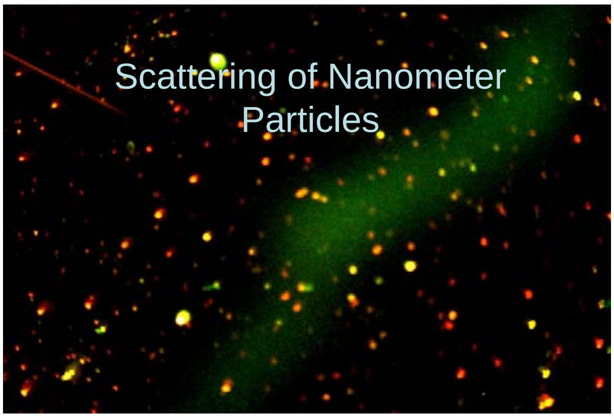
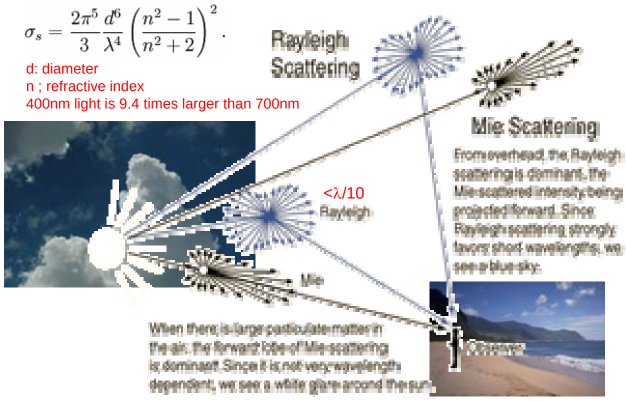


Rayleigh scattering cross-section

Why sky is blue ?

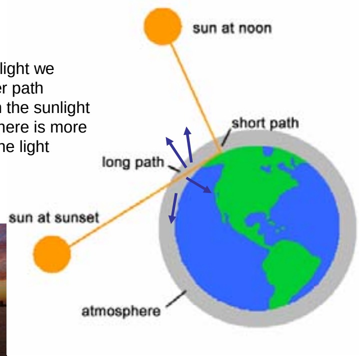
$$\sigma_s = \frac{2\pi^5}{3}\frac{d^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)^2 .$$

d: diameter
n ; refractive index
400nm light is 9.4 times larger than 700nm



Why sky is red ?

At sunset or sunrise, the sunlight we observe has traveled a longer path through the atmosphere than the sunlight we see at noon. Therefore, there is more scattering, and nearly all of the light direct from the sun is red.



Rayleigh scattering cross-section

$$\sigma_s = \frac{2\pi^5}{3}\frac{d^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)^2 .$$

$$n^2 = \varepsilon_r$$

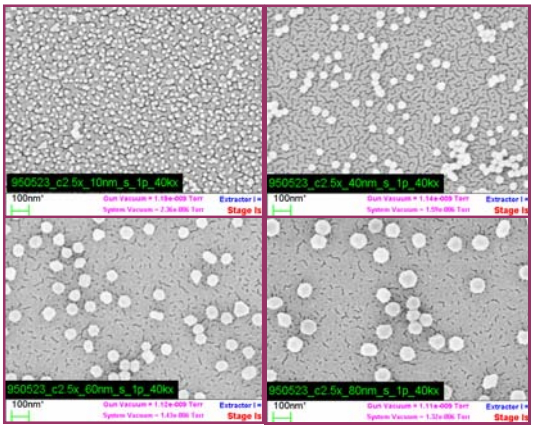
ε is negative for metals, when it approximates to -2, the scattering is greatly *enhanced !*

"Labors of the Months" (Norwich, England, ca. 1480).
(The ruby color is probably due to embedded gold nanoparticles.)

