

Single molecule blinking and localization based Superresolution Microscopy of Carbogenic Fluorescent NanoDots

A THESIS SUBMITTED

FOR THE DEGREE OF

Doctor of Philosophy

By

Navneet Chandra Verma



School of Basic Sciences

Indian Institute of Technology, Mandi

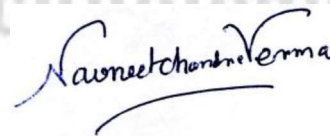
Himachal Pradesh 175001, INDIA

OCTOBER 2019



Declaration by the Research Scholar

This is to certify that the thesis entitled “Single molecule blinking and localization based Superresolution Microscopy of Carbogenic Fluorescent NanoDots”, submitted by me to the Indian Institute of Technology Mandi for the award of the degree of **Doctor of Philosophy (Ph.D)** is a bona fide record of research work carried out by me under the supervision of **Dr. Chayan K. Nandi**. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.



Navneet Chandra Verma

Date: 16-03-2020

Place: Mandi

Research Scholar
School of Basic Sciences
Indian Institute of Technology, Mandi
Himachal Pradesh -175001, India



Thesis Certificate

This is to certify that the thesis entitled “Single molecule blinking and localization based Superresolution Microscopy of Carbogenic Fluorescent NanoDots” submitted by **Mr. Navneet Chandra Verma** to the Indian Institute of Technology, Mandi for the award of the degree of **Doctor of Philosophy (Ph.D.)** is a bonafide record of research work carried out by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

Chayan Kanti Nandi

Dr. Chayan Kanti Nandi

Date: 16-03-2020

Place: Mandi

Associate Professor
School of Basic Science
Indian Institute of Technology, Mandi
Himachal Pradesh -175001, India

Dedicated to
My Beloved Parents and Family

Acknowledgments

I am feeling contentful to take this opportunity in thanking all those who have helped me in the successful completion of work leading to this thesis and in making my days memorable during my Ph.D. First and foremost, I would like to express my sincere gratitude to my supervisor, Dr. Chayan Kanti Nandi for his dexterous and never tiring guidance, which brought the best out of me during my research work. Especially, I thank him for introducing me to the fascinating world of single molecule techniques and helping me in the establishment of superresolution microscopy facility at IIT Mandi. Learning and working with him has been one of the most elevating and prolific practices of my life. I also wish to thank him and his wife, Mrs. Ishita Nandi for providing me all of their support and care in IIT Mandi and making me feel like a valued family member.

I express my sincere thanks to my institute IIT Mandi and its administration for all the facilities and necessary support provided for my research. I convey my sincere gratitude to my doctoral committee members, Dr. Aniruddha Chakraborty, Dr. Kaustav Mukherjee, Dr. Prasanth P. Jose and Dr. Dileep A. D. for their invaluable advice and encouragement. I thank all the faculty members in the Department of Chemistry for teaching me various exciting courses and keeping me motivated in my research. Especially, I would like to acknowledge Dr. Chayan Kanti Nandi, Dr. Amit Jaiswal and Dr. Prasanth P. Jose for enlightening me on various topics of science and research during my coursework with them. I am also grateful to the staff members and operators of various instruments for their help, support, and encouraging involvement in my research expeditions. I am grateful to the Department of Biotechnology, Government of India (DBT GOI) for providing me the junior research fellowship (JRF) for my research work. I am incredibly thankful to the Council of Scientific and Industrial Research, Government of India (CSIR GOI) for providing me the Senior Research Fellowship (CSIR SRF:9/1058(07)/17-EMR-1) during my thesis work.

It is a great pleasure to acknowledge Dr. Alexey Chizhik and Prof. Joerg Enderline at the University of Gottingen, Germany, for being a wonderful host and making my stay memorable at Gottingen. I am grateful to Dr. Eugenia Butkevich and Prof. Christoph Schmidt at the University of Gottingen, Germany, for suggesting me the excellent application of my samples in biology and conducting all the experiments, which is one of the essential chapters of this thesis. I am thankful to Dr. Eugenia Butkevich for her help, guidance, and all discussions about biology during my stay in Gottingen and enlighten me about the worms. I am grateful to Dr. Alexey Chizhik, Dr. Nareian Karedla, Dr. Ingo Gregor, Dr. Anna Chizhik, Dr. Roman Tsukanov, Mr. Sebastian Isbaner, Ms. Akshita Sharma, Mr. Steffen Mühle, Mr. Arindam Ghosh, Mr. Aditya Katti, Mr. Nazar Oleksiievets, Mr. Hongje Jang, Mr. Soheil Mojiri and Mr. Jan Christoph Thiele for being nice friends, helping me to learn new techniques and advance applications, during my stay in Gottingen.

