Frequency-comb-referenced quantitative phase imaging for high-sensitivity, high-speed, spectroscopic phase measurement

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QUANTITATIVE PHASE IMAGING FOR
HIGH-SENSITIVITY, HIGH-SPEED,
SPECTROSCOPIC PHASE MEASUREMENT

BOONRUANGKAN JEERANAN
SCHOOL OF MECHANICAL AND AEROSPACE
ENGINEERING

2019

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HIGH-SENSITIVITY, HIGH-SPEED,
SPECTROSCOPIC PHASE MEASUREMENT

BOONRUANGKAN JEERANAN

School of Mechanical and Aerospace Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the Doctor of Philosophy

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

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Jeeranan Boonruangkan

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

24-July-2019
.....
Date

.....
Nanyang Asst. Prof. KIM YOUNG-JIN

Authorship Attribution Statement

This thesis contains material from **4** paper(s) published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

Chapter 3 (**Sec. 3.1**) & Chapter 4 (**Sec. 4.1**) are published as Farrokhi, H., Boonruangkan, J., Chun, B.J., Rohith, T.M., Mishra, A., Toh, H.T., Yoon, H.S. & Kim, Y.J. Speckle reduction in quantitative phase imaging by generating spatially incoherent laser field at electroactive optical diffusers. *Optics express*, 25(10), 10791-10800 (2017). &

Chapter 3 (**Sec. 3.1**) is published as Rotational diffuser for speckle reduction in quantitative phase imaging. *Conference on Lasers and Electro-Optics/Pacific Rim* (p. *s1147*). Optical Society of America (2017).

The contributions of the co-authors are as follows:

- Asst/Prof Young-Jin Kim provided the initial project direction and edited the manuscript drafts.
- I performed all the laboratory work with the help of Dr. Hamid at the School of Mechanical and Aerospace engineering at NTU.
- Dr. Hamid Farrokhi and I mainly prepared the manuscript drafts
- I also analyzed the data with Dr. Hamid.
- Dr. Chun provided guidance of the experimental setup and helped to revise the manuscript.
- Mr. Rohith helped to revise the manuscript and provided some suggestion on the data analysis.
- Dr. Mishra Abhinay provided the biological sample which was conducted by Dr. Toh in the School of Biological Sciences under the supervision of prof. Yoon Ho Sup.

Chapter 3 (**Sec. 3.2**) & Chapter 4 (**Sec. 4.1**) is now in the submission process and will be published as Boonruangkan, J. *et al.* A frequency comb enables coherence-tailored, multi-wavelength, high-speed quantitative phase imaging with a high phase stability. (*Submitted to Nature Photonics*)

The contributions of the co-authors are as follows:

- Asst/Prof Young-Jin Kim provided the initial project direction and edited the manuscript drafts.
- I performed all the laboratory work at the School of Mechanical and Aerospace engineering at NTU.
- I performed the analysis on the experimental results.
- I wrote the drafts of the manuscript.
- Dr. Hamid helped to write one part of the manuscript.
- Mr. Rohit performed curve fitting for a surface plot of data.
- Dr. Toh provided the biological sample which was conducted in the School of Biological Sciences under the supervision of prof. Yoon Ho Sup.
- Mr. Samuel Kwok provided the zebrafish samples, was conducted in the Lee Kong Chian, School of Medicine under the supervision of prof. Tom James Carney.
- Prof. Seung-Woo Kim provided guidance in the data interpretation and direction of the project.

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The contributions of the co-authors are as follows:

- Asst/Prof Young-Jin Kim provided the initial project direction and edited the manuscript drafts.
- Dr. Hamid and Mr. Rohit mainly performed the laboratory work at the School of Mechanical and Aerospace engineering at NTU.
- I performed one experiment, collected and analyzed that data, and drew a conceptual figure.
- I help to revise the manuscript.
- Dr. Han helped to revise the manuscript.
- Prof. Seung-Woo Kim provided guidance in the data interpretation and planned the project.

1 Aug 2019

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Date

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Jeeranan Boonruangkan

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Abstract

QPI (Quantitative Phase Imaging) is an innovative optical imaging technique which has been widely used for study biological tissues and cells. Due to its non-invasive and quantitative manner, QPI has received much attention over conventional imaging techniques, which are labelled-based and qualitative manner. In QPI, the coherence of the optical field is the prerequisite for high-resolution imaging. However, the use of coherence inevitably accompanies phase ambiguity and coherent artifacts (i.e., speckles) which will degrade phase resolution and hinder successful phase reconstruction. Previous studies have proposed the use of low-coherence light sources to reduce the speckle effect for higher-sensitive phase imaging. However, they suffered from the small number of available photons per coherence volume, hence low visibility interferogram. As an alternative, partially coherent laser-like broadband light sources (i.e., super-luminescent laser diodes) have been proposed for moderate speckle level but it is not low enough for high-sensitivity phase imaging.

High-brightness and high-coherence continuous-wave lasers have been utilized in QPI for efficient light-specimen interaction and phase-sensitive interferometric imaging. However, the use of high-coherence light sources leads to severe speckles. To suppress the speckles in highly-coherent illumination, colloidal solutions, electro-mechanical mirrors, or liquid crystal diffusers have been introduced to the illumination path; they were accompanied by scattering power loss and additional vibration noise induced by mechanical moving parts.

Here, this research proposes a rotational optical diffuser as a compact, vibration-free, and effective method to suppress the speckle of high-coherence continuous-wave laser. The continuous-wave laser as a QPI's light source enables high-brightness phase imaging which could be realized in a compact setup with low-cost. Our speckle suppression method was demonstrated in an off-axis common-path QPI for high phase stability, high acquisition rate, and simplicity. The results showed that our method provided cleaner raw interferograms with background noise suppression of 1.36 dB. This speckle-less coherent illumination will offer high-brightness, high phase-sensitive interferograms over a large field-of-view which are required in many applications. However, a single continuous-wave laser source cannot satisfy phase-coherent multiple wavelengths for decoupling physical volume and refractive index so our first method can offer the advantages to some potential applications.

Therefore, this research further proposes a second speckle-suppression method to obtain highly-sensitive phase images and this method can provide multiple capabilities across a broad range of applications. In this method, the coherence characteristics of a partially coherence light source which is a frequency comb were controlled in wide ranges to optimize the speckle level for high visibility interferograms. The results show that the frequency comb provides a wide coherence tunability (L_c : 8.1 to 56.3 μm , V : 0.63 to 0.98) so as to form the best quality QPI interferogram in diverse biomedical applications. Also, this study demonstrates that the use of frequency comb as a light source in QPI, which is named here as the frequency-comb-referenced quantitative phase imaging (FCR-QPI), can provide phase-coherent multiple wavelengths for extracting physical height information from refractive index, and higher phase stability (Allan deviation: 2.39×10^{-3} at 10-s averaging time) at a higher speed up to 16.9 kHz. In addition, FCR-QPI was demonstrated as a new technique for study heart rate and blood flow development in an embryonic zebrafish. Therefore, FCR-QPI could be recognized as a remarkable technique for driving the future of biological and biomedical imaging.

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List of acronyms

AA	Aortic arches
AOM	Acousto-optic Modulator
ASE	Amplified spontaneous emission
AV	Atrioventricular
AVC	Atrioventricular canal
BPF	Band pass filter
BPM	Beat-per-minute
BS	Beam splitter
BW	Spectral bandwidth
C	Collimator
CA	Caudal artery
CCD	Charge coupled devices
CCV	Common cardinal vein
CL	Cylindrical lens
CM	Culture medium
CW	Continuous wave
CWT	Continuous wavelet transforms
CT	Computerized tomography

CTC	Circulating tumor cells
CV	Caudal vein
D	Diameter
DA	Diffusion angles
DA	Dorsal aorta
DC	Duct of Cuvier
DEA	Dielectric elastomer actuator
DHM	Digital holographic microscopy
DI	Deionized water
DIC	Differential interference contrast
DLAV	Dorsal longitudinal anastomotic vessel
DMEM	Dulbecco's modified eagle medium
DPM	Diffraction phase microscopy
DSTCI	Degrees of Spatiotemporal Coherence of Illumination
DT	Diffraction tomography
E	Electrode
EAP	Electro-active Polymer
ECLD	External-cavity Laser Diode

Er	Erbium
FBS	Fetal bovine serum
FC	Frequency comb
FCR-QPI	Frequency-comb-referenced Quantitative Phase Imaging
FCS	Fluorescence correlation spectroscopy
FFT	Fast fourier transform
FWHM	Full Width at Half Maximum
FOV	Field-of-view
Fps	Frames per second
FS	Femtosecond
HAZ	Heat-affected Zone
Hb	Hemoglobin
HPM	Hilbert phase microscopy
HWP	Half-wave Plate
IFFT	Inverse fast fourier transform
ISV	Intersegmental vessels
K	Number referring to pulse shape
L	Lens

LC	Liquid-crystal
LD	Laser diode
LED	Light-emitting diode
LSR	Laser speckle reducer
M	Mirror
MEM	Micro-electro-mechanical
MRI	Magnetic resonance imaging
NA	Numerical aperture
O	Objective lens
OA	Optical attenuator
OCM	Optical coherence microscopy
OCT	Optical coherence tomography
OD	Optical diffuser
OPD	Optical phase delay
OPL	Optical path length
OPT	Optical projection tomography
OVFT	Optical vector field tomography
P	Polarizer
PCF	Photonic crystal fibre

PCV	Posterior cardinal vein/ postcardinal vein
PD	Photodetector
<i>Pf</i>	<i>P. Falciparum</i>
PIV	Particle image velocimetry
PZT	Piezo-electric Transducer
QPI	Quantitative phase imaging
QPIU	Quantitative phase imaging unit
QPM	Quantitative phase microscopy
Rb	Rubidium
RBC	Red blood cell
RF	Radio-frequency
RI	Refractive index
RTPM	Regularized tomographic phase microscopy
SA	Segmental arteries
SCD	Sickle cell disease
SD	Standard deviation
SD/MD	Static diffuser/moving diffuser
SEM	Scanning electron microscopy

SHG	Second harmonic generator
SLD	Super-luminescent Laser Diode
SLIM	Spatial light interference microscopy
SL-QPM	Spatial-domain Low-coherence Quantitative Phase Microscopy
SMF	Single mode fiber
SNR	Signal-to-Noise Ratio
SV	Segmental vein
TL	Tube lens
TPM	Tomographic phase microscopy
Ti	Titanium
WBC	White blood cell
wDPM	White light diffraction phase microscopy
WHO	World health organization
WP	Wollaston prism

List of symbols

dB	Decibel
c	Speed of light
C	Speckle contrast
E	Electric field
f_o, ω_o	Frequency offset
f_r, ω_r	Repetition rate
h	Physical thickness/physical height
I	Intensity
L	Cavity length
L_c	Coherent length
M	Cellular dry mass
n	Refractive index
n_c	Refractive index of cell
n_m	Refractive index of medium
p	Pixel size
R, r	Radius
T	Seperation time

V	Visibility unit
V_g	Cavity's mean group velocity
ρ	Density
τ_c	Coherence time
λ	Wavelength of light
$\Delta\lambda$	Spectral width at FWHM
α, f_0	Spatial frequency
α_c	Specific protein increment
$\lambda_0/\lambda_{centre}$	Central wavelength
$\tilde{J}$	Mutual intensity
$\tilde{\mu}$	Complex coherence factor
γ	Degree of coherence
$\tilde{I}$	Mutual coherence function
Δn	Refractive index difference
Δt	Temporal width at FWHM
$\Delta\nu$	Spectral width at FWHM
$\Delta\phi$	Optical phase delay or phase shift

1 **Chapter 1**

2 **Introduction**

3

4

5 **1.1 Background**

6 Optical microscopy is a tool that has been used to study micro and nanostructures
7 such as biological cells which are the basic unit of living organisms. Since most
8 biological cells are optically transparent in visible light, the resulting image has
9 low visibility and contrast. Therefore, staining techniques have been introduced
10 to facilitate the visualization of the cells for higher contrast images. The localized
11 pigment from the staining will absorb the light so that the resulting image shows
12 higher contrast. However, staining techniques are not suitable for live-cell
13 imaging and are time-consuming; therefore, innovative fluorescence microscopy
14 was invented to facilitate live-cell imaging. Fluorescence microscopy uses
15 fluorophores for target-specific labeling of biological molecules to make the light
16 be absorbed and generate a high-contrast fluorescence image of biological cells
17 and their internal structures. However, the fluorophores will lose their capability
18 over long observation times and release reactive chemical species upon
19 photobleaching. These reactive chemical species can damage the cells through
20 their phototoxicity. Therefore, the use of non-invasive, label-free imaging
21 techniques have received much attention in live-cell studies because it is much
22 simpler and provides finer details of the cells in a non-destructive way. In the past

1 few decades, phase-contrast microscopy providing better image contrast by using
2 the phase shift rather than the absorption of light, has been widely introduced for
3 non-invasive and label-free live-cell imaging. Traditional phase-contrast
4 microscopy and differential interference contrast (DIC) microscopy provide
5 high-contrast of unlabeled cells [1, 2]; however, these techniques can only
6 provide qualitative cellular analysis, not quantitative analysis, because the
7 amplitude and phase information of the light are superposed to the interferogram.
8 The following research works have tried to separate amplitude and phase
9 information in phase-contrast microscopy, leading to the development of novel
10 quantitative phase microscopy (QPM) [3]. QPM has emerged as one of the
11 powerful optical phase imaging techniques for studying biological cells because
12 it provides non-invasive, label-free, and quantitative cellular analysis. The
13 technique is known as quantitative phase imaging (QPI). QPI has been widely
14 adopted for precision measurement of cellular morphology, refractive index, and
15 dry mass [3-6]; monitoring of inter-cellular communication of immune cells,
16 neurons, and cancerous cells [7-10]; and understanding the dynamics of red and
17 white blood cells [11-14].

18 Most QPI modalities reconstruct phase tomograms using a series of
19 interferograms. High-quality interferograms are, therefore, the prerequisite for
20 QPI. The use of high-coherence continuous-wave laser in QPI has received much
21 attention for obtaining high-quality interferograms because it offers high-
22 brightness phase imaging for retrieving finer phase information, which can be
23 realized in a compact setup with low-cost [15, 16]. However, the high coherence
24 of the lasers inevitably superimposes unexpected coherent noise – speckle,
25 diffraction, and parasitic interferograms – to the target interferograms, which

1 significantly hampers sensitive phase detection and successful phase
2 reconstruction [17, 18]. The coherent noise gets even severer when imaging
3 through the scattering media, e.g. biological tissues, plasmonic nanoparticles, and
4 non-ideal optics with surface roughness or contamination. To overcome the
5 problem of coherent noise in high coherence continuous-wave lasers, several
6 methods have been introduced to control the spatial coherence of the high-
7 coherence lasers such as colloidal solutions, electro-mechanical mirrors, or liquid
8 crystal diffusers [19-21]. However, they suffered from power loss and additional
9 vibration noise induced by mechanical moving parts. In our first study, we
10 propose a robust and cost-effective method to suppress the coherent speckle noise
11 in continuous-wave laser-QPI for phase-sensitive imaging. The proposed speckle
12 suppression in continuous-wave laser-QPI is compact, cost-effective, and allows
13 to tune the level of the suppressed speckle to optimize for the best results in QPI.

14 As an alternative to the use of high-coherence lasers coupled with
15 speckle-suppressed tools, low-coherence light sources have been used for the
16 speckle-less QPI; however, they have suffered from the small number of available
17 photons per coherence volume. Partially coherent laser-like broadband light
18 sources, such as amplified spontaneous emission sources and super-luminescent
19 laser diodes have been adopted but coherent speckles could not be sufficiently
20 reduced [22, 23]. Novel concepts of lasers, e.g., random lasers and degenerate
21 lasers, were recently reported as the promising means to generate an intermediate
22 degree of spatial coherence with suppressed speckle [24-26]. Because different
23 biomedical applications require different coherence levels in QPI, an ideal light
24 source for QPI should provide widely tunability in temporal coherence for high
25 visibility interferograms, spatial coherence for wide field-of-view, and

1 suppressed speckle for lower background noise. A single laser source (i.e.,
2 continuous-wave lasers), however, could not satisfy all these QPI requirements,
3 including (1) high temporal coherence for high-contrast interferogram, (2) high-
4 spatial coherence for large field-of-view, (3) low speckle for lower background
5 noise, (4) multiple wavelengths for distinguishing the physical sample volume
6 from refractive indices, and (5) high phase stability over a wide time span. In
7 addition, the inclusion of phase-coherent multiple wavelengths could enable
8 spectroscopic QPI for extracting physical height information from refractive
9 indices [27-29]; and higher phase stability could improve the QPI resolution. For
10 these purposes, this thesis introduces the first use of a frequency comb of
11 femtosecond laser as a QPI light source, which was named here as the frequency-
12 comb-referenced quantitative phase imaging (FCR-QPI). This thesis
13 demonstrates that FCR-QPI enables a wide coherence tunability for higher
14 visibility interferograms, phase-coherent multiple wavelengths for extracting
15 physical height information from the refractive index, and higher phase stability
16 for higher resolution QPI at a higher speed. Frequency comb refers to a mode-
17 locked femtosecond laser, which emits a repetitive pulse train with a few
18 hundreds of femtosecond pulse duration. In the frequency domain, the frequency
19 comb is comprised of equally spaced lines of the spectral components or “comb”.
20 The separation between each comb line (a repetition frequency) and the offset of
21 the comb (an offset frequency) are stabilized to an optical oscillator. Therefore,
22 it can be regarded as a well-stabilized multi-wavelength continuous-wave light
23 source.

