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Jaime Ortega Arroyo

Investigation of Nanoscopic Dynamics and Potentials by Interferometric Scattering Microscopy

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Jaime Ortega Arroyo

Investigation of Nanoscopic Dynamics and Potentials by Interferometric Scattering Microscopy

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

Springer

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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 detectingand 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).

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First of all, I would 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.

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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.

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