Doctoral Dissertation
Doctoral Program in Metrology (36^{th}cycle)

Beating Abbe's diffraction limit in optical microscopy via non classical photon statistics and structured illumination

By

Fabio Picariello

******

Supervisor(s):
Dr. Marco Genovese, Supervisor
Dr. Ivano Ruo Berchera, Co-Supervisor

Doctoral Examination Committee:
Prof. Milena D'Angelo, Università degli Studi di Bari
Prof. Stefano Olivares, Università degli Studi di Milano
Prof. Alessia Allevi, Università degli Studi dell'Insubria
Prof. Francesco Raffa, Politecnico di Torino
Dr. Marco Gramegna, Primo Ricercatore, INRIM

Politecnico di Torino
2024

Declaration

I hereby declare that, the contents and organization of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data.

Fabio Picariello
2024

* This dissertation is presented in partial fulfillment of the requirements for **Ph.D. degree** in the Graduate School of Politecnico di Torino (ScuDo).

Beating Abbe's diffraction limit in optical microscopy via non classical photon statistics and structured illumination

Fabio Picariello

Roughly two decades ago, it was demonstrated that the resolution limit posed by diffraction can be overcome, a breakthrough for science and medical research acknowledged by the Nobel Prize for chemistry in 2014. Since then, super-resolution optical far-field imaging has undergone tremendous evolution, resulting in the development of a wide range of methods especially in fluorescence microscopy. However, most of them require high optical power and are not suited for biological applications. A specific branch of methods that is also biocompatible employs the usage of emitters with either super-Poissonian bunched-light emission due to statistical fluctuations or sub-Poissonian antibunched-light, statistics typical of single photon sources. The ability of these techniques to beat the classical diffraction limit has been demonstrated both in wide-field and confocal microscopy, with scaling in the resolution of $\sqrt{j}$ where j is the measured correlation order. Another family of methods that have been demonstrated to achieve super-resolution relies on boosting the frequency information on the image plane. Normally, the total amount of frequency information that can be retrieved by an imaging setup is d_{Abbe}^{-1} where d_{Abbe} is the minimum resolvable distance in classic imaging. The Structured Illumination Microscopy (SIM) technique can overcome this limit by exploiting a sinusoidal light pattern to illuminate the sample, achieving twice the frequency information than a classical imaging setup. In recent years, it has been shown that it is feasible to integrate non-Poissonian super-resolution methods with Structured Illumination Microscopy. This integration offers the advantage of achieving a substantial enhancement in super-resolution by focusing on the analysis of a limited number of high-order correlations, scaling with $\sqrt{j}+j$.

This thesis aims to provide a comprehensive investigation into super-resolution techniques achieved through non-Poissonian statistics by elaborating a comprehensive quantum model valid for any non-Poissonian photon sources and in every illumination regime. The super-Poissonian photon statistics super-resolution model proposed in the literature is based on a semi-classical approach that overlooks quantum fluctuations, thereby significantly limiting resolution enhancement in low light conditions. Specifically, in low light scenarios, the quantization of light into photons becomes prominent, and only a fully quantum description can achieve optimal performance in optical imaging. Conversely, the sub-Poissonian super-resolution method reported in the literature relies on a mathematical model valid only with single-photon emitters that emit a single photon. Therefore, a comprehensive quantum description of a generalized non-Poissonian photon statistics super-resolution method is lacking. Developing such a model could greatly impact the advancement of super-resolution quantum microscopes,

potentially revolutionizing applications that require low power combined with high SNR and high resolution.

Furthermore, this thesis presents an extensive analysis of the SNR in the context of both sub and super-Poissonian super-resolution methods. The aim is to provide a guide for selecting the most effective detection schemes, photon sources, and experimental parameters, in order to reduce both the total data volume and acquisition time necessary for achieving non-Poissonian super-resolved images. Additionally, the thesis explores the integration of non-Poissonian super-resolution techniques with SIM, focusing on extremely low light levels.

The thesis will be divided into the following chapters:

Chapter 1 gives an insight into single photon emitters, focusing on their emission statistics and covering some examples of bio-compatible emitters used in fluorescence optical microscopy.

Chapter 2 explores the main limits of classical optical imaging and presents bio-compatible super-resolution techniques analyzed in the framework of the thesis.

Chapter 3 gives an insight into detectors used in fluorescence imaging.

Chapter 4 details the experimental setups implemented for the research conducted in this Ph.D. study.

Chapter 5 introduces a generalized quantum model for super-resolution developed in the framework of the Ph.D. and compare its performance with classical analysis reported in the literature.

Chapter 6 presents a detailed study of the SNR of sub and super-Poissonian emitters considering the most common detection schemes employed in biological optical imaging.

Chapter 7 reports simulations and experimental results over the combination of our generalized quantum model for super-resolution and Structured Illumination Microscopy.