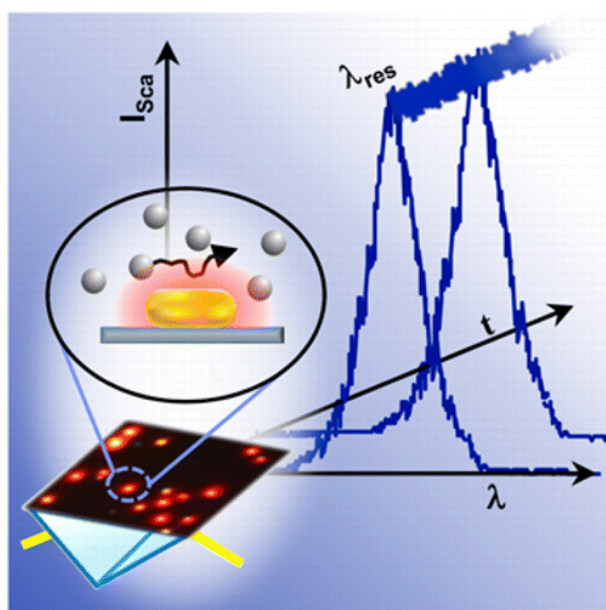




Summer Research Internship 2017



REPORT

submitted at the completion of

RESEARCH INTERNSHIP

at

SINGLE-MOLECULE GROUP, LION

Author :	Mudit Garg
Supervisor :	Prof. Dr. Michel Orrit
Daily Supervisor :	Biswajit Pradhan
Duration :	May-July 2017

Leiden, The Netherlands, July 20, 2017

Mudit Garg

Senior Undergraduate
Indian Institute of Technology Delhi
New Delhi, India, 110016

July 20, 2017

Acknowledgement

I would like to express my deep gratitude toward **Prof. Michel Orrit** for giving me this wonderful opportunity to spend my Summer at Single Molecule Group and for his constant guidance. I would like to thank **Biswajit Pradhan** for his daily supervision and constructive discussions .

This research internship was made possible by financial support from the **Leiden University** and I am thankful for that. I am certainly grateful to **Prof. Jan Aarts** for coordinating my summer internship and taking care of all logistics.

Special thanks to **MoNOS** group for accepting me in their family and helping me into smooth transition in Leiden. I've gained so much experience and knowledge here that can't be expressed in words.

Also I would like to express my gratitude toward **Prof. R.K. Soni**, the internal supervisor of this internship with his ever-helping nature and support.

Contents

1	Introduction	1
2	Theory	3
2.1	Fluorescence	3
2.2	Fluorescence Correlation Spectroscopy	4
2.3	Procedure	4
3	Results and Discussion	7
3.1	Passivation with 20 μ M Cysteamine	8
3.2	Passivation with 100 μ M Cysteamine	10
3.3	Conclusion	12

Chapter 1

Introduction

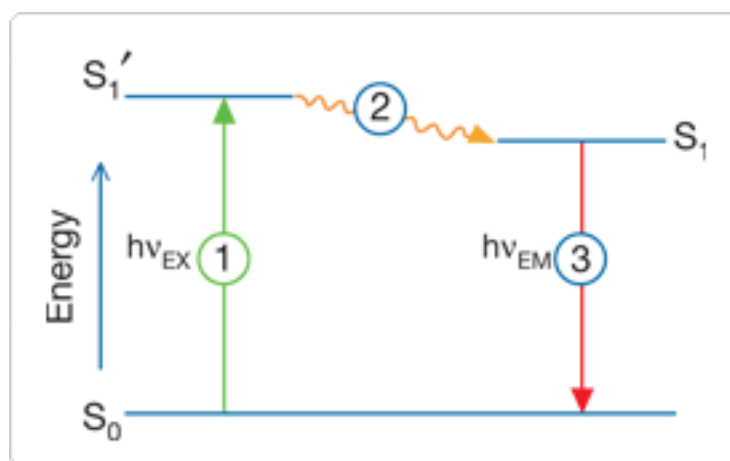
In this Internship I've used Fluorescence Correlation Spectroscopy to observe the single Gold nanorods(AuNR) as they interact with their nearby particles like proteins,silica nanoparticles.[1] .I first learned how to operate the system and how to make samples for the system.Then I learned how to take spectrum using luminescence with the help of 532nm green laser.By analyzing the spectrum I used appropriate AuNR to take their spectrum image with 660nm cobalt laser. Then I introduces BSA of different concentration. in environment of AuNR .I took time traces using two APDs and try to observe some bursts in that. There were some problems which I will discuss in Result section.

