Metallic Nanoparticles and Nanostructures for Bio-applications

J. B. Zhang[1a], L. Cheng[2], L. Y. L. Yung [3], S. S. Chua[1], J. Y. Sze[1], S. L. Zhu[4], T. C. Ayi[5] and R. Jeevaneswaran[1]

[1] Data Storage Institute, 5 Engineering Drive 1, Off Kent ridge crescent, NUS, Singapore

[2] Dept of Mechanical Engineering, National University of Singapore, Singapore

[3] Dept of Chemical & Biomolecular Engineering, National University of Singapore

[4] School of Electrical & Electronic Engineering, Nanyang Technological University, Singapore

[5] Defense Medical & Environmental Research Institute, DSO National Lab, Singapore

[a] Zhang_jingbo@dsi.a-star.edu.sg

Abstract

This paper elaborates on approaches of synthesis of Au and Ag nanoparticles (NPs), deposition of colloid onto glass substrate and encapsulation of NPs with silicon dioxide (SiO_2) thin shell. As one important bio application of metallic nanoparticles, both solution-based and substrate-based fluorescence enhancement tests are demonstrated.

Introduction

Appearing in stained color glass back to the 17th century, metallic nanoparticles (NPs) have gained great attention again in the new century in a variety of technologies. They are often referred to as nano-scatterers [1], nano-actuators [2] and nano-stoves [3]. Affected by the quantum confinement, NPs behave quite differently from the bulk and atomic state of the same material. They exhibit many extraordinary properties in terms of their responses to optical irradiation [4] and chemical reactions [5]. These properties are normally dependent on their shapes, sizes and compositions. They also exhibit large surface-to-volume ratio and high mechanical strength, which make NPs especially suitable for biochemical sensing [6] and optothermal local heating [3, 7].

Fluorescence technology is attributed to one of the most astonishing inventions and a vital tool in biochemistry and life sciences. As a result of rapid development in these fields, there have been endless quests and urges for higher and higher detection sensitivity. Among all the viable fluorescence enhancement approaches, the most promising one is Surface Plasmon (SP) enhanced fluorescence [8], also termed as Metal-Enhanced Fluorescence [9]. Other novel applications of metallic NPs and colloid deposited substrates include Surface Enhanced Raman Spectroscopy [10] and Immobilization of functionalized NPs to for nanopatterns [11] which would facilitate the integration of surface plasmon and MEMS optofluidic technologies.

This paper elaborates on synthesis of Au and Ag NPs, deposition of colloid onto glass slides and encapsulation of NPs with silicon dioxide (SiO_2) thin shell. Then it demonstrates their applications on Metal-Enhanced Fluorescence.

Localized Surface Plasmon Resonance – Origin of High Absorbance of Metallic Nanoparticles

Localized Surface Plasmon Resonance (LSPR) arises naturally from the scattering of light by metallic NPs. Such scattering phenomenon has an exact solution when the particle has spherical shape. In Mie's formulation, waves are expanded into infinite summations of normal modes, each weighted by coefficients of the respective order. The far-field cross-sections that describe the scattering effect are then expressed as summations over these coefficients. For particles

significantly smaller than the wavelength, phase retardation effect becomes negligible and so does the coefficients except that for the lowest order mode, the dipole plasmon mode [12]. The far-field cross-sections in this limit can be expressed as follows:

$$C_{scat} = \frac{8\pi}{3} k^4 a^6 \left| \frac{\varepsilon_e - \varepsilon_m}{\varepsilon_e + 2\varepsilon_m} \right|^2 \qquad C_{abs} = 4\pi k a^3 \, Im\left[\frac{\varepsilon_e - \varepsilon_m}{\varepsilon_e + 2\varepsilon_m} \right] \qquad C_{ext} = C_{abs} + C_{scat} \qquad (1)$$

The cross-sections simultaneously go to infinity as the common denominator vanishes. At the condition of $\varepsilon_e(\omega) = -2\varepsilon_m$, a peak appears in the absorption spectrum of metallic NPs.

