EE 412 Final Report

Functionalization & Optical Characterization of Colloidal Gold Nanoparticles on Planar Substrates

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1 INTRODUCTION

The phenomenon of surface plasmon resonance (SPR) in metallic nanoparticles is exciting for what it can reveal about the physics of nanoscale systems, and its applications photochemistry, EM detection and propagation, molecular sensing, etc.[1] Studies have shown that SPR properties can be tuned by controlling nanoparticle size[2] and arrangement as well as their chemical[3] and electrical[4] environment. In particular, when nanoparticles are close together, their plasmon modes couple to create a "hot spot", or strong enhancement of the electric field in the nanogap of the dimer.[5,6] These effects suggest ways to enhance photonics-based sensing techniques, as has been done with Surface-Enhanced Infra-Red Absorption Spectroscopy (SEIRA)[7] and Surface-Enhanced Raman Spectroscopy (SERS)[8,9]. It is of interest to develop capabilities in the SNF to functionalize nanoparticles with different morphologies and characterize their optical behavior.

Research in this area has mostly been on lithographically-defined or suspended colloidal nanoparticles (NP). However, in light of the Quantum Tunneling Electronic Probe (QTEP) project, we wanted to see if an orthogonal plasmonic detection method could be made compatible with the current electrical device—that is, a setup in which colloidal Au nanoparticles are attached to an underlying metallic (Pt) substrate via a non-conducting molecule such as ethanedithiol (EDT). To simplify the problem, instead of Pt/Ir tips, a Si(100) wafer was used as a planar substrate. First, 300 nm of thermal oxide was grown on it; then a thin titanium adhesion layer was evaporated, followed by platinum. As per the QTEP protocol, functionalization of the sample sites involved immersion in an ethanol solution of ethanedithiol followed by an aqueous solution of Au NP's.

We were mainly interested in exploring the capabilities of the Cytoviva Hyperspectral Imaging system in the NSIL to optically characterize our samples. While the original aim was to perform splits to study how NP size and spacing affects SPR peak position(s) and intensity, analysis along the way indicated the necessity of auxiliary imaging techniques to clarify the outcome of the functionalization step. The preliminary conclusion is that the protocol as utilized in QTEP was not successful in attaching Au nanoparticles to the Pt substrate.

The aims of this report are as follows:

- To document the methods and tools used to functionalize and characterize the samples
- To document the images and analysis that have led to the present conclusions
- To propose functionalization protocol and experimental design for future work on the project, based on the experience gained here

2 EXPERIMENTAL METHODS AND TOOLS

2.1 SAMPLE PREPARATION

Table 1 summarizes the steps used to prepare our samples of Au nanoparticles on platinum film on silicon <100> wafer. The tool names in the right-most column are as given in the SNF listing.

#	Process description	Tool
1	5:1:1 H_2O:H_2O_2:NH_4OH (10 minutes, 50°C), followed by dump/rinse	wbclean
2	50:1 HF (30 seconds, RT), followed by dump/rinse	wbclean
3	5:1:1 H_2O:H_2O_2:NH_4OH (10 minutes, 50°C), followed by dump/rinse	wbclean
4	Spin dry	wbclean
5	Wet thermal oxidation (42 minutes, 1000°C) to grow ~300 nm of oxide	thermco1-3
6	Deposit 10 nm of Ti	intlvac_evap
7	Deposit 200 nm of Pt	intlvac_evap
8	Place PDMS well plate onto Pt substrate. Ensure good adhesion.	
9	Pipette 400 µL of ethanedithiol in ethanol solution (~1:8000 volume ratio) into well. Leave for 24 h at room temperature.	Fume hood
10	Remove ethanedithiol solution. Using a pipette, rinse well with ethanol. Remove ethanol, then rinse well with DI water. Remove DI water.	Fume hood
11	Pipette 400 µL Au nanoparticle solution of desired concentration for required duration.	Fume hood
	Remove Au nanoparticle solution. Using a pipette, rinse well with DI water.	Fume hood
12	Peel off PDMS well plate from substrate. Blow dry surface using N_2 gun.	
13	Using a permanent marker to roughly outline the position of the wells so they can be easily located under the microscope.	
10	Optical characterization	Cytoviva, SEM, AFM

Table 1: Protocol for preparing Au nanoparticles on Pt film on Si wafer

The ethane-1,2-dithiol solution and Au nanoparticle colloidal solutions were obtained from Sigma Aldrich. Nanoparticles came stabilized in a Phosphate Buffered Saline to prevent aggregation, at concentrations of ~ 10^9 – 10^{12} particles / mL (depending on diameter). Different nanoparticle diameters (10, 50, 100 nm) were used, at different dilution factors (1x, 8x, 10x, 18x, 100x, 500x, 1000x). This will be described in the experimental outline section.

Alongside the Pt substrate functionalization, glass slides were also functionalized with Au NP's since the Cytoviva Hyperspectral tool is an established tool for imaging visualizing Au NP's in transmission dark field mode. This split serves as a control for what images / spectra of nanoparticles roughly look like, so we know what to search for. Literature search turned up two main methods for functionalizing colloidal Au NP's on biology-grade microscope glass slides:

1. Plasma treatment in argon gas for 360 s to increase the reactivity of the glass surface, followed by immersion in 4 x 10^{-4} mol/L solution of ethanedithiol for 24 h. After washing with methanol, slides are immersed into Au NP colloid solution for 24 h.[11]
2. Functionalization with (3-aminopropyl)triethoxysilane (APTES) in ethanol or methanol, followed by functionalization with Au NP's.[12]

Owing to the unavailability of argon plasma treatment, the second method was chosen. Table 2 shows the steps taken.

#	Process description	Tool
1	Place PDMS well plate onto glass slide. Ensure good adhesion.	
2	Pipette 400 µL of APTES in ethanol (or methanol) solution (1:10 volume ratio) into well. Leave for 24 h at room temperature.	Fume hood
3	Remove APTES solution. Using a pipette, rinse well with ethanol (or methanol), then rinse well with DI water. Remove DI water.	Fume hood
4	Pipette 400 µL Au nanoparticle solution of desired concentration for required duration.	Fume hood
5	Remove Au nanoparticle solution. Using a pipette, rinse well with DI water.	Fume hood
6	Peel off PDMS well plate from substrate. Blow dry surface using N_2 gun.	
7	Using a permanent marker to roughly outline the position of the wells so they can be easily located under the microscope.	
10	Optical characterization	Cytoviva, AFM

Table 2: Protocol for preparing Au nanoparticles on glass slides

It was found that the rinsing with ethanol / methanol in step 3 was to remove any physisorbed APTES, to prevent nanoparticle aggregation in the colloidal suspension during the next step. In this functionalization method, the gold nanoparticles bind to the intermediate organic chemical through their attraction to the positively charged amine (-NH_2) group in APTES, instead of a thiol group (-SH) as in ethanedithiol. Nonetheless, this method can be modified by functionalizing mercaptopropyltriethoxysilane (MPTES) onto glass instead using a similar protocol. Chain length can be increased by grafting mercaptoundecanoic acid (MUA) to APTES as shown in Figure 1.

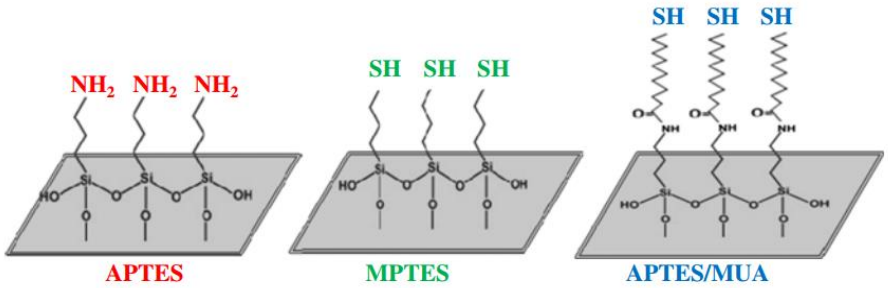


Fig. 1: Intermediate functionalization of glass with (a) APTES; (b) MPTES; (c) APTES/MUA

Figure 2. shows the functionalization setup with PDMS well plates on the metal / glass substrates.