24 Frequency-comb-referenced quantitative phase imaging (FCR-QPI) offers
25 phase-coherent multiple wavelengths which could enable spectroscopic QPI for

1 extracting the physical volume from the refractive index. With the use of FCR-
 2 QPI, the process of cellular death can be understood in term of the cellular
 3 morphological alteration as well as their change in biochemical components
 4 which is characterized via a cellular refractive index. Typically, refractive index
 5 is optical property of all existing material including the biological cells. In the
 6 cellular scale, refractive index can be distinguished between organelles, intra and
 7 extra fluid or different biochemical concentrations so it can reveal the cellular
 8 biochemical properties [30, 31]. In QPI, optical phase change or phase shift
 9 $\Delta\phi(x,y)$ will be produced by the variation of cellular refractive index (n_c) and
 10 physical cellular height (h) at any position of cell following this equation; $\Delta\phi(x,y)$
 11 $= (2\pi/\lambda) \cdot (n_c - n_m) \cdot h(x,y)$, where n_m is refractive index of surrounding media or
 12 culture media, and λ is the illumination wavelength [3]. From this aspect, QPI
 13 has been adopted to reveal the cellular refractive index. Several QPI techniques
 14 have been proposed to study the refractive index of various cells in different
 15 circumstances [32-41]; these approaches require a delicate preparation procedure,
 16 complicated experimental setup and demands (beam/sample scanning), and
 17 advanced/additional techniques for decoupling refractive index from the physical
 18 volume. By utilizing our phase-coherent multiple wavelength method, we can
 19 simply extract the refractive index from the physical volume to study the process
 20 of cellular death.

21 Additionally, this thesis proposes frequency-comb-referenced quantitative
 22 phase imaging for monitoring high-speed dynamic motions in biological samples
 23 which is a zebrafish embryo. Zebrafish have been used as a good cardiovascular
 24 model of human because they have ~70% of genes in common and ~84% of
 25 zebrafish's genes related to the genes found in human diseases [42, 43]. FCR-

1 QPI enables label-free, non-invasive, real-time monitoring of red blood cells flow
2 and heart rate in live zebrafish embryo will facilitate the process of drug testing,
3 toxic screening, and gene manipulation which is often conducted in an animal
4 model before testing in human.

5 In summary, we have developed a frequency-comb-referenced quantitative
6 phase imaging (FCR-QPI) technique which enabled wide coherence tunability
7 for high-contrast imaging, phase-coherent multiple wavelengths for a
8 spectroscopic measurement for study cellular morphology and intracellular
9 biochemical compositions with higher phase stability. Moreover, our technique
10 can be employed to measure high-speed dynamic motions at a cellular level in
11 the zebrafish embryo. Therefore, FCR-QPI can provide great impact on diverse
12 biomedical applications.

13 **1.2 Research objectives**

14 1) To suppress the speckle noise in high-coherence laser for high-brightness and
15 high-phase sensitive quantitative phase imaging.

16 2) To develop a frequency-comb-referenced quantitative phase imaging (FCR-
17 QPI) technique. FCR-QPI can fulfill all following QPI requirements; high-
18 temporal coherence for high-contrast interferogram, high-spatial coherence for
19 large field-of-view, low speckle for lower background noise, multiple
20 wavelengths for decoupling the physical volume from refractive indices, and high
21 phase stability over a wide time span.

22 3) To provide a non-invasive, high-speed motions measurement of heart rate and
23 blood flow development in a zebrafish embryo by using FCR-QPI.

1.3 Major contributions

1) *Speckle suppression for phase-sensitive QPI*: In this study, we proposed a rotational optical diffuser as a robust, cost-effective and practical method to suppress the speckle noise in high-coherence continuous-wave laser. Our method was demonstrated in an off-axis, common-path QPI for high-brightness, high-stability, high-data acquisition, and simplicity. In addition, the level of speckle suppression can be controlled by tuning the diffusion angles of the optical diffusers. The different speckle-suppression levels can be optimized for attaining high-visibility images under various coherent imaging conditions.

2) *FCR-QPI for high-visibility, high-speed, spectroscopic phase measurement with a high phase stability*: In this study, a frequency comb was used for the first time as a light source in quantitative phase imaging. This study demonstrates how a frequency comb can contribute to wide spatio-temporal coherence control in QPI, multi-wavelength QPI, and dynamic QPI. For the coherence control, we aim to reveal how one can optimize the spatio-temporal coherence of the frequency comb for the high-visibility interferogram. For the multi-wavelength QPI, we aim to monitor and understand the alternation in cellular morphology as well as spatiotemporal changes in their refractive indices. For the dynamic QPI, high-speed dynamic motion (up to ten kilohertz in frequency) in optical and biological samples were monitored and measured by utilizing a high-speed photodetector instead of conventional CCDs (charge-coupled device).

3) *Measurement of heart rate and blood flow in zebrafish by using FCR-QPI*. In this study, we proposed the first use of frequency-comb-referenced quantitative phase imaging (FCR-QPI) to measure heart rate and blood flow in a zebrafish

1 embryo. FCR-QPI was used to monitor the development of heart rate and blood
2 flow along with the development of the zebrafish.

3 **1.4 Scopes**

4 The major scopes of this research are summarized below:

- 5 ➤ Demonstrating a high-brightness, high-phase sensitive, speckle
6 suppressed QPI with high acquisition rate using a highly coherent
7 continuous-wave laser as the light source.
- 8 ➤ Demonstrating a frequency-comb-referenced quantitative phase imaging
9 (FCR-QPI) and realize wide coherence tunability for higher visibility
10 interferograms, phase-coherent multiple wavelengths for decoupling the
11 cellular refractive index and physical height, and higher phase stability
12 for higher sensitivity/resolution QPI at a higher speed.
- 13 ➤ Applying FCR-QPI for measuring heart rate and blood flow in a live
14 zebrafish embryo.

15 More detailed research contents are summarized as a flow chart as shown below:

Figure 1.1 Flow chart for the overall structure of this thesis

1.5 Organization of report

The organization of the thesis is as follows. Chapter 2 begins with the explanation of frequency comb and ultrafast lasers. The basic principle, technologies, and applications of the frequency comb and ultrafast lasers will be summarized. The principle of quantitative phase imaging (QPI) technique to form the image from

1 optically transparent specimens will then be explained and compared with the
2 conventional bright-field imaging techniques. The figure of merits in each QPI
3 method will be summarized to point out the performances of the QPI
4 configurations utilized in this research. The reviews of QPI for biological and
5 biomedical studies, refractive index measurement, and pathological diagnosis
6 will be provided. Chapter 2 also introduces the trend of using zebrafish embryos
7 in research and describes the cardiovascular anatomy of the zebrafish. In order to
8 compare our technique with the previous studies, the previous techniques of heart
9 rate and blood flow measurement in the zebrafish embryo will be reviewed and
10 discussed. Later, chapter 2 explains the coherence characteristics of the optical
11 fields in order to understand the formation of coherent speckle. Then, the
12 development of diffusers for suppressing the speckle will be discussed to
13 compare with our rotational optical diffuser. The last section of chapter 2
14 describes the analytical technique of QPI interferogram used in this research.
15 Chapter 3 presents the experimental designs and process of the sample
16 preparations. Chapter 4 reports and discusses the experimental results. Chapter 5
17 provides research conclusions and recommendations for the future works.

Chapter 2

Literature review

2.1 Ultrafast lasers

Ultrafast lasers (i.e., femtosecond and picosecond lasers) show good promises in a wide range of applications such as manufacturing, medical uses, security, etc. The discovery of chirped-pulse amplification leads to the development of ultrafast lasers [44]. Figure 2.1a depicts the principle of ultra-short pulse generation [45]. To obtain the ultra-short laser pulse, a Titanium:Sapphire (Ti:Sapphire) crystal is commonly used as an active medium because it provides a wide range of frequency generation. The relation between time and frequency of pulse should be considered to generate the ultrashort-pulse. In the other word, it is the relationship between temporal and spectral widths of the pulse which is written by $\Delta\nu\Delta t \geq K$, where $\Delta\nu$ is frequency or spectral width at Full Width at Half Maximum (FWHM), Δt is time or temporal width at FWHM, and K denotes the time-bandwidth product, which depends on the pulse shape (e.g., Gaussian, $K=0.44$). The minimum time duration of a pulse (Δt) can be calculated by $\Delta t \geq K(\lambda_0^2)/\Delta\lambda_c$, where $\Delta\lambda$ is a spectral width at FWHM between pulses, λ_0 is central wavelength, and c is the speed of light ($3 \cdot 10^8$ m/s). Frequency modes are the energy levels in the laser cavity. The number of frequency modes in one spatial laser confinement is related to the length of the laser cavity. To generate a short

1 pulse laser, the mode-locking mechanism must be accomplished. The mode-
2 locking mechanism will force all frequency modes to have a fixed relative phase
3 (constructive interference). Thus, it will create a high peak power of the pulse
4 [46]. After that, the short pulse can be amplified if a higher output power level is
5 required for certain applications. For example, high-power lasers with the power
6 of hundreds of watts or kilowatts are used in laser material processing.
7 Unamplified laser pulses can have an average power of ~140 watts in a mode-
8 locked solid-state laser [47].

9 Many kinds of the optical amplifier for ultrafast lasers are introduced: multi-
10 pass amplifier, regenerative amplifier, and fiber-based amplifier (See Figs. 2.5b
11 to 2.5d). The amplification principle requires a certain number of passes of the
12 beam through an active medium known as the multi-pass amplifier [48]. The
13 multi-pass amplifier requires broad gain bandwidth to enable a large number of
14 the passes without gain narrowing. From that reason, laser crystals (i.e.,
15 Ti:Sapphire) are used as gain media for the multi-pass amplifier. The regenerative
16 amplifier can be used instead of a conventional multi-pass amplifier. The
17 regenerative amplifier can be considered as one of the multi-pass amplifiers
18 which has multiple passes of the beams through the gain medium but the number
19 of passes is controlled by an optical switch, i.e., Pockels cell [49]. Pockels cell
20 is an electro-optics device which can rotate the polarization of the light by
21 applying a voltage on it. Pockels cell initially behaves like a quarter-wave plate.
22 When there are pulses passing in the laser cavity, the voltage is applied to the
23 Pockels cell to rotate the polarization state of the pulses. The change of
24 polarization state aids to keep the pulse inside the cavity until it reaches the
25 maximum saturation. Then, the second voltage is sent to eject the pulse from the

1 cavity. The regenerative amplifier can work with the laser pulses at kHz-
2 repetition rates, but it is not suitable with higher repetition rates (e.g., several
3 megahertz) of mode-locked lasers. For high pulse repetition rates, the fiber-based
4 amplifier (i.e., fiber-based optical parametric amplifier) is commonly used to
5 achieve high pulse energy [50, 51]. In this thesis, the frequency comb (FC-1500-
6 250-WG, M-VIS, Menlo Systems) utilizes fiber-based optical parametric
7 amplifier technology to boost the peak power up. The fiber-based optical
8 parametric amplifier uses nonlinear optical effects in optical fiber and can provide
9 a high gain as 70 dB [51]. High-frequency and high-intensity pump beam
10 transfers its energy to the lower-frequency and lower-intensity signal beam and
11 generates the third idler beam (excess energy). Typically, rare-earth-doped fibers
12 are used as gain media, e.g., Erbium-doped fiber amplifier (EDFA) and
13 Ytterbium-doped fiber amplifier (YDFA). Figure 2.1d shows the system design
14 of Erbium-doped fiber amplifier. The signal at 1550 nm wavelength combines
15 with the pump beam from the laser diode through a wavelength multiplexer.
16 Then, signal and pump beams are sent to amplify by the erbium-doped fiber.
17 Optical isolators are used to stabilize the amplification. Nowadays, the fiber-
18 based amplifier has become most famous technology for ultrafast amplifier due
19 to its compact, high output power, and wide tunability of the bandwidths.

20 After amplification process, pulses that have different frequencies will
21 propagate with the different velocities so-called a group velocity dispersion or
22 chirped pulse. In this case, the compression process must be done to create the
23 ultra-short pulse. Compression is achieved by making a long path for the previous
24 frequencies or frequencies that travel ahead, and a short path for each following

1 frequency. Therefore, all frequencies recombine at the end position to create the
 2 short pulse.

3
 4 **Figure 2.1 a**, Principle of chirp-pulse amplification for the ultrashort pulse generation
 5 Configurations of **b**, Multi-pass, **c**, regenerative, and **d**, fiber-based amplifiers [45, 47-
 6 49].

7 Ultrafast lasers are used in various applications. Since ultrashort pulses have
 8 enabled the observation of phenomena of short timescales (femtosecond to
 9 picosecond timescale) [52], they have been used for monitoring ultra-fast
 10 phenomena such as real-time molecular vibration and rotation [53]. Typically,
 11 the timescales of molecular vibration and molecular rotation are 10^{-13} s and 10^{-11}
 12 s, respectively. To observe these motions, the molecules should be excited on a
 13 timescale shorter than the vibrational and rotational periods. Therefore,
 14 femtosecond laser was implemented as a femtosecond transition-state
 15 spectroscopy (FTS) to activate and monitor these motions in a real time [53]. Two
 16 femtosecond lasers were used. One was used to excite the molecules to certain
 17 energy states (for vibration or rotation) and the other was used to probe the
 18 molecules. The signal was detected by a high-speed detector. Figure 2.2a shows

1 the vibrational signal of the excited molecules with a period of 300 femtoseconds;
2 this corresponds to the frequency of 3.3 THz in the frequency domain. The
3 rotational signal was obtained when the excited and probe pulses were parallelly
4 and perpendicularly polarized. The rotational recurrences occurred after 600
5 picoseconds and they were 180° out-of-phase. This result agrees well with the
6 theory. Both vibrational and rotational motions of the molecules could be
7 observed simultaneously because they occurred at different periods. With the use
8 of femtosecond laser, numerous studies of ultrafast dynamic were carried out
9 such as fast magnetization effects of superparamagnetic particles which
10 encountered timescale of a few picoseconds [54], ultrafast dynamic processes in
11 semiconductor materials (e.g., plasma oscillations of GaAs bulk material (up to
12 ~100 fs), and phonon-induced transition periods (up to ~115 fs)) [55].

13 In addition, ultrafast lasers play an important role in a laser machining due
14 to its unique performance. The ultrafast laser can minimize the thermal damage
15 of the ablated area hence the precise machining [56-59]. Figures 2.2b and 2.2c
16 show the scanning electron microscopy (SEM) images of holes drilled using
17 ultrafast femtosecond and nanosecond pulse widths, respectively. The sharp hole
18 was produced by the femtosecond laser due to the low generation of heat-affected
19 zone (HAZ) as shown in Fig. 2.2b. In contrast, the nanosecond laser generates
20 large HAZ, so the holes are ablated as shown in Fig. 2.2c. Moreover, the
21 femtosecond laser has been utilized as a low-coherence source in the low-
22 coherence scanning interferometer for measuring three-dimensional (3D) profile
23 of discontinuous step-structures in microelectronic industries [60]. The repetition
24 rate of the femtosecond laser was controlled by referring to a Rubidium (Rb)
25 atomic clock. By tuning the repetition rate of the femtosecond laser (i.e.,

1 99.37902000 to 99.37942000 MHz), the optical path difference (OPD) of the
 2 reference and measurement pulses were varied; hence the OPD scanning. The
 3 interferometric fringes were observed by a high-speed camera. Figure 2.2d shows
 4 the interferograms of step-structures scanning with the increment of 0.04-Hz
 5 repetition rate (corresponding to 60 nm scanning step) and 3D phase profile of
 6 the step-structures with a step height of $\sim 69.60 \mu\text{m}$. The OPD scanning speed of
 7 100 m/s and the scanning range of $20 \mu\text{m}$ could be achieved in the measurement.
 8 This technique can offer fast and precise measurement over a 14.5-mm field.

9 **Figure 2.2** Ultrafast laser technologies. Real-time observations of **a**, molecular vibration
 10 signal (10^{-13} s in time scale) showing 300 fs. Holes drilled by using laser pulse with the
 11 widths of **b**, 200 fs and **c**, 3.3 ns. **d**, Ultrafast laser as low-coherence source in scanning
 12 interferometry providing 3D phase map of the step-structures [53, 56, 60]
 13

14 2.2 Frequency comb

15 Ultra-short pulses emitted from the ultrafast laser is a sequence of pulses traveling
 16 with the separation time (T) of $T=V_g/2L$, where V_g is cavity's mean group velocity
 17 and L is cavity length [61]. However, all pulses are not identical because their
 18 pulse envelopes travel with V_g , while the carrier wave travels with its phase

1 velocity. After each round trip, the carrier shifts with respect to the pulse envelope
 2 by a phase angle, $\Delta\phi$ as shown in Fig. 2.3a. By performing the Fourier transform,
 3 the spectrum of this pulses train exhibits combs of laser modes that are separated
 4 by the repetition frequency (ω_r) and a continuous shift in each round trip creates
 5 a frequency offset (ω_o) as shown in Fig. 2.3b. Optical frequency of the n-th mode
 6 is expressed as $\omega_n = n \cdot \omega_r + \omega_o$. To obtain highly stable combs, both ω_r and ω_o are
 7 stabilized to a Radio-frequency (RF)-time standard such as a Rubidium (Rb)
 8 atomic oscillator. Therefore, an optical comb or a frequency comb of an ultrafast
 9 laser is a link to connect time standards to length standards as shown in Fig. 2.4
 10 [62].