Synthesis of Colloidal Gold and Silver Nanoparticles, Colloid Deposited Glass and Au@SiO$_2$

Synthesis of Au and Ag Colloids with Sodium Citrate Reduction Method. Au and Ag are selected to compose the NPs due to their large local EM-field enhancement capabilities. They can be synthesized routinely in our Lab with the standard sodium citrate reduction method [13]. The chemical equation of reducing Au^{3+} to Au^0 to synthesize citrate capped Au NPs is given below:

$$6AuCl_4^- + C_6H_8O_7 + 5H_2O \rightarrow 6CO_2 + 24Cl^- + 6Au^0 + 18H^+ \qquad (2)$$
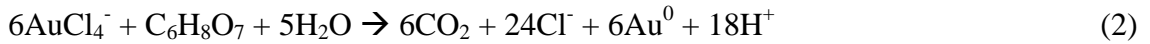
Where $C_6H_8O_7$ is the Citric acid which acts as a reducing agent at 109°C. The size of the Au NPs is inversely proportional to the amount of citrate added. STEM images of Au NPs, on copper grid, with different diameter are given from Figs. 1 to 4. Similarly, Ag colloid is reduced from $AgNO_3$.

Deposition of Au/Ag Colloid on Glass Substrates. Part of a cleaned glass slide is be functionalized with 3-aminopropyl-trimethoxysilane (APTES). Then, the functionalized glass slide is immersed in Au colloid, with estimated colloidal concentration of 1.5×10^7 particles per ml, for at least four hours. Hence, the Au NPs are immobilized onto the glass slides. For glass with higher surface coverage of NPs, more concentrated colloid (by removing 50~70% supernatant) can be used for deposition. Fig. 5 is a AFM graph of a glass slide with NPs, while Figs. 6 to 8 are SEM images of Au or Ag colloid deposited glass slides. Lastly, a SiO$_2$ film is sputtered over the randomly distributed NPs in an RF chamber of the Balzer sputtering system with the argon flow rate of 25 sccm. The SiO$_2$ layer is changed from 15 to 25 nm for Au NPs and 5 nm to 15 nm for Ag NPs.
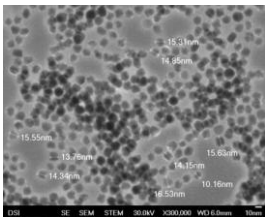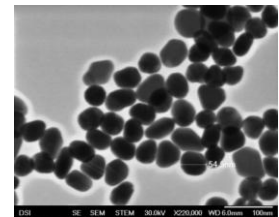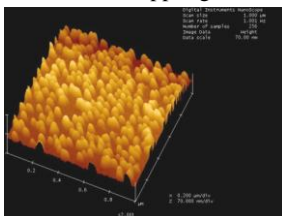

Fig. 1 Au NPs Dav = 14 nm NPs on copper grid
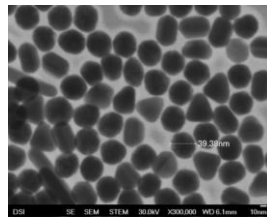
Fig. 2 Au NPs D$_{av}$ = 41 nm by direct synthesis
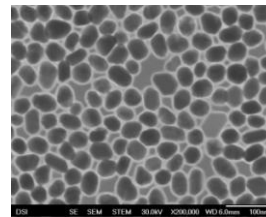
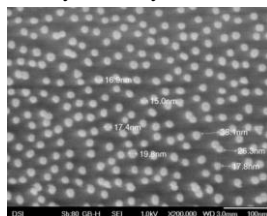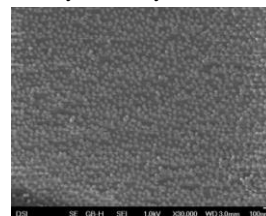**Fig 3:** Au NPs D$_{av}$ = 47.2 nm by direct synthesis
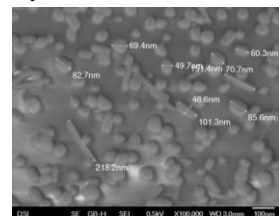
Fig 4: Au NPs D$_{av}$ = 53 nm by seed (14 nm) mediators