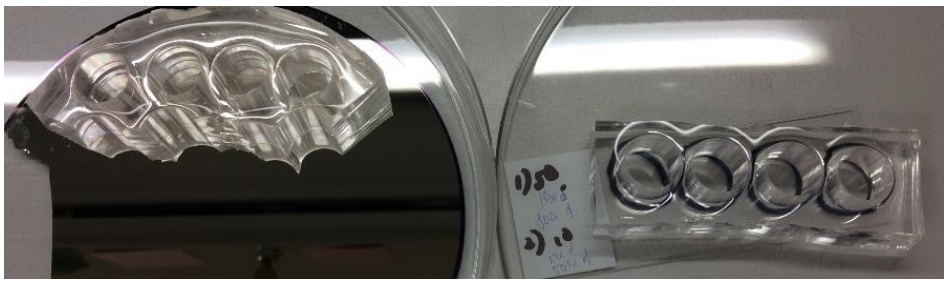


Fig. 2: Functionalization setup for Pt film on Si wafer (left) and glass slide (right)

2.2 Optical Characterization on Cytoviva Hyperspectral Imaging System

The basic components of CytoViva's Hyperspectral Microscope (Figure 3) include a high power halogen light source (1), liquid light guide (2), CytoViva patented nanoscale illumination optics (3) motorized stage (4), microscope stand with accessories (5), cooled color optical camera (6), and an imaging spectrograph (7) coupled to a sensitive digital camera (8). The halogen light source minimizes extraneous peaks present with other sources, like Xenon and metal halide light sources. The use of a light guide coupling with CytoViva's patented enhanced dark-field condenser provides extremely precise illumination directly onto the sample in dark field transmission mode. The concentration of light photons allows light scattered from nano-scale particles to be imaged and studied spectroscopically.

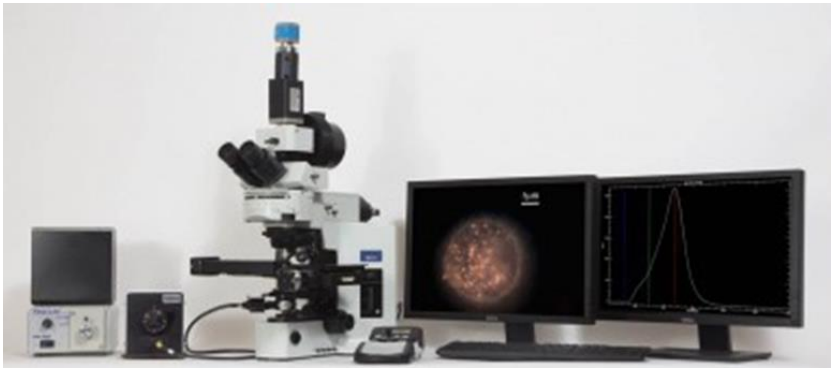


Fig. 3: Cytoviva hyperspectral imaging system

A hyperspectral image is created in a line scan or "pushbroom" fashion, by moving the sample across the field of view of the microscope and spectrograph via an automated translational microscope stage. Typically these spectral images are created in seconds or minutes, depending on the required exposure. Hyperspectral images appear very similar to a traditional optical image with one important difference. When observed via image analysis software, each pixel of a hyperspectral image provides the complete spectral response of that pixel's spatial area within the VNIR or SWIR spectral range. Figure 4 compares spectral images obtained for a pixel in an Au nanoparticle using a standard dark field microscope and the low-noise Cytoviva system.

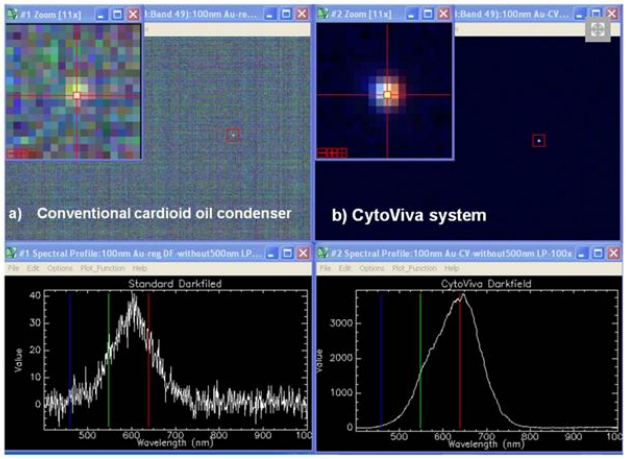


Fig. 4: Comparison of spectra from (a) standard dark field and (b) Cytoviva systems

2.3 EXPERIMENTAL OUTLINE

2.3.1 Samples & Controls

The following splits were done for the functionalization of Au nanoparticles on Pt substrate:

NP Size	NP dilution factor		NP Functionalization time
▪ 10 nm	▪ 1x	▪ 100x	▪ 2 h
▪ 50 nm	▪ 8x	▪ 500x	▪ 24 h
▪ 100 nm	▪ 10x	▪ 100x	
	▪ 18x		

The purpose of varying NP size is to see if this correlates to a shift in spectral peaks to longer wavelengths in the Cytoviva data, to confirm its utility in analyzing nanoparticle optical properties.

The purpose of varying dilution factor and functionalization time is to see if this changes the density of nanoparticle functionalization on the substrate, in a way that can also be reflected in the Cytoviva images. Density is also related to nanoparticle spacing, which has been shown to have a bearing on the position of the LSPR peak.

To serve as controls, the following samples were also prepared and imaged:

- Bare platinum (no subsequent functionalization)
- Platinum functionalized with ethanedithiol
    - One sample was left in air for 24 h, the other was immersed in water for 24 h
- Platinum functionalized with propanethiol for 24 h, followed by Au NP's. Propanethiol only has one thiol group which is expected to attach to Pt. So no Au NP binding expected.
- Platinum functionalized with ethanol for 24 h, followed by Au NP's

By comparing the images for the standard Pt → EDT → Au NP functionalizations with these controls, we can attribute any differences to differences in the procedure. Conversely, a lack of difference would indicate that the difference in procedure did not produce an observable effect.

The separate control performed on glass slides with the APTES in ethanol or methanol solution intermediate functionalization was similarly done with splits for nanoparticle size, dilution factor and functionalization time. More details for the experimental samples can be found in Tables 1 and 2 of the Appendix.

2.3.2 Characterization methods

Cytoviva Hyperspectral imaging formed the backbone of the optical characterization done. To image the Pt substrates, the standard objectives had to be swapped out for special dark-field objectives compatible with reflectance mode. Furthermore, it was found that the slide controlling how much of the reflected light is channeled to the eyepiece and to the camera needed to be toggled to a midway position in order to achieve a dark substrate background that enhanced contrast with the light scattered off particles on the substrate. Unfortunately, this limited the field of view, and may have introduced secondary imaging effects. Cytoviva was contacted regarding this issue, but did not have specific inputs to add.

To image the glass substrates, dark-field transmission mode was used. A drop of oil was placed on the condenser and the condenser raised until it made contact with the bottom of the glass slide, allowing light to be effectively coupled and focused onto the sample.

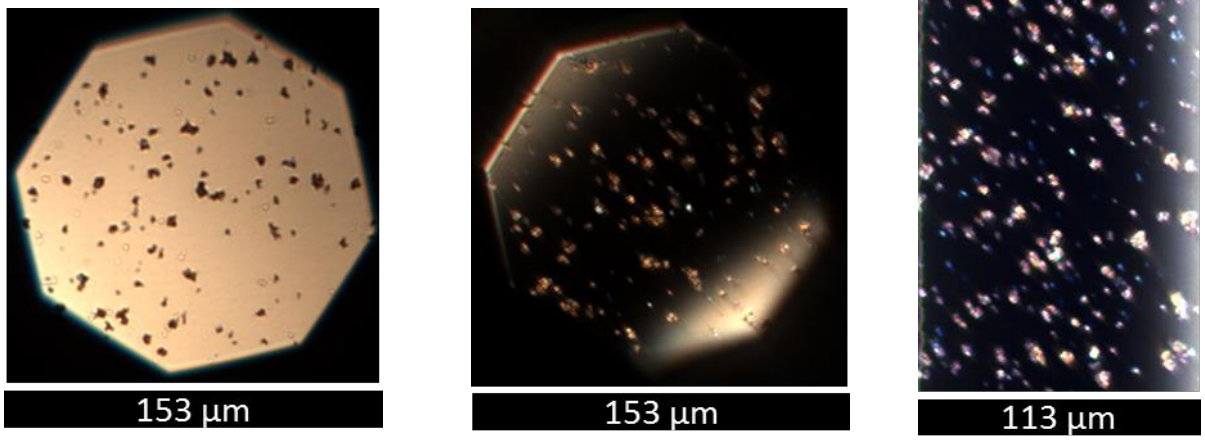
Exposure time and gain settings were toggled to obtain hyperspectral images with reasonable SNR. Different pixels on the captured images were sampled to obtain a range of optical spectra present in the sample. While this is by no means exhaustive, it does give a gauge of how particles on one sample might vary from another, through how they interact with light.

