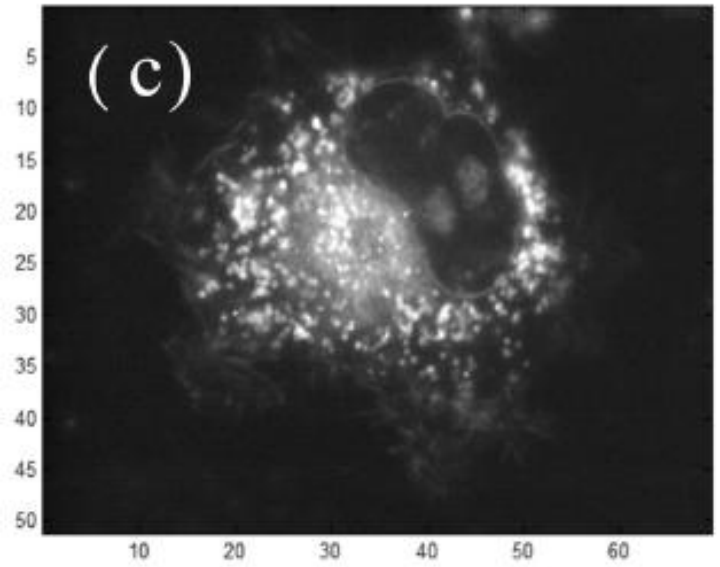
- Image was divided into the colours red (R), green (G) and blue (B).
- The (G+R)/2 image yielded a yellow image (Y) which had a stronger scattering intensity for AuNPs
- On the other hand, the organelles were brighter in the blue image.
- Image process of (Y-B), gives a grey image which is positive for AuNPs and negative for organelles.
- In grey image central position of every bright spot was recorded as the x-y position (xp, yp) of AuNPs.
- The z position (zp) for each AuNP was determined by finding the maximum scattering intensity.