Fig. 5 AFM image of Au deposited glass, D$_{av}$= 20 nm

Fig. 6 Au deposited glass D$_{av}$ = 17 nm

Fig. 7 Au deposited glass with high concentration D$_{av}$=60 nm

Fig. 8 Ag deposited glass NP D$_{av}$ = 65 nm

Synthesis of SiO$_2$ Encapsulated Nanoparticles (Au@SiO$_2$). The metallic NPs are to be used with proteins and fluorophores. If proteins bind directly to metallic surface, unfolding would occur. If fluorophore molecules attach to metal surface directly, they will be fully or partially quenched with much weaker fluorescence emission. Thus it is essential to have a spacer layer/shell with controlled thickness to maintain the optimum distance in between one NP and adjacent fluorophore

molecules. SiO$_2$ is selected because of its superior chemical stability and biomolecule affinity. Au@SiO$_2$ NPs with different shell thikness are shown in Figs 9 to 14. For 32.6 nm and 23.5 nm thick shells shown in Figs. 11 and Fig. 12, the silica coating process takes two days and TEOS are added in intervals. A total of 0.4 ml and 0.2 ml of 10 mM TEOS, dissolved in isopropanol, are added respectively into the isopropanol solution of citrate-capped 50nm Au NPs. The images shown in Figs. 10, 13, & 14 are obtained by one time addition of TEOS and gentle stirred for 15~18 hours.
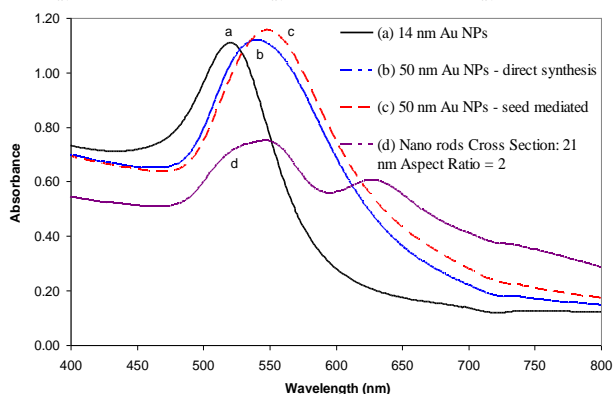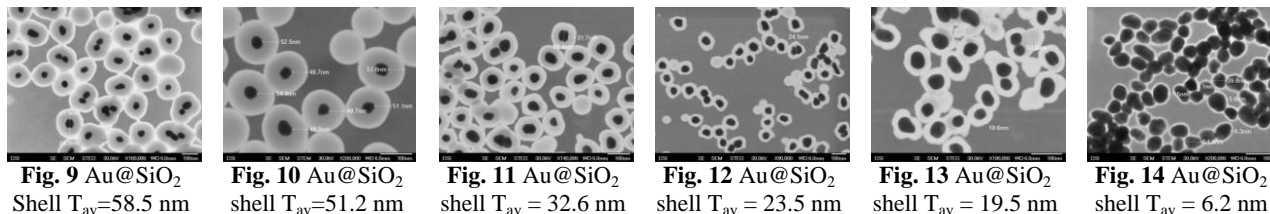

Fig. 9 Au@SiO$_2$ Shell T$_{av}$=58.5 nm

Fig. 10 Au@SiO$_2$ shell T$_{av}$=51.2 nm

Fig. 11 Au@SiO$_2$ shell T$_{av}$ = 32.6 nm

Fig. 12 Au@SiO$_2$ shell T$_{av}$ = 23.5 nm

Fig. 13 Au@SiO$_2$ shell T$_{av}$ = 19.5 nm

Fig. 14 Au@SiO$_2$ shell T$_{av}$ = 6.2 nm

Fig. 15 Absorption spectra of Au NPs with different diameters and one batch of nano rods
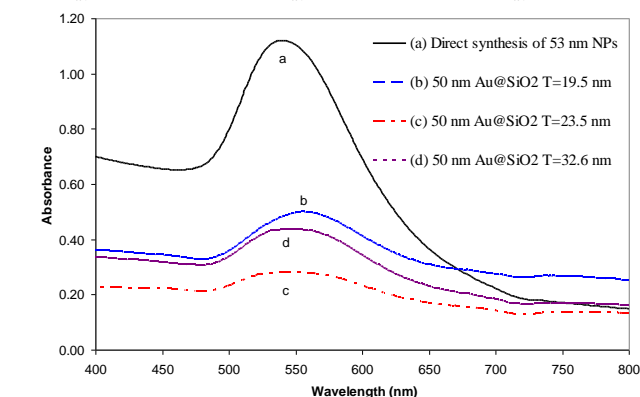