While Cytoviva images provide spectral and some sizing information (dimensions of particles can be estimated from the optical images), it lacks morphological data which are crucial to identifying the presence of nanoparticles of different sizes. As such, we complemented the characterization with Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) images, for a fuller picture of what is going on in the samples. (SEM was only be done on the Pt samples due to conductivity issues.) The findings are presented in next section.

3 Results and Discussion

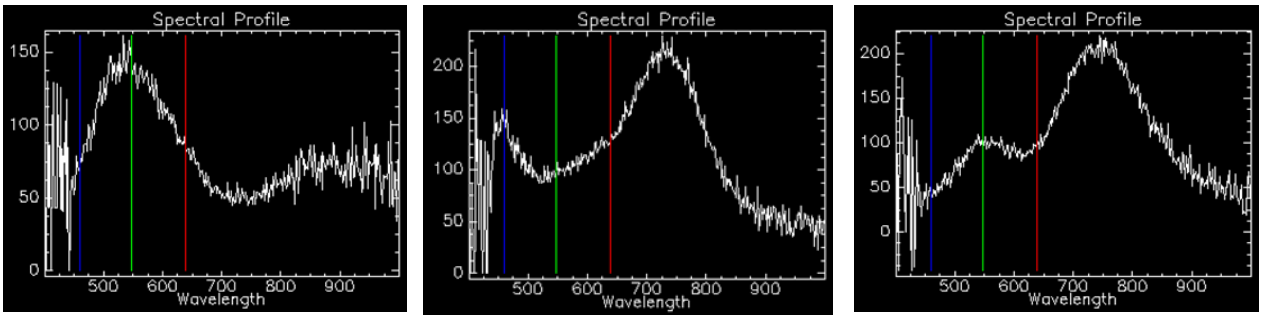
Groups of images (Cytoviva, AFM, SEM) for representative samples will be presented. We refer to samples by a shorthand that indicates the substrate and preparation procedure: e.g. Pt→Ethanedithiol→Au NP means the sample uses a platinum substrate and underwent functionalization in ethanedithiol / ethanol for 24 h, followed by Au nanoparticle solution.

3.1 Pt → Ethanedithiol → Au Nanoparticles



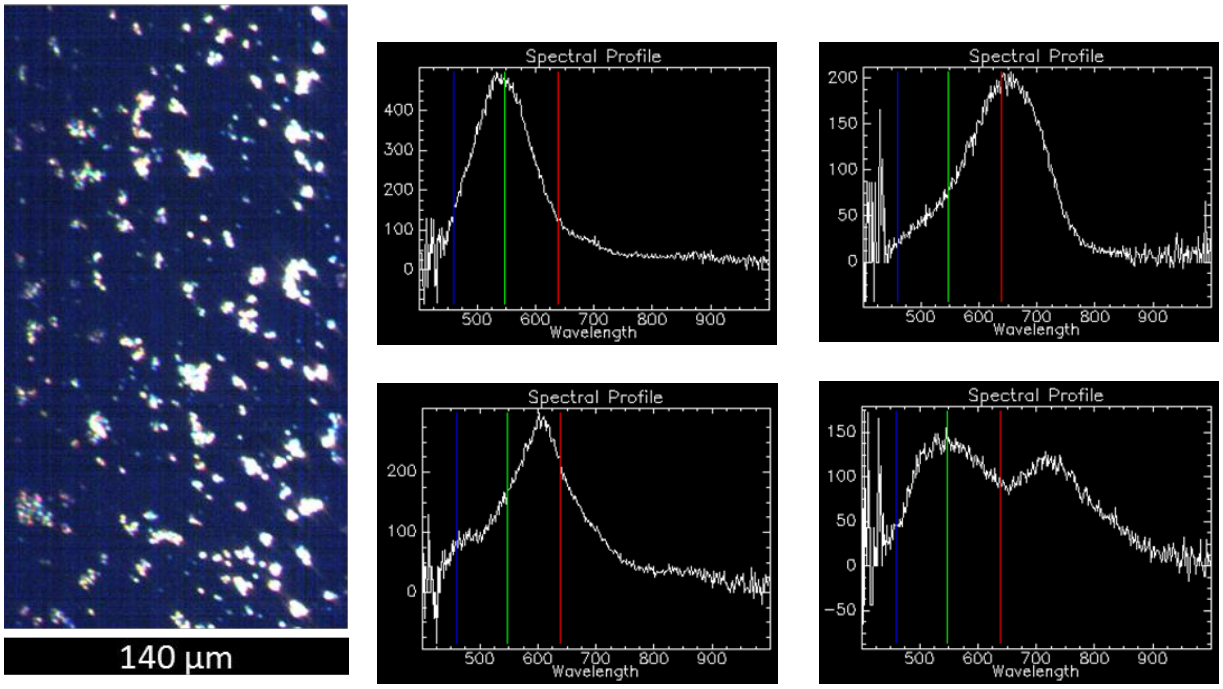
(a) Optical image (Bright-field) ***(b) Optical image (Dark-field)*** ***(c) Hyperspectral image***

Fig. 5: Cytoviva images: Pt→EDT→Au NP functionalization; 50nm NP, 1x dilution, 24 h



(a) Sample spectrum 1 *(b) Sample spectrum 2* *(c) Sample spectrum 3*

Fig. 5: Sample spectra: Pt→EDT→Au NP functionalization; 50nm NP, 1x dilution, 24 h



(a) Hyperspectral image *(b) Sample spectra*

Fig. 7: Cytoviva images: Pt→EDT→Au NP functionalization; 50nm NP, 8x dilution, 2 h

The optical micrographs in Figures 5 and 7a show the presence of clumps on the order of 1 -10 µm in size on the sample sites. No clear difference is visually detectable due to the different NP dilution factor and functionalization time—an observation consistent across all the Pt → EDT → Au NP splits. From Figures 6, 7b and 8, we observe peaks occurring frequently at: ~470, 550, 640, 750 nm, with peaks vary depending on the pixel in the bright clump. For Au NP's of ~ 50 nm diameter, the resonance peak in suspension is expected at 535 nm. However, the local refractive index close to the nanoparticle surface influences peak position as seen in Fig. 8b, and so attachment to a substrate is expected to shift this slightly toward longer wavelengths. A peak at ~ 550 nm might not be incompatible with this phenomenon. However, even if this were the case, the presence of the peaks at smaller and larger wavelengths would remain to be explained.

Another phenomenon is that peak position appears to evolve in a somewhat systematic way across large clumps. This is illustrated in the sequence of images in Figure 8. Starting from the

edge of a clump, we see a peak in the infrared region shifting towards smaller wavelengths, broadening, splitting into 2 (spectrum 12) and then 3 (spectrum 14), shifting and subsiding again.

