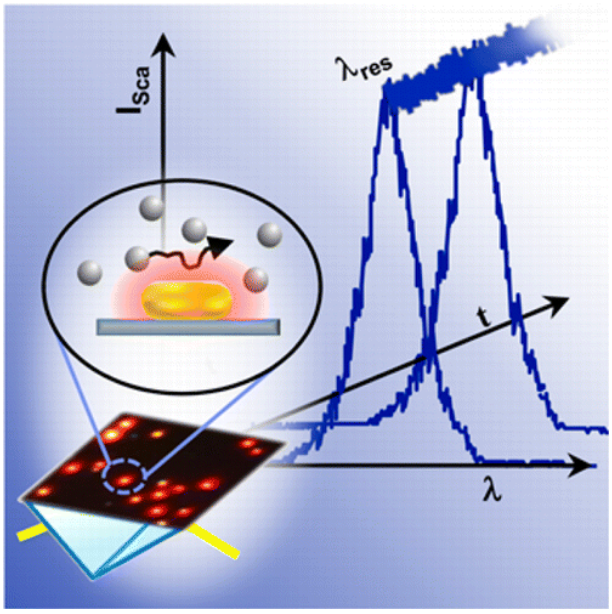

REPORT

submitted at the completion of

RESEARCH INTERNSHIP
at
SINGLE-MOLECULE GROUP,LION

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Contents

Chapter 1

Introduction

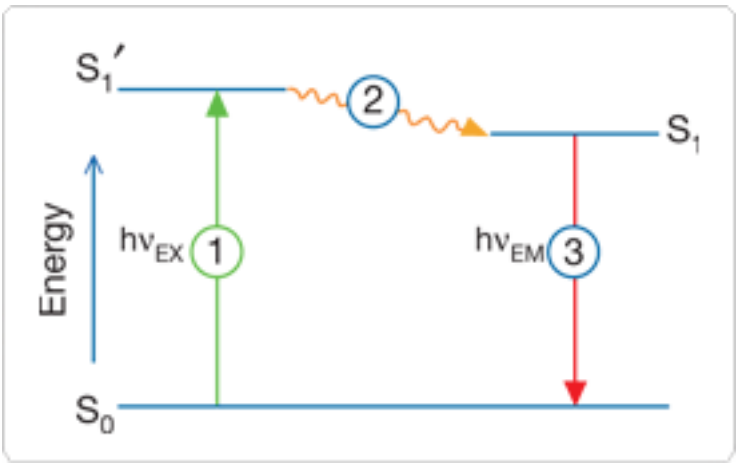
In this Internship I've used Fluorescence Correlation Spectroscopy to observe the single Gold nanorods(AuNR) as they interact with their nearby particles like proteins,silica nanoparticles.[1] .I first learned how to operate the system and how to make samples for the system.Then I learned how to take 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 Beam splitter,which focus on the sample.Then we gather the fluorescence light form this and send it to pin hole and via emissions filters and beam splitter 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 :

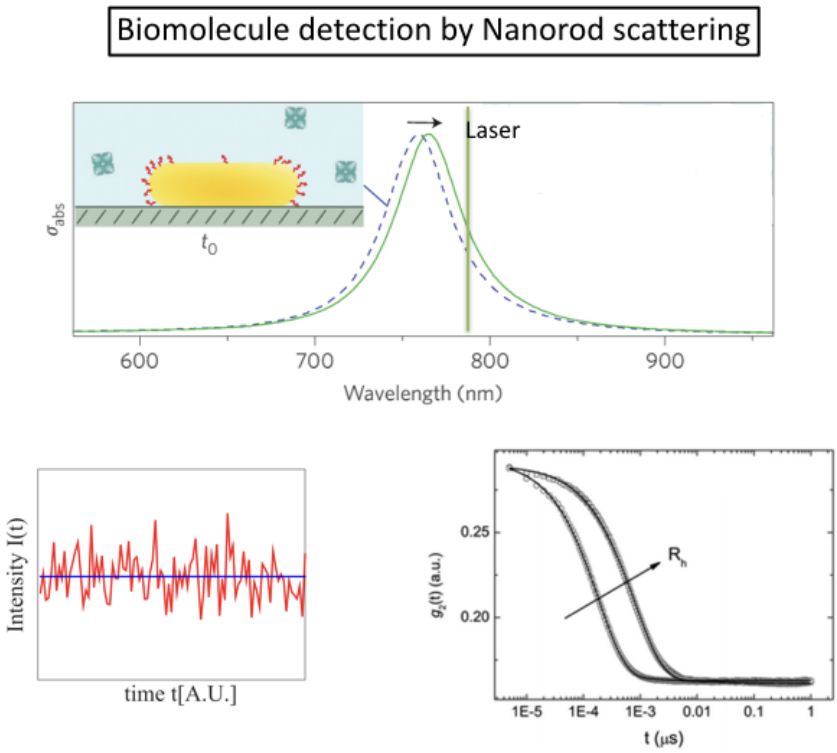
$$G(\tau) = \frac{\langle \delta I(t) \cdot \delta I(t+\tau) \rangle}{\langle I(t) \rangle^2} + 1$$

