UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Multi-photon Nonlinear Fluorescence Emission in Upconversion Nanoparticles for Super-Resolution Imaging

by Chaohao CHEN

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Doctor of Philosophy

under the supervision of Dr. Fan Wang, Prof. Dayong Jin, Dr. Qian Peter Su, Dr. Peter Reece

University of Technology Sydney
Faculty of Science

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Certificate of Original Authorship

I, Chaohao CHEN declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences, Faculty of Science, at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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For my lover

Rui Zhao

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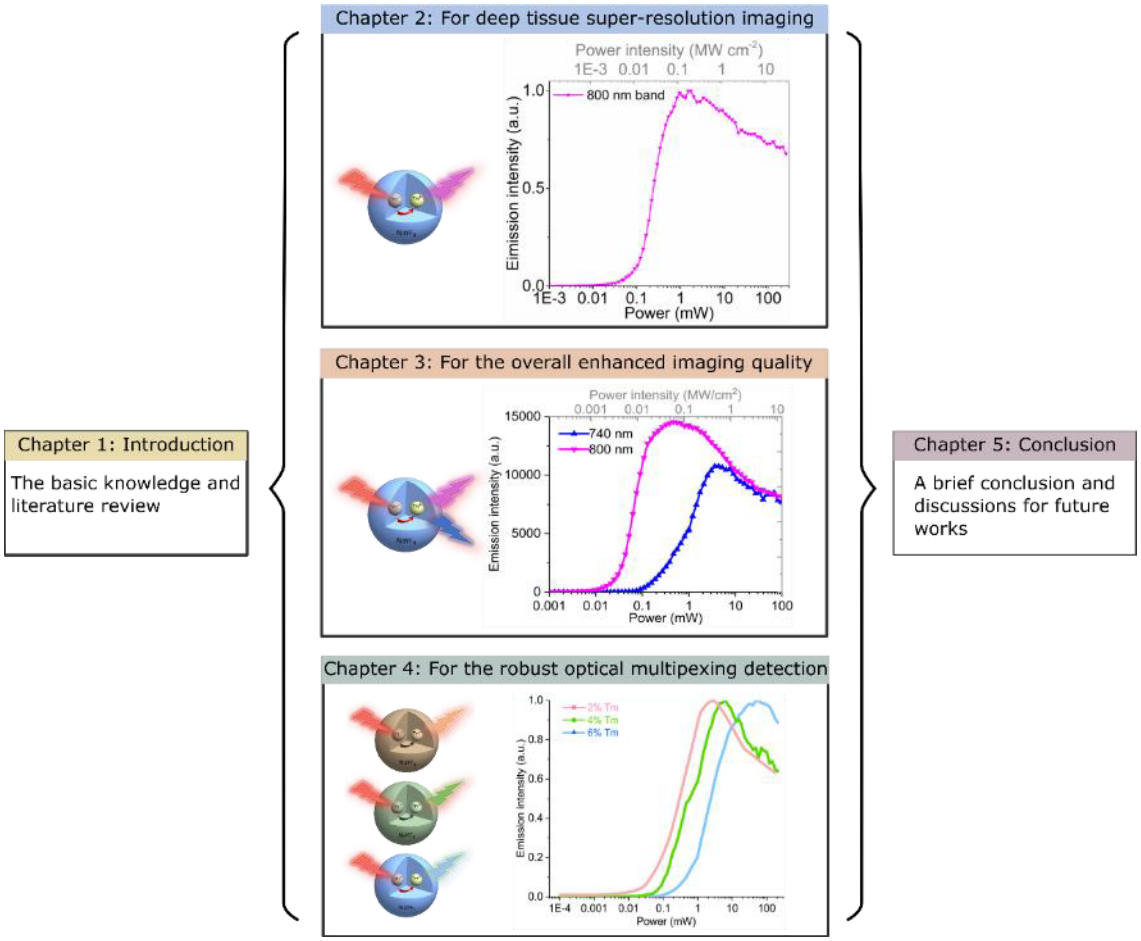
Format of Thesis

This thesis consists of five chapters. Chapter 1 is the introduction and in Chapters 2-4 the research results are the focus on applying optical nonlinear response curves of upconversion nanoparticles. The objective is to implement new modalities of super-resolution microscopies. Chapter 5 concludes this thesis with a summation of the main themes covered in this investigation. This thesis flowchart is illustrated below.