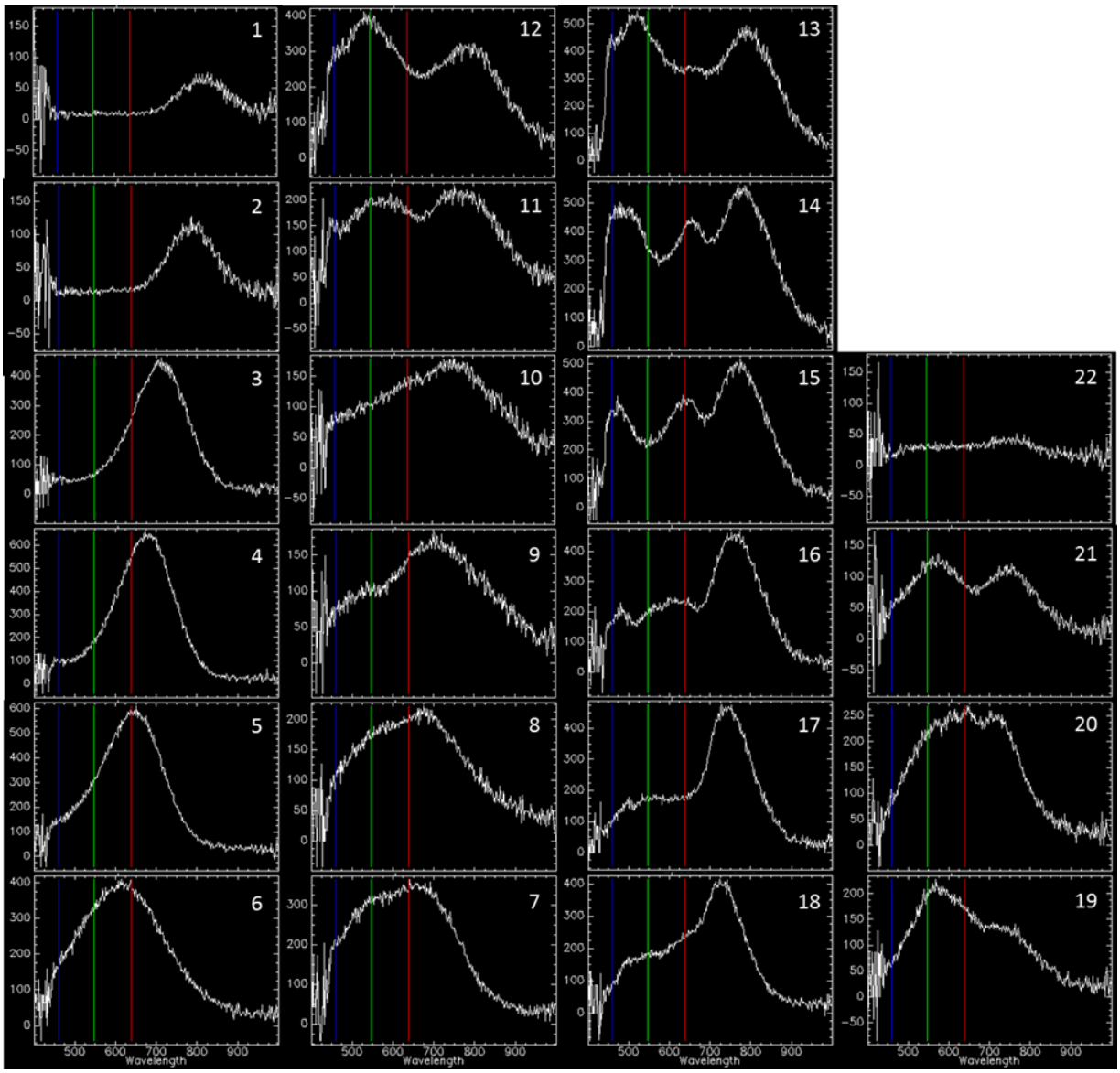
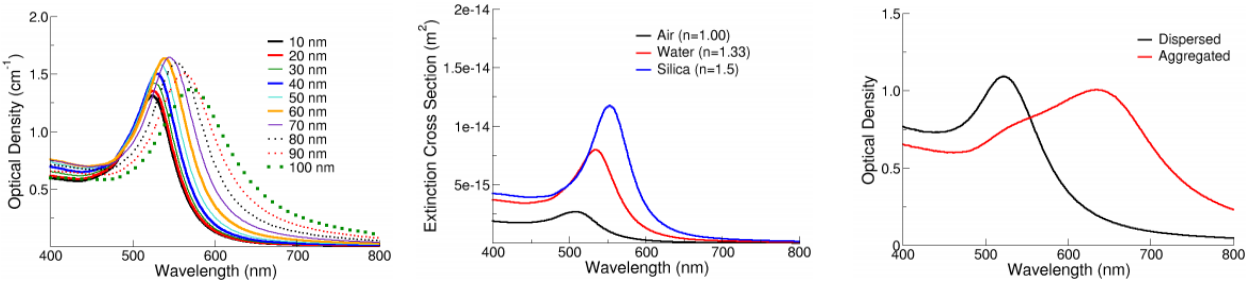


Fig. 8: Evolution of spectra across a cluster (spatial sequence given by numbers) for Pt→EDT→Au NP functionalization; 50nm NP, 8x dilution, 24 h

Several possibilities exist to explain these two observations. The distinct peaks in three different regions of the spectrum could be due to:

- A non-Au nanoparticle material that scatters light resonantly at these wavelengths. Resonance would then be due to is due to constructive interference of light scattered from different interfaces material. Deviations of peak position from a mean at different points in the sample might be explained by heterogeneity in the material structure / composition, resulting in variations in optical thickness.[13]
- Au nanoparticles aggregating together, resulting in a broader peak at a longer wavelength, as illustrated in Figure 9c.

- Au nanoparticles being attached in close proximity to each other, giving rise to a longitudinal coupled mode at a longer wavelength in addition to the original peak at ~ 550 nm. This is shown in Figure 10, for dimers of nanoparticles with ~ 26.5 nm diameter.[10]
- The peaks in the emission spectrum of fluorescent light. While the main source of sample illumination was from the Cytoviva halogen lamp and was normalized out, the spectrum of the fluorescent ceiling light was not and could have influenced the observations. Figure 11 shows that the 550 nm peak in our measurements and the emission spectrum seem to match up. However, the match with other peaks is not as close; also, the 550 nm peak was not observed throughout the sample, so we can probably rule this factor out.



(a) Effect of NP size ***(b) Effect of local refractive index*** ***(c) Effect of aggregation***

Fig. 9: Au nanoparticle resonance peak shifts due to different effects

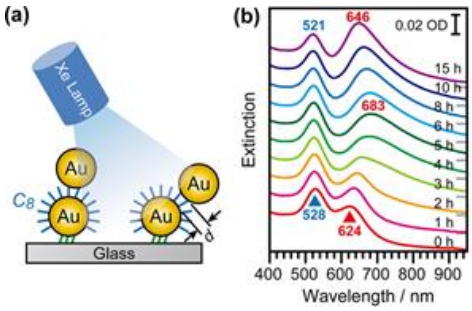


Fig. 10: Spectral shifts of Au NP dimers with increasing irradiation time, causing nanogap decrease

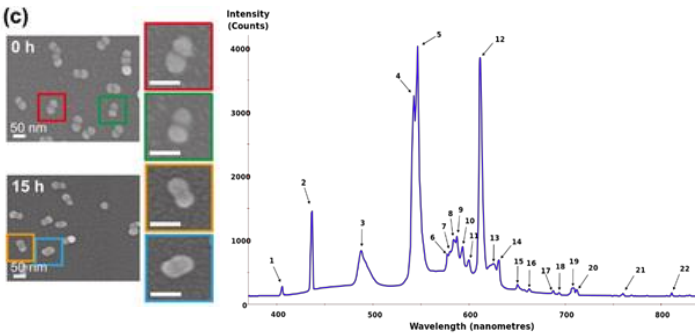
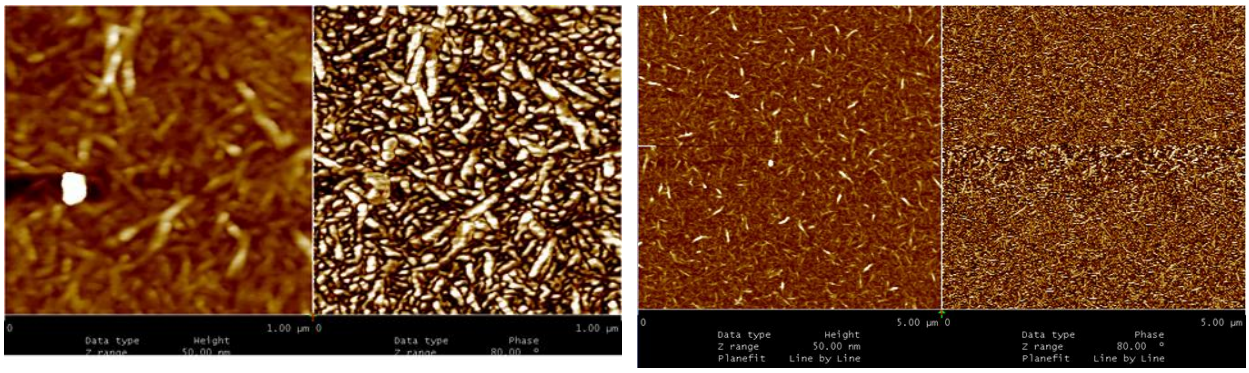


Fig. 11: Emission spectrum of standard fluorescent lamp

The first three factors could also explain why peaks were seen to evolve with pixel position across a clump—this could be due to changes in optical thickness across the material in the clump, or pixel position relative to the surface of a single or multiple Au nanoparticles. Peak variations might also arise from variations in spacing between nanoparticles at different positions and variations in nanoparticle size in the commercial solution (though this is unlikely to be able to explain the full range of shift). Chromatic aberration at boundaries of the clump and between sub-particles in clump is another possible explanation. This lens effect results in “fringes" of color along boundaries that separate dark and bright parts of the image, because each color in the optical spectrum cannot be focused at a single common point.

