

**Springer Theses**

Recognizing Outstanding Ph.D. Research

Jaime Ortega Arroyo

# Investigation of Nanoscopic Dynamics and Potentials by Interferometric Scattering Microscopy



Springer

# **Springer Theses**

Recognizing Outstanding Ph.D. Research

## **Aims and Scope**

The series “Springer Theses” brings together a selection of the very best Ph.D. theses from around the world and across the physical sciences. Nominated and endorsed by two recognized specialists, each published volume has been selected for its scientific excellence and the high impact of its contents for the pertinent field of research. For greater accessibility to non-specialists, the published versions include an extended introduction, as well as a foreword by the student’s supervisor explaining the special relevance of the work for the field. As a whole, the series will provide a valuable resource both for newcomers to the research fields described, and for other scientists seeking detailed background information on special questions. Finally, it provides an accredited documentation of the valuable contributions made by today’s younger generation of scientists.

### **Theses are accepted into the series by invited nomination only and must fulfill all of the following criteria**

- They must be written in good English.
- The topic should fall within the confines of Chemistry, Physics, Earth Sciences, Engineering and related interdisciplinary fields such as Materials, Nanoscience, Chemical Engineering, Complex Systems and Biophysics.
- The work reported in the thesis must represent a significant scientific advance.
- If the thesis includes previously published material, permission to reproduce this must be gained from the respective copyright holder.
- They must have been examined and passed during the 12 months prior to nomination.
- Each thesis should include a foreword by the supervisor outlining the significance of its content.
- The theses should have a clearly defined structure including an introduction accessible to scientists not expert in that particular field.

More information about this series at <http://www.springer.com/series/8790>

Jaime Ortega Arroyo

# Investigation of Nanoscopic Dynamics and Potentials by Interferometric Scattering Microscopy

Doctoral Thesis accepted by  
the University of Oxford, Oxford, UK

*Author*

Dr. Jaime Ortega Arroyo  
ICFO—The Institute of Photonic  
Sciences  
Barcelona  
Spain

*Supervisor*

Prof. Dr. Philipp Kukura  
Physical and Theoretical Chemistry  
Laboratory  
University of Oxford  
Oxford  
UK

ISSN 2190-5053

Springer Theses

ISBN 978-3-319-77094-9

<https://doi.org/10.1007/978-3-319-77095-6>

ISSN 2190-5061 (electronic)

ISBN 978-3-319-77095-6 (eBook)

Library of Congress Control Number: 2018934395

© Springer International Publishing AG 2018

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Springer imprint is published by Springer Nature

The registered company is Springer International Publishing AG

The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

*I would like to dedicate this thesis to my  
loving parents.*

# Supervisor's Foreword

This work describes a breakthrough in optical microscopy in terms of sensitivity and the application of the resulting novel capabilities to a broad range of questions in nanoscience. Microscopy, by definition, is concerned with visualising structure and dynamics on ever decreasing length and timescales. With that comes a need for increasing both temporal resolution and sensitivity with the clear goal of visualising, and thereby studying matter down to the single molecule level. This hurdle was initially taken almost three decades ago in cryogenic environments and has subsequently evolved into an almost standard methodology with far-reaching applications. Prior to the work of Dr. Ortega-Arroyo, optical detection of single molecules has been exclusively limited to resonant detection of chromophores with large absorption cross sections and strong light matter interactions. In this work, Dr. Ortega-Arroyo demonstrates that interferometric scattering microscopy (iSCAT) is capable of detecting and tracking single proteins in solution, and he then applies this unique level of sensitivity to both ultraprecise and rapid single-particle tracking, as well as monitoring self-assembly relevant to the origin of life.

The difficulty associated with detecting single molecules optically without relying on fluorescence comes down to a combination of the discrepancy in physical size of biomolecules, the diffraction limit, and the need to identify the molecule of interest on top of a very large background caused by the environment. The status quo, until this work, has been that it would be impossible to detect single biomolecules with light scattering alone, partially due to the very small signals observed using extinction in previous studies of dye molecules. What Dr. Ortega-Arroyo has shown here, is that, possibly surprisingly, single protein molecules can produce an imaging contrast on the order of a tenth of a percent of the reflected light intensity in an inverted microscope, and that such a signal can be readily detected. The work presented in Chap. 6 I believe, will become an important landmark in optical microscopy, not necessarily because of what was learned about the molecular motor myosin 5a, but because of its implications of what one can study with light.

