Università di Genova | Dipartimento di Fisica | iit Istituto Italiano di Tecnologia

PhD Program in Physics and Nanoscience

Curriculum: Bio-Nanoscience

Student Name: Muhammad Waseem Ashraf

Student No. 4621629, XXXIV Cycle

Principal Investigator: Alberto Diaspro

Dear Riccardo,

During the past one year of my PhD, I have attended and passed the exams of following courses:

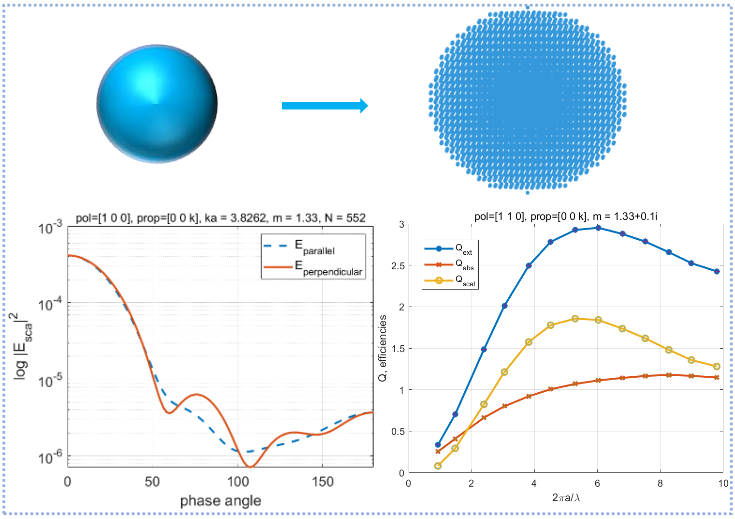
No.	Course Title	Teacher	Lesson Location
1.	Electronics and data acquisition	Flavio Fontanelli	Dept. of Physics, UniGE
2.	Quantum optics: from photons to electrons	Dario Ferraro	Dept. of Physics, UniGE
3.	Nanophotonic devices: from fabrication to applications	Andrea Toma	IIT, via Morego Genova
4.	Lasers and applications	Marti Duocastella	IIT, via Morego Genova
5.	Fluorescence Super-Resolution Microscopy: Basis, Applications and Perspectives	Giuseppe Vicidomini	IIT, via Morego Genova
6.	The optical microscope: optics, image formation and resolution	Alberto Diaspro	IIT, via Morego Genova
Schools and Workshops			
1.	IVSLA International School on Nanoscale Optical Microscopy	11-14th June 2019, Venice, Italy	
2.	5th NIC@IIT advanced microscopy practical workshop	3-6th December 2018, IIT Genova	
Presentation in Conference			
1.	Submitted abstract for poster presentation in Biophysical Society Annual Meeting	15-19th Feb 2020, California U.S.	

Research Activity:

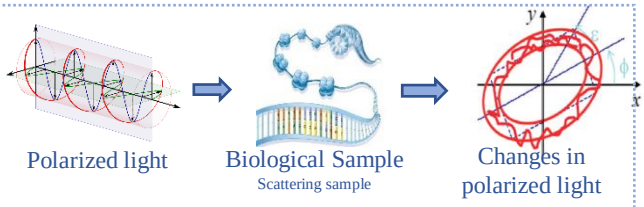
My project is to know about chromatin organization and its dynamics in interphase[1]. Various approaches have been reported to study chromatin organization. The widely used approaches are based on optical microscopy, spectroscopic techniques[2], super-resolution methods[3,4], fluorescence based methods[5], light scattering and polarimetry[7,8]. The resolution of optical microscopy techniques is limited by Abbe's diffraction limit around 250nm. The super resolution microscopy techniques can image beyond this diffraction limit around 10nm down to nucleosome level. However, the investigation of chromatin DNA by super resolution methods requires development of fluorescent probes for specific labelling that impose a challenge. Therefore, to demonstrate the chromatin organization without the requirement of fluorescent labelling we need new approaches that can image at the nucleosome level.

I have been working to develop label-free non-invasive microscopy approach based on polarized light scattering. When the polarized light interact with some biological sample, measuring the scattered light we can infer about the size, shape, birefringence, dichroism, molecular orientation of that sample. The 4x4 Mueller-matrix[9,10] is the mathematical description that demonstrates all these polarization properties of biological samples. Interpretation of polarization properties from Mueller matrix (MM) elements requires the development of new theoretical models. The different elements of MM demonstrate different polarization properties of the samples. The M_{03} element of Mueller matrix, known as circular intensity differential scattering (CIDS), has particularly been reported to describe chiral properties of the samples. In our lab we have been working to design CIDS Mueller matrix experimental setup to study chromatin DNA. Diaspro and Aymeric[13] has already reported CIDS result for the chromosomes. As my role in this project is to model chromatin DNA numerically, I have been working on computing electromagnetic scattering of polarized light from the biological samples. To this end, I have worked on numerical techniques particularly the discrete dipole approximation[14] (DDA) and Mie theory. I have written DDA codes using MATLAB. These DDA codes can solve various scattering problems and are able to calculate the scattering cross-section, absorption, extinction spectra, phase function and Mueller matrix. The analytical results exist for spheres so I have validated the working accuracy of DDA codes. I have calculated scattering from silica and polystyrene microspheres. The scattering images have been obtained for the silica microspheres experimentally. The simulation results for silica microspheres are in correlation with experimental results. The microspheres scattering results serve for the calibration of experimental setup that we designed to study chromatin DNA organization. Next, we will obtain simulated images of chromatin DNA and experimental measurements of different population of cells.

Discrete dipole approximation



Polarized light scattering



$$S_{out} = M_{sample} S_{in}$$

Mueller matrix

$$M_{sample} = \begin{pmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{pmatrix}$$

Reference articles:

1. Kornberg et.al, Vol. 98, 3, Cell, 1999.
2. Weidemann et.al, Vol. 334, J. Mol. Biol. 2003.
3. Lothar et.al, Nature Cell Biology, 2019.
4. Hell et.al, Proc. Natl. Acad. Sci. 2005.
5. Scherise et.al, Vol. 3121, J. Mol. Cell. Card. 2012.
6. Wood et.al, Vol. 333, 6040, 307, Science, 2011.
7. Bustamante, Ph.D. Uni. of California, 1980.
8. Muller et.al, Phys. Med. Biol. 64 045016, 2019.
9. Diaspro et.al, IEEE Trans. Biomed. Eng. 1991.
10. RMA Azzam, Vol. 3121, SPIE, 1997.
11. Shapiro et.al, J. Chem. Phys. 1994.
12. Arteaga et.al, Applied Optics, 2014.
13. Aymeric et.al, OSA Continuum, 2018.
14. Draine et.al, J. Opt. Soc. Am. A, 1994.