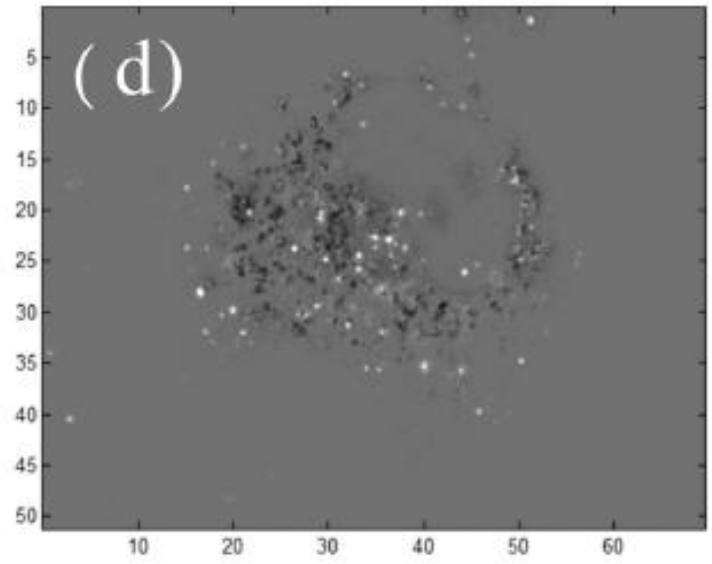
Image processing



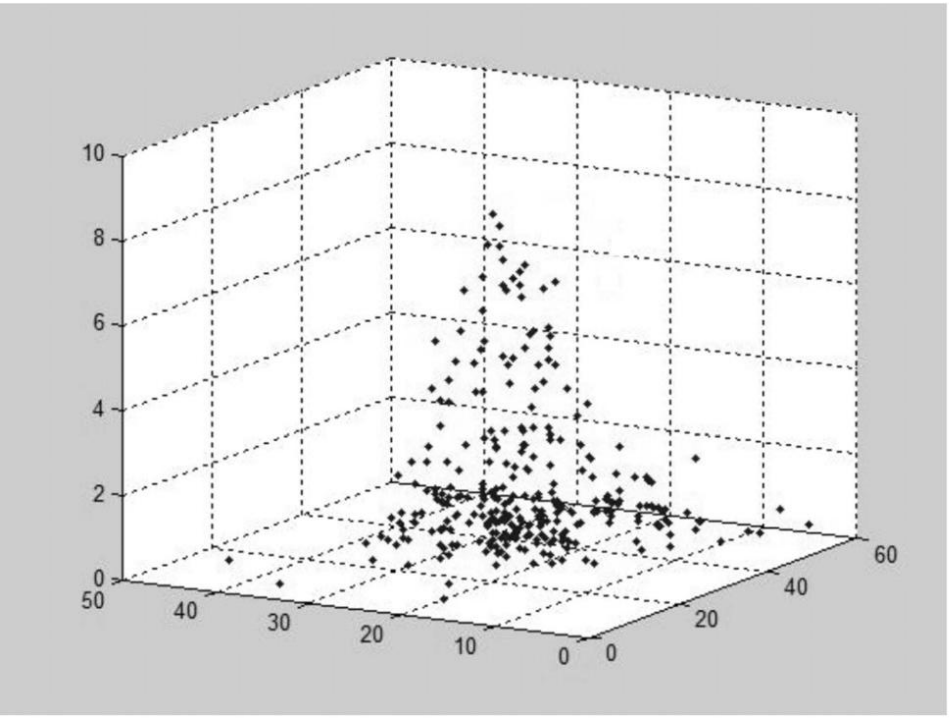


(a) Color
(b) Yellow
(c) Blue
(d) (Y-B)