Dr. Ortega-Arroyo then demonstrates the versatility of scattering, rather than fluorescence-based detection with single-molecule sensitivity. On the one hand, he shows that it can be used to achieve either very high-speed (Chap. 4) or very high precision (Chap. 5) single particle tracking of very small metallic nanoparticle labels. Importantly, it is the improved imaging capabilities that provide key information on how lipids communicate across bilayer membranes and the molecular mechanism behind the remarkable processivity of myosin 5a. On the other hand, Dr. Ortega-Arroyo pushes the sensitivity of the technique even further to directly detects objects as small as single micelles consisting only of a few hundred lipid molecules (Chap. 7) and thereby performs in-situ monitoring of an autocatalytic process relevant to the origin of life and subsequent assembly processes.

This work represents a detailed account of the first label-free detection of single biomolecules in solution and presents a variety of examples of what these capabilities may enable in terms of studying biological and chemical systems. As such, the impact of this work will not be limited to those interested in developing more powerful microscopes, but equally to those who are looking for novel methods to enable measurements that are currently difficult or impossible to do. I am delighted to see it published in the Springer Thesis series.

Oxford, UK  
January 2018

Prof. Dr. Philipp Kukura

# Abstract

The advent of single-particle tracking and super-resolution imaging techniques has brought forth a revolution in the field of single-molecule optical microscopy. This thesis details the development and subsequent implementation of the technique known as interferometric scattering microscopy as a novel single-molecule tool to study nanoscopic dynamics and their underlying potentials. Specifically, Chap. 2 lays out the theoretical framework and draws comparisons between this technique and other state-of-the-art single-molecule optical approaches. Chapter 3 provides a detailed description for the design and implementation of an interferometric scattering microscope including alignment, instrumentation, hardware interfacing, image processing and respective characterisation to achieve the highest levels of performance. The following two chapters use model systems, namely the diffusion of receptor GM1 in a supported lipid bilayer and the movement of molecular motor myosin 5a, to demonstrate the intrinsic shot-noise-limited nature of the technique, its ability to decouple the temporal resolution from localisation precision, and highlight the importance of taking both parameters into consideration when drawing conclusions about the dynamics of each model system. Chapter 6 provides a proof-of-concept study on the limits of sensitivity and demonstrates for the first time the all-optical label-free imaging, detection and tracking of a single protein. In the last chapter, interferometric scattering microscopy is used to quantitatively study dynamic heterogeneous systems in situ at the single-particle level and thus serves as a proof of principle for future label-free studies beyond the realms of biophysics.

**Parts of this thesis have been published in the following journal articles:**

**Chapter 2:**

- Ortega Arroyo, J. & Kukura, P. Interferometric scattering microscopy (iSCAT): new frontiers in ultrafast and ultrasensitive optical microscopy. *Phys. Chem. Chem. Phys.* 14, 15625–15636 (2012).
- Ortega Arroyo, J. & Kukura, P. Non-fluorescent schemes for single-molecule detection, imaging and spectroscopy. *Nat. Photon.* 10, 11–17 (2015).

**Chapter 3:**

- Ortega Arroyo, J., Cole, D., & Kukura, P. Interferometric scattering microscopy and its combination with single-molecule fluorescence imaging. *Nat. Protoc.* 11, 617–633, (2015).

**Chapter 4:**

- Spillane, K. M.\*, Ortega Arroyo, J.\*, de Wit, G., Eggeling, C., Ewers, H., Wallace, M.W. & Kukura, P. Interleaflet coupling and molecular pinning causes anomalous diffusion in bilayer membranes. *Nano Lett.* 14, 5390–5397 (2014).

**Chapter 5:**

- Andrecka, J.\*, Ortega Arroyo, J.\*, de Wit, G., Fineberg, A., MacKinnon, L., Young, G., Takagi, Y., Sellers, J.R. & Kukura, P. Structural dynamics of myosin 5 during processive motion revealed by interferometric scattering microscopy. *eLife.* 4, e05413 (2015).