Gold nanoparticles, 20nm, 40nm ,60nm, 80nm in diameter

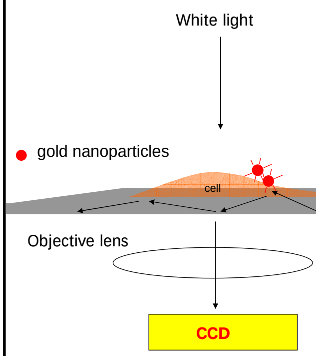
P-K, Wei

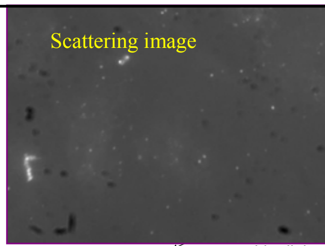
Nano golds

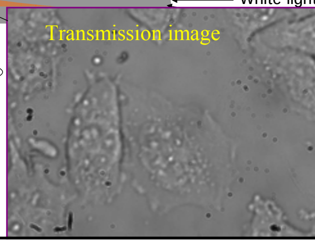


P-K, Wei

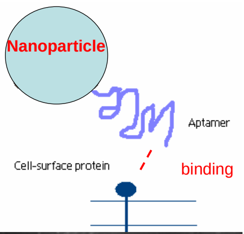
Application in the cell imaging

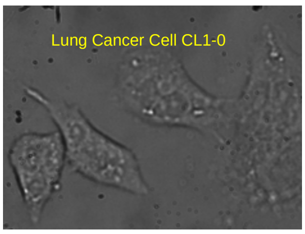


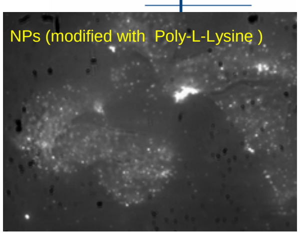


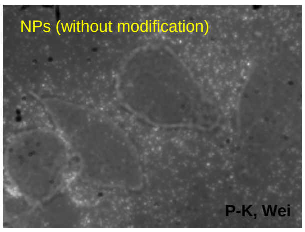


P-K, Wei

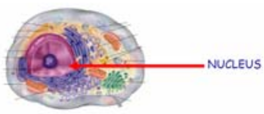






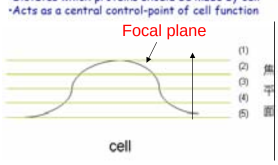


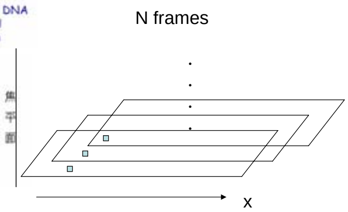
P-K, Wei



·Stores genetic material (genes) in the form of DNA
·Dictates which proteins should be made by cell
·Acts as a central control-point of cell function