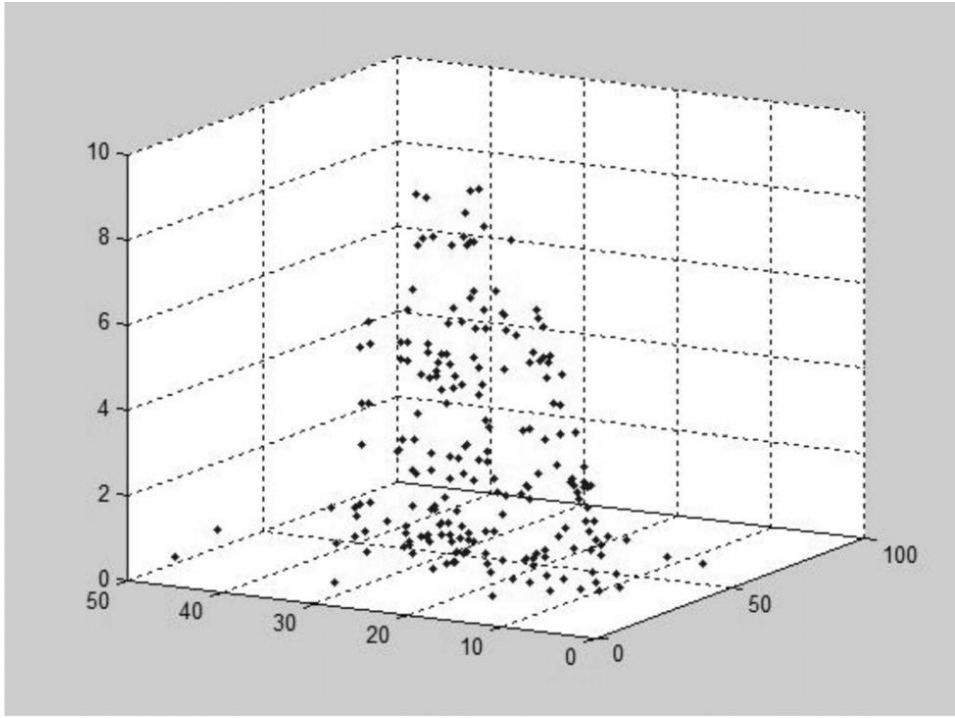




3D distribution of AuNPs



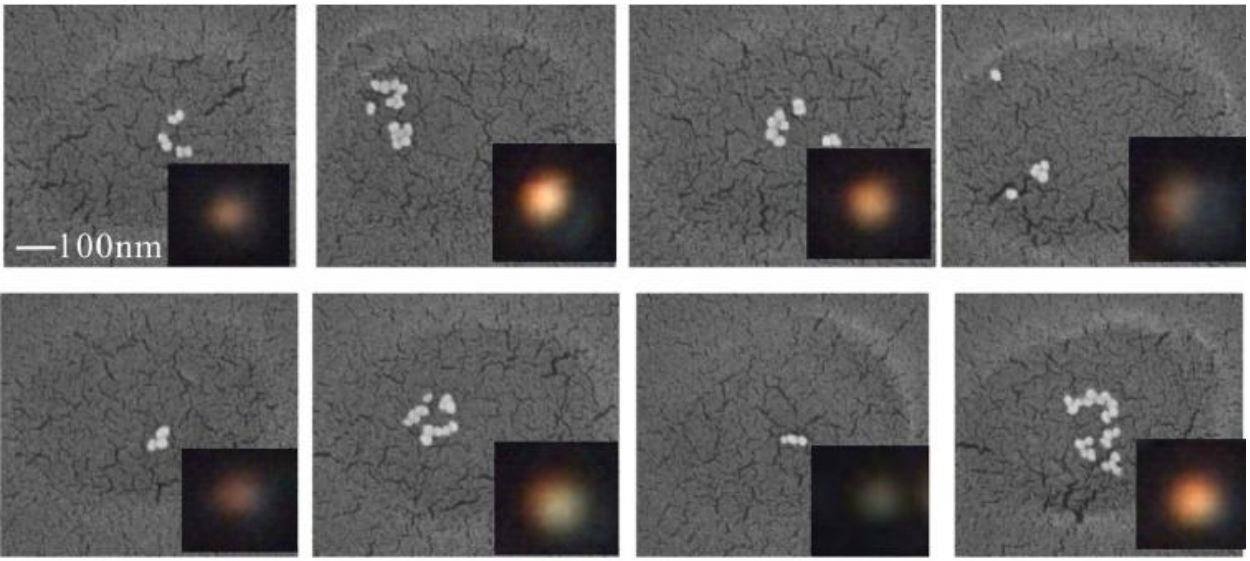
45 nm



70 nm

Relation between the scattering optical intensity and #(AuNPs) in the vesicle.

- 500-nm-diameter holes were prepared in a transparent film to mimic the vesicles and coated on a glass substrate.
- The sample was dipped in the AuNP solution for six and then washed to measure the scattering images in water.
- Then dried sample was observed by the SEM to identify the number of AuNPs in each hole.