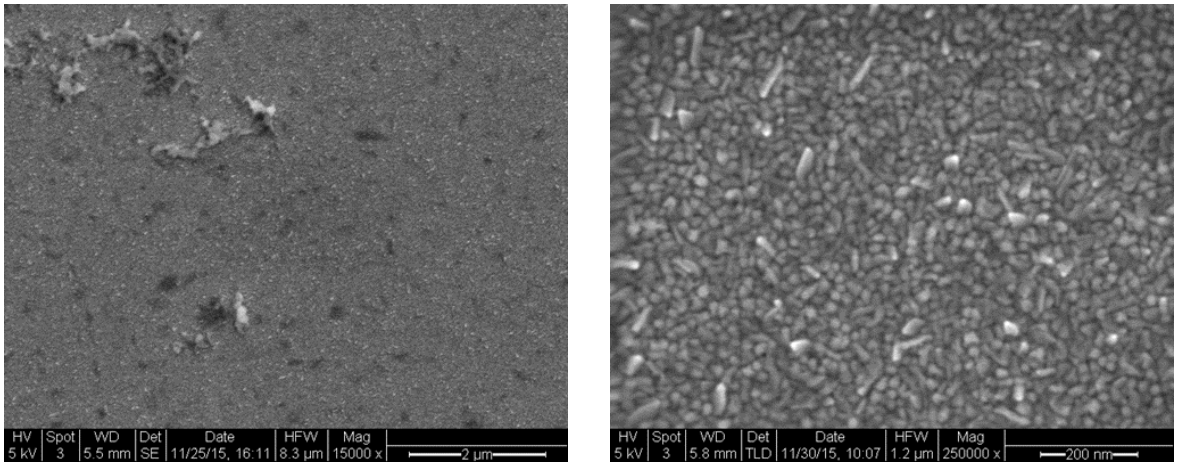
Underlying all these speculations is the question of what the bright clumps observed are—if they are indeed Au NP aggregates or some form of impurity—and if so how this was introduced. To answer this, controls are needed to delineate the contributions from the Pt substrate and the

ethanedithiol and Au nanoparticle functionalization steps, as will be presented. AFM and SEM measurements were also conducted to study the surface topography. These are shown in Figures 12 and 13. Note that these show a much smaller length scale of the sample than the Cytoviva optical images. At this scale, clumps on the order of 1 µm are visible in some images, but more striking than that is the background structure roughly spherical and oblong grains.



(a) Z & phase images, 1 x 1 µm²scan size ***(b) Z & phase images, 5 x 5 µm²scan size***

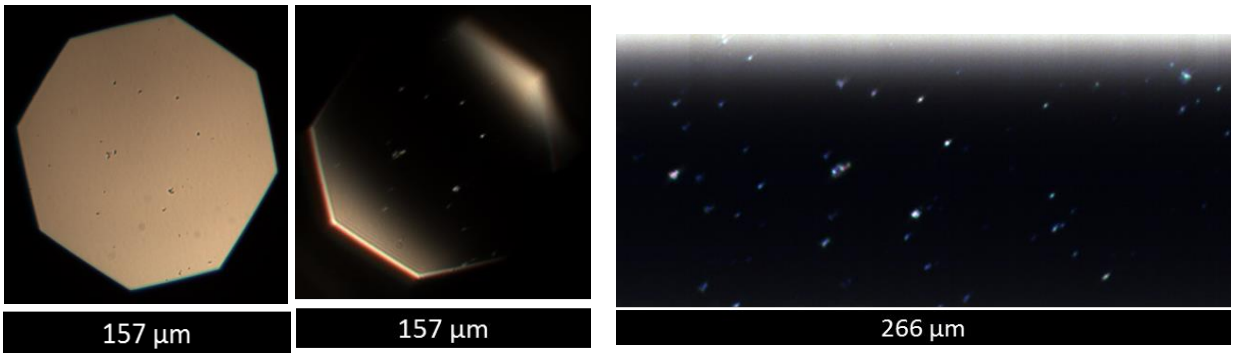
Fig. 12: AFM images for Pt→EDT→Au NP functionalization; 50nm NP, 1x dilution, 24 h



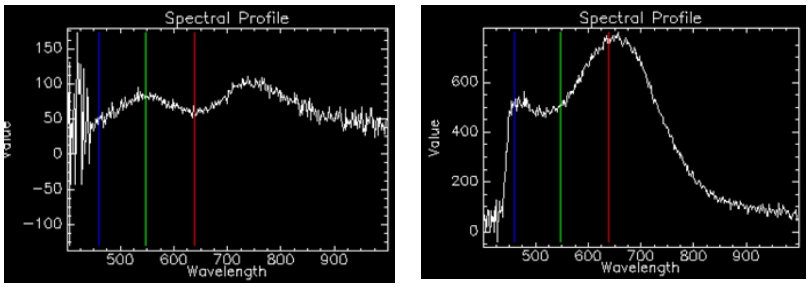
(a) 15000x magnification ***(b) 250000x magnification***

Fig. 13: SEM images for Pt→EDT→Au NP functionalization; 50nm NP, 1x dilution, 24 h

3.2 Pt → Ethanedithiol (no Au Nanoparticle Functionalization)

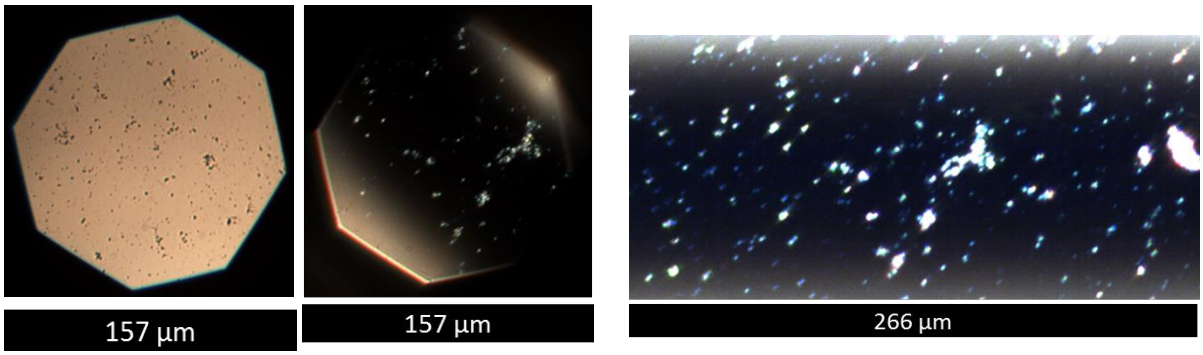


(a) Optical image (Bright-field) ***(b) Optical image (Dark-field)*** ***(c) Hyperspectral image***

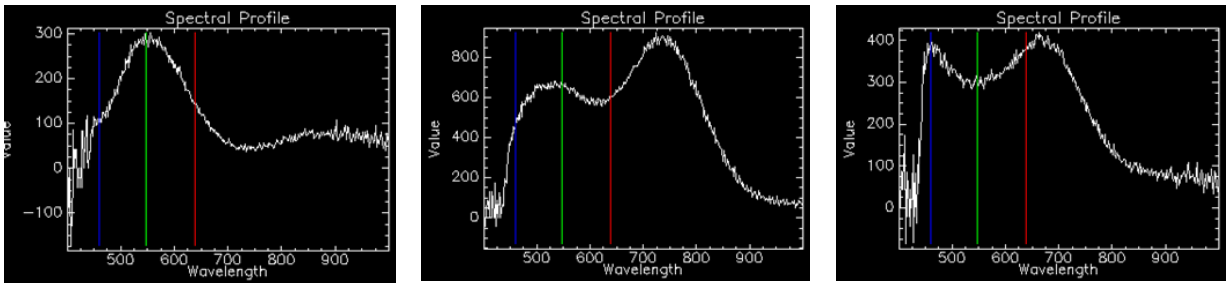


(d) Sample spectrum 1 *(e) Sample spectrum 2*

Fig. 14: Cytoviva images for Pt→Ethanedithiol→DI water (24 h)

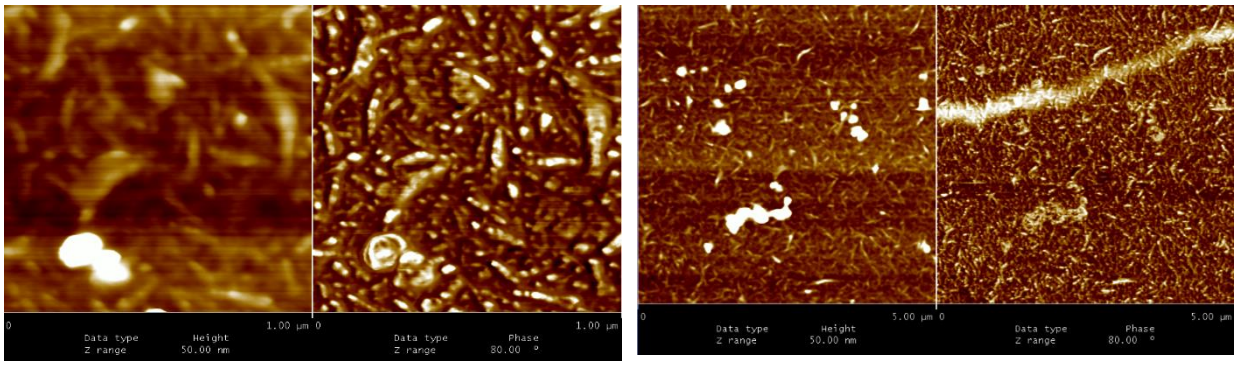


(a) Optical image (Bright-field) *(b) Optical image (Dark-field)* *(c) Hyperspectral image*