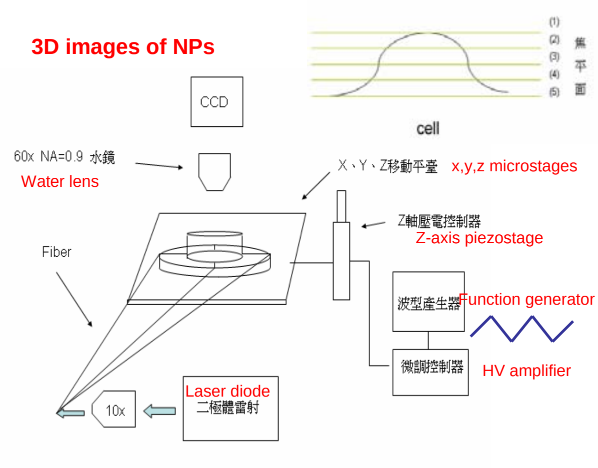
Varying focal plane + Image processing



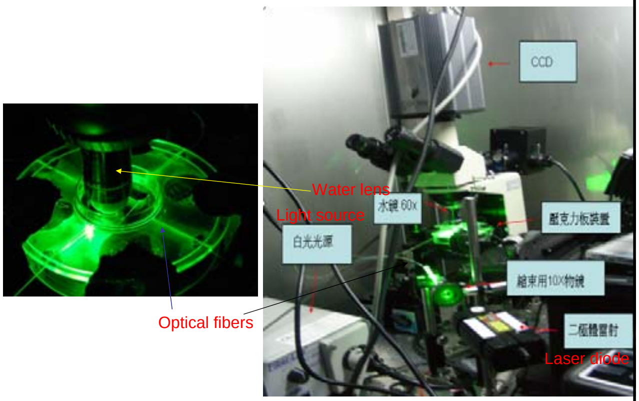


Some cells like fiberblasts have very good adhesion to the surface. However, most cells on the dishes are like mountains with heights over tens of microns. The length is beyond the depth of focus of an optical microscope with high magnification.
How can we obtain the nanoparticles image on a whole cell?

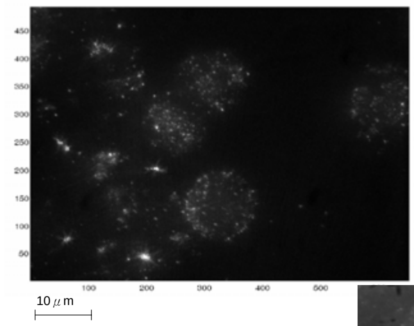
3D images of NPs



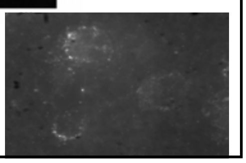
Setup of a 3-D NPs image system



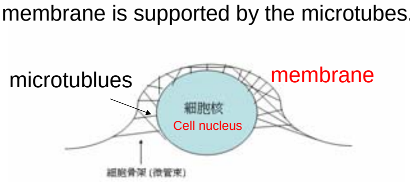
Varying focal planes + Image processing



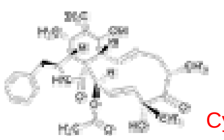
Only at one focal plane



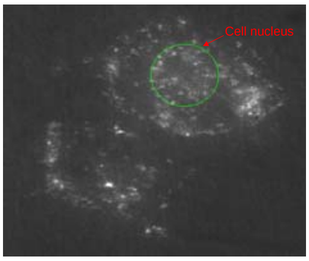
Cell membrane is supported by the microtubes.



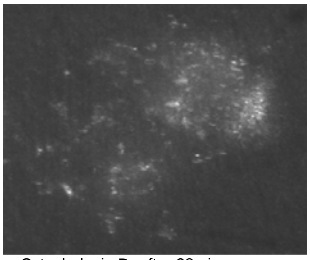
Cytochalasin D can dissolve the microtublues and then makes the collapse of the cell membrane



Cytochalasin D



Cytochalasin D, after 3mins



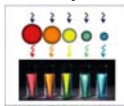
Cytochalasin D, after 28mins

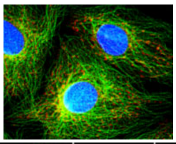
10 μm

Quantum dots (QDs)

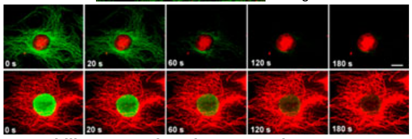
Advantages:

1.change emitting colors by sizes
2.no photobleaching
3.excite multicolors using a single laser source





Double-labeling of mitochondria and microtubules in NIH 3T3 cells. The mitochondria were labeled with human anti-mitochondria antibodies, goat anti-human IgG-biotin and



Photostability comparison between Qdot 605 and Alexa 488 conjugates.

http://www.qdots.com/live/index.asp

Disadvantages of ODs labeling.
1. Toxic to the cells
2. Surface modification is not easy
3. Expensive

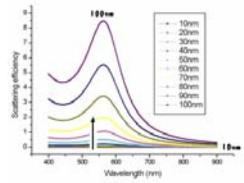
Advantages of nanogold labeling
1. No photobleaching。
2. No toxicity。
3. Good biocompatibility, easy for surface modification
4. Can be used for phototherapy

Disadvantages of nanogold labeling:
Scattering light has the same wavelength with the incident wave, dark-field illumination is necessary