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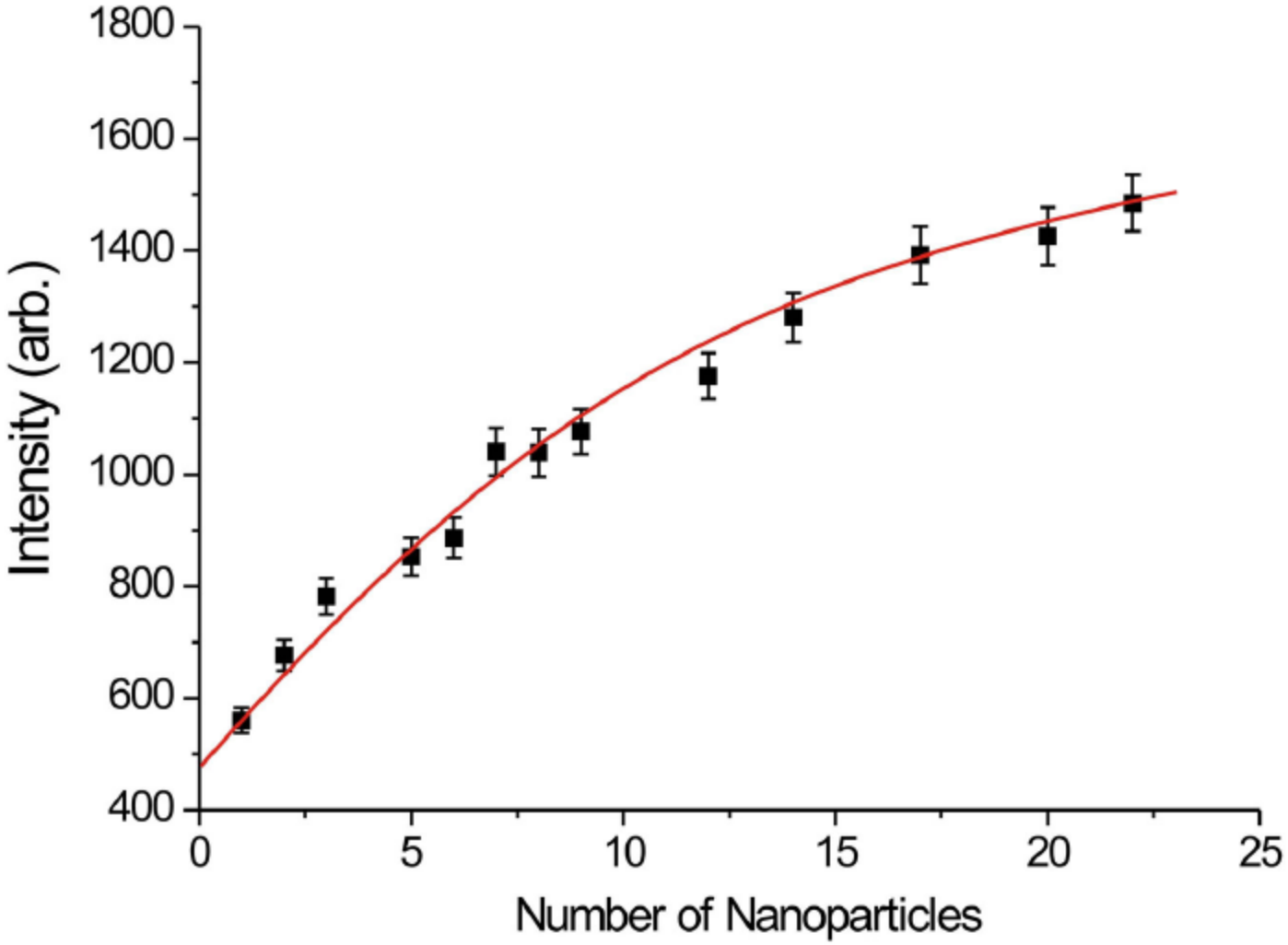
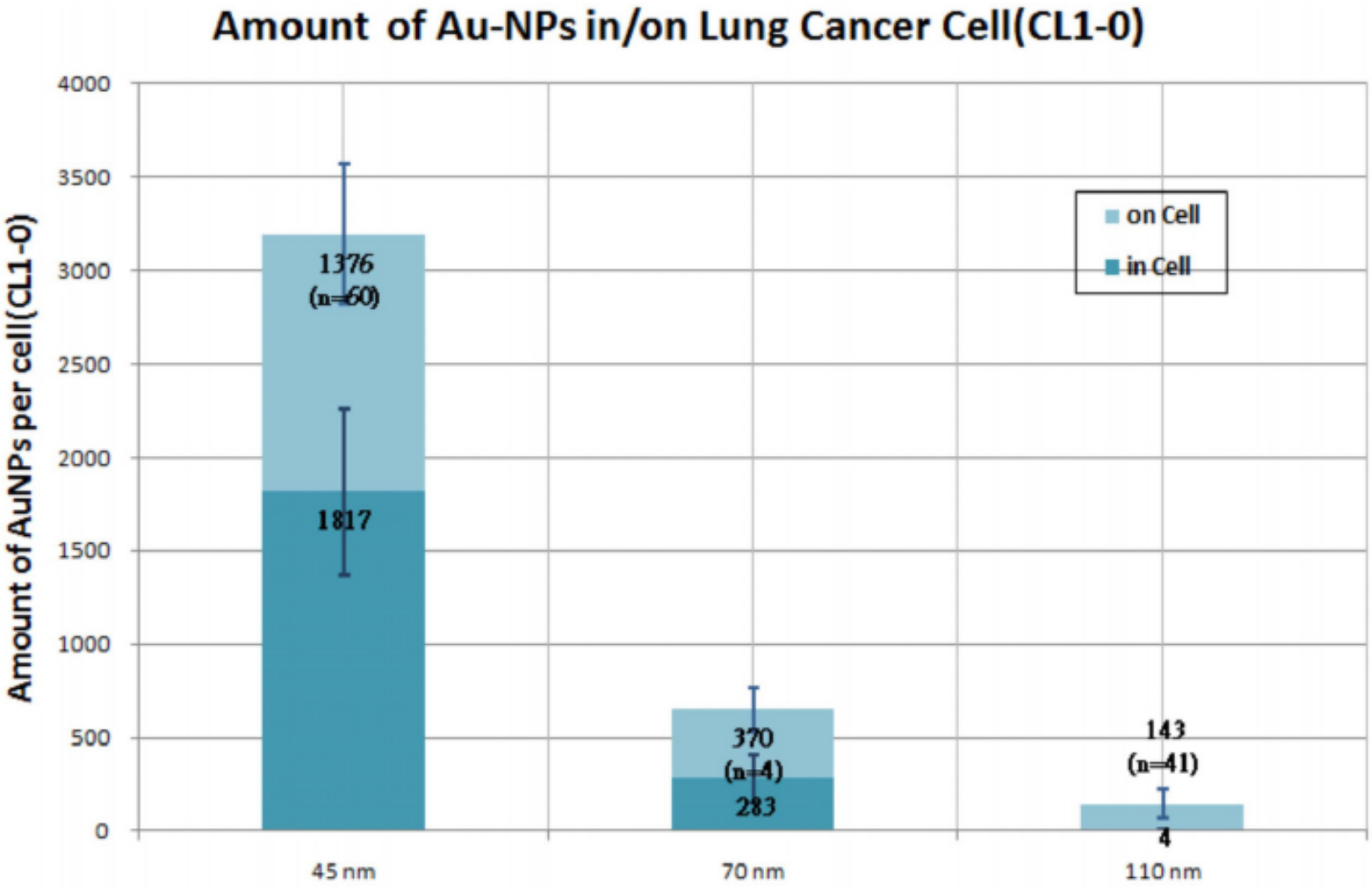