It is given in more details in reference [3]

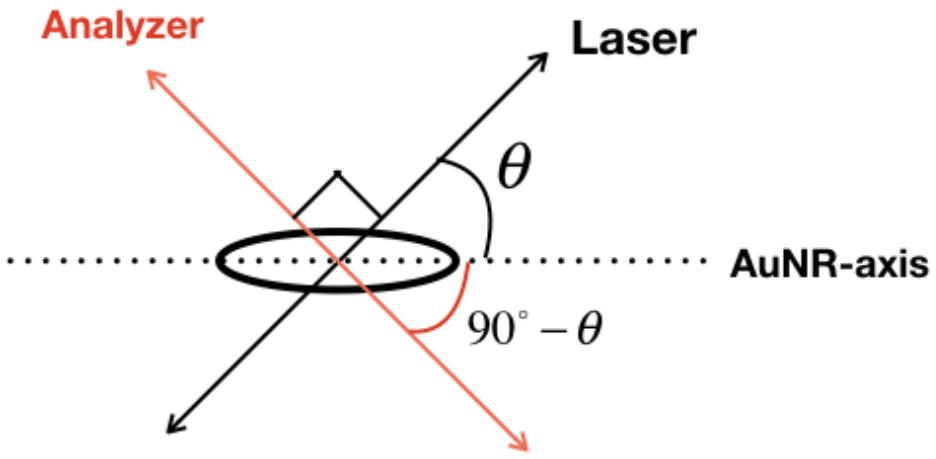
2.3 Scattering Correlation Spectroscopy

In this technique[1] we monitor an immobilized single AuNR using scattering from the white light using the TIE configuration.We use flow cell to control the environment of the AuNR. Then we introduce diffusers such as small gold.We measure the shift in spectra of the AuNR as diffusers enter and leaves in its vicinity.Then we take autocorrelation of $\lambda(t)$ in to find out diffusion coefficient D.Then using stokes einstein relation $D = \frac{k_B T}{6\pi\eta r}$ we can find viscosity and diffuser conc. of other diffusers if we know it for one using their respective D.

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



Shift in spectrum & correlation due to protein in the vicinity of AuNR[4]



Laser is at an angle θ with axis of AuNR and perpendicular to analyzer

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which is extinguishing the reflection part ($I_{reflection} == I_L \cdot \cos 90^\circ = 0$) .so only scattering along the axis survive and result in final intensity.

$$I_{total} = Reflection + AuNR - scat = 0 + I_{scatAuNR} \cdot \cos(90^\circ - \theta)$$

$$I_{total} = I_{scatAuNR} \cdot \sin(\theta)$$

2.4 Procedure

This is my full procedure in the internship:

A. **Sample Preparation**

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.