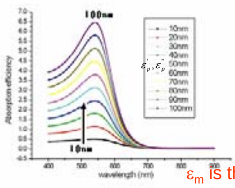
scattering

$$C_{sca}(\omega)=4\pi r^2\times\frac{32}{3}\pi^4\left(\frac{r}{\lambda}\right)^4\varepsilon_m^2\frac{\left[\varepsilon'_P(\omega)-\varepsilon_m\right]^2+\varepsilon''^2_P(\omega)}{\left[\varepsilon'_P(\omega)+2\varepsilon_m\right]^2+\varepsilon''^2_P(\omega)}$$

$$\sigma_s=\frac{2\pi^5}{3}\frac{d^6}{\lambda^4}\left(\frac{n^2-1}{n^2+2}\right)^2.$$



absorption

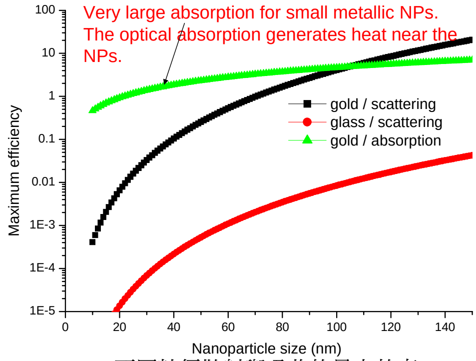


ε_p' and ε_p"are real part and imaginary part of the metal. Maximum absorption and scattering occur at $\varepsilon'_p = -2\varepsilon_m$。

ε_m is the dielectric of the surrounding medium(1 for air)

$$C_{abs}(\omega)=4\pi r^2\times 6\pi\left(\frac{r}{\lambda}\right)\varepsilon_m^{3/2}\frac{\varepsilon''_P(\omega)}{\left[\varepsilon'_P(\omega)+2\varepsilon_m\right]^2+\varepsilon''^2_P(\omega)}$$

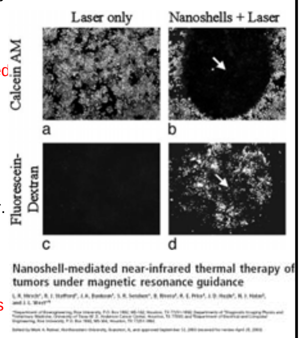
Scattering and Absorption of NPs



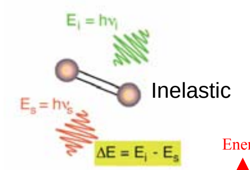
不同粒徑散射與吸收的最大效率

Nanoparticles hunt down and kill tumors - Biomedicine - Brief Article Science News, Dec 13, 2003

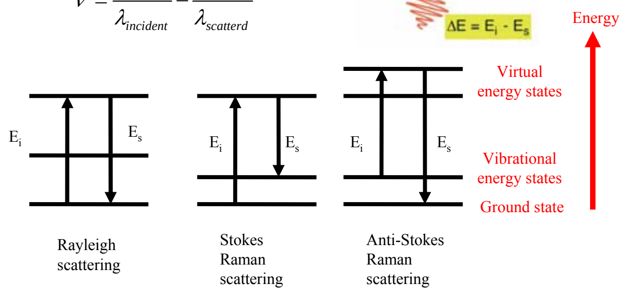
An innovative therapy that uses gold nanoparticles to destroy tumors could someday offer patients a new weapon against cancer. Researchers at Rice University in Houston injected gold-coated silica spheres into mouse tumors. Light shined onto the particles triggered the release of heat that destroyed the cancer cells.
Because this phototherapy would be less invasive than surgery, it could offer an alternative to typical cancer treatments,
Each particle, which the researchers call a nanoshell, measures about 130nm in diameter. The team designed the nanoshells to absorb near-infrared light, which can penetrate tissue without damaging it. After injecting the nanoshells into the mouse tumors, The resulting temperature rise of nearly 40 degrees was enough to cause irreversible tissue damage.