Chapter 2

Theory

2.1 Fluorescence

Fluorescence is the property of substance where it absorbs light at certain wavelength and emit light at relatively longer wavelength, Therefore have lower energy than the absorbed one. Molecule is termed as fluorophores or fluorescent dyes. It happens in three stages : First Excitation then Excited state Lifetime and finally Fluorescence Emission. This is displayed using Jablonski diagram. More details are in this reference [2]



Jablonski diagram: Three stages of Fluorescence [2]

2.2 Fluorescence Correlation Spectroscopy

Fluorescence Correlation Spectroscopy(FCS) is a technique which can be used to monitor single molecule using specific optical setup with an inverted confocal microscope. In this setup we send laser beam to this microscope's objective via dichroic mirrors, which focus on the sample. Then we gather the fluorescence light from this and send it to pin hole and via emissions filters and dichroic which use few nanomolars of fluorescence in order to detect single molecule. Then we use two APD detectors which are getting light from 50/50 beam splitter then take their cross correlation. Formula for normalized autocorrelation of intensity i.e. $I(t)$ is :

$$G(\tau) = \frac{\langle I(t) \cdot I(t + \tau) \rangle}{\langle I(t) \rangle^2}$$

and if we define $\delta I(t) = I(t) - \langle I(t) \rangle$ then $G(\tau)$ can be written as :

$$G(\tau) = \frac{\langle \delta I(t) \cdot \delta I(t + \tau) \rangle}{\langle I(t) \rangle^2} + 1$$

It is given in more details in reference [3]

2.3 Procedure

This is my full procedure in the internship

- I I first prepared sample using stock AuNR solution which were coated with CTAB to avoid aggregates
- II I only chose those solution which have surface plasmon resonance around 650 so that they right swing at 660nm.
- III then remove CTAB using centrifugation. This is necessary so that AuNR could stuck glass slides.
- IV then I sonicated the sample to avoid aggregates.
- V After that I used two different things to put AuNR sample
 - i Flow cell with rectangular slide
 - ii Circular holder with circular slides
- VI Also used two different procedure for introducing AuNR to glass slides

- i Spin coating method
 - ii sucking in AuNR solution into flow cell
- VII After doing all that I took scattering image using 660nm red laser at focus for finding out positions of AuNR.
- VIII Then I took spectrum using 532nm green laser for those AuNR which were excited by 660nm.
- IX After doing this process I chose those AuNRs which had SPR either right swing or left wing at 660nm.
- X then I passivated the AuNRs with either Thioglycolic acid or Cysteamine to avoid physical contact between protein and AuNR.
- XI Then after leaving it for overnight I introduces different conc. of BSA protein.
- XII Took their time traces using two APDs and found out their cross correlation in order to find diffusion time.
- XIII and finally using Stokes Einstein relation $D = \frac{k_B T}{6\pi\eta r}$ to find out size of protein.