Fig. 16 Absorption spectra of Au@SiO$_2$ NPs with different shell thickness show peak red shift from the peak of Au NPs

Demonstration of Metal-Enhanced Fluorescence (MEF)

A fluorophore molecule in the excited state acts as an oscillating dipole. The average distance between a metallic NP and nearby fluorophore molecules governs the behavior of adjacent fluorophores for either being quenched or enhanced. The interaction has a number of useful effects that lead to increases in Quantum Yield (QY), photo stability and resonance energy transfer range and also the decrease in lifetime. These changes can result in increased sensitivity and signal-to-noise ratio. To demonstrate this effect, fluorescence enhancement experiments with above described colloids and colloid deposited glass substrate are conducted in both dry state and wet state.
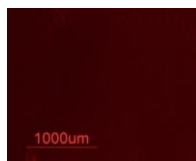
Dry State. We immobilize Au or Ag NPs on APTES functionalized SiO$_2$ thin film which is sputtered on top of NPs with varying thickness from 5 nm to 30 nm on glass slide. The glass slides are then incubated with dye conjugated BSA overnight in darkness at 4°C. After washing, the fluorescence emmision of BSA-Rhodamine B, physisorptioned on the glass slide, is observed and an image is captured by a fluorescence microscope with attached CCD. The image shown in Fig. 17 is taken on a glass slide with 53.0 nm Au NPs covered by 25nm SiO$_2$ film. The fluorescence image in Fig. 18 is taken from the control with the same BSA-Rhodamine B solution being absorbed on bare glass after overnight incubation. Using an image processing S/W named ImageJ, comparation of line intensity profiles from Figs. 17 and 18 prove that the fluorescence emission from Rhodamine B is enhanced to 3 times than the emission from the control.

Wet State. SiO$_2$ encapsulated Au or Ag colloid with 2 to 5 times of the original concentration is incubated in darkness with the BSA-dye solution of varying relative concentrations for at least 30 minutes in a microwell plate. Control solution is kept in a separate well on the same microwell plate. The fluorescence image in Fig 19, captured by the fluorescenc microscope, is compared with the control in Fig. 20 and the intensity profiles are analyzed with ImageJ. The results show enhancement of about 2 times. The fluorescence emission spectrum is also scanned by a microplate reader. The enhancement factor at emission peak agrees with that from the fluorescence images.
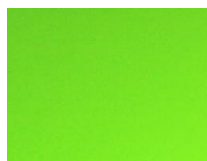
There is a huge potential for higher fluorescence enhancement by changing all the variables in narrower intervals. The variables include size, shape and composition of NPs, SiO_2 shell/film thickness, approaches of dye adhesion to NPs, pH of solutions and temperature during incubation.
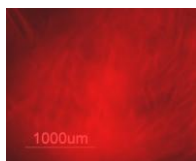
Fig. 17 Dry State BSA-Rhodamine B on glass with 53 nm Au NP covered by 25 nm SiO_2 film, 24 hour incubation	**Fig. 18** Dry state control BSA-Rhodamine B with the same concentration, incubated on glass 24 hours	**Fig. 19** Wet State mixed solution of Au@SiO_2 and BSA-FITC, 24 hour incubation, D_{Au} = 17 nm, T_{SiO2}= 25 nm	**Fig. 20** Wet state control BSA-FITC solution with the same concentration, incubated on glass 24 hours

Summary

In this paper, we report our effort and approaches for controlling the size of Au and Ag NPs in chemical reduction assays, and the thickness of the SiO_2 shell during the synthesizing process of Au@SiO2, as well as the thickness of the sputtered thin film on colloid deposited glass substrate. We also report the application of MEF using metallic nano colloids and colloid-deposited glass. Both solution-based and glass-based fluorescence tests show promising fluorescence enhancement.

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