Raman sacttering



$$\bar{\nu} = \frac{1}{\lambda_{incident}} - \frac{1}{\lambda_{scatterd}}$$

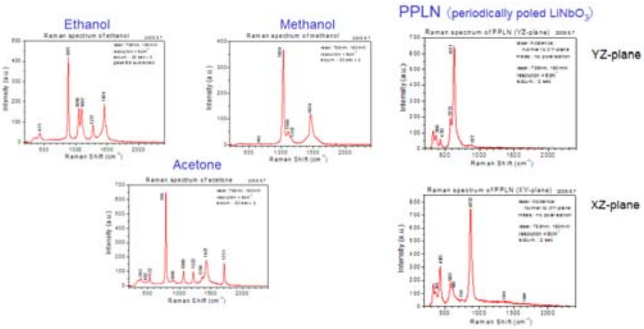


Ref: Thomas Huser"Introduction to Surface-enhanced Raman Spectroscopy", 2007.

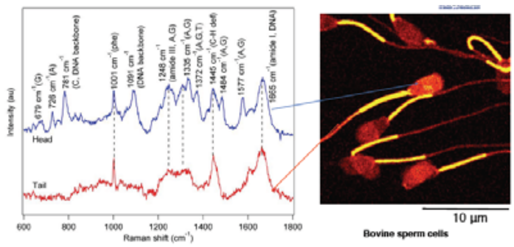
Advantages of Raman spectroscopy

- Fingerprint spectra (molecular identity)
- Information about 3d structural changes (orientation, conformation)
- Information about intermolecular interactions
- Combine microscopy

Different molecules are characterized by their own, unique Raman signature.



Ref: http://www.enwaveopt.com/spectra.htm, Enwave Optronics Inc.



- non-destructive, non-invasive
- works in-situ and in-vitro for biological samples
- works under a wide range of conditions: (temperature, pressure)

Ref: Thomas Huser"Introduction to Surface-enhanced Raman Spectroscopy", 2007.

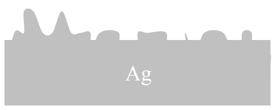
So, if Raman spectroscopy is so powerful and has been around for 70 years - why is it not used more often?

Raman scattering, however, is extremely inefficient

Only 1 in 10^7 incident photons are Raman scattered

SERS(surface enhancement Raman scattering)

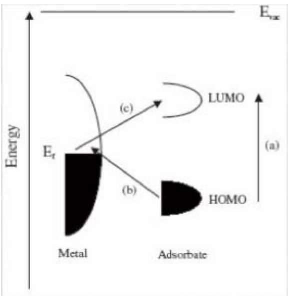
In 1977, Jeanmaire reported an interesting finding, sliver surfaces give Raman enhancements in the range of 10^3~10^8



The enhancement mechanisms are roughly divided into chemical enhancement and electromagnetic enhancement

Ref: Jeanmaire,Van Duyne, J. Electroanal. Chem.,84,1, 1977.

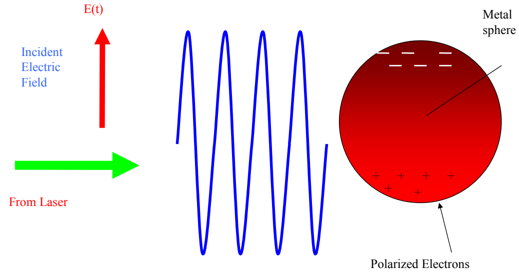
Chemical enhancement



Chemical enhancement has been estimated to contribute a factor of up to 100 to the SERS enhancement.

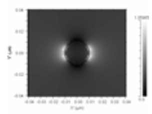
Ref:Campion, et al., Chem. Soc. Rev., 27, 241, 1998.