B. **Putting AuNRs on glass slide**

I After that I used two different things to put AuNR sample

i Flow cell with rectangular slide

ii Circular holder with circular slides

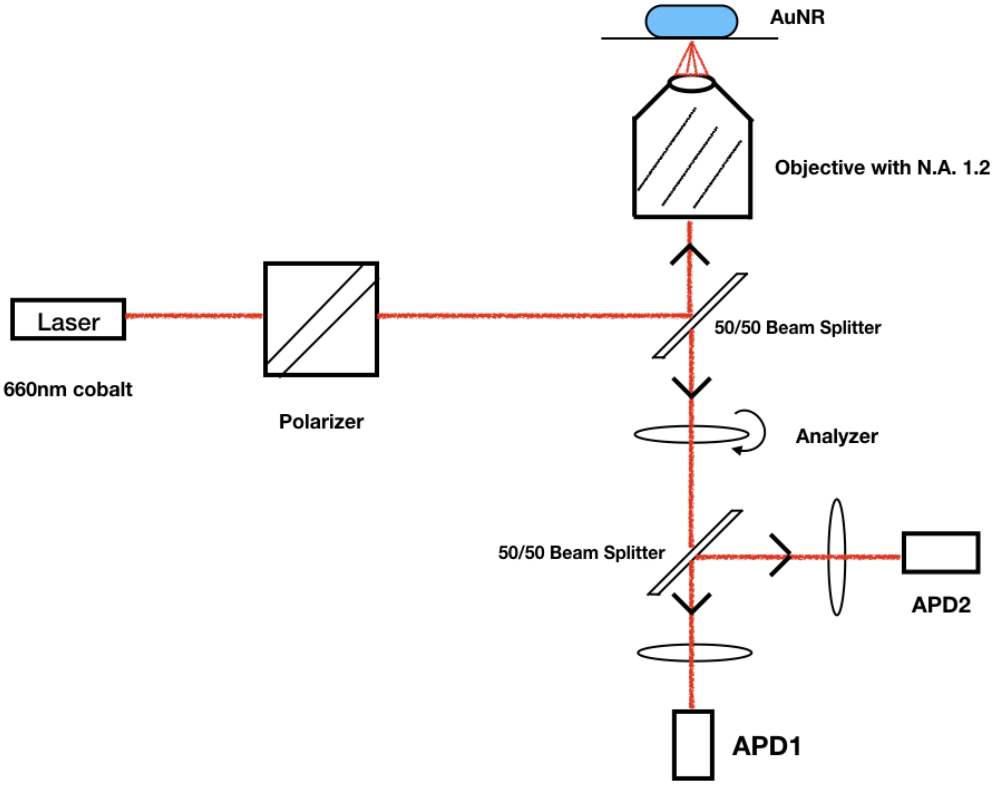
II Also used two different procedure for introducing AuNR to glass slides

i Spin coating method

ii Flowing AuNR solution into flow cell

C. **Experimenting**

I So I used this setup for experimenting



In this Experimental setup laser is going through polarizer then using 50/50 beam splitter going to AuNR though microscope and coming back through analyzer which is then again splitting by 50/50 beam splitter to APDs.

II I took scattering image using 660nm red laser at focus for finding out positions of AuNR.

III Then I took spectrum using 532nm green laser for those AuNR which were excited by 660nm.

IV After doing this process I chose those AuNRs which had SPR either right swing or left wing at 660nm.

V then I passivated the AuNRs with either Thioglycolic acid or Cysteamine to avoid physical contact between protein and AuNR.

VI Then after leaving it for overnight I introduces different conc. of BSA protein.

VII Took their time traces using two APDs.

D. **Analysis**

I I found out their cross correlations using time traces in order to find diffusion time.

II and finally using Stokes Einstein relation to find out size of protein.

Chapter 3

Results and Discussion

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

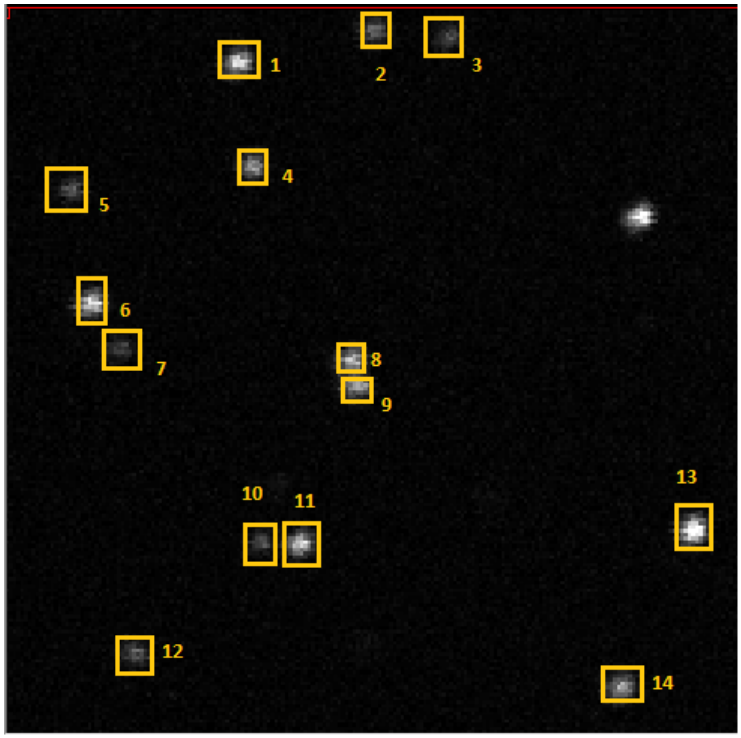
1. Time constraint of my internship

2. initially laser wasn't stable

3. Not able to prevent attaching of BSA to AuNR

Due to this time constraint I spent my initial time learning the technique 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 accelerating 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 20μM Cysteamine and in second case with 100 μM Thioglycolic acid.