(a) Sample spectrum 1 *(b) Sample spectrum 2* *(c) Sample spectrum 3*

Fig. 15: Cytoviva images for Pt→Ethanedithiol→Left in air (24 h)



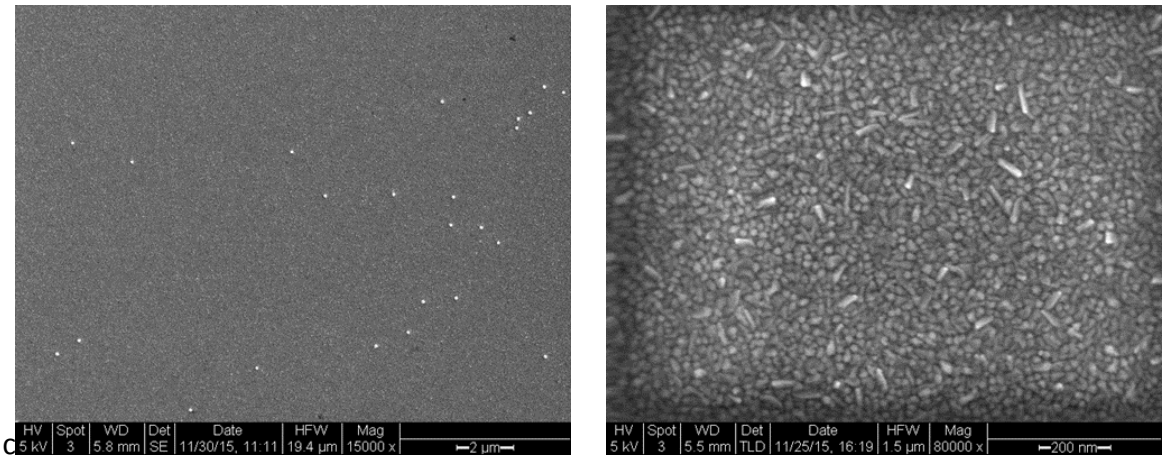
(a) Z & phase images, 1 x 1 µm²scan size *(b) Z & phase images, 5 x 5 µm²scan size*

Fig. 16: AFM images for Pt→EDT→Left in air (24 h)

From Figures 14 and 15, we observe that clumps are also present in the hyperspectral images of the samples without Au NP functionalization. Spectral peaks in the similar ranges seem to be

observed, but these now appear broader. The Pt→Ethanedithiol sample that was kept in DI water after EDT functionalization also shows fewer clumps than that left in air for 24 h. This suggests that the clumps observed in these samples are impurities introduced with the EDT functionalization. Immersing the samples in water might have reduced this either by preventing impurities from getting onto the sample from air, or prompting whatever impurities were introduced by the EDT functionalization, or EDT itself, to desorb. Figure 16 shows that the oblong structures seen in the AFM and SEM images for the standard Au NP functionalizations are also present in the samples where NP's have not been added. Clearly, the background structures are not due to nanoparticles.

3.3 BARE PLATINUM SUBSTRATE



(a) 15000x magnification ***(b) 80000x magnification***

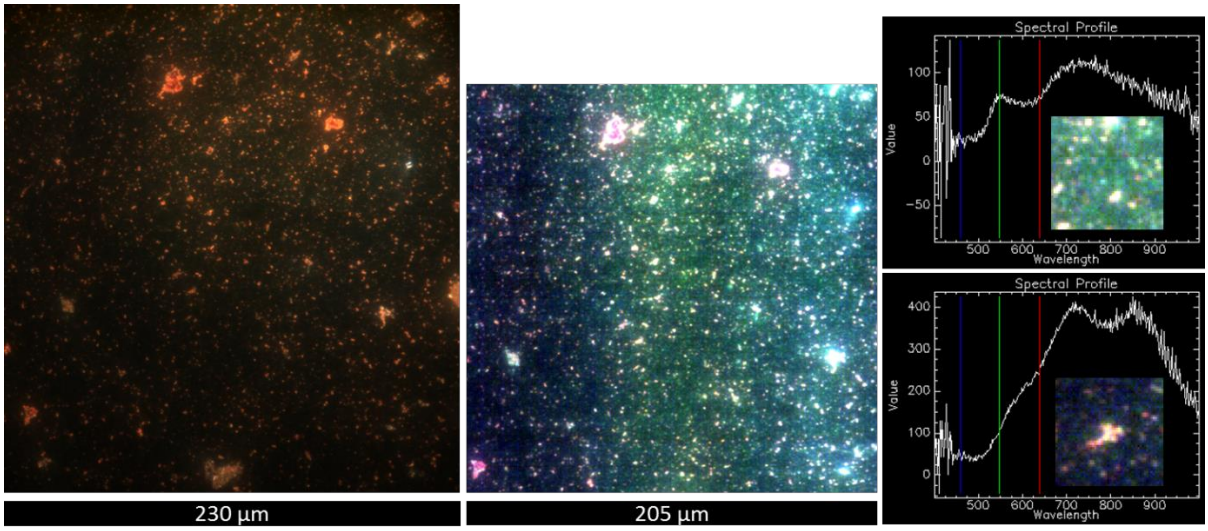
Fig. 17: SEM images of bare platinum substrate

From the SEM image of bare platinum in Figure 17, we can probably conclude that the structures are native to the Pt deposition itself. However, it is not clear what the source of this morphology is. The data presented so far also points to limitations of the Cytoviva system in dark-field reflectance mode for resolving different materials and features on the Pt samples. First of all, it is unable to resolve light scattered from features below a certain roughness—the oblong background structure (dimensions ~ 10 nm) seen under AFM and SEM does not show up in the Cytoviva image. Having said that, it is possible that Au nanoparticles of the same dimensions would scatter light much more strongly than these Pt features and be visible. Furthermore, it is hard to distinguish between the spectra of the clumps belonging to samples that underwent Pt→EDT→Au NP functionalization versus those that did not go through the Au NP step. And if they did arise due to different materials with similar peak positions, there is no way of telling.

Nonetheless, the clumps appear superficially similar in both bright-field and dark-field optical images. Neither do they appear under AFM and SEM to be composed of units with the spherical morphology of identifiable diameter, as we would expect with Au nanoparticles. While it possible that interaction with the Pt surface may have changed its appearance (e.g. the nanoparticles may have become embedded), this seems unlikely. The more plausible conclusion to draw seems to be that the Au NP functionalization was not successful after all.

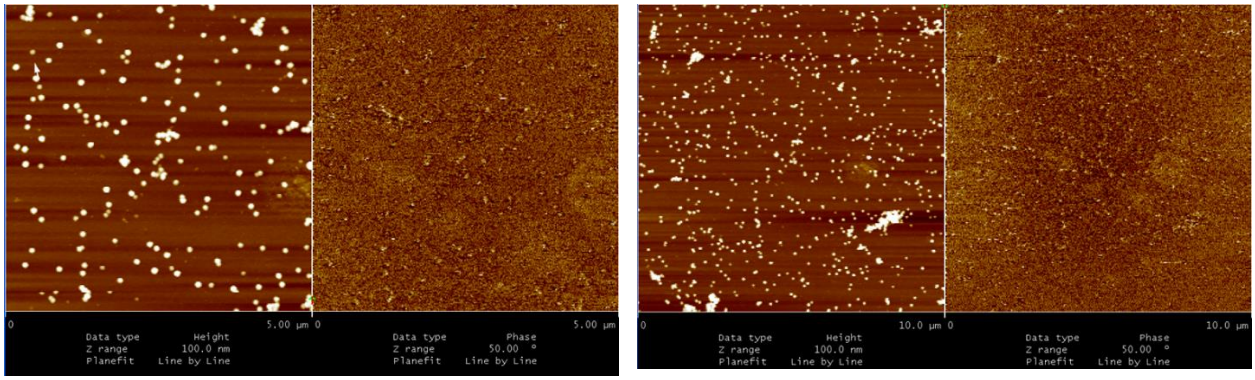
3.4 Glass → APTES / Ethanol → Au NP

Figures 18 and 19 show the Cytoviva optical images and spectra, as well as the AFM plots, for glass which has been functionalized with Au nanoparticles via a SAM of APTES. While peaks at ~ 550 and 750 nm were observed here, as before on the Pt samples, a salient difference is that the 550 nm peak is consistently present in all the pixels in the hyperspectral image which appear greenish, even when they do not fall directly onto bright 'clumps'. On the AFM images, spherical