Electromagnetic enhancement



ref: K. Kneipp et al., Phys. Rev. Lett., 78, 1667, 1997.

$$^{em}G_{SER}(r,\nu) \propto \left| \frac{E(r,\nu)}{E_{inc}(\nu)} \right|^4$$



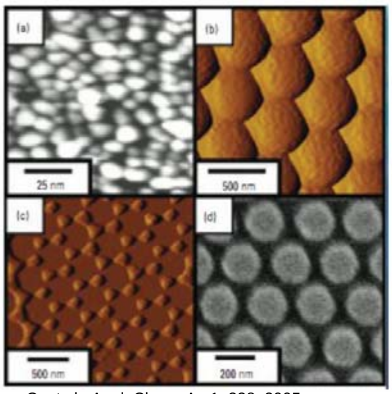
Substrates

Rough sliver film on glass

Sliver coating on top of ps beads

Nanosphere lithography

Sliver columns produced by e-beam lithography



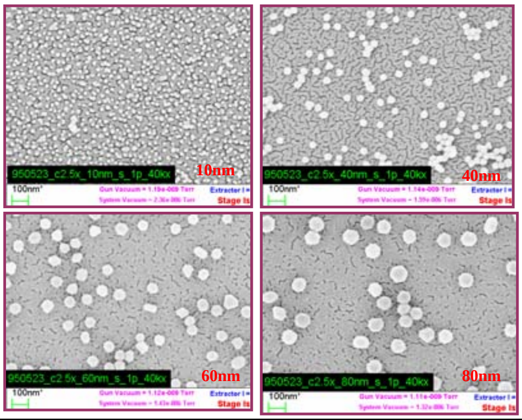
Ref: Haynes, C. et al., Anal. Chem. A., 1, 338, 2005.

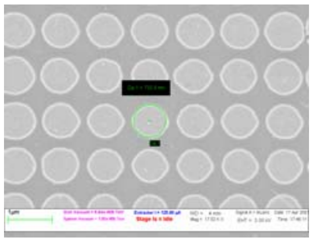
Observations

Surface-enhanced Raman spectroscopy required:

- specific metals (e.g. Au, Ag, Cu, Pt, ...)
- surfaces with roughness on the nanometer scale
- certain molecules provided much higher Raman intensities (mostly molecules with carbon double-bonds) N, S. Benzene.