Chapter 3

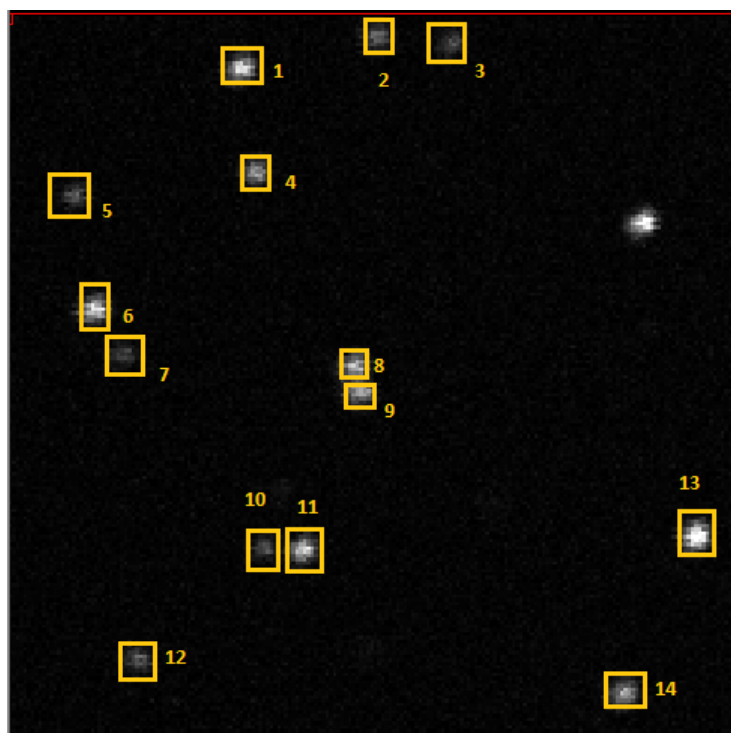
Results and Discussion

As I laid out my procedure in previous chapters, I was not able to achieve them perfectly this was due to these things:

1. Time constraint of my internship
2. initially laser wasn't stable
3. Not able to prevent attaching of BSA to AuNR

Due to this time constraint I spent my intail time learnig the tecnique and how to make samples. After that we found out that our 670nm laser is not stable, so we ordered a new one 660nm cobalt laser but even after acclearating the procedure it took 3 weeks to arrive. even after finally setting up the laser which was stable. we weren't able to avoid attaching of BSA even after the passivation. So I would like to share my two days of result in which laser was stable. So in first case I passivated with 20 μ M Cysteamine and in second case with 100 μ M Thioglycolic acid.

3.1 Passivation with 20 μ M Cysteamine

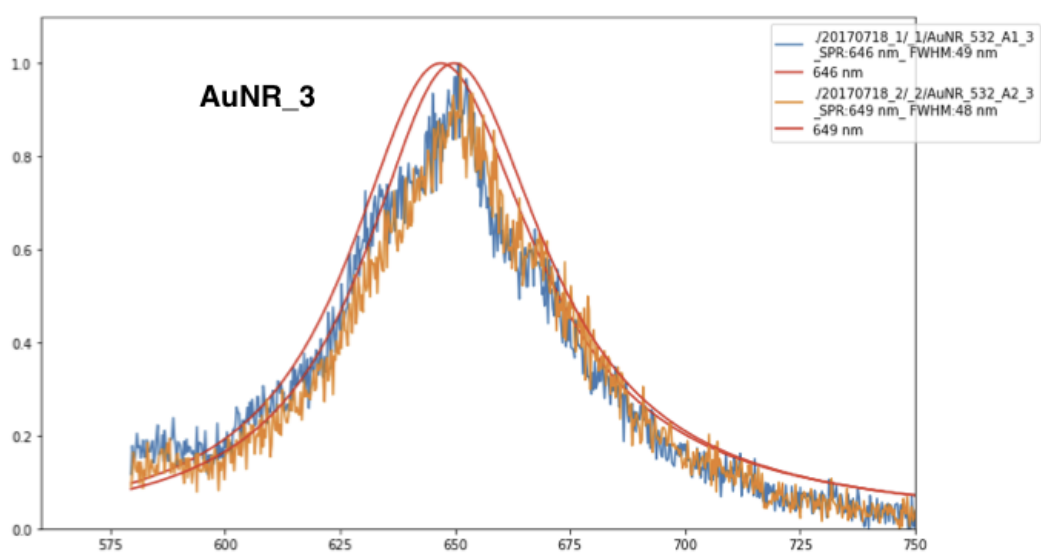
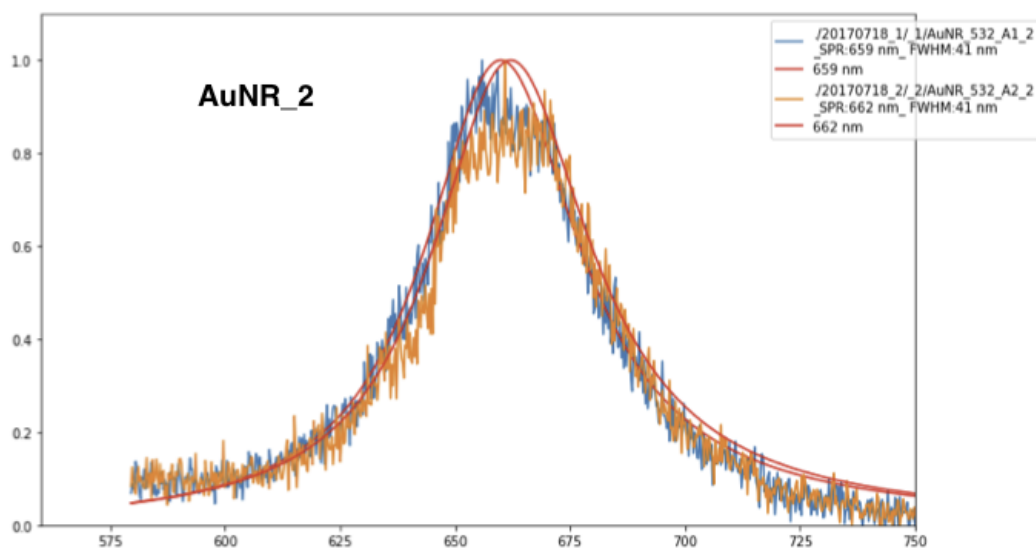


