doi:10.1017/S1431927616012368
Microsc. Microanal. 22 (Suppl 5), 2016

Fluorescently Labeled Silica Coated Metal Nanoparticles as Fiducial Markers for Correlative Light and Electron Microscopy

Jantina Fokkema[1,2], Gerhard A. Blab[1], Andries Meijerink[2] and Hans C. Gerritsen[1]

[1.] Soft Condensed Matter & Biophysics, Debye Institute and EMMEΦ, Utrecht University, The Netherlands.
[2.] Condensed Matter & Interfaces, Department of Chemistry, Utrecht University, The Netherlands.

The field of correlative microscopy where, for example, light and electron microscopy are combined has been growing very quickly in the past couple of years. In order to gain additional information from combining these two techniques, it is very important to properly overlay (or register) the images obtained with the two modalities. A fiducial marker that is clearly visible in both modalities helps to increase the ease and accuracy of this process.

Such a fiducial marker would need to be a particle or nanocomposite that provides us with high contrast for electron microscopy. Furthermore, it is also important that the particle is bright as well as photostable for light microscopy. For an integrated approach the fluorescence has to be preserved even under the cryo and vacuum conditions of an electron microscope. A final and very important requirement is the size of the particle. As a simple fiducial marker, a large particle diameter of 50-100 nm would be acceptable. However, if we also want to be able to use the particles for immunolabeling the total diameter should be as small as possible, ideally below 50 nm.

In this work, gold nanoparticles coated with a thin layer of fluorescently labeled silica, are presented as fiducial markers. Here the metallic core provides the contrast for electron microscopy while the fluorophores embedded in the silica shell provide us with the fluorescence we need. Proof of principle experiments are conducted demonstrating that we can successfully use this type of particles as fiducial markers for image registration in a typical biological samples.

The plasmonic properties of gold nanoparticles can offer a second advantage since the interactions of a plasmonic particle with a nearby fluorophore can result in quenching or enhancement of emission. This phenomenon has been studied extensively, and the important parameters determining whether fluorescence is quenched or enhanced have been identified [1]. These parameters include the size and shape of the metal particle, the separation between the fluorophore and the metal and the spectral overlap between the spectra of the fluorophore and the plasmon resonance peaks of the metal particle. We will explore the parameter space and present preliminary results that will allow us to successfully design and produce plasmon enhanced fiducial markers.

References:

[1] H Mertens, AF Koenderink and A Polman, Physical review B **76** (2007), p. 115123.

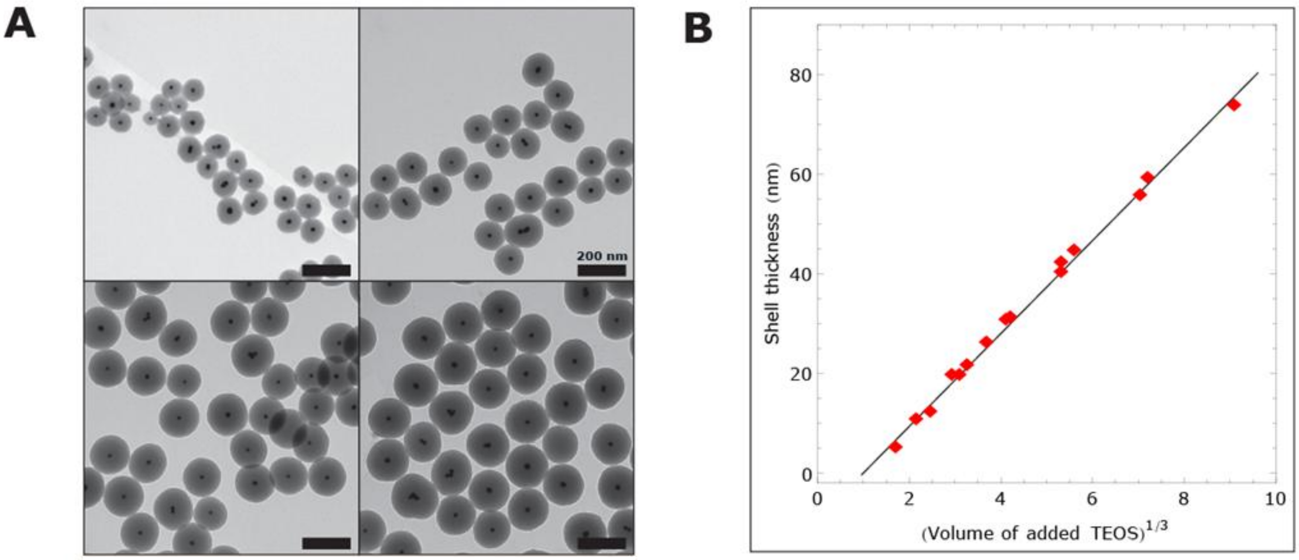

Figure 1. 15 nm diameter gold nanoparticles coated with different silica thicknesses. In (A) TEM pictures of particles with different total diameters are included. In (B) it is demonstrated that there is a linearly dependency between the silica shell thickness and the cube root of the volume of added silica precursors, TEOS, indicating that we can controllably synthesize particles with different total diameters.

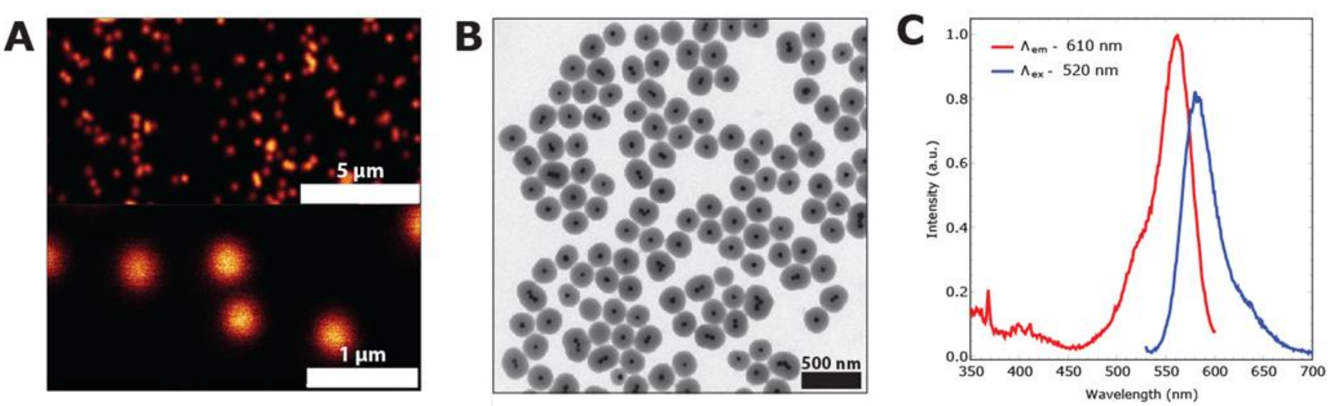

Figure 2. Gold nanoparticles coated with a Rhodamine B labeled silica shell can be visualized in light and electron microscopy. (A) Confocal images of the particles; (B) A TEM image of the particles; (C) Excitation and emission spectra of the particles measured in ethanol.

https://doi.org/10.1017/S1431927616012368 Published online by Cambridge University Press