3.1 Passivation with 20μM Cysteamine



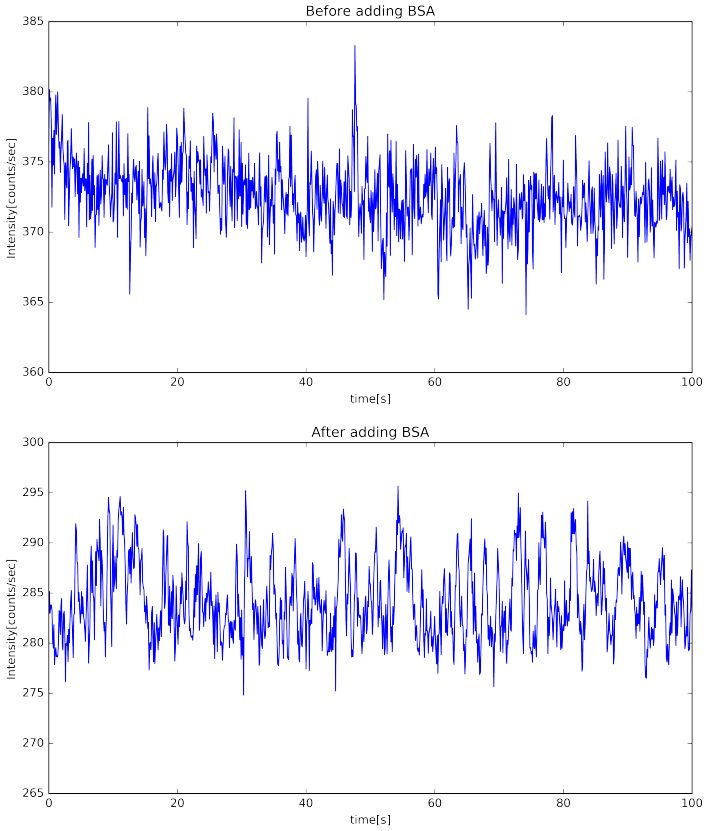
Numbering according to order of taking spectrum

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.

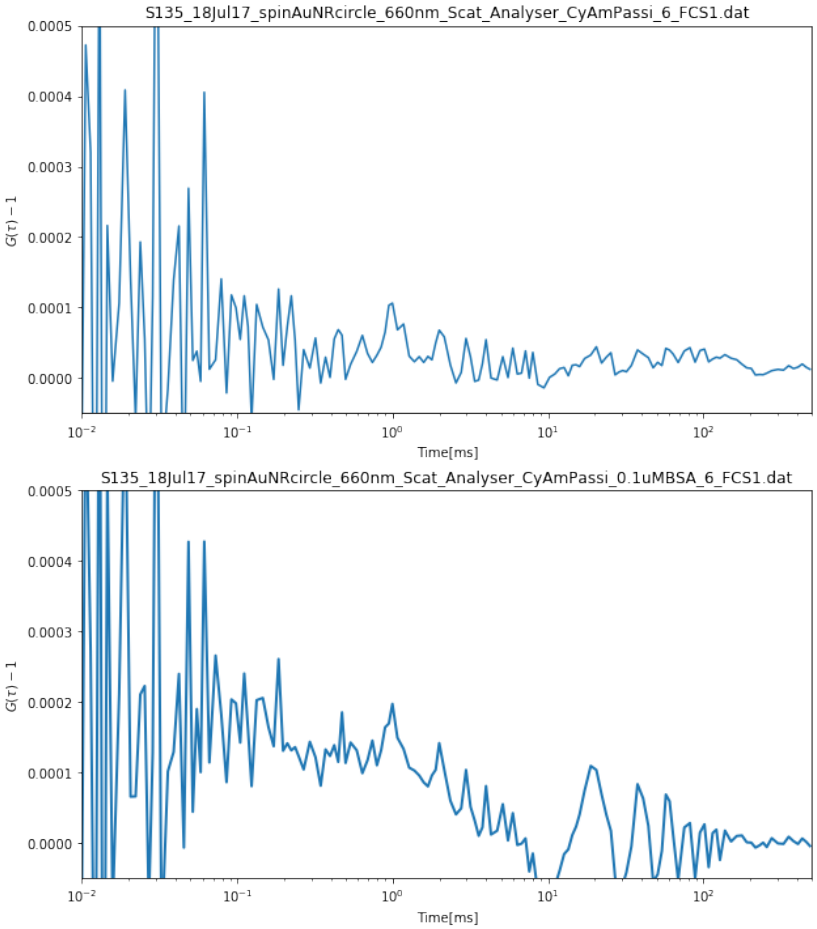
AuNR	Before 0.01uM_BSA		After 0.01uM_BSA	
	SPR(nm)	FWHM(nm)	SPR(nm)	FWHM(nm)
2	659	41	662	40
3	646	49	649	47
5	670	40	672	39
6	685	42	687	42
8	656	45	658	44
10	646	50	649	48
12	670	42	672	41
13	672	40	674	40
14	670	40	672	40
15	665	41	667	40