11 **Figure 2.3** Pulse trains emitted by an ultrafast laser and its spectrum. **a**, The carrier wave
 12 (blue) shifts by a phase angle ($\Delta\phi$) with respect to the pulse envelope (red). **b**, The
 13 spectrum showing optical combs with frequency offset of ω_o and repetition frequency of
 14 ω_r [61].

Figure 2.4 Frequency comb of a femtosecond laser as a link between time and length standards [62].

Frequency comb provides millions of well-defined Continuous-wave (CW) optical modes over a broad spectral bandwidth with a high phase coherence referenced to a time standard can be exploited as a light source in a high-precision measurement. For example, the frequency comb enables high-speed and high-precision within a large-measurement range [62, 63]. G. Wang *et al.* reported that frequency-comb-referenced multi-wavelength interferometry can provide rapid and precise absolute distance measurement over a few meters (See Figs. 2.5a and 2.5b) [63]. Output frequency of tunable external-cavity laser diode (ECLD) precisely tuned to the selected modes of the frequency comb provide the improvement of measurement speed and precision. Also, the frequency-comb-referenced multi-wavelength interferometry has been reported to measure 3D profiles of discontinuous surfaces [64, 65]. The large step heights (500 μm and 1800 μm) can be measured without phase ambiguity. Frequency comb as a wavelength ruler are beneficial for wavelength-sensitive measurement such as

1 calibration of the absolute length of gauge blocks and absolute long-distance
2 measurement [64, 65]

Figure 2.5 Frequency-comb-referenced multi-wavelength interferometry. **a**, Configuration of multi-wavelength generation. Four wavelengths of ECLD tuned to the frequency comb for the multi-wavelength interferometry. **b**, Absolute distance measurement by frequency-comb-referenced multi-wavelength interferometry [62, 63]

Moreover, the use of such a frequency comb has led to remarkable advances in high-resolution spectroscopy, the broadband calibration of astronomical spectrographs, time/frequency transfer over long distances, absolute laser ranging, and the inter-comparison of atomic clocks [66-73] as shown in Fig. 2.6.

Figure 2.6a shows time and frequency transfer between remote locations (site A and site B) via a two-way exchange of frequency comb [66]. Pulse trains emitted from the frequency combs sharing a common optical clock through optical fibres at site A and site B, but with different repetition frequencies ($f_r \approx 100$ MHz for site A and 100.003 MHz for site B). The pulse trains emitting from the frequency comb at each site traveled over a 2-km free-space to the other site. 100-MHz balanced detectors were used to detect the signal. The path variation or timing difference between two pulse trains generated an interferogram. The

1 timing difference in the two ways was interpreted from the interferograms in the
2 spectral domain as a slope of power spectral density. With the use of the phase-
3 locking in the frequency comb, the uncertainty of measurement represented by
4 Allan deviation was below than 1×10^{-18} at 1000-s averaging time. This level of
5 accuracy was the highest compared to the previous works which had a
6 femtosecond-level timing jitter at highest accuracy of 1×10^{-17} .

7 The equally spaced lines of spectral components of the frequency comb are
8 an ideal calibrator for an astronomical spectrograph as shown in Figure 2.6b [67].
9 A Yb-doped fibre frequency comb emitted the pulse trains at a repetition rate of
10 250 MHz. To generate a broad supercontinuum, the frequency of the pulse trains
11 was doubling via a second-harmonic generation (SHG) process and was
12 spectrally broadening by a photonic crystal fibre (PCF). The supercontinuum was
13 coupled to a multimode fibre. A dynamic scrambler was attached to the fibre to
14 increase the coverage of the lines and prevent the multi-path interferences. Then,
15 the supercontinuum was coupled to Accuracy Radial velocity Planet Searcher
16 (HARPS) spectrograph via two multimode fibres; one channel was used to
17 receive the starlight and the other was used for the calibration. The comb lines
18 spacing was tuned by adjusting the Fabry–Pérot cavities to prevent the
19 overlapping of the calibrating lines in the resulting images. The photon maps at
20 various wavelength orders were acquired by a charge-coupled-device (CCD)
21 detector as shown in ‘Channel B’ of Fig. 2.6b. A thorium spectral lamp was used
22 for comparison as shown in ‘Channel A’ of Fig 2.6b. The lines of the thorium
23 lamp were sparse and vague, so it did not suitable to use for a non-linear
24 calibration curve. Meanwhile, the comb lines were sharp and tightly located with

1 equal space. In addition, the frequency comb could enable long-term stability of
2 the calibration because of low photon noise and stable lines.

3 Time-of-flight of light pulses has been used as a tool for distance
4 measurement with a micrometer precision. Lee *et. al.* proposed a phase-locking
5 mechanism of the femtosecond laser pulses for improving the measurement
6 precision to nanometer regime [68]. By phase-locking of pulse repetition rates to
7 the rubidium atomic clock, a 0.7-km distance could be measured with 7-nm
8 accuracy (Allan deviation at 1-s averaging time). Figure 2.6c shows the system
9 configuration of the time-of-flight measurement of the femtosecond laser pulses.
10 Pulse train was split into two by a polarization beam splitter. One pulse train
11 directly transmitted to the target mirror (measurement pulse), then reflected and
12 recombined with the other pulse train (reference pulse). The temporal offset
13 between two pulse trains was quantified using optical-cross-correlation crystals.
14 The second-harmonic sub-pulses, one is forward propagation, and the other is
15 backward propagation, generating from the crystals were converted into electrical
16 signals via a pair of photodetectors. A balanced cross-correlation (BCC) of the
17 two main pulses were obtained at the D.C. peak of the resulting electrical signal.
18 The balanced cross-correlation signal had a linear relationship with the temporal
19 offset of the reference and measurement pulses. To enhance a measurement
20 precision, it was necessary to achieve the premeditated zero-offset overlapping
21 between those two pulses. This could be done by precisely controlling the
22 repetition rates of the pulse trains (i.e., phase-locking to the time standard) . With
23 the use of Rb atomic clock as a time standard, the pulse repetition rate was
24 controlled with 10^{-12} -precision, so as to improve the measurement precision.

1 Optical distance measurement offers contactless measurement with high
2 resolution. The frequency comb was used to measure 240-m distance with a
3 resolution of 50 μm (See Fig. 2.6d) [69]. The frequency comb emitted the pulse
4 trains at a repetition rate of 50 MHz. After driving the second-harmonic
5 generation, the beam was split into two beams by a beam splitter; one was used
6 as a reference beam and the other was travelled 240-m distance in the optical
7 tunnel. Phase difference (ϕ) at specified electric frequency between reference and
8 measurement beams was measured by a phasemeter before converting to a
9 distance (D) via $D = (N + \phi/2\pi) c / (2fn_g)$, where N is the integer part of phase, c is the
10 speed of light (3×10^8 m/s), f is the frequency that used for the measurement, and
11 n_g is the group refractive index. There are some advantages of using the frequency
12 comb over conventional light sources (i.e., continuous-wave lasers). The high
13 frequency up to gigahertz, which could offer by the frequency comb, was utilized
14 for the high stability of measurement. In addition, a wide range of the frequencies
15 in the frequency comb could be utilized for the two-color measurement; at the
16 same time, could be realized within a simple optical setup.

Figure 2.6 Frequency comb utilized for various advanced technologies. A frequency comb for **a**, time/frequency transfer over long distances **b**, calibration of the spectrograph **c**, absolute laser ranging, and **d**, high-accuracy distance measurement [66-69].

A frequency comb is a mode-locked fs pulse laser, which emits a repetitive pulse train with a few hundreds of fs pulse duration, corresponding to tens of μm in length; this time-domain nature can make it work as a low-coherence white-light source. In the frequency-domain, a frequency comb can be regarded as a combination of highly coherent well-stabilized multi-wavelength continuous-wave (CW) light sources. Consequently, frequency comb interestingly contains two competing characteristics of the precedent QPI light sources, low-coherence white-light sources and high-coherence CW lasers. Furthermore, it also provides broad wavelength spectrum by supercontinuum generation at highly nonlinear fibres, and high spatial coherence via its well-defined spatial mode profile. If we

1 carefully tailor the superior optical characteristics of a frequency comb, it can
2 work as an alternative QPI's light source offering multiple capabilities: coherent
3 speckle suppression, multiple phase-coherent wavelengths, high phase stability,
4 and high wavelength accuracy.

5 **2.3 Quantitative phase imaging (QPI)**

6 Quantitative phase imaging has emerged as an optical imaging technique to study
7 biological cells and tissues. It offers non-invasive, label-free and quantitative
8 measurements of physical and optical thickness, volume, and refractive index,
9 which cannot be realized by other conventional bright-field imaging techniques
10 [3, 74]. QPI utilizes the principle of interferometry to measure both, amplitude
11 and phase of the optical field, whereas the conventional bright-field imaging
12 measures only the amplitude of the optical field, as shown in Fig. 2.7a. Since
13 most biological samples are optically transparent, the resulting images have low
14 visibility and contrast. Therefore, staining techniques are often required to
15 facilitate visualization. However, these transparent biological samples can alter
16 the phase of the optical field because of their spatial variation in physical
17 thickness and refractive index and known as phase objects. The phase objects
18 create an optical phase delay (OPD) or phase shift ($\Delta\phi$) of the optical field; QPI
19 exploits this information as image contrast and quantitative measurement of
20 physical thickness and refractive index. In QPI, another optical field (reference
21 wave) is delivered to interfere with the object field to preserve both phase and
22 amplitude in the resulting field (See Fig. 2.7b). Then, a detector records the
23 intensity of the interference of the QPI interferogram. For an off-axis QPI setup,
24 the resulting intensity $I(x,y)$ is defined by the following equation [75];

1

$$I(x,y) = I_0 + I_1(x,y) + 2\sqrt{I_0 I_1(x,y)} \cos[\alpha x + \Delta\phi(x,y)]$$

2

3

4

5

6

7

8

9

where I_0 and I_1 are the intensities of the reference wave and object field, respectively, α is the spatial frequency related to the angle introduced between two fields or an off-axis angle (θ), $\alpha = 2\pi \sin(\theta)/\lambda$, and λ is the wavelength of the light. Then, numerical approaches (i.e., Fourier transform and phase unwrapping techniques) are employed to decouple the $\Delta\phi$ from the $I(x,y)$. As a result, the OPD represented as a phase map, $\Delta\phi(x,y)$ of the sample is retrieved. The physical thickness (h), the RI of the samples (n_c) and the refractive index of surrounding medium (n_m) can be calculated from the OPD via $\Delta\phi(x,y) = (2\pi/\lambda) \cdot (n_c - n_m) \cdot h(x,y)$.

10

11

12

13

Figure 2.7 Principles of bright-field imaging and QPI. **a**, Bright-field image contains the amplitude of the optical field. **b**, Interferogram contains the amplitude and phase of the optical field. **c**, Off-axis QPI configuration. [74, 75]

14

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Therefore, QPI provides full-field distribution of OPD referenced to light wavelength. QPI enables us to attain a sub-wavelength precision with quantitative analysis in phase-sensitive cellular imaging, which could not have been realized in traditional phase-sensitive microscopes, such as phase-contrast microscopy and differential interference contrast microscopy, which only support higher contrast cellular analysis not with a quantitative [1, 2]. There are several QPI

1 methods and each method has own figure of merit as shown in Table 2.1 [76].
 2 The off-axis QPI method offers a high acquisition rate, enabling high-speed
 3 measurement. A phase-shifting method provides high transverse resolution. The
 4 common-path method gives high temporal sensitivity or high temporal stability.
 5 White-light QPI provides less speckle effect for better spatial resolution. QPI
 6 techniques which combine those methods are seeking to combine the benefits.
 7 For example, diffraction phase microscopy (DPM) utilizes both off-axis and
 8 common-path methods, and can offer high acquisition rates and a high temporal
 9 sensitivity.

10 **Table 2.1** Figure of merit in QPI methods [76]

	Acquisition rate	Transverse resolution	Temporal sensitivity	Spatial sensitivity
Off-axis	X			
Phase- shifting		X		
Common- path			X	
White light				X

11

12 In this thesis, two configurations of QPI were employed for two light sources;
 13 (1) an off-axis, common-path interferometer for a continuous-wave laser diode,
 14 and (2) a modified version of Michelson's interferometer for a frequency comb.
 15 This update configuration was addressed for matching the OPD for forming the
 16 low-coherence interferogram within the short coherence length of the frequency
 17 comb. The off-axis, common-path interferometer employed in our work was the

1 one that first introduced by Lee *et al.* [77] (See Fig. 2.8). The combination of
2 these two methods offers fast data acquisition and high phase stability. The setup
3 consists of illumination, microscopy and quantitative phase imaging unit (QPIU)
4 as shown in Fig. 2.8. Light from a continuous-wave laser diode was delivered to
5 illuminate the sample. The transmitted light was then directed to the QPIU which
6 was comprised of three components: a half-wave plate ($\lambda/2$), a Wollaston prism
7 (WP), and a linear polarizer (L). The half-wave plate rotated polarization of the
8 beam by 45° . Then, the Wollaston prism split the beam into its different
9 polarization compounds (vertical and horizontal polarization). In addition,
10 polarizer at 45° alignment generated the visible interference of the two beams on
11 the CCD camera. To obtain the image with sufficient spatial resolution, the carrier
12 spatial frequency must be sufficient. The spatial frequency depends on the
13 wavelength of the light. Additionally, pixel size (p) should access the spatial
14 frequency but there is a limitation. It cannot access the spatial frequency higher
15 than $(1/2p)$. In QPIU, coherent length of illumination light is a significant
16 parameter to define the existing interferogram. WP denotes the angle (θ)
17 separation, and D is the distance between the image plane and the separation
18 point. In order to get the usable interferogram, coherent length should be satisfied
19 with the following equation; *coherent length* $\geq D\sin(\theta)$.

1

2 **Figure 2.8** Off-axis common-path QPI setup. **a**, Microscopy constructed with QPIU **b**,
 3 Photo of QPIU. [77]

4 Michelson's interferometer is a common off-axis QPI technique which
 5 commonly used to measure the distance or length with sub-wavelength precision
 6 [78]. Michelson's interferometer consists of a light source, a beam splitter (BS),
 7 two mirrors (M_1 and M_2), and a detector (See Fig. 2.9). Light that transmits
 8 through the beam splitter will be equally split their intensities (I_1 and I_2) onto two
 9 mirrors, then reflected back to interfere at the detector (I_3). One mirror can be
 10 slightly tilted to tune the spatial frequency. In this research, we utilized a modified
 11 Michelson-type version satisfying both off-axis and common-path geometry,
 12 which can provide both benefits of a high data acquisition rate and high phase
 13 stability in the time domain.

Figure 2.9 Michelson's interferometer configuration [78]

2.3.1 QPI for biological and biomedical studies

A non-invasive manner of the QPI technique will provide the study of live-cell in a longer observation time (without photobleaching or phototoxicity). Innovative fluorescence microscopies have been proposed for a better resolution with less photobleaching and phototoxicity effect (i.e., spinning-disk confocal microscopy and light-sheet fluorescence microscopy), but they are not good enough to study the live-cell in long observation time, compared with the QPI. QPI can provide a 3D reconstruction of the cell without a scanning scheme (i.e., Hilbert-phase microscopy), whereas the confocal microscopy requires a scanning scheme to obtain the 3D image. The scanning speed will limit the temporal resolution of the system, so the study of fast dynamic phenomena in the cell is difficult. QPI can offer real-time monitoring by implementing high-speed detectors (i.e., CMOS camera), so it is suitable for observing the fast dynamic phenomena. In addition, QPI can provide quantitative assessments of the cell such as cell volume, cellular height, cellular dry mass, cellular refractive index,

1 etc., which are valuable for study the cellular pathology. The comparison of QPI
 2 and other imaging techniques are shown in Table 2.2.