I would also take this opportunity to thank Dr. Viswanath Balakrishnan, Dr. Jaspreet Kaur Randhawa, and Dr. Neha Garg at IIT Mandi for their enthusiastic involvement in my research and sharing of their fantastic knowledge and skills. Research would have been less exciting without the enthusiastic involvement of inquisitive minds with whom I was personally attached. I wish to thank my previous and current research group members Dr. Charu Dwivedi, Dr. Abhishek Chaudhary, Dr. Abhishek Gupta, Dr. Syamantak Khan, Ms. Chethana, Mr. Aditya Yadav, Mr. Pushpendra Mani Mishra, Mr. Prashant Gupta, Ms. Akshita Sharma, Ms. Sanjhal Jain, Mr. Kush Kaushik, Mr. Shivendra Singh, Ms. Richa Garg, Mr.

Neeraj Soni, Ms. Gayatri Batra, Ms. Sakshi Tyagi, Ms. Diksha Sharma and Mr. Bhola Nath Gupta for their support and encouragement during my research work. Especially, I would like to thank Mr. Aditya Yadav, Ms. Chethana and Mr. Pushpendra Mani Mishra for their constant help in the correction of my thesis.

I wish to convey my heartfelt gratitude to Syamantak for his motivation and sharing of his ideas with me and providing me support as a true friend. I am grateful to Abhishek for his guidance and for keeping me motivated all the time. I am thankful to Chethana for her support in all my ups and downs, being an active research partner and friend in need. My sincere thanks to my friends who always enthusiastically shared their knowledge and helped me learn more. I express my gratitude to Dr. Pawan Kumar, Mr. Ashish Tiwari and Mr. Ashutosh Singh for being excellent research collaborators and friends as well. It is said that good friends are not easy to find, but I have been lucky to have many. Thanks to Dr. Sougata Sinha, Dr. Suneel, Dr. Abhishek Kumar Gupta, Dr. Gourab Dey, Dr. Syamantak Khan, Dr. Debarati Banerjee, Dr. Abhishek Gupta, Dr. Jalim Singh, Dr. Pankaj Gour, Mr. Sultan Alam, Mr. Fauzul Mobin, Mr. Arpit Baradwaj and Mr. Rituporno Gogoi for making my early days at IIT Mandi truly memorable. Again, I would thank Mr. Aditya Yadav, Ms. Chethana, Mr. Ashish Tiwari, Mr. Nishith Mohan, Ms. Diksha Gambhir, and Ms. Bidisha Bishwas for making my final days at IIT Mandi genuinely memorable. I would like to acknowledge Mr. Kishore and his family for serving the exotic and customizable tea every evening. I want to acknowledge Dr. Ashvini Sharma, Dr. Efosa Obazee, Dr. Ashish Bauguna, Dr. Saurabh Singh and Mr. Bhuwan Upadhyay for being my friends and great roommates I ever had.

I am incredibly grateful to the peer-reviewers of my accepted and rejected research papers. It would have been impossible for me to carry out my research successfully and write this thesis today without their critical assessment of my work and valuable suggestions to improve them. I am also grateful to the editors for accepting my research papers in their esteemed journals, giving me an opportunity to communicate my research findings. I want to convey my gratitude to the organizers of various national and international conferences, which I attended in the last few years, to provide me the opportunity to present my research with talks and posters. I also thank the organizers of the ‘MRSI, Bangalore’ and ‘CRSI, Kanpur’ for recognizing my research with special awards.

I wish to remember and thank my supervisor at J.N.U. Delhi Prof. Pradipta Bandyopadhyay for sowing the seeds of research in me with his encouragement and precious guidance. I want to thank some of my closest friends, including Dr. Ravikant Tripathi, Dr. K.C. Bhamu, Mr. Chanda Kishor Maurya, Mr. Rohit Sharma, Mr. Shashank Shekhar, and Ms. Sonia Verma for their unfailing support to date despite staying in different parts of the world. I want to thank Ms. Sapna for her encouragement and support in some of my difficult phases. My sincere thanks to Dr. Soumya Poddar for being a wonderful host and tour guide during my stay in California to attend the International Conference: Advanced Functional Materials 2017, UCLA.

Last but not least, I am, needless to mention, indebted to my parents, my beloved brother, and sister-in-law. I want to express my gratitude to both of my loving sisters and both brothers-in-law. I am grateful to have my family without whose constant encouragement and boundless support this day of mine would have remained a dream.