Numbering according to order of taking spectrum

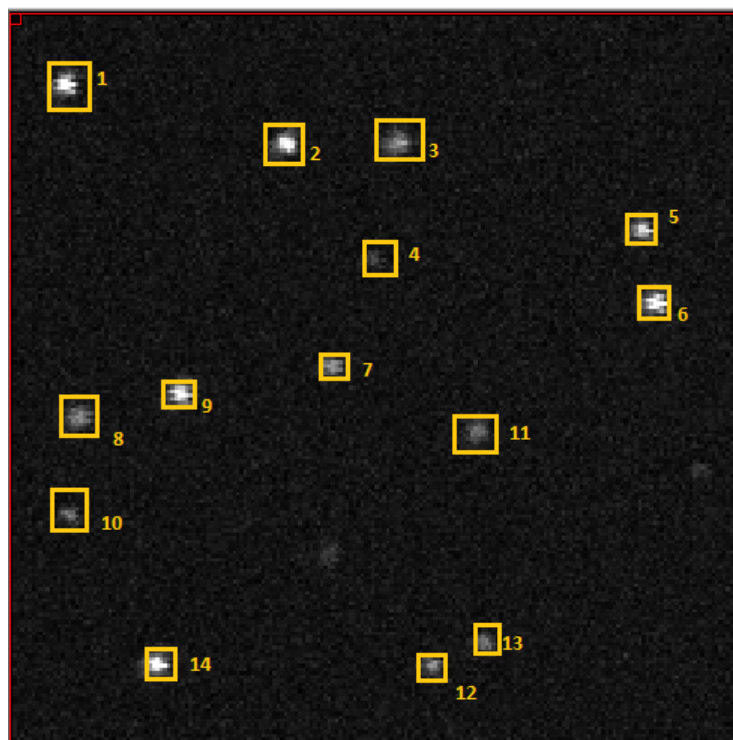
Out of these fifteen only ten i.e. 2,3,5,6,8,10,12,13,14,15 had right spectrum. then I introduced 100nM BSA. Then took 300 second time traces. After doing that I again took the spectra and found out that there was a shift of 2-3nm in SPR and decrease of around 1nm in FWHM.

AuNR	Before 0.01uM_BSA		After 0.01uM_BSA	
	SPR(nm)	FWHM(nm)	SPR(nm)	FWHM(nm)
2	659	41	662	40
3	646	49	649	47
5	670	40	672	39
6	685	42	687	42
8	656	45	658	44
10	646	50	649	48
12	670	42	672	41
13	672	40	674	40
14	670	40	672	40
15	665	41	667	40