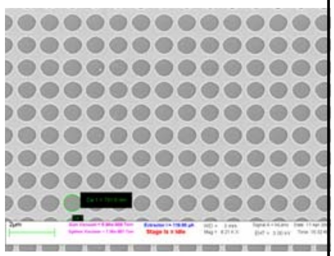
Nano gold particles



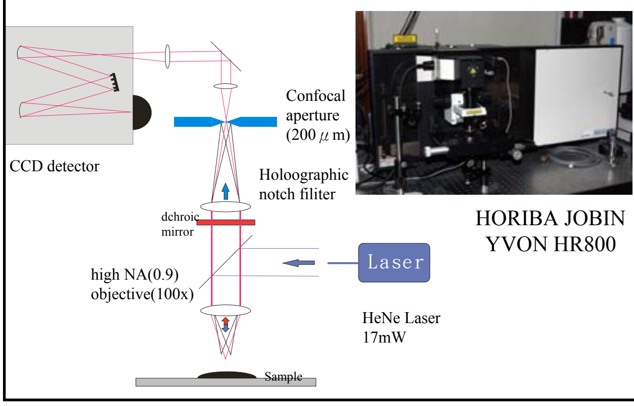


Cylinders diameter:124-800nm ;1 μm

Holes diameter:180-800nm ;1 μm



Micro-Raman Spectrometer



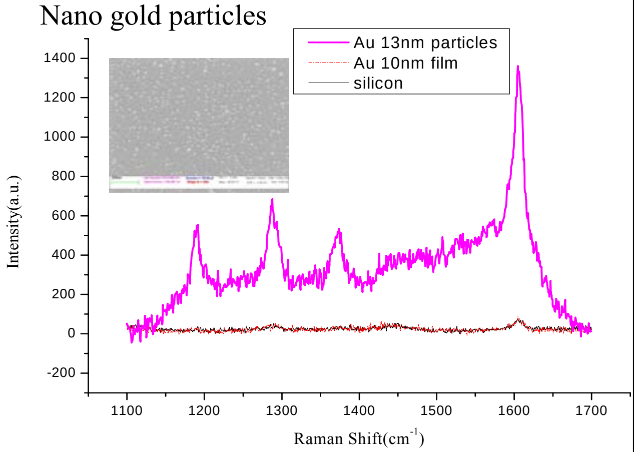
RESULTS

1.SEM images of Nano metallic surface

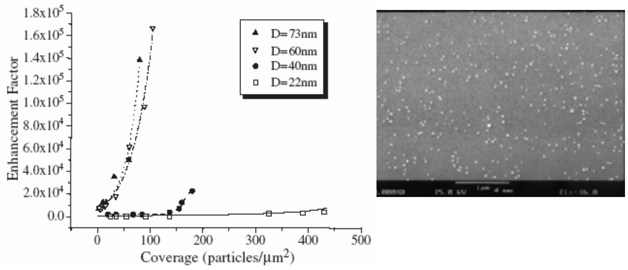
2.Nano metallic structures for SERS

- Nano particle gold assembles on a silicon surface
- Nano cylinders & holes
- Dewetting Au & Ag
- Sliver particles on dewetting Au

Nano gold particles

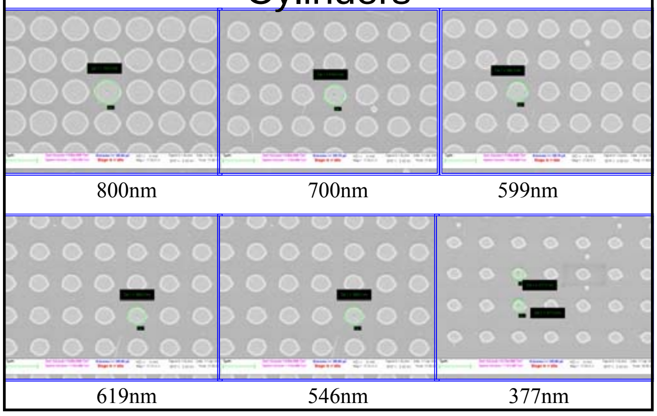


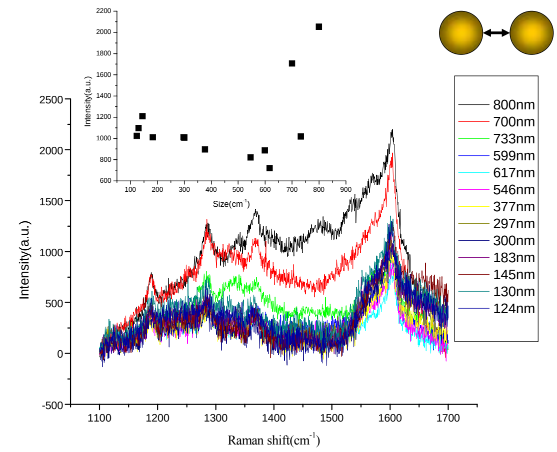
Nano gold particles assembles on a silicon surface



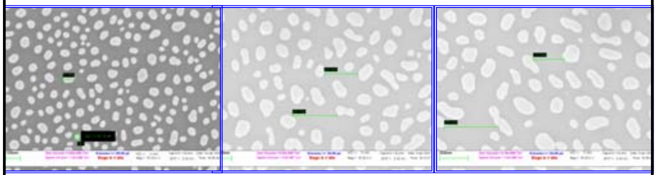
Ref:Zihua Zhu, Tao Zhu and Zhongfan Liu ,Nanotechology, 15,357, 2004.