3 **Table 2.2.** Comparison of imaging techniques

Imaging techniques		Limitations	Comparison to QPI
Bright-field microscopy		Straining technique	Label-free
Fluorescence microscopy	Wide-field microscopy	- Phototoxicity and photobleaching - Not suitable for live-cell imaging in a long observation time	- Non-invasive - Live-cell imaging in a long observation time
	Confocal laser scanning microscopy	- Light scattering from the refractive index variation limit the penetration depth ~200-300 μm [1] - Difficult for fast dynamic motion observations or real-time imaging (frame rate < 100 Hz for the field-of-view of 60 μm $\times$ 60 μm) [2]	- Higher penetration depth (around 1-3 mm) by optical coherence tomography [4] - High measurement speed by single-shot measurement (e.g., phase-shifting interferometry) [5]
	Spinning-disk confocal microscopy	Image collection requires 10-15 seconds and low frame rate (50-60 frames per second) [1, 3]	Fast dynamic motion observations or real-time imaging by optional high-speed camera
	Light-sheet fluorescence microscopy	The complex of experimental setting and configurations	Realized in a simple experimental configuration

4

5

1 At an early stage of QPI development, optical coherence tomography
2 (OCT) has been introduced as a point-scanning, cross-sectional imaging
3 technique based on low-coherence interferometry [79]. OCT can provide depth-
4 resolved high-resolution imaging in deep tissue structures such as human retina
5 structures and blood vessels (See Figs. 2.10a and 2.10b). The penetration depth
6 of the OCT limits its capability to perform only with specific types of biological
7 tissues. Typically, the depth ranges exceed a few millimeters (1-3 mm) cannot be
8 explored by the OCT [80]. The penetration depth of the OCT is limited by the
9 multiple light scattering from the refractive index variation of multi-layer tissues.
10 Despite the limit of penetration depth range, OCT plays important clinical roles
11 in ophthalmology, dermatology, and cardiology [81]. OCT has been used to study
12 human retina, choroidal layer containing blood vessels at a penetration depth up
13 to 500 μm [82], epidermis (the outmost layer of skin), some hair follicles, and
14 sebaceous glands at dermis (the middle layer of skin) at a penetration depth up to
15 2 mm [83, 84]. Several techniques have been introduced to the OCT to increase
16 the penetration depth such as spectral domain OCT, long-wavelength OCT, dual-
17 axis OCT, and the use of contrast agents. Compared to a conventional time-
18 domain OCT, spectral-domain OCT provides higher acquisition speed, higher
19 resolution, and greater penetration depth [85]. The spectral-domain OCT
20 provided an image of brain tissue at a deeper region of 2 mm, whereas the time-
21 domain OCT provided an image of brain tissue below 1 mm-deep [86].
22 Wavefront-shaping OCT, one of the spectral-domain OCTs which controls the
23 shape of the incident beam, can enhance the signal-to-noise ratio (SNR) by
24 factors of 3-7 compared to a conventional OCT, so as to enhance the penetration
25 range [87].

1 The penetration range is governed by the wavelength used. Previous
2 studies suggested that long-wavelength regions (> 1000 nm) could minimize the
3 multiple scattering effect from the tissues to increase the penetration depth ranges
4 [88, 89]. OCT operating at 1300-nm wavelength region provided an image at
5 deeper skin layer compared to OCT operating at 800-nm wavelength region. [84].
6 The penetration depth also increased with the increasing wavelength used in the
7 OCT. A 1700-nm OCT system provided the penetration depth of 3 mm in
8 intralipid solution, whereas a 1300-nm OCT system provided the penetration
9 depth of 2.5 mm. Swept source OCT with a tunable spectral range from 1590 to
10 1750 nm provided greater penetration depth compared to a conventional 1300-
11 nm OCT system (a tuning range of 1260 -1380 nm) [88]. A 1700-nm OCT system
12 was used to study the mouse brain at 1.8 mm deep [90]. Dual-axis OCT which
13 separated the illumination and detection beams could enhance the depth
14 resolution to 2 mm in the skin tissue [91]. The offset angle between illumination
15 and detection beams rejected the out-of-plane photons, so it improved the depth
16 of fields. The use of gold nanoparticles as contrast agent could improve the SNR
17 at the same penetration depth of *ex vivo* embryo culture [92]. Many derivatives
18 of the OCT have been developed to improve the performance for diverse
19 applications such as Doppler OCT [93], Polarization-sensitive OCT [94], and
20 optical coherence microscopy (OCM), a better lateral resolution version of OCT
21 [95].

Figure 2.10 Optical coherence tomographs of **a**, human retina and **b**, human artery [79].

Figure 2.11 Digital holographic microscopy for biological studies. **a**, Topographic phase map of neurons cell, and **b**, fibroblast cells treated with 200 μM etoposide. **c**, 3D height map of RBC. **d**, Phase variation of human pancreatic carcinoma cells exposed to toxin [96-99].

Later, another QPI technique has been developed which is a digital holographic microscopy (DHM). DHM provides a full-field, 3D holographic imaging and has been used to monitor cellular morphology and its alteration such as neuron cells, fibroblast cells, red blood cells (RBC), and carcinoma cells (See Figs. 2.11a to 2.11d) [96-99].

Up to date, several QPI techniques have been introduced to measure the dynamic phenomena of cells such as the height and the membrane fluctuation in

1 RBC [72, 100, 101], and intracellular transport in the neuron cells [70] as shown
 2 in Figs. 2.12a and 2.12b. Also, QPI allows quantifying the hemoglobin
 3 concentration in RBC [72], dry mass of HeLa cells [87], surface area, cell volume,
 4 sphericity, height of neurons cell [88], and cellular refractive index [89] as shown
 5 in Figs. 2.12c to 2.12g.

7 **Figure 2.12** QPI for study dynamic phenomena in various biological cells. **a**, Height map
 8 and height fluctuation of RBC. **b**, Intracellular transport in microglia cells measured and
 9 represented by a dispersion curve, and **c**, hemoglobin concentration map of RBC. **d**,
 10 Membrane fluctuation of RBC in healthy and diabetic conditions. **e**, Dry mass of HeLa
 11 cell with temporal stability. **f**, Quantitative data analysis of neuron cells; surface area,
 12 volume, sphericity, height, dry mass density, and dry mass. **g**, Refractive index map of
 13 HeLa cell in the metaphase [70, 72, 100-104].

14 2.3.2 QPI for refractive index measurement

15 Cellular RI has been used to investigate the biochemical properties and cellular
 16 functions [30, 31]. In QPI, the physical thickness and the index are confined in
 17 the OPD. To split those two parameters, several techniques have been introduced

1 to operate with QPI. L. C. Claire *et al.* proposed quantitative phase-amplitude
 2 microscopy and confocal microscopy to measure the refractive index (RI) of
 3 airway smooth muscle (ASM) cells [32]. Confocal microscopy was used to
 4 measure the cellular height, while quantitative phase-amplitude microscopy was
 5 used to measure the OPD as shown in Fig. 2.13a. As a result, the cellular RI can
 6 be calculated at any region of interest. The average RI of the cytoplasm and RI
 7 of the nucleus are shown in Fig. 2.13a. N. Cardenas *et al.* introduced an optical
 8 tweezer to measure the physical thickness of the cell providing a better axial
 9 resolution comparing to the confocal microscopies [33]. N. Lue *et al.* measured
 10 the average cellular RI with the help of microfluidic devices and the use of two
 11 media [34]. Hilbert phase microscopy (HPM) was used to measure the phase
 12 difference as well as the refractive index of the HeLa cell. HPM is off-axis
 13 interferometry, which can provide the phase images with a single-shot
 14 measurement. The spatial carrier frequency of the interference fringes is created
 15 by introducing a tilt angle between reference and sample beams. The phase
 16 information is retrieved from the spatial carrier fringe patterns via Hilbert
 17 transform. To find the cellular refractive index independently from the cell
 18 thickness, first, the phase differences of microchannel filled up with water
 19 ($\Delta\phi_{channel-water}$) and microchannel filled up with culture medium ($\Delta\phi_{channel-cm}$) were
 20 measured by HPM. Second, the refractive index of culture medium (n_{cm}) was
 21 calculated via the following equation; $n_{cm} = n_{channel} + (\Delta\phi_{channel-cm} / \Delta\phi_{channel-water}) \cdot (n_{channel} - n_{water})$, where $n_{channel}$ is the refractive index of the microchannel
 22 ($n_{channel} = 1.430$) and n_{water} is the refractive index of water ($n_{water} = 1.333$). After
 23 that, HPM was used to measure the HeLa cell which was confined in the
 24 microchannel filled up with the culture medium ($\Delta\phi_{channel-cell}$). Figure 2.13b

1 presents the phase reconstruction of the HeLa cell in the microchannel and its
2 cross-sectional line showing the measuring phase differences of the cell and
3 culture medium. The HeLa cell which was confined in the microchannel provided
4 the assumption that the cell thickness was equal to the microchannel height.
5 Therefore, the refractive index of cell could be calculated by the following
6 equation; $n_{cell} = n_{cm} + (\Delta\phi_{channel-cell} / \Delta\phi_{channel-cm}) \cdot (n_{channel} - n_{cm})$. The average RI of the
7 HeLa cells was measured as 1.384 ± 0.0018 (n = 17 cells).

8 **Figure 2.13** QPI coupled with height measuring methods for extracting the RI
9 information. **a**, Confocal surface plot of the airway smooth muscle cell (ASM) thickness
10 and phase map. The average RIs at each selected region are calculated and displayed. **b**,
11 Topographic phase map of the HeLa cell confined in the microchannel and its cross-
12 sectional line [32-34].
13

1 Due to the complex experimental setting and multiple procedures to split the
2 physical thickness and refractive index (RI) information, the combination of
3 tomography and holography, known as tomographic phase microscopy (TPM),
4 has been introduced to alleviate these problems. W. Choi *et al.* demonstrated the
5 TPM for high-resolution quantitative three-dimensional (3D) RI measurements
6 of suspended or substrate-attached cells [4]. TPM has been widely used as a tool
7 to provide 3D RI mapping or RI tomography of RBC and HeLa cell [4, 13, 35]
8 as shown in Figs. 2.14a and 2.14b. Recently, TPM has been employed for a
9 single-cell analysis which is a tomographic flow cytometry [105] as shown in Fig.
10 2.14c. However, TPM's setup can induce mechanical vibration and plane-by-
11 plane sectioning can increase temporal phase noise.

12
13 **Figure 2.14** Tomographic phase microscopy for RI measurement and single-cell
14 analysis. Refractive index tomogram of **a**, RBC, and **b**, HeLa cells. **c**, Topographic flow
15 cytometry for a single RBC analysis [4, 13, 105]

1 As an alternative, the measurement of RI dispersion using spectroscopic
 2 phase microscopy or quantitative dispersion phase microscopy have been
 3 proposed as simple and effective methods to evaluate the cellular RI [29, 104,
 4 106-107]. The dispersion measurement of biomolecules in the cells can reveal
 5 intracellular biochemical compositions such as hemoglobin concentration in red
 6 blood cells [29]. D. Fu *et al.* introduced a dispersion phase microscopy to measure
 7 the RI dispersion of live HeLa cells [106]. Dual-wavelength (310 and 400 nm)
 8 was used to illuminate the HeLa cells and optical path lengths (OPL) can be
 9 measured at two wavelengths. Figure 2.15a shows the OPL map of HeLa cells at
 10 310 and 400 nm. The RI dispersion or the RI ratio of the OPLs measured at two
 11 wavelengths was calculated via $\int OPL(x,y;310\text{ nm})dxdy / (\int OPL(x,y;400\text{ nm})dxdy)$
 12 as shown in RI ratio map in Fig. 2.15a. Also, cellular dry mass (M) was calculated
 13 by $M = \int OPL(x,y)dxdy / \alpha$, where α is the specific refractive index increment of
 14 the protein ($\alpha_c = 0.191\text{ mL/g}$). The calculated cellular dry mass, project area
 15 (μm^2), and average dispersion ratio are shown in Fig. 2.15b. The average RI ratio
 16 was calculated as 1.088 for the 54 HeLa cells which is almost similar to the
 17 calibrated dispersion of pure protein solution of 1.089. This confirms the fact that
 18 approximately 60% of cellular dry mass is comprised of the proteins. The
 19 standard deviation of the RI ratio ($SD = 0.013$) was higher than the cells to cells
 20 variations. This is because the speckle noise degrades the dispersion information.
 21 Therefore, choosing the region of illumination wavelengths is a simple solution
 22 to enhance the dispersion signal. However, changing the wavelength range is not
 23 sufficient as the standard deviation of the RI ratio is still high. To overcome this
 24 problem, Y. Sung *et al.* developed a regularized tomographic phase microscopy
 25 (RTPM) [104]. RTPM utilizes an iterative reconstruction to recover the missing-

1 cone artifacts, the limited angular coverage of the incident beam, which causes
 2 the underestimation of refractive index. The iterative reconstruction can be done
 3 by applying two constraints: positivity and piecewise-smoothness constraints.
 4 Positivity constraint requires that the refractive index of the cell is higher than
 5 that of the culture medium, which is always justified. The piecewise-smoothness
 6 constraint requires the smooth variation of the refractive index of the cellular
 7 organelles (e.g., nucleus), but rough variation at the cellular boundary. This is
 8 justified, because the mean refractive index of the cellular organelles can be
 9 distinct from the surrounding cytoplasm. By applying these two constraints to the
 10 iterative reconstruction of tomographic data, the accuracy of refractive index
 11 measurement is improved. Figure 2.15c shows three-dimensional refractive index
 12 measurement of human colorectal adenocarcinoma cell line (HT-29) performing
 13 cell division which are measured at $\lambda_1 = 325$ nm and $\lambda_2 = 442$ nm. In addition, the
 14 dispersion ratio (D) was calculated as $D(\lambda_1, \lambda_2) = \Delta n_{\lambda_1} / \Delta n_{\lambda_2}$, where Δn is the
 15 refractive index difference between cell and culture medium at $\lambda_1 = 325$, and λ_2
 16 $= 442$ nm. The refractive index of chromosomes inside nucleus is larger than that
 17 of the cytoplasm during cell division at two wavelengths:
 18 $RI_{chromosome} = 1.367 \pm 0.004$ (442 nm) and 1.382 ± 0.004 (325 nm),
 19 $RI_{cytoplasm} = 1.356 \pm 0.009$ (442 nm) and 1.369 ± 0.008 (325 nm) as shown in Figs.
 20 2.15d. Similarly, the dispersion ratio of chromosome is larger than that of the
 21 cytoplasm ($D_{chromosome} = 1.088 \pm 0.076$, $D_{cytoplasm} = 0.984 \pm 0.056$) as shown in
 22 2.15e. This is because the DNA and proteins are dense in the chromosome during
 23 the cell division.

24 We have summarized the advantages and disadvantages of each QPI
 25 technique for measuring the cellular RI in Table 2.3

Figure 2.15 RI dispersion measurement. **a**, Optical path length of HeLa cells measured at 300 and 410 nm and their RI ratio map. **b**, Scattered plot of dry mass, project area, and average RI ratio. Tomographic phase microscopy for RI dispersion measurement. **c**, RI tomogram of human colorectal adenocarcinoma cell line (HT-29) measured at (i) 325 nm and (ii) 442 nm. **d**, Refractive index of chromosome of HT-29 cells measured at (i) 325 nm and (ii) 442 nm, and cytoplasm measured at (iii) 325 nm and (iv) 442 nm. **e**, Dispersion parameters (D) of chromosome and cytoplasm [104, 106].

Table 2.3 QPI techniques for RI measurement

Techniques	Pros	Con
QPI + method to decouple cellular height and RI	- Easy to calculate RI of cell	- Additional procedures or tools needed - Time-consuming
Tomographic phase microscopy	- 3D RI mapping of a single cell within one technique	- Low temporal sensitivity - Complex setup - Time-consuming
QPI with multiple wavelengths to extract the RI dispersion	- Compact, fast, and easy in term of setup and numerical processing	- Multi-wavelength sources needed - Not actual RI, but RI ratio

1 **2.3.3 QPI for pathological diagnosis and future clinical** 2 **applications**

3 QPI can be employed in various pathological studies. In biomedicine, it could be
4 applied for clinical studies [75, 108]. QPI was used to study a malignant cell (an
5 abnormal cell growth) which is a major cause of a well-known cancer. Cancer
6 causes an enormous number of mortalities worldwide. According to World
7 Health Organization's (WHO's) report in 2015, 8.8 million people died from
8 various type cancers e.g., lung cancer, liver cancer, breast cancer, etc. [109]
9 Tissue biopsy is the most common technique to diagnose the cancer disease.
10 However, the determination of the disease via tissue biopsy is required
11 specialists, and it is a qualitative inspection. Therefore, it is a specialized, low-
12 throughput and time-consuming method. Other diagnostic techniques for cancer
13 detection are X-ray imaging, magnetic resonance imaging (MRI), and
14 computerized tomography (CT) scan. But due to their high-cost, these methods
15 are not suitable for the early screenings.

16 Alternatively, QPI has been used to evaluate the pathological states and
17 progression of the cancer disease via study either on tissues or single living cells.
18 Z. Wang *et al.* introduced a spatial light interference microscopy (SLIM) to
19 measure an unstained prostate cancer tissue biopsy section [110]. Figure 2.16a
20 shows the results from SLIM against conventional hematoxylin and eosin (H&E)
21 staining technique imaging under bright-field microscopy with the mark of
22 normal (green) and malignant (red) areas on the tissue section. To provide
23 quantitative inspection, the histograms of the marked areas are calculated as
24 shown in Fig. 2.16a. The certified pathologist confirmed that the region of normal

1 and malignant cells in the phase image matched well with the H&E stained image.
2 This shows that QPI can offer a quantitative analysis for the cancer diagnosis. N.
3 Lue *et al.* reported that the different RI found in the different mouse organ tissues
4 by using HPM [111]. They also showed that RI can be used as a marker of
5 lysosomal storage disease found in the mouse's liver. This motivates the study of
6 the cellular and tissue RI to evaluate the normal and malignant cells/tissues.
7 Thereafter, P. Wang *et al.* proposed a spatial-domain low-coherence quantitative
8 phase microscopy (SL-QPM) to measure RI of normal and malignant cell
9 nucleuses in the tissue slices [112]. The RI of cell nucleus of malignant tissue
10 was larger than the normal and uninvolved tissues as shown in Fig. 2.16b. This
11 result indicates that RI can serve as a marker of cancer disease which has been
12 reported in many studies [110, 113-114]. Additionally, the cancerous cells can be
13 distinguished by their physical properties. Y. Bishitz *et al.* studied the cellular
14 mass, volume, and density of the circulating tumor cells (CTC) which mostly
15 present in the blood of cancer patients [115]. The dynamic of the cell membrane
16 or the temporal fluctuation of the cell membrane can be used to distinguish
17 normal and malignant cells [116]. Y. Bishitz *et al.* measured the membrane
18 fluctuation of a normal rat intestinal epithelial cell (IEC-18) and a cancerous (R1)
19 cell as shown in Fig. 2.16c. Standard deviation of the membrane fluctuation in
20 the cancerous cells is higher than in the normal cells. They also measured the
21 membrane fluctuation in primary (SW-480) and metastatic (SW-620) human
22 colorectal carcinoma cells. Similarly, a higher fluctuation (higher standard
23 deviation) was found in the cancerous cells as shown in Fig. 2.16c. This study
24 points out that the membrane fluctuation could be used to characterize the

1 cancerous cells. Also, M. Mir *et al.* used SLIM to monitor the dynamic growth
 2 of the cancerous cells to carry out drug testing [117].