Change in Spectrum before and after introduction of BSA



3.2 Passivation with 100 μ M Cysteamine

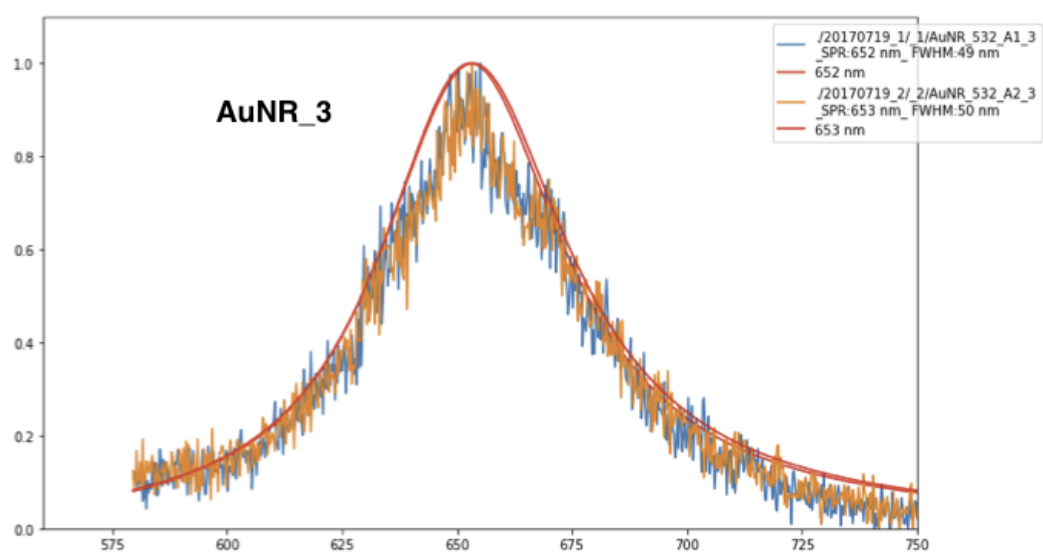
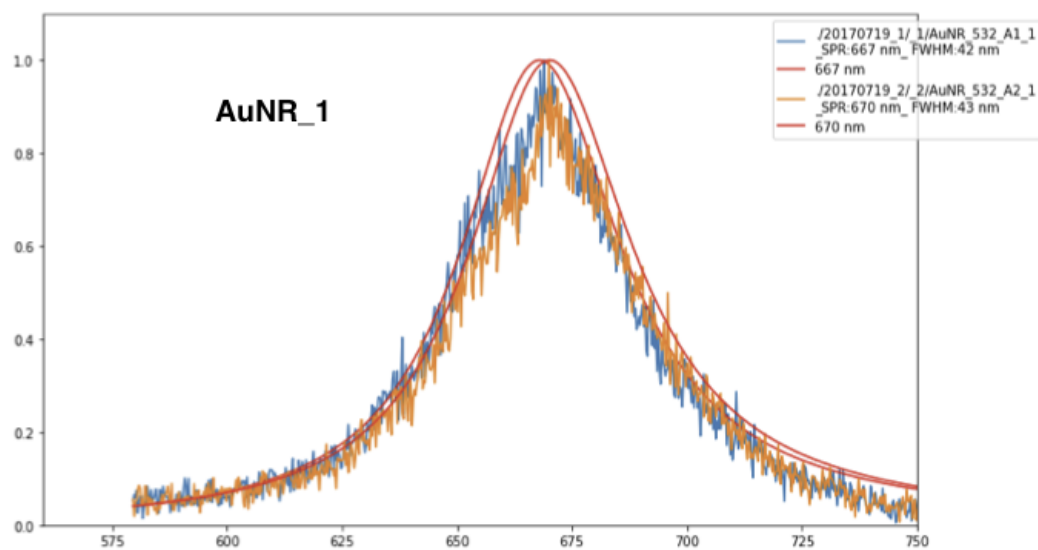


Numbering according to order of taking spectrum

Out of these fourteen only nine i.e. 1,3,4,6,7,10,11,12,13 had right spectrum. then I introduced 1nM BSA. Then took 600 second time traces. After doing that I again took the spectra and found out that there was a shift of 2-3nm in SPR and but his time increase of around 1nm in FWHM.

AuNR	Before 1nM_BSA		After 1nM_BSA	
	SPR(nm)	FWHM(nm)	SPR(nm)	FWHM(nm)
1	667	42	670	41
3	652	49	653	50
4	627	56	630	58
6	667	40	669	41
7	644	51	646	53
10	642	49	643	49
11	655	44	656	45
12	641	48	642	50
13	637	52	640	52

Change in Spectrum before and after introduction of BSA



3.3 Conclusion

References

- [1] V. Wulf, F. Knoch, T. Speck, and C. Sönnichsen, *Gold Nanorods as Plasmonic Sensors for Particle Diffusion*, The Journal of Physical Chemistry Letters **7**, 4951 (2016), PMID: 27934054.
- [2] <https://www.thermofisher.com/nl/en/home/references/molecular-probes-the-handbook/introduction-to-fluorescence-techniques.html>.
- [3] E. Haustein and P. Schwille, *Fluorescence Correlation Spectroscopy: Novel Variations of an Established Technique*, Annual Review of Biophysics and Biomolecular Structure **36**, 151 (2007).