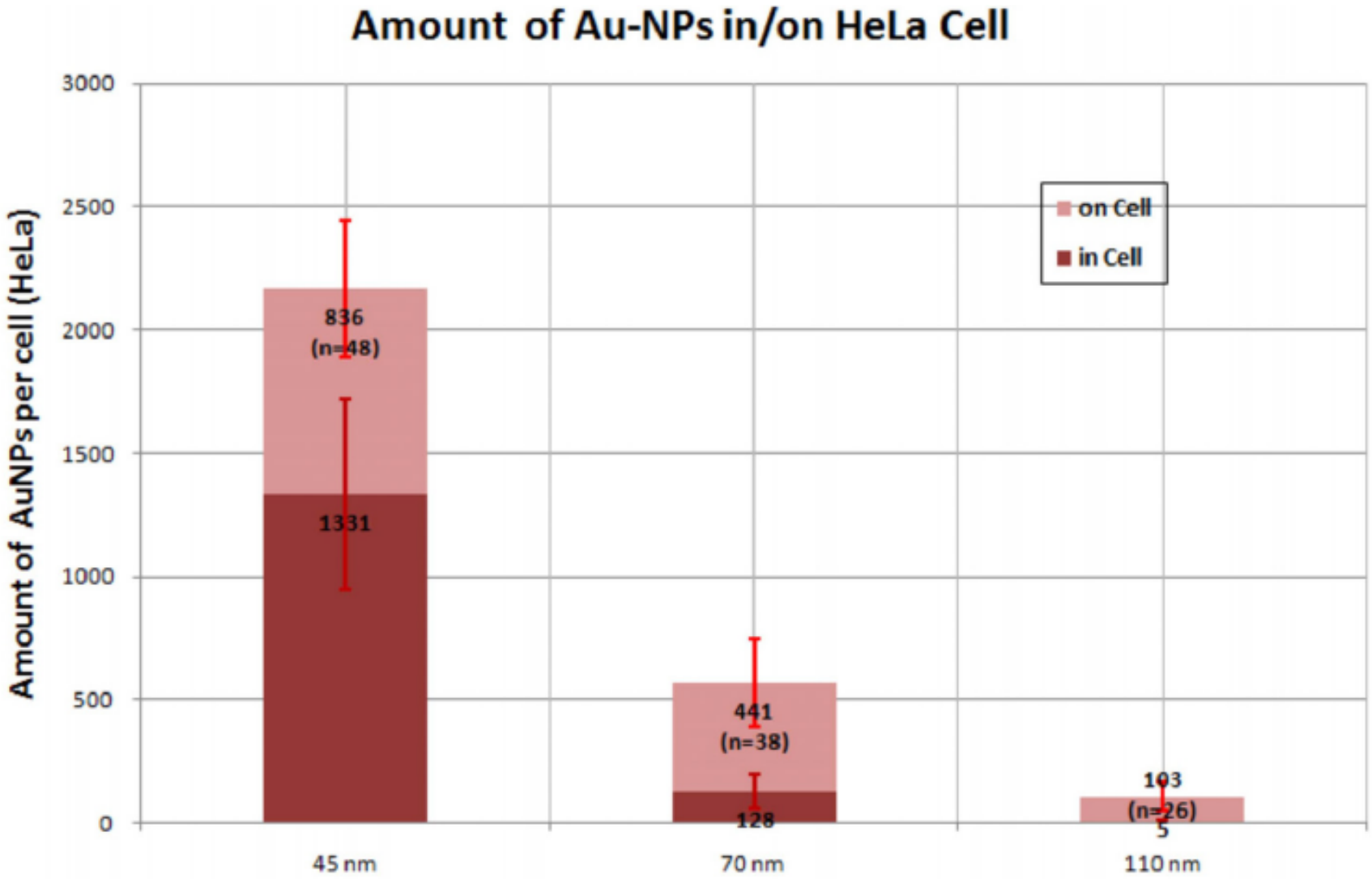
Quantitative calculation of the endocytosis

- The scattering intensity has increased with the AuNP number in the aggregate.
- Using the scattering curve and the measured scattering intensity I(xp, yp, zp) for the AuNP aggregates, we can quantitatively estimate the AuNP numbers at each (xp, yp, zp) position.

Quantitative calculation of the endocytosis



Quantitative calculation of the endocytosis



Conclusions

- The total amount is consistent with the result measured by using inductively coupled plasma atomic emission spectroscopy and transmission electron microscopy.
- The optimal diameter for AuNPs falls in the range of 40-60 nm for reasonable values of membrane bending rigidity and ligand-receptor binding energy.
- The proposed 3D scattering method is suited only for medium-sized AuNPs.
- This proposed method is very useful for long-term tracking of the process of endocytosis without any labelling.
- Particle size of 45 nm has the highest efficiency for drug delivery by AuNPs.
- Large AuNPs which remain bound to the cell membrane can be used to reconstruct the morphology of the cell.

Thank you