4 **Figure 2.16** QPI for study the pathological states of the cancerous cells. **a**, 2D map of
 5 phase, scattering length, scattering angle (cosine), and mean²/variance of a prostate
 6 cancer tissue biopsy section comparing to H&E stained tissue image. Normal and
 7 malignant cell areas are marked. Histogram of the marked areas. Scattered plot of normal
 8 and malignant areas of 11 different tissue sections. **b**, Stained images and RI maps of
 9 cell nuclei from normal, uninvolved, and malignant breast epithelial cells. Calculated
 10 average RIs are plotted from different cells. **c**, SLIM for measuring membrane
 11 fluctuation of the normal and malignant intestinal epithelial cell. **c-i**, Raw interferogram,
 12 and **c-ii**, the optical path length of normal rat intestinal epithelial cell (IEC-18). **c-iii**,
 13 Standard deviations of the optical path length in a time domain represented membrane
 14 fluctuation for normal (IEC-18) and cancerous (R1) rat intestinal epithelial cells, and **c-**
 15 **iv**, primary (SW-480) and metastatic (SW-620) human colorectal carcinoma cells. [110,
 16 112, 116]

1 In the field of hematology, QPI can be used to screen red blood cell disorders
2 in sickle cell disease (SCD) as shown in Fig. 2.17a [118], anemia, and diabetes
3 [13, 119]. Y. Park *et al.* introduced a common-path diffraction optical
4 tomography (cDOT) where DPM combined with diffraction tomography (DT) to
5 measure the iron deficiency in the red blood cells (RBC) which is found in
6 anaemia [119]. Figure 2.17b shows the 3D RI tomograms of healthy and iron
7 deficiency RBC which has smaller haemoglobin (Hb) concentration. Moreover,
8 QPI was used to screen one of malaria's parasites; *Plasmodium falciparum* (*P.*
9 *falciparum*)-infected RBC [120]. Y. Park *et al.* investigated the RI, Hb
10 concentration and membrane fluctuation of *P. falciparum*-invaded human RBC
11 (*Pf*-RBC) using TPM. Figure 2.17c shows the 3D refractive index maps and Hb
12 concentration of *Pf*-RBC at every pathological states comparing to the healthy
13 RBC. Healthy RBC shows homogeneous distribution in refractive index, while
14 the infected RBC shows non-homogeneous refractive index in their cytoplasm.
15 At the final stage, the refractive index of the infected RBC decreased from the
16 initial stage (healthy stage) (from 1.399 ± 0.006 to 1.373 ± 0.006). Besides, QPI
17 was used to study white blood cells (WBC) (See Fig. 2.17d), and neuron cell-
18 associated neurotoxin in Parkinson's disease via measuring the cellular
19 morphology, dry mass, and refractive index [121-125].

Figure 2.17 QPI in the field of hematology. **a**, Tomograms and membrane fluctuation maps of healthy RBC and RBC from sickle cell disease (SCD) patient types I-IV. **b-I**, 3D RI tomogram of healthy RBC, and **b-II**, iron deficiency RBC. 3D RI maps of *Pf*-RBCs reveal the morphological alteration, the refractive index and the Hb concentration of **c-I**, healthy RBC, **c-II**, ring stage, **c-III**, trophozoite stage, and **c-IV**, schizont stage. **d**, Refractive index maps at various z-planes and the refractive index tomogram of the white blood cell [118-121].

In summary, QPI has been adopted in some fields of biomedicine. Table 2.4 and Table 2.5 provides a comparison of the conventional techniques and QPI techniques for screening cancer and malaria diseases.

1 **Table 2.4** Comparison of cancer diagnostic techniques

Cancer detection techniques	Pros	Con
Conventional technique (Stained tissue biopsy & bright-field imaging) [126]	<ul style="list-style-type: none"> - Reliable 	<ul style="list-style-type: none"> - Qualitative inspection - Time-consuming - Decision-making by specialists
QPI technique	<ul style="list-style-type: none"> - Simple and fast (no staining & computational based analysis) - Quantitative inspection - Not require specialists 	<ul style="list-style-type: none"> - New methods (many trials or tests required)

2

3 **Table 2.5** Comparison of malaria diagnostic techniques

Malaria detection techniques	Pros	Con
Conventional technique (Stained RBC & bright-field imaging) [127, 128]	<ul style="list-style-type: none"> - Reliable 	<ul style="list-style-type: none"> - Qualitative inspection - Staining requirement - Time-consuming - Decision-making by specialists
QPI technique	<ul style="list-style-type: none"> - Simple and fast (no staining & computational based analysis) - Quantitative inspection - Not require specialists 	<ul style="list-style-type: none"> - New methods (many trails or tests required)

4

5

1 **2.4 Heart rate and blood flow in zebrafish embryo**

2 In this study, the zebrafish in an embryonic stage was chosen as a sample for a
3 demonstration of bio-motions measurement. FCR-QPI was used to evaluate the
4 dynamic motions in the zebrafish's cardiovascular system; heart rate and blood
5 flow. In this section, we will review the previous methods that have been
6 introduced to study the heart rate and blood flow in the zebrafish embryos.

7 **2.4.1 Trends of using zebrafish in research**

8 Zebrafish (*Danio rerio*) has been used as a good model for study human
9 cardiovascular system due to their high optical transparency during the
10 embryonic stage, their rapid organ development, and easy laboratory
11 maintenance. Moreover, ~70% of human genes have true orthologues in the
12 zebrafish, and ~84% of human genes associated with the disease are also found
13 in the zebrafish [42, 43]. Zebrafish in the embryonic and larval stages have been
14 used as an animal model for study human cardiovascular functions and diseases
15 [129, 130], toxicological and drug screening [131, 132], human disease-
16 associated genes [133-135] (i.e., human hematologic disorders [136, 137]). The
17 number of studies that include the use of zebrafish dramatically increase over past
18 30 years as shown in the rapid growth of publications in the field (See Fig. 2.18a).
19 [138] Studies in the field of health-science increasingly use the zebrafish as an
20 animal model for study human diseases as shown Fig. 2.18b which presents a
21 trend [139]. This indicates that zebrafish is one of the good animal models up to
22 now.

Figure 2.18 Trend of using zebrafish in research. **a**, The number of publications using zebrafish as a model increasing from 1970 to 2016. **b**, Trend analysis of “zebrafish” and “disease” words co-occur in PudMed publications from 1995 to 2015 [138, 139].

2.4.2 Cardiovascular anatomy in zebrafish embryo

In embryonic zebrafish, heart is the first organ to form and function [140]. By 22 hours-post-fertilization (hpf), a primary heart tube is found with a cardiac contraction. After the heart tube formation, cardiac looping occurs to bend the heart tube into a curvature. As a result, heart chambers; ventricle and atrium become morphologically distinguishable from 30 hpf as shown in Fig. 2.19a. The heart of adult fish has two chambers with a heart valve. The heart valve is known for preventing the retrograde flow. At an early stage of a heart valve formation,

1 endocardial cells form a single layer of cell at atrioventricular canal (AVC) by 48
2 hpf. By 105 hpf, the heart valve (atrioventricular valve) is completely formed in
3 the zebrafish. In addition, as the heart develops from the heart tube to chambers,
4 heart changes from a peristaltic-like pump to a beating heart which has
5 contraction–relaxation cycles. The heart rate (beat-per-minute, bpm) increases
6 with fish development. Figure 2.19b shows the heart of transgenic
7 *Tg(Tp1:EGFP);Tg(my17:dsRed)* zebrafish at 48-50 hpf.

9 **Figure 2.19** Morphological development of zebrafish's heart. **a**, Heart development in
10 embryonic zebrafish **b**, Two chambers heart of zebrafish at 72 hpf. A: atrium, AVC:
11 atrioventricular canal AV: atrioventricular, V: ventricle, and OFT: out flow tract [141,
12 142].

13 Blood starts circulating at 24 hpf in a single circulatory loop (See Fig. 2.20a);
14 blood exits the heart via aortic arches (AA) to the cranial trunk's vessel known
15 as a dorsal aorta (DA); then continue to flow to the tail [143-145]. At a portion of
16 a single caudal to an anal pore, the dorsal aorta named as caudal artery (CA).
17 After that, blood returns at the tail via the caudal vein (CV) which is the braided
18 plexus vessels not a single vessel like DA or CA. Blood continues to flow to the
19 trunk via a single distinct posterior cardinal vein or postcardinal vein (PCV); then
20 to a duct of Cuvier (DC) or a common cardinal veins (CCV), which spreads across
21 the yolk cell and enters to the heart via sinus venosus. From 22 to 40 hpf, the
22 sprouts start (later named as segmental arteries and segmental vein) growing from

1 DA and PCV and form a future dorsal longitudinal anastomotic vessel (DLAV).
 2 At 48 hpf, blood flows in the intersegmental vessels (ISV) after completed the
 3 formation of segmental arteries (SA), segmental veins (SV), and DLAV (See Figs.
 4 2.20b and 2.20c).

5 **Figure 2.20** Morphological development of zebrafish's blood vessels. Vascular anatomy
 6 in **a**, 24 hpf, and **b**, 48 hpf zebrafish. **c**, vascular morphogenesis in zebrafish from 22 hpf
 7 to 48 hpf. AA: aortic arch. AVC: atrioventricular canal, CA: caudal artery, CCV:
 8 common cardinal vein, CV: caudal vein, DA: dorsal aorta, DC: duct of Cuvier, DLAV:
 9 dorsal longitudinal anastomotic vessel, DV: dorsal vein, H: heart, ISV: intersegmental
 10 blood vessels, LDA: lateral dorsal aorta, PCV: posterior cardinal vein. SA: segmental
 11 arteries, SV: segmental vein [143-145].
 12

13 **2.4.3 Previous techniques for blood flow and heart rate** 14 **measurement**

15 Several techniques have been introduced to measure heart rate and blood flow in
 16 the zebrafish embryos [146-155, 158]. Bright-field microscopy operated with a
 17 high-speed camera is a common technique for measuring velocity of RBCs flow
 18 in the blood vessels [146, 147]. Figure 2.21a-i shows a bright-field image of blood
 19 flow in the zebrafish at 48 hpf. Particle image velocimetry (PIV) was employed

1 to calculate the blood velocity by tracking the RBCs flow in main trunk vessels;
2 dorsal aorta (DA) and postcardinal vein (PCV) as shown in Fig. 2.21a-ii. It shows
3 that the blood velocity in DA is higher than PCV. Due to the transparent or
4 semitransparent nature of the biological cells, the resolution obtained by this
5 technique is low and not applicable for some measurements. Thus, fluorescence
6 imaging techniques have been proposed for the better contrast and higher
7 resolution of the transparent cells and tissues (i.e., RBCs and blood vessel walls)
8 as shown in Fig. 2.21b [148, 149]. Transgenic zebrafish lines expressing the
9 fluorescent proteins, $Tg(kdrl:GFP)^{la116};Tg(gata1:dsRed)^{sd2}$ are commonly
10 requested for the fluorescent imaging. Fig. 2.21b-i shows the EGFP-expression
11 in the transgenic zebrafish's blood vessels. Watkins *et al.* measured the dynamic
12 change of blood velocity when zebrafish (at 72 hpf) exposed to thromboxane A₂
13 analogue (U-46619) which has effects on the blood vessels such as smooth-
14 muscle contraction and endothelial dysfunction. A high-speed CMOS camera
15 was used to capture the RBC flows at a rate of 486 frames per second (fps) and
16 PIV was used to calculate the blood velocities from time-series images. Figure
17 2.21b-ii shows the measured blood velocities of the zebrafish at 72 hpf with U-
18 46619 treatment and without U-46619 treatment. It shows that blood velocity
19 increased when treated with U-46619. Moreover, heart rate was calculated by
20 estimating the time interval of the pulsatile blood flow. The heart rate was higher
21 in the older fish; 137 ± 5 bpm at 72 hpf and 162 ± 7 bpm at 120 hpf. On the other
22 hand, the mean blood velocity was higher in the younger fish; 605 ± 63 $\mu\text{m/s}$ at
23 72 hpf and 527 ± 39 $\mu\text{m/s}$ at 120 hpf. This technique can offer a better contrast,
24 good resolution, and high-speed measurement; however, it is invasive since it

- 1 requires transgenic fish lines as contrast agents, so that the genetic modifications
- 2 have to be made in the fish species.

3
4 **Figure 2.21** Measurement of blood flow in zebrafish via cellular tracking in bright-field
5 and fluorescence imaging. **a-i**, Bright-field image of blood flow in 48 hpf zebrafish. **a-**
6 **ii**, Blood velocity versus time in the DA (red) and PCV (blue). **b-i**, EGFP-expression in
7 a transgenic zebrafish's blood vessels. **b-ii**, Blood velocity in the DA without U46619
8 treatment (baseline) and with U46619 treatment. **c-i**, Autofluorescence expressed from
9 zebrafish at 72 hpf. **c-ii**, Fluorescence intensity extracted from the cross-sectional line in
10 **c-i**. Blood velocity in the DA and CV when **c-iii**, systolic, and **c-iv**, diastolic phase of
11 heart [146, 148, 150].

12 Several non-invasive techniques (i.e., without fluorescent agents or probes)
13 offering a high spatial resolution have been proposed. Fluorescence correlation
14 spectroscopy (FCS) was used to measure autofluorescence signal fluctuation
15 which was quantified as blood velocity in non-transgenic fish lines (wild-type
16 zebrafish) [150-152]. Autofluorescence signal can be generated from the proteins
17 (i.e., Hb in RBC) in the zebrafish's blood when excited by a light source with a
18 specific excitation wavelength and power (i.e., 488 nm laser line with 100 μ W
19 power) as shown in Fig. 2.21c-i. Fluorescence intensity profile was extracted
20 from the line in Fig. 2.21c-i (See Fig. 2.21c-ii). The signal of fluorescence

1 intensity fluctuation represents the dynamic blood flow. The signal was detected
2 by an Avalanche photodetector (0.6-1 kHz). Blood flow during systolic (heart
3 contraction) and diastolic (heart relaxation) phases were measured in dorsal aorta
4 (DA) and cardinal vein (CV) as shown in Figs. 2c-iii and 2c-iv. This technique is
5 non-invasive and offers high-spatial resolution but there are some limitations.
6 This technique suffered from low autofluorescence signal from the blood so that
7 the zebrafish need to be treated by a chemical solution (PTU, 0.003% 1-phenyl-
8 2-thiourea in 10% Hank's saline) to prevent the pigment formation at 18 hpf.
9 Meanwhile, the autofluorescence signal is stronger at the tissue region (i.e., egg
10 yolk) so it can degrade the resultant fluorescence signal. L. Fieramonti *et al.*
11 introduced an optical vector field tomography (OVFT), which utilizes optical
12 projection tomography (OPT) and a high-speed detector to provide a label-free,
13 3D blood velocity maps [153]. Bright-field images were collected from each
14 angle of the rotating zebrafish sample (See Fig. 2.22a); then a filtered back-
15 projection algorithm was performed to reconstruct the 3D image of the sample as
16 shown in Fig. 2.22b-i. PIV method was applied to calculate the blood velocity in
17 planar velocity maps. The 3D blood velocity vector fields were reconstructed
18 from four orthogonal planar views as shown in Fig. 2.22b-ii. Figure 2.22b-iii
19 shows the plots of blood velocities in the caudal artery (CA) and caudal vein (CV)
20 retrieved from the selected two vectors. OVFT is able to provide 3D blood
21 velocity vector fields in a large volume of the observation. However, a point-
22 scanning of the rotating sample in this technique is not suitable for time-resolved
23 imaging.

Figure 2.22 Optical vector field tomography measuring 3D blood velocity vector fields. **a**, Experimental configuration of OVFT. **b-i**, 3D reconstruction of blood vessels. **b-ii**, 3D blood velocity vector fields. **b-iii**, Blood velocity versus time from selected two vectors of CA and CV. CV: Caudal vein, CA: Caudal artery, VTAs: Vertebral arteries, and DLAVs: Dorsal longitudinal anastomotic vessels [153].