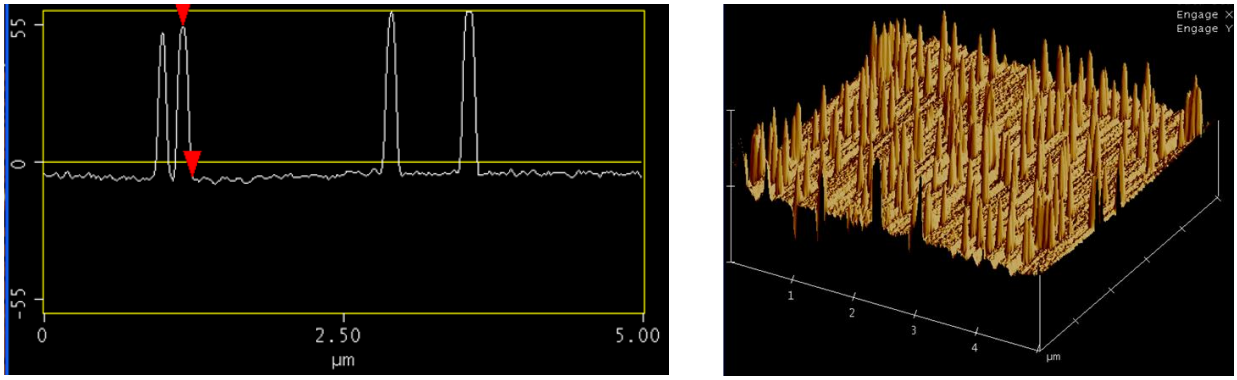


(a) Optical image *(b) Hyperspectral image* *(c) Sample spectra*

Fig. 18: Cytoviva images for Glass→APTES/Ethanol→Au NP functionalization; 50 nm, 1x dilution, 24 h



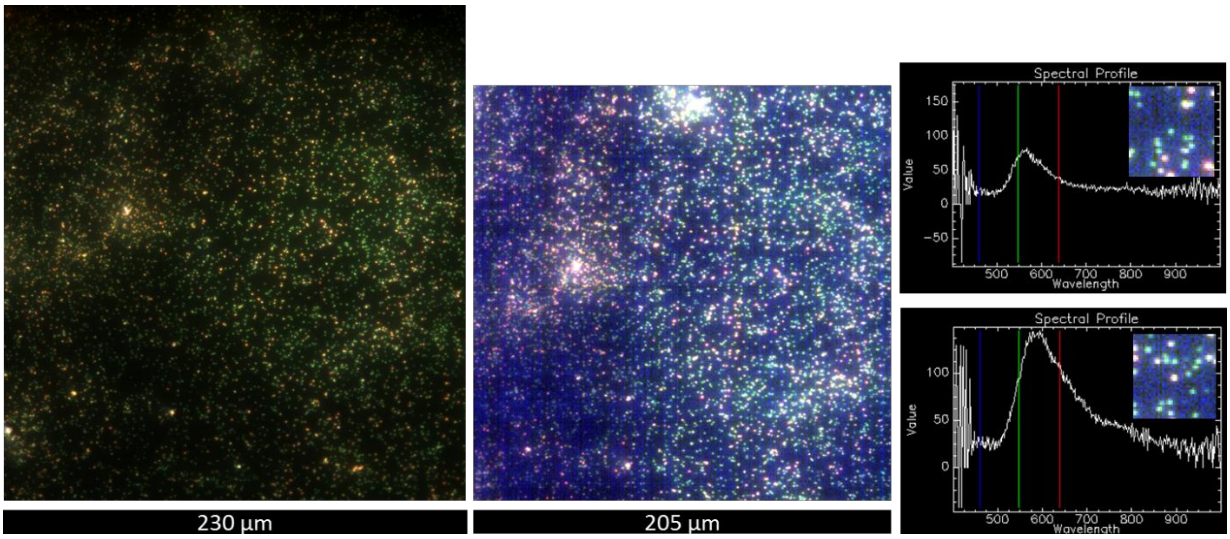
(a) Z & phase images, 5 x 5 um² scan size *(b) Z & phase images, 10 x 10 um² scan size*



(c) Line section of 5 x 5 um² scan *(d) 3D plot of 5 x 5 um² scan*

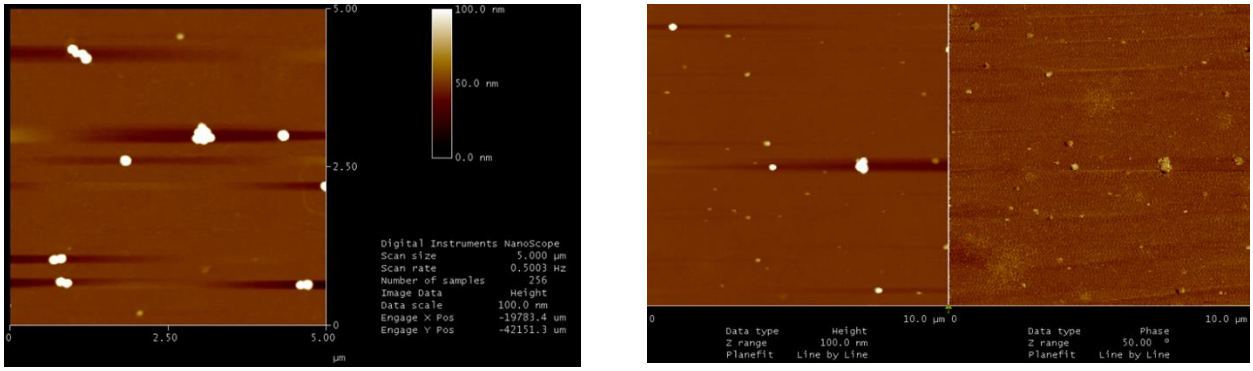
Fig. 19: AFM images for Glass→APTES/Ethanol→Au NP functionalization, 50 nm, 1x dilution, 24 h

structures of ~ 50 nm height are clearly distinguished, providing strong evidence for the link between the successful functionalization of 50 nm NP's to a high surface density, and the spectral peak at 550 nm. A split for a functionalization of 100 nm nanoparticles on glass yields similar results, but with a clear shift of the spectral peak to ~ 570 nm as expected, and spherical particles of ~ 100 nm height seen on the AFM images. These results provide confirmation that the nanoparticle solutions used were viable, and that we were not in fact seeing NP's on platinum.

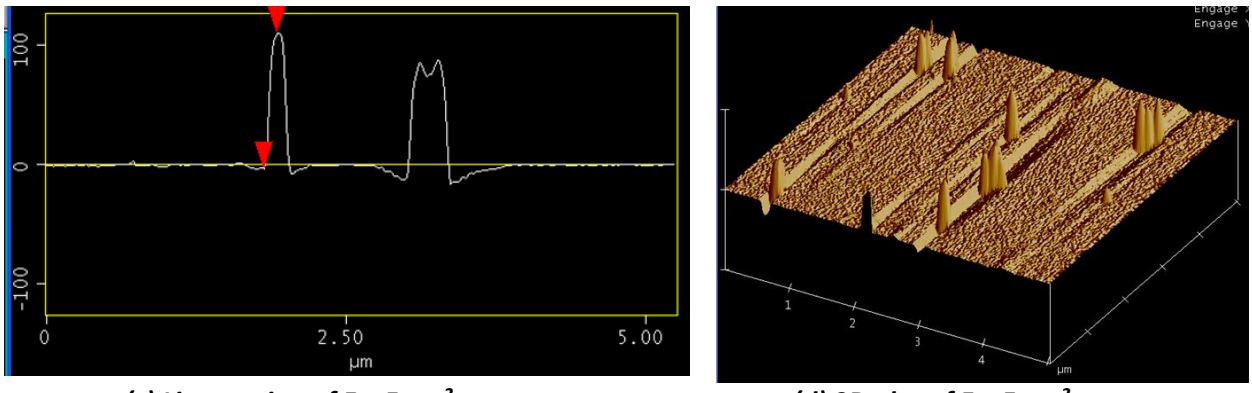


(a) Optical image *(b) Hyperspectral image* *(c) Sample spectra*

Fig. 20: Cytoviva images for Glass→APTES/Ethanol→Au NP functionalization; 100 nm, 1x dilution, 24h