This thesis summarizes the key results from my PhD research program, in which the topic covered is super-resolution microscopy and nanoparticle-based super-resolution microscopy. My PhD sets out to develop new super-resolution microscopy methods using advanced nanomaterials. The main bulk of the framework developed for this thesis consists of three results chapters. The first chapter describes the use of a near-infrared nonlinear response curve from a single nanoparticle to develop a deep tissue super-resolution imaging method. The second results chapter takes advantages of the two response curves from two emission bands of a single nanoparticle, which yields two

things: the overall improved image quality; and increased imaging speed. In the third chapter concerning the results, I explore the multiple emission curves derived from different nanoparticles for robust optical multiplexing detection. Lastly, the conclusion and discussions for future research are provided.

List of Publications

- Articles

1. **Chen, C.**, Liu, B., Liu, Y., Liao, J., Wang, F. & Jin, D. Fourier domain heterochromatic fusion for single beam scanning super-resolution microscopy. (Under revision)
2. **Chen, C.**[†], Wang, F.[†], … & Jin, D. Multi-photon near-infrared emission saturation nanoscopy using upconversion nanoparticles. *Nature Communications (2018),* 9:3290.
3. **Chen, C.**, Liu, Z. & Jin, D. Bypassing the limit in volumetric imaging of mesoscale specimens. *Advanced Photonics (2019)* 1.2 : 020502.
4. Liu, B., **Chen, C.**, Di, X., Wang, F. & Jin, D. Upconversion nonlinear structured illumination microscopy. *Nano Letter (2020)* 20, 7, 4775-4781.
5. Liao, J., Jin, D, **Chen, C.**, Li, Y. & Zhou, J. Helix shape power-dependent properties of single upconversion nanoparticles. *Journal of Physical Chemistry Letters (2020)* 11, 8, 2883-2890.
6. Liu, Y., Wang, F., Liu, H., Fang, G., Wen., **Chen, C.** … & Jin, D. Super-Resolution Mapping of Single Nanoparticles inside Tumor Spheroids. *Small (2020)* 10.1002. 201905572.
7. Clarke, C., Liu, D., Wang, F., Liu, Y., **Chen, C.**, ... & Jin, D. Large-scale dewetting assembly of gold nanoparticles for plasmonic enhanced upconversion nanoparticles. *Nanoscale (2018)* 10 (14), 6270-6276.

- Publication in the conference proceeding

8. **Chen, C.,** Wang, F…. & Jin, D. Upconversion nanoparticles assisted multi-photon fluorescence saturation microscopy. In Nanoscale Imaging, Sensing, and Actuation for Biomedical Applications XVI (Vol. 10891, p. 108910S). International Society for Optics and Photonics. SPIE 2019

- Patents

9. Wang, F., Jin, D. & **Chen, C.** A super-resolution imaging method, a plurality of upconversion nanoparticle fluorophores, and a super-resolution scanning microscope. WO2020028942A1, 2020-02-13. WIPO (PCT)
10. **Chen, C.,** Jin, D. & Wang, F. A super-resolution imaging method, a plurality of upconversion nanoparticle fluorophores, and a super-resolution scanning microscope. AU2018902855A0, 2018-08-16, (Australian Patent)

([1,2,8,9,10] are closely related to my PhD program)

Table of Symbols

λ	Acoustic radiation force	α	Objective aperture angle
n	Refractive index	I_{max}	Maximum STED beam power
I_{sat}	Saturation intensity	hv	Photon energy
σ	Cross-section of excitation	τ_{fl}	Fluorescence lifetime
T_1	The metastable dark triplet state	N	Total captured number of photons
s	The standard deviation of Gaussian function	b	The standard deviation of the background noise
I_{FED}	Image of FED	I_{Gau}	Image of a Gaussian beam
I_{Dou}	Image of a doughnut beam	r	A normalising coefficient
R_{tr}	The transmission ratio	P	The beam power
r_o	The loss rate of the objective lens	t_τ	The exposure time
A	The area of the focused laser spot	f	The pulse frequency
t_p	The pulse duration	α_λ	The attenuation coefficient
l	The path length	h_o	The FWHM of confocal PSF
ς	The saturation factor	k_{BA}	The carrier transition rate
σ_{TPA}	The molecular cross-section	$h_{em}(x)$	PSF of emission
$h_{exc}(x)$	PSF of the excitation beam	η	Emission response curve
$h_c(x)$	PSF of the confocal system	PSF_{Gau}	Gaussian PSF
PSF_{Dou}	Doughnut PSF	OTF_{eff}	Processed effective OTF
I_{eff}	Processed effective image	OTF_{Gau}	Gaussian OTF
OTF_{Dou}	Doughnut OTF		