D. Donnarumma *et al.* proposed a laser Doppler digital holography which employs both digital holography and laser Doppler techniques to measure the blood velocity in a large volume of the observation [154, 155]. Holographic laser Doppler imaging or Doppler OCT have been widely used to measure the velocities in the biological samples. [156, 157]. The main concept is Doppler shift can directly be translated to the scattered RBC velocity. Experimental configuration of holographic Doppler microscopy is shown in Fig. 2.23a. Light from the laser ($\lambda = 650$ nm) was split into illumination and referenced arms by a beam splitter (BS). Acousto-optic modulators (AOM) were used to control the frequency of the reference arm. Beam at the illumination arm was split into two different angles to illuminate at the zebrafish trunk. The scattered light from the

1 zebrafish was collected by the microscope objective lens ($NA = 0.25$, $M = 10$).
2 The beams from sample and reference interfered at the camera; then multiple
3 holograms from different frequency was recorded. Holograms of the RBCs in the
4 blood vessels were reconstructed over 80 frames. Figure 2.23b shows the average
5 intensity of reconstructed blood flow at different z-planes. To obtain the
6 holograms of the moving RBCs, the frequency offset of the reference beam is set
7 to zero hence two phases hologram. Only the scattered signal from moving
8 objects will be taken into an account. By calculating 250 frames of the moving
9 RBCs, the reconstructed image of RBCs flow was obtained as shown in Fig.
10 2.23c. PIV was applied to calculate the velocities of RBCs. The average velocity
11 versus time of clustered RBCs and individual RBC were presented in Fig. 2.23d
12 (light grey and dark grey lines) with the sinusoidal curve fitting (black line). This
13 technique can provide 3D RBCs flow with individual RBC velocity in a larger
14 area of the observation. However, this technique has complex multi-beam
15 configuration and low spatial resolution. In addition, the images were acquired
16 by plane-by-plane optical sectioning which is not suitable for time-resolved
17 imaging.

1

2 **Figure 2.23** Laser Doppler digital holography for blood flow measurement. **a**,
3 Experimental configuration. **b**, Average intensity of reconstructed blood flow images at
4 different planes ($z = 0, +26.7$, and $+53.3 \mu\text{m}$). **c**, Reconstructed image of RBCs flow with
5 $z = 0$. **d**, RBC velocity versus time; average velocity (grey), individual velocity (dark
6 grey) and waveform fitting of the velocity (black) [154, 155].

7 Recently, pupil-engineered localization technique has been introduced for
8 monitoring blood flow in an extremely large area (up to $600 \mu\text{m}$ in axial range)
9 with sub-100 nm precision and sub-1 ms temporal resolution [158]. Fluorescent
10 microbeads ($D = 1 \mu\text{m}$) was injected to the zebrafish's blood vessel and used as a
11 tracer. The reconstructed 3D tracer trajectories in both CV and ISV is shown in
12 Fig. 2.24a. Figure 2.24b shows the velocities of the tracer in CA and its Fourier
13 spectrum corresponding to the heart rate of the zebrafish (2 Hz). The advantages
14 of this technique are a larger detection area and suitable for the thick samples.
15 However, the fluorescent beads injection is an invasive process which can cause
16 the toxicity in a live sample.

Figure 2.24 Pupil-engineered localization microscopy for blood flow measurement. **a**, Reconstructed 3D tracer trajectories in intersegmental vessels (ISV) and caudal vein (CV) of zebrafish at 72 hpf. **b**, Velocity of the tracers represents the blood velocity in caudal artery (CA) and its Fourier spectrum representing the heart rate [158].

To overcome those problems and limitations, we propose a simple, non-invasive and high-resolution FCR-QPI technique to measure the heart rate and blood flow in the zebrafish embryo. The comparison of previous techniques and our FCR-QPI technique is presented in Table 2.6.

✓ refers that the studies have been demonstrated in the literature

X refers that the studies have not been demonstrated in the literature

1 **Table 2.6** Comparison of previous literature studies of zebrafish and this study

	Label-free, non-invasive	Detection speed	Fish development	Single RBC analysis	Cons
Bright-field imaging	Yes	2,000 fps	√	X	-Low spatial resolution
Fluorescence imaging	No	486 fps	√	X	-Required genetically modified fish
Fluorescence correlation spectroscopy (FCS)	Yes	0.6–1 kHz	X	X	-Low signal at the blood -Required a excitation source
Optical vector field tomography (OVFT)	Yes	80 fps	X	X	-Required sample rotation
Pupil-engineered localization microscopy	No	26.5 fps	X	X	-Fluorescent bead injections
Doppler optical coherent tomography (DOCT)	Yes	25 fps	X	X	-Complex multi-beam configuration -Low spatial resolution
Doppler Digital Holography	Yes	110 or 220 fps	X	√	-Complex multi-beam configuration -Low spatial resolution
Our FCR-QPI	Yes	86 fps and 5 kHz	√	√	-Small observation area

2.5 Coherent characteristics of the optical fields

In QPI, the coherence of the optical field is the prerequisite for high-resolution phase imaging [17]. Coherence is a measure of an optical correlation between two points in the space (spatial coherence) and along the propagations in the time domain (temporal coherence). By using a classical formula of Young's double-slit, we can simply explain the relationship between coherence and interference. At the detector, the intensity of the interference pattern can be expressed as

$$I = I_1 + I_2 + 2\sqrt{I_1 I_2} \Re\{\gamma_{12}(\tau)\} = I_1 + I_2 + 2\sqrt{I_1 I_2} |\gamma_{12}(\tau)| \cos(\phi_{\gamma_{12}}(\tau))$$

where $|\gamma_{12}(\tau)|$ defines the coherence degrees of the illumination which is $0 \leq |\gamma_{12}(\tau)| \leq 1$. If $|\gamma_{12}(\tau)| = 0$, the waves are completely incoherent, and the interference pattern is lost which means the phase information ($\phi_{\gamma_{12}}(\tau)$), is also lost. On the other hand, if $|\gamma_{12}(\tau)| = 1$, the waves are completely coherent, and the interference can occur in infinite time or distance. If $0 \leq |\gamma_{12}(\tau)| \leq 1$, the waves are partially coherent, and the interference can occur within a finite distance (See **Appendix B** for the deeper details of coherence). Even though high coherence of the optical field is needed for the existing interference over a wide field-of-view (FOV) and for high visibility, however, it also induces the unwanted coherent artifacts (e.g., speckle) which hinder the successful phase reconstruction and degrade the phase resolution in QPI [159]. For example, high-coherence CW laser is often used as a light source for high phase-sensitive imaging but also has high speckle noise in the resulting interferogram [160-162]. Low-coherence sources (i.e., LEDs) employed spatial filtering methods (i.e., pinholes) to increase the spatial coherence have received attention for providing a speckle-less phase

1 image in an extended FOV [18, 26, 163-164]. However, they also suffered from
2 the low-visibility interferogram which will degrade the quality of phase
3 reconstruction. It is known that speckle noise can be reduced by
4 destroying/controlling the spatial coherence of the optical field. This idea leads
5 to the invention of many static and dynamic diffractive diffusers (Refer **Sec. 2.6**
6 for more details). So far, several QPI methods attempt to optimize spatial
7 coherence, temporal coherence, and speckle level for obtaining high quality
8 interferograms; however, each method owns benefit for their specific applications
9 [165-168] (Refer **Results Sec. 4.2.1** for 3D coherence cloud). S. Seungwoo *et al.*
10 proposed a method to control various spatial and temporal coherences of
11 illumination for obtaining the best quality interferogram [153]. However, the
12 experimental setting and systematic control have remained a problem. Since
13 different applications require different coherence levels, an ideal light source for
14 QPI should provide wide tunability in spatio-temporal coherence which means
15 both spatial and temporal coherences should be separately controlled to obtain
16 the speckle-less interferograms. Therefore, we proposed a frequency comb as a
17 partially coherent light source for QPI to enable the coherence tunability for high-
18 sensitivity phase imaging in a broad range of applications. Frequency comb can
19 be regarded as a fully or partially coherent light source. It can be regarded as a
20 fully coherent light source because all of the optical modes over a broad spectral
21 bandwidth of the phase-stabilized frequency comb are phase coherent with each
22 other [186, 187]. However, the broad supercontinuum of the frequency comb
23 forms a repetitive coherence envelope over the coherent phase carriers, which
24 could be also regarded as the low coherence envelope as widely known
25 broadband light sources for optical coherence tomography (OCT). Therefore, the

1 frequency comb is a highly coherent light source per each mode in the time
2 domain; however, at the same time, it is a temporally low coherent light source if
3 all the modes of the frequency comb works as a whole. Hence, the frequency
4 comb is a partially coherent light source (spatially high but temporally low
5 coherent light source)

6 **2.6 Speckle suppression based on diffuser technologies**

7 In this section, the previous studies of the speckle suppression in laser imaging
8 based on conventional diffuser technologies are provided as the comparison of
9 our proposed method, which is electroactive micro-optic diffuser. A generation
10 of the uncorrelated speckle pattern is one method to suppress the speckles [170,
11 171]. Various studies have utilized this method to develop static or moving
12 diffuser tools for suppressing the speckle noise [19-21, 172-175]. A. L. Andreev
13 *et al.* proposed a scattering liquid-crystal (LC) cell [19]. A helix ferroelectric LC
14 cell with a defined orientation was fabricated and embed with electrodes. After
15 applying on external electric field to the cell, the LC molecules generated the
16 refractive index variations from the changes in the scattering indicatrix positions.
17 In order to create a random phase modulation for the speckle suppression,
18 simultaneous action of low- and high-frequency controlled voltages were applied
19 to the LC cell. This technique can suppress the speckle noise at a moderate level,
20 but it involves complex experimental setup and operation. A microscanning
21 mirror combined with a random phase plate was introduced for rapid phase
22 modulation as shown in Fig. 2.25a [20]. Micromirrors were scanned to steer the
23 incoming beam into different spatial positions on the random phase plate which
24 eventually resulted in the speckle suppression in the resulting image (See Figs.

1 2.25a-i and 2.25a-ii). However, to scan the mirrors with a higher rate than CCD's
2 integration time, higher voltage with higher frequency was required (up to 50
3 $V_{\text{peak-to-peak}}$) and the method leads to optical-power loss. To minimize this power
4 consumption and reduce the power loss, colloidal solution or colloid dispersion
5 was proposed as a diffused solution as shown in Fig. 2.25b [21]. Colloid particles
6 immersed in a solution generally have Brownian motion which will act as a
7 moving diffuser for suppressing the speckle without external power consumption.
8 However, this technique is not suitable for compact experimental systems and it
9 is hard to control the outcomes. S. Subramaniam *et al.* developed micro-electro-
10 mechanical (MEM) diffuser devices [172]. The moving surface is a reflective
11 metal layer having random height distributions as shown in Fig. 2.25c. A voltage
12 was applied to induce displacements of the diffusers. Nevertheless, this technique
13 has multiple and complex procedures to fabricate the metal layer on the moving
14 surface and requires mechanical moving parts generating vibration which
15 mitigates the advantages.

17 **Figure 2.25** Diffuser technologies for speckle suppression. **a**, A scanning micromirror
18 scatted the light onto the phase plate enhancing the random walk of amplitude and phase

1 of the light. **b**, Colloidal solution scattered the light delivered by a single mode fiber
2 (SMF). **c**, MEM diffuser devices having a metal surface with random height to reflect
3 and diffuse the light [19-21, 172].

4 Here, we propose an electroactive micro-optic diffuser comprising of static
5 and moving diffusers as a laser speckle reducer (LSR) to suppress the different
6 speckle levels in high-coherence CW laser as well as in the frequency comb. The
7 optical diffusers used in this research are double diffusers which were first
8 introduced by S. Lowenthal and D. Joyeux [176]. Our optical diffusers have
9 uniform intensity transmittance, but they are two independent phase-modulated
10 parts. One contributed by a static diffuser (ϕ_0) and another contributed by a
11 moving diffuser (ϕ_d). When the incident plane wave is passing through the
12 diffuser, it produces a transmitted field of the form; $a(\alpha, \beta; t) =$
13 $a_0 e^{j\phi_0(\alpha, \beta)} e^{j\phi_d(\alpha - \vartheta t, \beta)}$, where a_0 is a constant and the diffuser is assumed to
14 moving in the positive α direction with the speed ϑ . As the diffuser rotates and
15 scatters the incident wave, any point on the object that is contributing to the image
16 point will experience an altered phase of the illumination varying with time t .
17 The time-varying random walk of the field amplitudes and phases of the speckles
18 are also created on each image point. If the integration time of an imaging sensor
19 is slower than the rotating speed ϑ of the LSR, the speckle effect will be averaged
20 out in the resulting images (Refer **Methods Sec. 3.1.2** for more details).

2.7 Interferogram analysis by Fourier Transform and phase unwrapping methods

In this research, we utilize off-axis QPI configurations which exploit the spatial modulation of the light. To retrieve the phase map from the resulting interferogram, we implement Fourier transform and Goldstein's phase-unwrapping techniques, which will be discussed below. At the detector, the intensity of the interferogram is defined as

$$g(x,y) = a(x,y) + b(x,y)\cos[2\pi f_0 x + \phi(x,y)] \quad (1)$$

where $g(x,y)$, $a(x,y)$, and $b(x,y)$ are the intensity of overall interferogram, background intensity, and fringe amplitude, f_0 defines by the spatial frequency, and $\phi(x,y)$ is the phase which we want to extract from the interferogram. The spatial frequency allows us to isolate the phase term by performing a Fast Fourier transform (FFT). As a result, the signals are located at the different positions in the frequency domain as shown in Fig. 2.26a and defined by the following equation

$$G(x,y) = A(f,y) + C(f-f_0,y) + C^*(f+f_0,y) \quad (3)$$

where A and C denote the Fourier spectra and f is the spatial frequency. The phase term confined in both spectra carrier part $C(f-f_0,y)$ or $C^*(f+f_0,y)$ is selected out. (See Fig. 2.26b), and then transverse backed into the image domain by inverse Fourier transform method according to the following equation

$$\log[c(x,y)] = \log[(1/2)b(x,y) + i\phi(x,y)] \quad (3)$$

1 Here, phase, $\phi(x,y)$ can be obtained from an imaginary part of the equation
2 (3). However, the acquired phase is in $-\pi$ to π range which often called the
3 wrapped phase. The method of phase unwrapping is required to connect them
4 into the factor of 2π as a continuous phase map (See Fig. 2.26c) [177].

5
6 **Figure 2.26** Process of phase reconstruction from the interferogram. **a**, Spatial frequency
7 after FFT process **b**, One spectral carrier selection **c**, Discontinuity of phase between
8 $-\pi$ to π are connected with phase unwrapping method [177].

9 For this purpose, this research utilizes Gold-stein's phase-unwrapping method
10 [178]. This method has three main processes; (1) Phase residue, (2) Branch cut,
11 and (3) Flood fill. Phase residue is the process that finds the difference of phase
12 one by one of four points around each point. The sum of these four different
13 values is called Residue. Residues are collected in “plus” for positive residue and
14 “minus” for negative residue. After completed finding all the residues, Branch
15 cuts is a process that connects the nearby residues and avoids the discontinuity.
16 The box is used to search plus or minus residue area and create the path between
17 the same sign residues. Flood fill is the last step which integrates the overall phase
18 without disturbing the cut path. The unwrapping was done in a 2D manner.

Chapter 3

Experimental design

3.1 Speckle suppression for phase-sensitive QPI

3.1.1 Off-axis common-path QPI configuration

Our speckle suppression method was employed in an off-axis common-path quantitative phase imaging (QPI). Figure 3.1a shows the experimental layout for speckle suppression in a high-coherence laser. The output beam from a continuous-wave laser diode (LD, $\lambda = 638$ nm) was focused onto an rotational optical diffuser; the resulting beam was collected and collimated by a plano-convex lens (L1 and L2). Three different diffusers were used in our study; (1) diffuser-1: $DA = 1^\circ$, (2) diffuser-2: $DA = 6^\circ$, and (3) diffuser-3: $DA = 12^\circ$. The diffuser is regarded as an infinite number of small point sources, each with numerical aperture of the diffusion angle. We placed the diffuser at the focal point of L1 and L2. Then, the collimated beam was transmitted through the sample, which was positioned on an inverted microscope (IX73, Olympus). An objective lens ($NA = 0.7$, $M = 60$) was used for the imaging. QPI part was comprised of three optical components: a half-waveplate, a Wollaston prism, and a linear polarizer. The vertically polarized beam passes through the half-wave plate, resulting in a polarization rotation by 45° . Then, a Wollaston prism splits the beam into two beams propagating at slightly different angles. The linear polarizer located after the Wollaston prism is aligned at 45° with respect to the two split

1 beams. This makes the two beams to interfere with each other at the image plane,
 2 where a CCD camera records the interferogram. The half-wave plate can be
 3 rotated for balancing the intensities at the two polarization states, which results
 4 in high visibility of the interferogram. Figure 3.1b shows that a cleaner raw
 5 interferogram of a microbead is attained when using the optical diffuser for
 6 speckle suppression.

7
 8 **Figure 3.1** Speckle suppression of high-coherence laser in an off-axis common-path QPI.
 9 **a**, Experimental layout. **b**, Interferogram of microbead before and after using the optical
 10 diffuser for speckle suppression. LD: CW laser diode, C: collimator, OA: optical
 11 attenuator, I: iris, L: plano-convex lens, OD: optical diffuser, O: objective lens, M:
 12 mirror, TL: tube lens, HWP: half-wave plate, WP: Wollaston prism, P: polarizer, CCD:
 13 charged-couple device.