**Chapter 6:**

- Ortega Arroyo, J., Andrecka, J., Billington, N., Takagi, Y., Sellers, J. R. & Kukura, P. Label-free, All-optical detection, imaging, and tracking of a single protein. *Nano Lett.* 14, 2065–2070 (2014).

**Chapter 7:**

- Ortega Arroyo, J., Bissette, A., Kukura, P. & Fletcher, S. Visualization of the spontaneous emergence of a complex, dynamic, and autocatalytic system. *Proc. Natl. Acad. Sci. U.S.A.* 113, 11122–11126, (2016).

# Acknowledgements

My life as a graduate student, both inside and outside the laboratory environment, has been moulded by many people, whom without their selfless contributions and support would not have made my time in Oxford a truly memorable and rewarding experience. This thesis is both an acknowledgement and a celebration to those wonderful people, whom I am glad to count all as good friends and the funding agency that made it possible: CONACyT.

First of all, I would like to thank Prof. Philipp Kukura for going far and beyond the role of a graduate supervisor, and instead taking the role of a friend and a mentor. Throughout the past years, he has provided me with much more than guidance and fun projects, as he created an environment and gave me the liberty to pursue my passion for finding things out. Even at times when projects refused to give in, I admired his confidence to know that “in the end, it all works out”. Moreover I consider it being a privilege to have been part of the first generation and witness how the group matured from just three members to more than a dozen nowadays.

Next I want to acknowledge Matz Liebel, who as part of the first generation spent endless hours in the laboratory with me, learning, building optical contraptions, exchanging fruitful ideas of what the future directions of the field should be and, above all, having fun doing experiments as if the pursuit of knowledge and scientific research were a game to be enjoyed, rather than a job that had to be done for academics sake. I would then like to thank Dr. Joanna Andrecka, who earned the pseudonym of Mama Joanna (MJ), for her passionate care for every member of the group and for always looking out for us both academically and personally. I will never forget the thrill we shared in every project we worked together on, especially on the label-free detection of a protein. We made a great team, and Chaps. 5 and 6 in the thesis are a testament to that they are not mine alone—they are ours.

Probably, the most unexpected and most fascinating projects of all, the origin of life (Chap. 7), would not have been possible without the vision and passion of Andrew Bissette and Prof. Stephen Fletcher and lest not forget the synthesis and characterisation of the necessary reagents. Together, we pushed the technique to

new frontiers we did not believe was possible, all while having an absolute fantastic time. I would also like to thank both Gabrielle de Wit and Dr. Katelyn Spillane together with David Marshall (MW group), Dr. Oliver Castel (MW group), Prof. Christian Eggeling and Prof. Mark Wallace for the many fruitful collaborations and discussions on membrane biophysics that led to the results presented in Chap. 4.

Although now belonging to a different field, I want to thank Aleksandar Sebesta for sharing those long hours in the laboratory with me, motivating each other while doing very long and unforgiving experiments, having many passionate discussions and above all being a great friend. Furthermore, I want to express my gratitude and my utmost respect to Dr. Alexander Weigel as a source of knowledge of optics and a role model, whose vision of science research I cherish and commend: as a quest for knowledge that should be performed meticulously and be judged by its quality and not by the popularity of the topic—an element that unfortunately plagues today's science research.

I would also like to thank Prof. James R. Sellers (Jim), Dr. Yasuharu Takagi (Harry) and Prof. Keir Neuman for receiving me with arms wide open at the National Institutes of Health and making me feel at home, away from home. I will never forget that first conference in the San Francisco and every single visit to the NIH. Jim's and Harry's support in the myosin project was critical as they provided us with the purified motor protein constructs and more importantly, they had the patience and faith to entrust their work to us.

As whole, working in the Kukura group for the past years has been an incredible experience, and every single member of it has contributed to it regardless of the choice of project. Christoph Schnedermann, Dr. Torsten Wende, Dr. Alex Duarte and Dr. Jongmin Lim, although our projects are quite different, we always shared a fascination for what each other worked on, attempted to understand what is going on and asked questions that have lead to very interesting developments. I look forward to the many exciting results that are to come from Dan, Gavin and Adam as they have been wonderful students. In addition, it has been a privilege to have such excellent part IIs, especially like Dorcas Tan, my first part II student.