Change in Spectrum before and after introduction of BSA

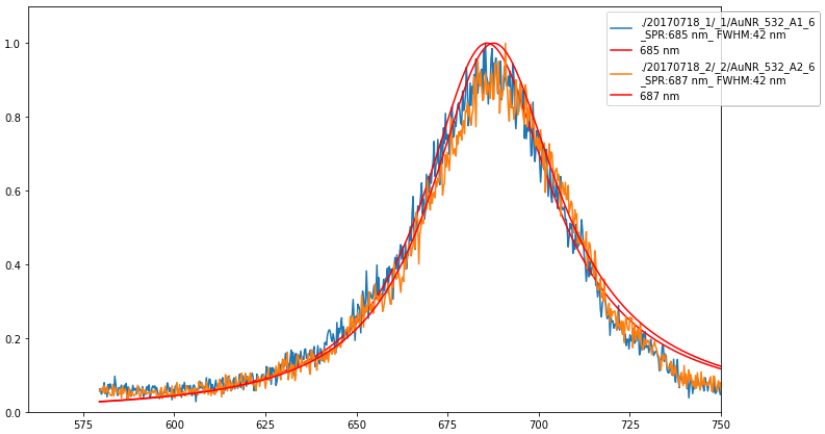
I would particularly discuss one of the AuNR which was showing some correlation i.e. AuNR6



Time Trace of 6 before and after BSA.As we can see from time traces that due to BSA there are fluctuations in this

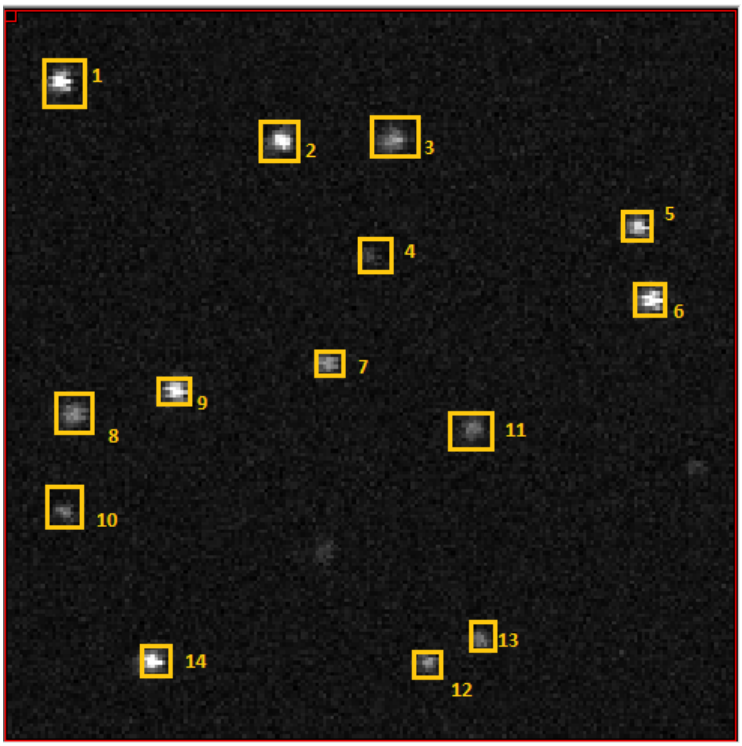


FCS correlations of AuNR6 before and after BSA.There is some correlation due to the some sticking of BSA



Spectrum shift in AuNR6 before and after BSA.

3.2 Passivation with 100μM 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

First conclusion is that we're experimentally able to detect protein sticking with our theory.Second conclusion is that due to only maximum 3-4 % increase in time trace after introduction of BSA ,FCS correlation is too noisy which hinders our ability to analyze.

3.4 Outlook

We can use good detectors which have low noises such as photodiode to get better idea from correlations.Also we can passivate AuNR better to avoid sticking with protein.

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).

[4] P. Zijlstra, P. M. R. Paulo, and M. Orrit, *Optical detection of single non-absorbing molecules using the surface plasmon resonance of a gold nanorod*, Nat Nano **7**, 379 (2012).