Abbreviations

UCNPs	upconversion nanoparticles
NIRES	near-infrared emission saturation
PSFs	point speared functions
FWHM	full width at half maximum
SEM	scanning electron microscopy
TEM	transmission electron microscopy
TIRF	total internal reflection fluorescence
SNOM	scanning near-field optical microscopy
RESOLFT	reversible saturable or switchable optical fluorescence transitions
STED	stimulated emission depletion
GSD	ground-state depletion
SIM	structured illumination microscopy
PALM	photoactivated localisation microscopy
STORM	stochastic optical reconstruction microscopy
OTF	optical transfer functions
SPEM	saturated pattern excitation microscopy
SMLM	single-molecule localisation microscopy
FRC	Fourier ring correlation
SOFI	super-resolution optical fluctuation imaging
PAINT	point accumulation for imaging in nanoscale topography
QDots	quantum dots
CDots	carbon dots
PDots	polymer dots
AIE	aggregation-induced emission

UV	ultraviolet
ACQ	aggregation caused quenching
FED	fluorescence emission difference
MPLSM	multi-photon laser scanning microscopy
NIR	near-infrared
CW	continuous-wave
SW	single wavelength
VPP	vortex phase plate
HWP	half-wave plate
PBS	polarised beam splitter
DM	dichroic mirror
QWP	quarter-wave plate
BPF	bandpass filters
MMF	multimode fibre
SPAD	single-photon counting avalanche photodiode
OA	oleic acid
PFA	Paraformaldehyde
DFT	discrete Fourier transform
FFT	fast Fourier transform
THF	tetrahydrofuran
BSA	bovine serum albumin
BSA	bovine serum albumin
ISM	imaging scanning microscopy
LLS	lattice light sheet

Table of Contents

List of Figures

List of Tables

Abstract

Due to the unique optical properties gained by converting near-infrared light to shorter wavelength emissions, upconversion nanoparticles (UCNPs) have attracted considerable interest. Their superior features, including their multi-wavelength emissions, optical uniformity, background suppression, photostability and deep penetration depth through the tissue, make them extremely suitable for biological and biomedical applications. By taking advantage of their multi-photon nonlinear emissions in UNCPs, the goal of this thesis is to develop UCNPs-based super-resolution microscopy methods to address the challenges currently facing nanoscopy, for instance complexity, stability, limited penetration through the tissue and low throughput. The methods being investigated in this thesis make concrete the specific advantages in terms of image depth, speed, overall quality, and multiplexing potentials. To unlock a new mode of deep tissue super-resolution imaging, I first developed the near-infrared emission saturation (NIRES) nanoscopy by taking advantage of near-infrared-in and near-infrared-out optical nonlinear response curve from a single upconversion nanoparticle. This approach only requires two orders of magnitude that are lower than the excitation intensity, which is generally required for conventional multi-photon dyes. This work achieves a super-resolution of sub 50 nm, less than $1/20^{th}$ of the excitation wavelength, and can image single UCNP through a 93 μm thick liver tissue.

To improve the overall imaging quality and simplify the system setups, I further exploited the distinct nonlinear photon response curves from the two emission bands in UCNP, and explored an opportunity for a tightly focused doughnut excitation to generate distinct spectral dependent point speared functions (PSFs). With controllable PSFs from multi-channel emissions by the excitation power density, this work presents the possibility of achieving super-resolution imaging under saturated fluorescence excitation via PSF engineering. Moreover, I developed a multicolour Fourier fusion algorithm to enlarge the optical system's frequency shifting ability, and yield an enhanced imaging quality at a higher imaging speed. By realising the uniform and distinct nonlinear emission curves from different nanoparticles, this work posits a new optical encoding dimension for multiplexing imaging. Proposed here is a robust PSF engineering strategy to extract emitter properties. This work extends the multiplexing capacity of UCNPs and offers new

opportunities for their applications. These methods are my contributions to the search for a stable, viable, and multifunctional optical imaging modality for the nanoscale context.