14 3.1.2 Introduction to electroactive optical diffuser

15 An undesirable background speckle across the image, originating from the highly
 16 coherent laser source, causes severe information loss in the image as shown in

1 Fig. 3.2a. Coherent speckles can far suppressed by tailoring the illumination
 2 wavefronts at the target specimen. A representative image for the speckle
 3 suppression is shown in Fig. 3.2b. Multiple coherent beams with different
 4 propagation vectors (multiple k-factor) are focused to the sample so the scattered
 5 and diffracted wavefronts are washed out. Here, we introduce a compact moving
 6 diffuser based on the electro-active polymer (EAP) to reduce the speckle noise.
 7 Figure 3.2c shows a concept of the electroactive optical diffuser for the speckle
 8 suppression. Because the diffuser rotates and scatters the impinging collimated
 9 laser beam (λ_1, k_1), any point on the sample experiences a changing phase of the
 10 illumination ($\lambda_1, k_1 \sim k_N$). For any given point in the image plane, therefore, the
 11 region of the sample contributing to the formation of a specific image point will
 12 experience almost random walk of time-varying field amplitudes and phases. As
 13 a consequence, the speckle intensity at a point on the image plane changes with
 14 time; then, the image sensor (i.e., CCD) captures the integrated intensity over its
 15 exposure time. Their motion will destroy both, the spatial and the temporal
 16 coherence of the light which leads to an averaged-out speckle pattern. EAP
 17 consists of four independent dielectric elastomer actuators (DEA). By applying
 18 the voltage, four DEAs induce the displacement of the micro-particles inside the
 19 diffuser in both directions of the x- and y-axis. To generate the circular motion,
 20 two of all four electrodes (E1, E2, E3, and E4) are activated at one time. By
 21 placing these four electrodes with 90° respect to each other, a circular oscillation
 22 is achieved by controlling the signal for all of them in quadrature. The static and
 23 moving diffusers are combined here to provide more effective speckle
 24 suppression by minimizing the increased beam divergence. Three electroactive
 25 optical diffusers with three different particle sizes and diffusion angles are

1 implement in this study (LSR-3000 Series, manufactured by Optotune); diffuser-
 2 1: DA = 1°, diffuser-2: DA = 6°, and diffuser-3: DA = 12°. Their specifications
 3 are shown in the Table 3.1.

4
 5 **Figure 3.2** Electroactive optical diffuser for speckle-suppressed imaging. **a**, Spatially
 6 coherent illumination with well-defined wavefronts causes strong background speckle.
 7 **b**, By angle-diversifying incoming wavefronts to a sample, coherent speckles can be
 8 suppressed. **c**, Conceptual image of speckle reduction using an electroactive optical
 9 diffuser, with four electrodes (E1, E2, E3, and E4) for rotational diffusion angle control.
 10 OD: optical diffuser, E: electrode, and L: lens.

1 **Table 3.1:** Specification of the optical diffusers (OD) used in this research

	OD, DA = 1°	OD, DA = 6°	OD, DA = 12 °
Particle size (μm)	100	30	10
Number of independent point sources per unit area (for double diffuser)	254.8	2830.8	25477.8
Rotation speed (Hz), (mm/s) @ r = 300 μm, $V=2\pi r \cdot f$	300 (562.2)	300 (562.2)	300 (562.2)
Diffusion angle of the overall diffuser	1°	6°	12°
Effective aperture (mm)	5	5	5

2

3 **3.1.3 Interferogram analysis**

4 Figure 3.3 shows the analytical technique to provide the phase data as a
5 reconstructed phase map of an interferogram. The resulting interferogram of a
6 polystyrene microbead immersed in oil is shown in Fig. 3.3a. Phase
7 reconstruction are performed by the process which consisted of 4 modules; (1)
8 2D Fast Fourier Transform (FFT) module, (2) cropping, shifting, and zero-
9 padding, (3) 2D inverse FFT (IFFT), and (4) Goldstein's phase unwrapping, linear
10 slope subtraction, and phase map reconstruction module (See Figs. 3.3b-1, 3.3b-
11 2, 3.3b-3, and 3.3b-4). To study the effect of the optical diffuser in QPI, a FFT
12 power spectrum is extracted by a line passing through the three frequency peaks
13 (one DC at the center and two AC peaks) in Fig. 3.3c-1. The data of three peaks

are plotted on logarithmic and linear scales (See Figs. 3.3c-2 and 3.3c-3). The linewidth and signal-to-noise ratio (SNR) of the 1st harmonic peak in the Fourier domain represent the suppression of background noise. To analyze the linewidth and SNR of the QPI carrier peak, the cross-sectional lines of the 1st harmonic peak are taken at varied angles; this approach could provide signal's linewidths and SNRs at various angles.

Figure 3.3 Analytical technique of the interferogram and process of phase reconstruction. **a**, Interferogram of polystyrene bead immersed in microscope oil. **b**, Algorithm for quantitative phase map reconstruction from the interferogram. **c**, Algorithm for FFT line extraction in logarithmic and linear scale.

3.2 FCR-QPI for high-visibility, high-speed, spectroscopic phase measurement with a high phase stability

3.2.1 Frequency-comb-referenced QPI configuration

Figure 3.4a illustrates the system layout of frequency-comb-referenced quantitative phase imaging (FCR-QPI). An Er-doped fiber frequency comb (FC-1500-250-WG, M-VIS, Menlo Systems) emits an ultra-short pulse train at a repetition rate of 250 MHz (at a centre wavelength of 1,550 nm) (See Sec. 3.4.1 for more details). Before broadening the spectra, the spectra must be converted to near-infrared range (at a center wavelength of 780 nm) by a non-linear second-harmonic-generation (SHG) unit. Then, the beam is coupled to a photonic crystal fiber (PCF) which leads to a broad supercontinuum over 520 to 980 nm. The output supercontinuum was delivered to an illumination path having a coherence control unit and wavelength selection unit and then to a Michelson's type QPI to study the role of frequency comb in; (1) coherence control in QPI, (2) multi-wavelength QPI, and (3) dynamic QPI (See Figs. 3.4a and 3.4b). For the coherence control, the spatial coherence of the frequency comb was controlled by tuning the different diffusion angles ($DA = 1^\circ, 6^\circ$ and 12°) of a micro-optic optical diffuser as a laser speckle reducer (LSR). The temporal coherence was controlled by a band-pass-filters (BPF) with various spectral bandwidths ($BW = 40, 25,$ and 10 nm) (See Fig. 3.4a-I). For the multi-wavelength QPI, the output beam was delivered to the BPFs with various center wavelengths ($\lambda_{\text{center}} = 700, 750,$ and 800 nm, $BW = 10$ nm) to provide multiple wavelengths for the illumination which could enable spectroscopic phase measurements (See Fig. 3.4a-II). For the dynamic QPI, a programable spatial filter was introduced to

1 select the point-of-interests in the sample to be measured by a high-speed
 2 photodetector. The photodetector was installed to measure higher-speed dynamic
 3 motions up to MHz which could not be achieved by the conventional CCD
 4 camera (See Fig. 3.4a-III).

5
 6 **Figure 3.4** Frequency-comb-referenced quantitative phase imaging (FCR-QPI). **a**, FCR-
 7 QPI for (I) coherence control in QPI, (II) multi-wavelength QPI, and (III) dynamic QPI.
 8 **b**, Experimental configuration. An Er-doped fibre frequency comb (the repetition rate
 9 and offset frequency of the comb are detected by f:2f interferometer and phase-locking
 10 via automatic phase-locked-loops to a standard Rb atomic clock) is wavelength-down-
 11 converted to near-infrared range by second-harmonic-generation and spectrally
 12 broadened at PCF, resulting in low temporal and high spatial coherence. For temporal
 13 coherence control, the combination of a diffraction grating and a slit was used for tunable
 14 band-pass-filtering with different centre wavelength and spectral bandwidth. For spatial
 15 coherence control, the laser speckle reducer (LSR) with different diffusion angle (DA)
 16 was installed in the beam path. The spatio-temporal coherence-controlled beam was then

delivered to the sample on an inverted microscope and the resulting sample phase information was analyzed at a QPI unit with a CCD and a high-speed PD. CL: cylindrical lens, DG: diffraction grating, M: mirror, C: collimator, OL: objective lens, TL: tube lens, L: lens, BS: beam splitter, PSF: programmable spatial filter, CCD: charge-couple device, PD: photodetector.

3.2.2 Experimental detail for coherence control in QPI

The frequency comb was delivered to the band-pass filters (BPF) with various spectral bandwidths ($\lambda_{\text{centre}} = 750$ nm, BW = 40, 25, and 10 nm) for the temporal coherence control. Then, the light was focused onto the laser speckle reducers (LSR) with various diffusion angles (DA = 1°, 6°, and 12°) for the spatial coherence control. Before the light delivery to the polystyrene microbeads immersed in index-matching oil, a scattering film (polycarbonate film, $\rho = 1.2$ g/cm³, DA = 1°, manufactured by Edmund Optics) was placed in the illumination path. The light diffracted from the microbead was collected by an objective lens (NA = 0.7, M = 60) in an inverted microscope. Then, the light was transmitted to the Michelson-type QPI unit. The resulting interferograms of the microbead at various spatial and temporal coherences of the illumination were recorded by the CCD camera. The spatial coherence, temporal coherence, and speckle contrast were experimentally quantified (refer to Sec. 3.3.6)

3.2.3 Experimental detail for multi-wavelength QPI

Three wavelength segments of the frequency comb were extracted by the band-pass filters with different central wavelengths ($\lambda_{\text{centre}} = 700, 750$, and 800 nm, BW = 10 nm) to illuminate the MCF-7 human breast cancer cells. The scattering film (polycarbonate film, $\rho = 1.2$ g/cm³, DA = 0.5°, manufactured by Edmund Optics)

1 was placed in the illumination path to create more scattering environment which
 2 possibly can be found in biological environments or plasmonic sample. Then, the
 3 laser speckle reducer with diffusion angle ($DA = 6^\circ$) was used here to suppress
 4 the speckle noise. After illuminating the cells, scattered light was collected by the
 5 objective lens ($NA = 0.7$, $M = 60$), and then transmitted to the QPI unit. Time-
 6 lapse interferograms of the cell at each wavelength were recorded with a time
 7 interval of 20 minutes for 9 hours. The refractive index (RI) ratio at two
 8 illumination wavelengths of 700 (λ_1) and 800 nm (λ_3) was calculated by $n_{l,3}$ -
 9 $n_{m1}/n_3 - n_{m3} = \lambda_1 \cdot \Delta\phi_1 / \lambda_3 \cdot \Delta\phi_3$, where $n_{l,3}$ are the refractive indices of the cell
 10 measured at 700 and 800 nm, $n_{m1,m3}$ is the refractive index of the culture media
 11 measured at 700 and 800 nm, and $\Delta\phi_{l,3}$ is the optical phase difference measured
 12 at 700 and 800 nm. Note that the RI ratio was expressed by the RI difference of
 13 the cell and culture media.

14 **3.2.4 Experimental detail for dynamic QPI**

15 High-speed dynamic motions of an optical fibre and micro-particles are measured
 16 in a quantitative manner with FCR-QPI. Figure 3.5a-1 shows the experimental
 17 configuration of FCR-QPI for measuring a high-frequency periodic physical
 18 motion of an optical fibre; the same system was also used to measure the
 19 oscillating micro-particles. The external coating layer of the optical fibre (a single
 20 mode fibre (SMF-28), $D_{core} = 8.2 \mu\text{m}$, $D_{cladding} = 125 \mu\text{m}$, $n_{core} = 1.452$, $n_{cladding} =$
 21 1.447) was removed. One end of the fiber was mounted to the piezo-electric
 22 transducer (PZT, Piezoelectric benders, manufactured by Thorlabs) and the other
 23 end ($\sim 5 \text{ mm}$) was immersed in the index-matching medium (glycerol/water:
 24 90/10) filled in the cover slit channels as shown in Fig. 3.5a-2. By applying the

1 sinusoidal electric field to the PZT (~ 10 V), the optical fiber was oscillated
2 perpendicular to the illumination beam from the frequency comb (See Fig. 3.5a-
3 2). The resulting phase modulated signal was collected by the objective lens (NA
4 $= 0.4$, $M = 10$) and delivered to the QPI unit. A programmable spatial filter and a
5 high-speed photodetector were used in this setup instead of CCDs for measuring
6 the higher speed motions of oscillating signal from our points of interest. To
7 obtain the position-dependent-phase modulation (at core, cladding, and between
8 core and cladding) which resulted from the difference in the refractive index, the
9 central position of the modulation were shift to the core, cladding, and
10 background medium as shown in the schematic drawing (See Fig. 3.5a-3) as well
11 as in the interferogram of the fibre (See Fig. 3.5a-4). To demonstrate high-speed
12 measurement capability, PZT's modulation frequency was tuned from 1 Hz to 4
13 kHz. Due to the upper-frequency limit of the PZT, the last phase-modulated-
14 signal was measured at 4 kHz. To measure higher-speed motion, we measure the
15 moving micro-particles (size ~ 10 μm) embedded inside the laser speckle reducer
16 (LSR) with diffusion angle (DA) of 12° (See Fig. 3.5b). LSR utilizes dielectric
17 elastomer actuators (DEA) as a rotational mechanism. By applying the voltage to
18 the electrodes of DEAs (activated LSR), micro-particles were oscillated with its
19 fundamental frequency of 297 Hz. Light from the frequency comb was focused
20 onto activated LSR, then the phase-modulated-signal was detected by the
21 photodetector.

Figure 3.5 FCR-QPI for measuring high-speed dynamic motions of the oscillating fibre and micro-particles. **a-1**, 3D schematic of the system configuration. **a-2**, A sinusoidal voltage applied to PZT results in the lateral movement of an optical fiber in an immersing medium. **a-3**, Input sinusoidal signal-induced-periodic modulation at the different positions of the optical fiber sample. Five different positions were observed; cladding (left), cladding-core (left), core, cladding-core (right), and cladding (right). **a-4**, Interferogram of the optical fibre. **b**, Micro-particles moving inside the dielectric polymer of the laser speckle reducer.

3.2.5 Experimental detail for phase stability

To evaluate the phase stability for high measurement precision, we quantitatively analyzed the coherent speckle in the recorded interferograms at low update rate (by a CCD camera) and high update rate (by a photodetector). Light from frequency comb was delivered to illuminate the microbeads; then scattering beam from the microbeads was collected by an objective lens ($NA = 1.25$, $M = 100$, oil immersion) and was transmitted to the QPI unit. The interferograms of microbeads were recorded by a CCD camera for 400 s (at 12 fps). For the comparative study, the same set of experiment was done using continuous-wave

1 laser diode. Phase instability in the time-lapse interferograms obtained using
2 frequency comb and continuous-wave laser diode were characterized in time- and
3 frequency-domains and analyzed by Allan deviations. For the higher update rate,
4 we quantified the phase instability from the following three light sources: a
5 continuous-wave laser diode (CW LD), a femtosecond pulse laser without
6 frequency stabilization (FS), and a frequency comb (FC) with full frequency
7 stabilization. Light from those light sources was delivered to an amplified Si
8 photodetector (PDA36A-EC, Thorlabs) through the system. Phase instability
9 signal was recorded by an oscilloscope and Allan deviations were derived at
10 various averaging times to quantitatively evaluate the measurement precision and
11 update rate.

12 **3.2.6 Quantification of spatio-temporal coherence and speckle** 13 **contrast**

14 **Spatial coherence**

15 To quantify the spatial coherence of the frequency comb and continuous-wave
16 laser diode, we used Young's double slit experiment, as shown in Fig. 3.6a (slit
17 width = 10 μm and slit separation = 50 μm). Light from the frequency comb
18 transmitted through the laser speckle reducers with various diffusion angles
19 (LSR, DA = 1°, 6° and 12°) and then passed through the bandpass filters with
20 different bandwidths (λ_{center} = 750 nm, BW = 40, 25, and 10 nm). The collimated
21 beam was delivered to the double slit. The resulting two diffracted beams
22 propagated through the free space, which is long enough to generate the far-field
23 Fraunhofer diffraction and interference pattern on a CCD camera. The visibility

1 of the interference pattern, which is a broad envelope in the shape of Bessel
 2 functions, was used as a measure of the spatial coherence for each illumination
 3 condition. The spatial coherence can be best explained if the light is emitted from
 4 the temporally coherent light source (monochromatic light source). Practically,
 5 the temporal and spatial coherences cannot be separately investigated by
 6 experiment. Therefore, the results of fringe visibility (V) shown here are the effect
 7 of both spatial and temporal coherences, which are determined by different
 8 spectral bandwidths and diffusion angles. However, the spatial coherence can be
 9 still defined as the visibility (V) that is obtained at specific coherence lengths,
 10 e.g., $V = 0.88$ at $L_c = 8.1 \mu\text{m}$. To calculate the spatial coherence, the mutual
 11 coherence function (γ) from the interference fringes was obtained. The degree
 12 of coherence between two electric fields, E_1 and E_2 , is defined as $\langle E_1 E_2^* \rangle / \sqrt{I_1 I_2}$,
 13 where $I_1 = |E_1|^2$, and $I_2 = |E_2|^2$. In this measurement, the intensity on the two
 14 slits is equal ($E_1 = E_2$). This reduces γ to the $\gamma = V = (I_{\max} - I_{\min}) / (I_{\max} + I_{\min})$,
 15 where $I_{\max}$ and $I_{\min}$ are the maximum and minimum intensities of the interference
 16 fringes. Figures 3.6b to 3.6f show the resulting interference patterns at various
 17 illuminations. The visibility of the interferogram (V) was calculated and
 18 represented as the spatial coherence, which was obtained at any specific temporal
 19 coherence. The spatial coherence was reduced from 0.88 to 0.63 V when using
 20 the optical diffusers with higher diffusion angle (from no LSR to LSR, $\text{DA} = 12^\circ$,
 21 for no BPF). Similar to the continuous-wave laser diode, the spatial coherence
 22 was reduced from 0.98 (no LSR) to 0.68 (LSR, $\text{DA} = 12^\circ$). The visibility was
 23 increased from 0.88 to 0.98 V when using the band-pass filters with narrower
 24 bandwidth (from no BPF to BPF, $\text{BW} = 10 \text{ nm}$, for no LSR). This could be the
 25 influence of the increasing temporal coherence.