Cylinders

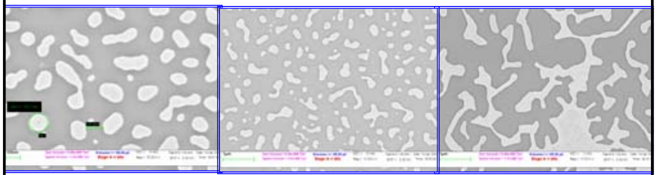




Dewetting Au 400℃

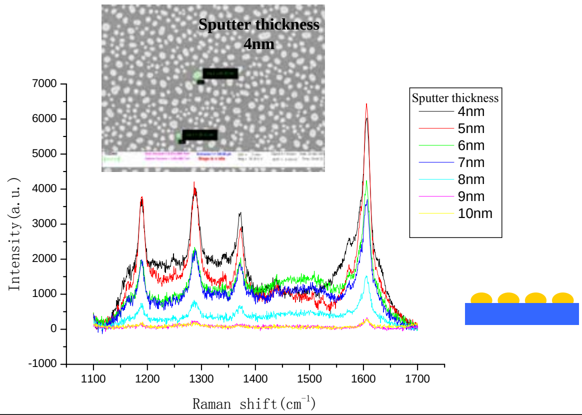


5nm400 ℃ | 6nm400 ℃ | 7nm400 ℃

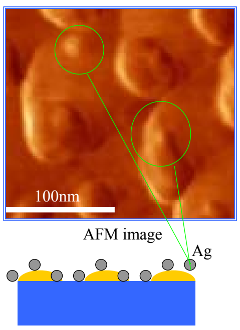


8nm400 ℃ | 9nm400 ℃ | 10nm400 ℃

Dewetting Au 400℃ for SERS

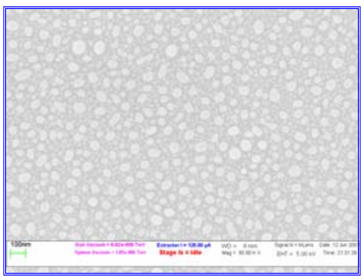


Sliver particles on dewetting Au



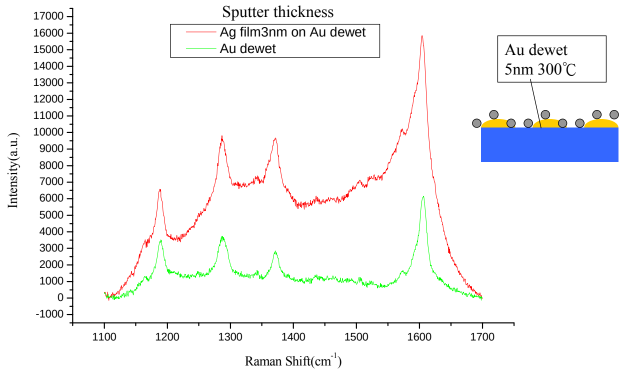
AFM image

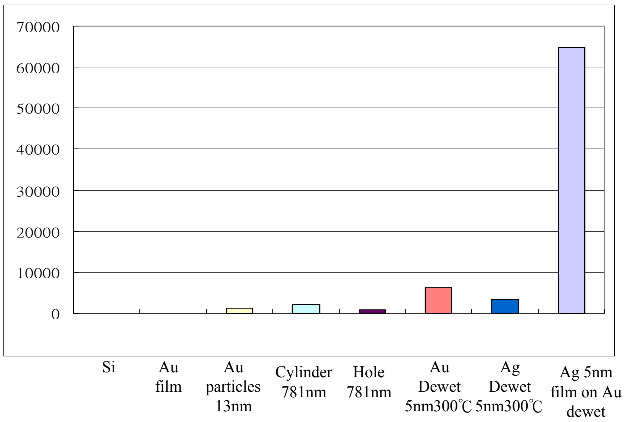
point discharge



SEM image

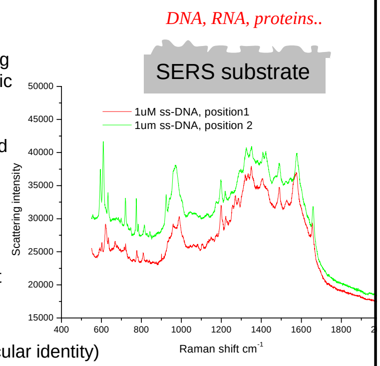
Sliver NPs on dewetting Au





Conclusions:

1. The absorption and scattering is greatly enhanced in metallic nanoparticles.
2. Gold nanoparticle is a good candidate for bio-labeling and therapy.
3. Substrate with nanometer roughness can greatly enhance the Raman signals, (SERS)
4. Raman signals are important for biological studies:



1. Fingerprint spectra (molecular identity)
2. Information about 3d structural changes (orientation, conformation)
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