(a) Z images, 5 x 5 um² scan size *(b) Z & phase images, 10 x 10 um² scan size*

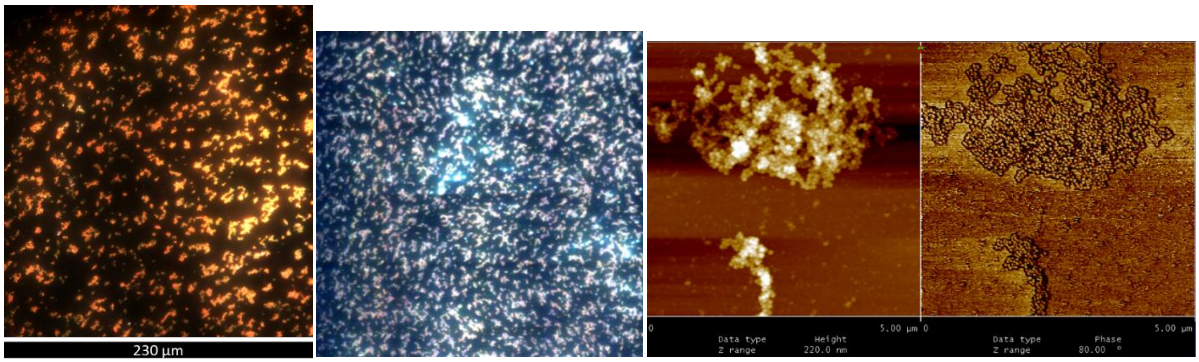


(c) Line section of 5 x 5 um² scan *(d) 3D plot of 5 x 5 um² scan*

Fig. 21: AFM images for Glass→APTES/Ethanol→Au NP functionalization, 100 nm, 1x dilution, 24 h

3.5 Glass → APTES / Methanol (w/o sufficient washing) → Au NP

Finally, we present incidental data from an experimental split in the glass functionalizations whereby the intermediate functionalization involved APTES dissolved in methanol rather than ethanol. APTES residue left on the slide was not fully washed off prior to the Au nanoparticle functionalization step, resulting in severe aggregation as seen in Figure 20. However, even here, the spherical forms of the nanoparticles are clearly visible in the AFM image. Thus, it can be expected that if functionalization were successful on the Pt substrate, we should be able to distinguish the nanoparticles by AFM.



(a) Optical image (b) Hyperspectral image (c) AFM Z & phase images, 5 x 5 um² scan size

Fig. 20: Optical and AFM images for Glass → APTES/Methanol → Au NP functionalization Aggregation exhibited due to insufficient washing after APTES functionalization.

4 Conclusions & Proposal for Future Work

4.1 Summary of Findings & Contributions to Date

- Demonstrated the use of the Cytoviva hyperspectral tool for capturing dark-field images and analyzing particle spectra in both transmission and reflectance modes. In the literature, reflectance mode is not yet established yet as a method for characterizing nanoparticles.
- Demonstrated functionalization of Au nanoparticles of different sizes on glass slides by corroborating Cytoviva and AFM results. Peak dependence on size can be distinguished.
- Developed a protocol for functionalization on evaporated films on Si wafers, with multiple splits attempted for nanoparticle size, dilution factor, and functionalization time.
- Observed distinct and shifting spectral peaks for clump-like particles found on Pt samples. Put forward suggestions for possible causes, though this is yet to be resolved.
- Observed interesting oblong-shaped formations in the background under SEM and AFM, attributable to the Pt film, though the cause of this is yet unclear.
- Observed that the optical and morphological characteristics of the Pt samples do not vary much for these different Au NP functionalization splits. In fact, they are similar to Pt samples which have not been exposed to Au NP's at all, suggesting that Au NP did not attach.
- Inferred that since the splits for the Au NP functionalization steps did not result in Au NP attachment, it is likely that the problem lies with the ethanedithiol functionalization.

4.2 PROPOSAL FOR CONTINUATION OF PROJECT

4.2.1 Possible Improvements

A likely reason for why the ethanedithiol functionalization may not have been effective is that both thiol groups on the molecule may have ended up binding to the platinum substrate, leaving no free groups to bond with the Au nanoparticles. The planar geometry of the substrate makes this more likely to occur than with the non-planar QTEP substrate. A lower concentration of ethanedithiol in the ethanol solution used is likely to discourage the molecule from binding to the substrate from both ends. However, the threshold concentration for this needs to be determined through experimentation.

Turning the problem on its head, it would be worth considering functionalizing ethanedithiol onto the Au nanoparticles first, and then functionalizing the Pt substrate by immersing it in a solution of this.[14] This would avoid the tricky SAM issue of double binding of the flexible ethanedithiol molecule to the substrate. The curvature of the Au nanoparticles is likely to make double binding onto a single Au nanoparticle less likely. The challenge is to minimize the formation of a matrix of colloidal Au nanoparticles linked via ethanedithiol molecules.

Alternatively, the ethanedithiol SAM can be replaced with a bifunctional molecule that is more rigid. Examples include longer alkanedithiols and 4,4-biphenyldithiol (BPDT).[15] Other routes proposed to bind gold nanoparticles to metallic substrates[12] include the use of diisocyanides and SH-terminated aryl diazonium salts deposited by electrochemical reduction of the diazonium.[18] An amine-terminated monolayer could also be employed. This would require a thiol end group to bind to the platinum and an amine group to coordinate with the Au nanoparticle. A possible option is 1,4-aminothiophenol.[19] The main limitations on these alternatives is that a larger molecule would come at the cost of signal deterioration in the QTEP context, as the tunneling electrons are more likely to get scattered as the cross the molecule to the bulk Pt electrode.

Finally, if needed, alternative characterization tools could be brought to bear to probe for the presence of Au NP's on the samples. UV-vis-infrared spectroscopy is typically used for optical characterization of samples, but it generates bulk extinction spectra with limited spatial resolution. Reflectance Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Photoelectron Spectroscopy (XPS) could be used to monitor changes in surface binding and composition that might be associated with successful nanoparticle functionalization.

4.2.2 Suggested Experimental Design

From the work presented, the importance of careful controls is clear. With the dual aim of finding a dilute enough EDT solution to allow successful binding of Au NP's, as well as seeing if Cytoviva can be used to image Au NP's functionalized to planar Pt, we could proceed thus:

Characterization	Negative controls	Pt → EDT → Au NP	Pt → BPDT → Au NP
AFM imaging	Pt only	Concentration splits:	Mix with EDT in
Cytoviva imaging	Pt →EDT (Conc. splits) Pt →BPDT	1x, 10x, 100x, 500x, 1000x present dilution	different ratios to test effect on Au NP density

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6 APPENDIX

Table 1: Samples and characterization done on Pt substrate

			CYTOVIVA IMAGING *Dark field Reflectance*	CYTOVIVA IMAGING *Dark field Transmission*	AFM IMAGING	SEM IMAGING
Pt only						
Pt → EDT	Left in air					
	Left in water					
Pt → PpT → GNP						
Pt → ethanol → GNP		1x dilution				
Pt → EDT → GNP	10 nm NP 24h	1x dilution				
		10x dilution				
		100x dilution				
		10^3x dilution				
	50 nm NP 24h	1x dilution				
		8x dilution				
		10x dilution				
		18x dilution				
		100x dilution				
		10^3x dilution				
	50 nm NP 2h	1x dilution				
		8x dilution				
		18x dilution				

Table 2: Samples and characterization done on glass slide

			CYTOVIVA IMAGING *Dark field Reflectance*	CYTOVIVA IMAGING *Dark field Transmission*	AFM IMAGING	SEM IMAGING
Glass						
Glass → APTES / methanol						
Glass → APTES / methanol → GNP	50 nm NP 24h	1x dilution				
		8x dilution				
		18x dilution				
	50 nm NP 2h	1x dilution				
		8x dilution				
		18x dilution				
	100 nm NP 24 h	1x dilution				
	100 nm NP 2h	1x dilution				
Glass → APTES / ethanol						
Glass → APTES / ethanol → GNP	50 nm NP 24h	1x dilution				
		8x dilution				
		18x dilution				
	50 nm NP 2h	1x dilution				
		8x dilution				
		18x dilution				
	100 nm NP 24 h	1x dilution				
	100 nm NP 2h	1x dilution				

EDT = Ethanedithiol in ethanol solution
Ppt = Propanethiol in ethanol solution
APTES = (3-Aminopropyl)triethoxysilane