Table of Contents

1	Introduction.....	1
1.1	Abstract	2
1.2	Objective and Overview of the Thesis	3
1.3	Superresolution Microscopy in Brief	3
1.4	Probes for STORM Microscopy	8
1.4.1	Fluorescent proteins.....	8
1.4.2	Organic dyes.....	9
1.4.3	Fluorescent nanomaterials	10
1.5	Fluorescent Carbon Nanodots	11
1.5.1	Single molecule measurements of FNDs	14
1.5.2	Superresolution microscopy using FNDs.....	16
1.6	Overview of the Chapters.....	18
1.7	References.....	21
2	Materials and Methods.....	27
2.1	Materials	28
2.2	Synthesis and Purification Methods	29
2.2.1	Synthesis of FNDs from potato dextrose agar (FNDs1)	29
2.2.2	Synthesis of FNDs from chitosan (FNDs2).....	30
2.2.3	Synthesis of FNDs from sodium citrate and sodium thiosulphate (FNDs3)	30
2.2.4	Synthesis of orange emissive FNDs from citric acid and urea (FNDs4).....	31
2.2.5	Synthesis of orange emissive Phalloidin-FNDs conjugates	32
2.2.6	Centrifugation.....	32
2.2.7	Dialysis.....	33
2.2.8	Separation of fluorescent components from FNDs	34
2.2.9	Polymerization of actin filament and FNDs labeling	34
2.2.10	Synthesis of FNDs labeled self-assembled polymer ring structure	35
2.3	Characterization Techniques	35
2.3.1	Transmission electron microscopy (TEM)	35
2.3.2	X- Ray diffraction (XRD).....	36
2.3.3	Atomic force microscopy (AFM)	37
2.3.4	X-ray photoelectron spectroscopy (XPS).....	38
2.3.5	Raman spectroscopy	39
2.3.6	UV-Vis spectroscopy.....	39

2.3.7	Fourier transform infrared spectroscopy (FTIR).....	40
2.3.8	Fluorescence spectroscopy	40
2.3.9	Quantum yield (QY) calculation.....	41
2.4	Fluorescence Microscopy and Single Molecule Measurements.....	42
2.4.1	Total internal reflection fluorescence microscopy	42
2.4.2	Single molecule transients, blinking and photoswitching study	43
2.4.3	Single molecule imaging	44
2.4.4	Fluorescence correlation spectroscopy (FCS).....	44
2.4.5	Cell culture	45
2.4.6	Cytotoxicity test of FNDs	45
2.4.7	Confocal imaging of mammalian cells	46
2.5	References	47
3	Single Molecule Analysis of FNDs Towards Localization Based Superresolution Nanoscopy	49
3.1	Abstract	50
3.2	Introduction.....	50
3.3	Results and Discussion.....	51
3.3.1	Characterization of FNDs	51
3.3.2	Multicolor confocal imaging of FNDs in bacterial and human cells	53
3.3.3	Photoswitching and laser power dependence of FNDs	62
3.4	Single Particle Fluorescence blinking Studies	65
3.4.1	Single molecule time trace, photon counts and localization method.....	65
3.4.2	Single particle blinking and electron transfer mechanism	66
3.4.1	Blinking rate and photon budget.....	69
3.4.2	Single particle localization microscopy of FNDs	72
3.5	Conclusion	74
3.6	References	74
4	FNDs as Efficient Fluorescent Probe for STORM Nanoscopy in Actin Filament and Self-assembled Soft Matter	77
4.1	Abstract	78
4.2	Introduction.....	78
4.3	Results and Discussion.....	79
4.3.1	Synthesis and characterization details of FNDs.....	79
4.4	Single Particle Analysis of FNDs.....	81
4.4.1	Single molecule measurement method	81
4.4.2	Single molecule time trace, photon counts, and localization	83
4.5	Single particle localization microscopy of FNDs.....	89
4.6	Superresolution Microscopy of F-actin and Artificial Rings.....	90

4.7	Conclusion	95
4.8	References	95
5	FNDs for Specific Intracellular and Tissue Labeling	98
5.1	Abstract	99
5.2	Introduction.....	99
5.3	Results and Discussion.....	100
5.3.1	Characterization of FNDs	100
5.3.2	FNDs-phalloidin conjugation	106
5.3.3	Staining of cells, tissues and <i>C. elegans</i> worm with FNDs-phalloidin	106
5.3.4	Confocal microscope	107
5.4	Conclusion	116
5.5	References	116
6	Cellular Actin specific FNDs as a Probe for Superresolution SRRF and Electron Microscopy	120
6.1	Abstract	121
6.2	Introduction.....	121
6.3	Results and Discussion.....	122
6.3.1	Characterization of FNDs	122
6.3.2	Single molecule time trace and photon counts.....	125
6.3.3	Single molecule localization.....	125
6.3.4	Single particle analysis of FNDs.....	127
6.3.5	SRRF microscopy of FNDs labeled cells	130
6.3.6	TEM of FNDs labeled cells	134
6.4	Conclusion	136
6.5	References	136
7	Paving the Path to the Future of FNDs	139
7.1	Abstract	140
7.2	Introduction.....	140
7.3	Fallacies and Artefacts in FNDs.....	142
7.4	Importance of Purification of the FNDs.....	145
7.5	Guidelines to Characterize FNDs.....	146
7.6	Conclusion	149
7.7	References	149
8	Conclusion and Outlook.....	153
8.1	Summary and Overall Conclusion of the Present Thesis	154
8.2	The Future Prospects of the FNDs	155
9	Appendix.....	158