Beyond the academic aspect, I am forever grateful to Anna Jones, for selflessly supporting me day after day and putting up with my long hours in the laboratory and during the write-up. I do not know how the thesis would have been completed without her motivation and help. Similarly, the Aston crew always kept my moral high and helped me keep the fine work-life balance.

A special mention goes to my former supervisor Prof. Edward Grant, who encouraged me to leave my comfort zone in UBC (University of British Columbia) and pointed me into a new direction that ended up being the Kukura group. I will never forget his advise that no matter what you do, as long as you are having fun and are happy, that such choice could not have been wrong, but rather the best possible outcome.

Last but not least, I want to thank my parents, whose everlasting support and complete freedom to choose my path have led me to this very point in time. They may have instilled that bug for finding how things work out, for doing science, but more importantly they allowed me to discover it on my own terms.

The future holds many more challenges and uncertainties; however, I am convinced that the success that is yet to follow will be founded on the experiences and interactions I had with all those people mentioned above.

# Contents

<b>1</b>	<b>Introduction</b>	1
	References	4
<b>2</b>	<b>Non-fluorescent Single-Molecule Approaches to Optical Microscopy</b>	7
2.1	Introduction	7
2.2	Single-Particle Tracking	8
2.3	Scattering Detection: An Alternative to Fluorescence	12
2.4	Interferometric Scattering	17
2.4.1	Confocal Detection	18
2.4.2	Non-scanned Wide-Field Detection	19
2.4.3	Confocal Beam Scanning Wide-Field Detection	20
2.5	Applications	21
2.5.1	Lateral Single-Particle Tracking	21
2.5.2	Axial Localisation via Interferometry	23
2.5.3	Label-Free Imaging	28
2.6	Conclusion and Outlook	29
	References	31
<b>3</b>	<b>Experimental Methods</b>	37
3.1	Experimental Optics and Hardware	37
3.1.1	Interferometric Scattering Channel	39
3.1.2	Focus Control Feedback Channel	42
3.1.3	Single-Molecule Fluorescence Channel	43
3.1.4	Sample Stage Stabilisation	43
3.1.5	Camera Characterisation	44
3.1.6	Operation and Synchronisation of the Acousto-Optic Beam Deflector	46
3.1.7	Data Acquisition	47

3.2	Experimental iSCAT Microscopy . . . . .	48
3.2.1	Image Processing . . . . .	48
3.2.2	Spot Detection . . . . .	52
3.2.3	Localisation . . . . .	53
3.2.4	Trajectory Linking . . . . .	54
3.2.5	Assessment of Localisation Precision . . . . .	55
3.2.6	Self-referencing . . . . .	55
	References . . . . .	57
<b>4</b>	<b>Anomalous Diffusion Due to Interleaflet Coupling and Molecular Pinning . . . . .</b>	<b>59</b>
4.1	Introduction . . . . .	59
4.2	Experimental Methods . . . . .	61
4.2.1	Materials . . . . .	61
4.2.2	Vesicle Preparation . . . . .	62
4.2.3	Substrate Preparation . . . . .	62
4.2.4	Supported Lipid Bilayer Formation . . . . .	62
4.2.5	Instrument Setup Parameters . . . . .	63
4.3	Experimental Results . . . . .	63
4.3.1	GM1 Undergoes Anomalous Diffusion in Supported Lipid Bilayers . . . . .	63
4.3.2	Transient Confinement Causes Anomalous Diffusion . . . . .	66
4.3.3	Concentration Dependent Dynamics of Transient Binding . . . . .	69
4.3.4	Recovery of Brownian Motion upon Tuning Substrate Interactions and Interleaflet Coupling . . . . .	70
4.4	Discussion . . . . .	72
4.4.1	Importance of Simultaneous Localisation Precision and Time Resolution . . . . .	72
4.4.2	Thermal and Optical Force Considerations . . . . .	73
4.4.3	Membrane Defects, Labelling Artefacts, and CTxB Induced Aggregation Do Not Cause Transient Binding . . . . .	74
4.4.4	Transient Binding Requires Substrate Interaction and Interleaflet Coupling . . . . .	75
4.4.5	Multiple CTxB-GM1 Interactions Result in Ring-Like Structures . . . . .	75
4.4.6	A Model of Transient Binding: Molecular Pinning . . . . .	76
4.5	Conclusion and Outlook . . . . .	77
	References . . . . .	77
<b>5</b>	<b>Structural Dynamics of Myosin 5a . . . . .</b>	<b>81</b>
5.1	Introduction . . . . .	81
5.2	Experimental Methods . . . . .	83

5.2.1	Sample Preparation . . . . .	83
5.2.2	Experimental Setup . . . . .	84
5.3	Experimental Results . . . . .	84
5.3.1	N-Terminus Labelling Does Not Perturb the Kinetics of Myosin 5a . . . . .	84
5.3.2	During Myosin Movement the Motor Domain Undergoes a Transition Between Two Distinct States . . . . .	85
5.3.3	The Labelled Motor Domain Moves in Three Dimensions . . . . .	86
5.3.4	A Conformational Change in the Motor Domain Accompanies the Power Stroke . . . . .	88
5.3.5	Myosin Steps via a Single, Spatially-Constrained Transient State . . . . .	91
5.3.6	Transient States Occur on the Same Side of Actin for Each Head Domain . . . . .	95
5.3.7	The Diffusion Rate of the Unbound Labelled Head Is Comparable to the Frame Time of 1ms . . . . .	96
5.4	Discussion . . . . .	97
5.4.1	Association Between an N-Terminus Rotation and the Lever Arm Motion . . . . .	97
5.4.2	Sub-steps Along Actin and Leading Head Detachment Do Not Significantly Contribute to the Mechanochemical Cycle . . . . .	98
5.4.3	Myosin Preferentially Walks in a Plane Perpendicular to the Glass Surface . . . . .	99
5.4.4	Structurally Constrained Diffusion Leads to Unidirectional Motion . . . . .	101
5.4.5	Relationship Between the Transient State and the AB Transition . . . . .	103
5.4.6	Directionality of the Symmetric Hand-Over-Hand Mechanism . . . . .	104
5.5	Conclusion and Outlook . . . . .	105
	References . . . . .	106
<b>6</b>	<b>All Optical Label-Free Detection, Imaging and Tracking of Single Proteins . . . . .</b>	<b>111</b>
6.1	Introduction . . . . .	111
6.2	Experimental Methods . . . . .	113
6.2.1	Experimental Setup Parameters . . . . .	113
6.2.2	Sample Preparation . . . . .	113
6.3	Experimental Results . . . . .	113
6.3.1	Label-Free Detection of Actin Filaments . . . . .	113
6.3.2	Label-Free Detection of Single Proteins . . . . .	114

6.3.3	Detection Sensitivity of iSCAT . . . . .	116
6.3.4	Comparison of Single-Molecule Fluorescence and iSCAT Imaging . . . . .	117
6.3.5	Observation of Single-Molecule ATP-Dependent Kinetics . . . . .	119
6.3.6	Nanometric Tracking of Individual Myosin Molecules . . . . .	119
6.4	Discussion . . . . .	120
6.5	Conclusion and Outlook . . . . .	121
	References . . . . .	122
<b>7</b>	<b>Single-Molecule Chemical Dynamics: Direct Observation of Physical Autocatalysis . . . . .</b>	<b>125</b>
7.1	Introduction . . . . .	125
7.2	Experimental Methods . . . . .	126
7.2.1	Experimental Setup Parameters . . . . .	126
7.2.2	Sample Preparation . . . . .	126
7.3	Results and Discussion . . . . .	127
7.3.1	Detection of Micellar Aggregates as the Product of the Chemical Reaction . . . . .	127
7.3.2	Super-Resolution Imaging of the Progress of the Reaction . . . . .	129
7.3.3	Direct Observation of Bilayer Formation . . . . .	131
7.3.4	Characterisation of the Reaction Kinetics . . . . .	132
7.3.5	Observation of Physical Autocatalysis in Situ . . . . .	133
7.3.6	Interfacial Dynamics: Surface Interactions Lead to Different Mechanistic Pathways of Product Formation . . . . .	136
7.3.7	Complex Phenomena in the Oil Phase . . . . .	137
7.4	Conclusion and Outlook . . . . .	138
	References . . . . .	139
<b>8</b>	<b>Outlook . . . . .</b>	<b>141</b>
	Reference . . . . .	142