Figure 3.6 Spatial and temporal coherence characteristics of the light source. **a**, Young's double-slit experiment. **b**, **c**, **d**, **e**, Resulting interference fringes showing the spatial coherence of a coherence-controlled frequency comb and **f**, a continuous-wave (CW) laser diode.

Temporal coherence

Temporal coherence was evaluated by the coherence length, L_c ; $L_c = \lambda^2/n\Delta\lambda$, where λ is the central wavelength, $\Delta\lambda$ is the spectral bandwidth in full-width-half-maximum (FWHM), and n is the refractive index of the medium [17]. The temporal coherence of the frequency comb without a band-pass filter and with band-pass filters with the bandwidth of 40, 25, and 10 nm at FWHM were calculated to 8.1, 14.0, 22.5, and 56.3 μm , respectively. These numbers were also experimentally verified with the QPI interferograms. The temporal coherence lengths were measured to 7.9, 15.0, 22.5, and 56.3 μm , respectively, which agrees well with the calculation. A continuous-wave laser diode was confirmed to have a coherence length of $\sim 20 \text{ mm}$ using a Michelson homodyne interferometer with the reference arm scanning.

1 **Speckle contrast**

2 To obtain the speckle contrast at various spatio-temporal coherence of the
3 illumination, a 1963 US Air Force resolution test chart was imaged in the
4 presence of simulated speckle noise as shown in Fig. 3.7a. The speckle noise was
5 generated by placing a scattering film (polycarbonate film, $\rho = 1.2 \text{ g/cm}^3$, DA =
6 0.5°) before the light illuminating the test chart. Spatial and temporal coherences
7 of the frequency comb (FC) were controlled by various diffusion angles of the
8 laser speckle reducer (LSR, DA = 1° , 6° , and 12°) and various spectral bandwidths
9 (BW) of the bandpass filter (BPF, $\lambda_{\text{centre}} = 750 \text{ nm}$, BW = 40 nm, 25 nm, and 10
10 nm). The spatial coherence of the continuous-wave laser diode was controlled for
11 the comparative study. Figures 3.7b to 3.7f show the resulting images at various
12 coherences of the illumination. The intensity variation of the representative
13 background area (dashed white rectangle in Fig. 3.7b) was used to calculate the
14 speckle contrast and the standard deviation. The intensity of all images was
15 normalized before the analysis. The speckle contrast (C) was calculated by $C =$
16 $SD_I / \langle I \rangle$, where SD_I is the standard deviation of the intensity, and $\langle I \rangle$ is the
17 average intensity for each image. As a result, the speckle contrast and the standard
18 deviation were decreased by the use of optical diffuser and found to be lowest
19 when using high diffusion angle (DA = 12°). Figure 3.7b shows that the speckle
20 contrast (C) and standard deviation (SD) were tailored down in the frequency
21 comb without the band-pass filter from speckle contrasts = 0.52 to 0.29 and
22 standard deviations = 0.25 to 0.13 (from no LSR to LSR, DA = 12°). Similarly,
23 this trend can be observed in the frequency comb with the band-pass filters and
24 in the continuous-wave laser diode. However, when using the band-pass filter with

1 narrower bandwidth (BW), the speckle contrasts increased from 0.29 to 0.39 and
 2 the standard deviations increased from 0.13 to 0.17 (from no BPF to BPF, BW =
 3 10 nm, for LSR, DA = 12°). This results in an increase in temporal coherence
 4 length which eventually induces the coherent speckle in the images. The lowest
 5 speckle contrast and standard deviation were found in the frequency comb
 6 without band-pass filter and with laser speckle reducer with diffusion angle of
 7 12° (speckle contrast = 0.29 and standard deviation = 0.13) as shown in Fig. 3.7b.

8
 9 **Figure 3.7** Speckle contrast measurement. **a**, Resolution test chart imaging. **b, c, d, e**,
 10 Resulting images illuminated by a coherence-controlled frequency comb and **f**, a
 11 continuous-wave (CW) laser diode.

3.3 Measurement of heart rate and blood flow in zebrafish by using FCR-QPI

We have previously demonstrated that frequency-comb-referenced quantitative phase imaging (FCR-QPI) can be employed to measure high-speed dynamic motions of micro-objects (e.g., optical fibres). However, no demonstrations of dynamic motions measurement in biological samples were provided. In this study demonstrates that FCR-QPI can be employed to monitor dynamic motions of heart beat and blood flow in zebrafish embryos. To understand the development of zebrafish's cardiovascular system, we measured heart rate and blood flow in a developing zebrafish from 24 to 56 hpf (hours-post-fertilization). Figure 3.8a depicts bright-field image of zebrafish at 24 hpf with its heart and mid-trunk blood vessels; dorsal aorta (DA) and postcardinal vein (PCV). At 24 hpf, red blood cells (RBC) flow individually inside the vessel; then, at 48 hpf, the development of blood stem cells and heart chambers drastically increase the overall number of RBCs and the speed of RBC flow [46]. To understand this development, we monitor the heart and RBCs need to be in real time. For this purpose, light from frequency comb was delivered to illuminate the heart and mid-trunk blood vessels of a live zebrafish as shown in Fig. 3.8c. The development of heart morphology, heart rate, RBC velocity, number of RBC, RBC size, and pulsatility of blood pulse were quantitatively analyzed.

Figure 3.8 FCR-QPI for dynamic motions measurement in zebrafish. **a**, Bright-field image of zebrafish at 24 hpf showing heart and blood vessels (DA and PCV). **b-1**, QPI image of the RBCs flow in the mid-trunk vessel of zebrafish at 48 hpf. **b-2**, Light from the frequency comb illuminated to RBCs flow in the blood vessel. **c**, Experimental configuration of FCR-QPI. L: lens, M: mirror, C: collimator, S: sample, O: objective lens, TL: tube lens, BS: beam splitter, I: iris/programmable spatial filter, CCD: charge-couple device, PD: photodetector.

3.4 Materials and sample preparation

3.4.1 Frequency comb

A spectrally broadened visible frequency comb (FC-1500-250-WG, M-VIS, Menlo Systems) was employed as the light source. The master oscillator was an Er-doped fibre femtosecond laser having a repetition rate of 250 MHz, a centre wavelength of 1550 nm, and a spectral bandwidth of ~25 nm. The power was amplified with two Er-doped fibre amplifiers (EDFAs); one was used for frequency stabilisation and the other for nonlinear spectral broadening. At the frequency stabilisation part, the repetition frequency (f_r) and carrier-envelope

1 offset frequency (f_{CEO}) were phase-locked to a reference Rb atomic clock (FS725,
2 Stanford Research Systems) for establishing a frequency comb, with the aid of a
3 f - $2f$ interferometer and phase-locked control loops. At the spectral broadening
4 part, the pulses were first wavelength converted to a near-infrared range of ~780
5 nm by second harmonic generation (with an optical power ~250 mW), and then
6 coupled to a photonic crystal fibre to have a broad supercontinuum over 620 to
7 980 nm with an optical power of ~120 mW.

8 **3.4.2 Polystyrene microbeads**

9 Polystyrene beads (Diameter = $10 \pm 0.66 \mu\text{m}$, distribution = $4 \times 10^6/\text{ml}$, $n_{\text{microbead}}$
10 = 1.579 @ $\lambda = 750 \text{ nm}$, manufactured by SPI supplies), suspension in Deionized
11 water (DI) water, was prepared on glass slide following these procedures; First,
12 polystyrene bead solution ($4 \mu\text{l}$) was diluted in mixture solution containing 50-
13 50% ethanol and DI water. Second, diluted polystyrene ($5 \mu\text{l}$) solution was
14 dropped on cleaned glass slide. Next, polystyrene sample was dried by placing
15 glass slide on hotplate (70°C) for 3 minutes. Finally, immersion oil ($n_{\text{immersion oil}} =$
16 1.493 @ $\lambda = 750 \text{ nm}$) was dropped on dried polystyrene sample for the refractive
17 index matching; then sandwiched under a cover slip. The microbead sample was
18 sealed to prevent evaporation by putting epoxy glue around the cover slip.

19 **3.4.3 MCF-7 breast cancer cells**

20 MCF-7 cells were cultured in Dulbecco's modified Eagle's media (DMEM) with
21 10% (v/v) Fetal Bovine Serum (Gibco, CA) containing growth factors (i.e.,
22 protein). Penicillin (100 units/ml) and streptomycin antibiotics (100 $\mu\text{g}/\text{ml}$,
23 Hyclone, CA) were added to the media to prevent bacterial contamination. The

1 cells were grown in a 37°C incubator with 5% CO₂, 95% humidity. Cell were
2 passaged every 3-4 days. 5,000 cells were seeded on the 12 mm coverslip of a
3 quartz based dish (Iwaki, Japan) and allowed to grow for 72 hours before
4 changing with fresh media for the experiment.

5 **3.4.4 Embryonic zebrafish**

6 Zebrafish embryos were derived from natural matings of AB wild-type adults,
7 housed in the LKC Medicine Zebrafish Facility, NTU. Zebrafish housing, mating
8 and embryo manipulations were performed under the oversight of the NTU
9 Institutional Animal Care and Use Committee (IACUC) under IACUC Protocol
10 Number A18002 and complied with the National Advisory Committee For
11 Laboratory Animal Research (NACLAR) Guidelines set out by the Agri-Food
12 and Veterinary Authority (AVA) of Singapore. Embryos were collected and
13 maintained in E3 media (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, 0.33 mM
14 MgSO₄, 10⁻⁵% Methylene Blue). Embryos were dechorionated with
15 watchmakers' forceps and then anaesthetized with 0.02% Tricaine (buffered to
16 pH = 7.0). Embryos were added into 0.7% molten low melting agarose
17 maintained at ~42 °C. Vacuum grease was used to draw a border around the edges
18 of a standard microscope glass slide. Embryos were pipetted onto the glass slide
19 inside the vacuum grease border and orientated using a fine gel loading tip. After
20 the gel solidified, the vacuum grease-bordered area was then flooded with E3
21 media and a long glass coverslip was used to cover the mounted specimens (~5
22 embryos/slide).

1 **Chapter 4**

2 **Results and discussion**

3

4

5 **4.1 Speckle suppression for phase-sensitive QPI**

6 **4.1.1 State-of-the-art light sources for optical imaging**

7 An optical diffuser diffuses the incoming light in order to suppress speckles;
8 meanwhile, it also reduces the brightness or intensity of the transmitted light. In
9 this section, we provided a state-of-the-art light source offering high-brightness
10 and effective speckle suppression (See Fig. 4.1). Figure 4.1 depicts the photon
11 degeneracy versus speckle contrast of various coherent light sources. Thermal
12 light sources (halogen, xenon, and mercury lamps) and light emitting diodes
13 (LEDs) have been widely used as the partially coherent light sources after the
14 spatial filtering through a small-diameter pinhole ($\sim 5\text{--}50\text{ }\mu\text{m}$); spatial filtering
15 provides a higher spatial coherence, and therefore, enables the formation of
16 higher visibility interferograms over a larger FOV. However, this improvement
17 in the spatial coherence is accompanied by severe optical power attenuation (from
18 several W to a few mW). Therefore, a bright coherent laser illumination with
19 suppressed coherent artifacts is preferred for efficient optical imaging in optically
20 turbid media, having micro/nano scatterers inside. Previous studies for speckle
21 noise control are summarized in Fig. 4.1, with the speckle contrast level (related

1 to spatial coherence) as the horizontal axis and the photon degeneracy (related to
2 temporal coherence and brightness) as the vertical axis. High photon degeneracy
3 (δ), the number of photons per a unit coherence volume, is crucial for an efficient
4 light-sample interaction hence fast and precise measurement. Coherent photons
5 from different volumes cannot interfere with one another so do not generate
6 speckles; therefore, a light source with many bright coherence volumes enables
7 speckle-free sensitive imaging over a large FOV. In this respect, the limitations
8 of low-coherence light sources and coherent lasers are clear. Low-coherence light
9 sources suffer from the small number of photons per coherence volume (low
10 photon degeneracy) as shown as thermal sources (T), spatially filtered white light
11 (SFWL), and light emitting diodes (LEDs) in Fig. 4.1. On the other hand,
12 conventional lasers having a large number of photons per unit volume (high
13 photon degeneracy) generate unexpected coherent artifacts as shown as
14 narrowband laser (NBL) in Fig. 4.1. These are the two extreme cases located in
15 the left bottom (T, SFWL, and LEDs) and right top (NBL) of the Fig. 4.1. As the
16 alternatives to the low-coherence light sources, broadband laser-like sources,
17 such as amplified spontaneous emission sources and super-luminescent laser
18 diodes have been adopted [23], and labeled as ASE in Fig. 4.1; however, they
19 still preserve a high-level of spatial coherence, and therefore coherent speckles
20 cannot be sufficiently reduced [24]. In order to suppress the effective spatial
21 coherence of conventional lasers, moving diffusers, colloidal solutions, micro
22 electro-mechanical mirrors, or chiral nematic liquid crystal diffusers [19-21, 159,
23 160] have been introduced to the illumination path; these techniques, however,
24 require mechanical moving parts, generating vibration noise or require longer
25 acquisition times, which negates much of the advantages (See CNLC in Fig. 4.1).

1 The concepts of random lasers (RL) [24, 25], and degenerate laser (DGL-PD)
2 [26], were recently demonstrated as promising means to generate an intermediate
3 degree of spatial coherence, indicated as RL and DGL-PD (6 mm and 120 μm) in
4 Fig. 4.1; however, these suffer from high lasing thresholds, low collection
5 efficiencies, and large footprints.

6

7 **Figure 4.1** State-of-the-art light sources for optical imaging; classified by photon
8 degeneracy and speckle contrast level. ‘Investigation zone’ indicates the tunable
9 temporal and spatial coherence area demonstrated in this investigation. NBL:
10 narrowband laser [24,179], BBL: broadband laser [24, 166, 180], ASE: amplified
11 spontaneous emission [24, 25], RL: random laser [24, 25], LEDs: light emitting diodes
12 [24, 179, 181], T: thermal sources [24, 179, 181], SFWL: spatially filtered white light
13 [24, 164, 179, 181], DGL: degenerate laser with pinhole inside the cavity [26], CNLC:
14 chiral nematic liquid crystal [175], LD w/wo SD/MD: a laser diode with and without a
15 static diffuser and a moving diffuser with a specific diffusion angle (DA).

4.1.2 Speckle suppression in QPI

We demonstrated speckle suppression in QPI by imaging a transparent microbead sample using common-path phase shifting QPI. A 10- μm polystyrene bead was initially illuminated without optical diffuser. The resulting interferogram and its Fast Fourier Transform (FFT) spectrum are shown in Figs. 4.2a-1 and 4.2b-1. Moreover, the phase map retrieving via FFT and phase unwrapping process (based on Goldstein algorithm) are shown in Fig. 4.2c-1. To obtain the effect of speckle suppression, three different optical diffusers comprising of static and moving diffusers (SD/MD) were placed in the illumination path and the resulting interferograms were analyzed by the aforementioned FFT and phase mapping techniques. The resulting interferograms (See Figs. 4.2a-2, 4.2a-3, and 4.2a-4), FFT power spectra (Figs. 4.2b-2, 4.2b-3, and 4.2b-4), and topographic phase maps (Figs. 4.2c-2, 4.2c-3, and 4.2c-4), show the speckle suppression after using the optical diffuser. In the interferogram when the optical diffuser is used, the background area is clean, indicating the speckle suppression, and getting even 'cleaner' with the larger diffusion angle (SD/MD, $\text{DA} = 12^\circ$). The standard deviations of the background were calculated and taken as a quantitative measurement for the background speckle noise suppression. It was shown that the speckle was suppressed from 0.20 (for no SD/MD) to 0.04 (for SD/MD, $\text{DA} = 12^\circ$) by increasing the diffusion angle (Figs. 4.2c-1 to 4.2c-4). In Fourier domain (FFT spectrum), high order harmonic peaks are presented in the background, showing the reduction of speckle noise by the optical diffuser. The topographic phase maps (Figs. 4.2c-1, 4.2c-2, 4.2c-3, and 4.2c-4) provide a smoother background for optical diffusers with larger diffusion angles. In addition, the information loss due to the speckle suppression process was

1 investigated by sectioned 2D phase profiles as shown in Fig. 4.2d. It shows that
 2 the measured height of polystyrene bead with and without optical diffuser are
 3 slightly different (3.4% variation). Therefore, we find that the proposed speckle
 4 reduction method did not negates much of the phase measurement accuracy.
 5 Moreover, with a higher DA of the optical diffuser, the phase line gets smoother
 6 at the outer side and sharper at the periphery of the bead. We also demonstrate
 7 our speckle suppression method in non-coherent imaging (See **Appendix C.1**),
 8 and Young's double slit experiment (See **Appendix C.2**).

9
 10 **Figure 4.2** Speckle suppression in the interferograms of microbeads. QPI interferogram
 11 of a 10 μm polystyrene bead with **a-1**, no SD/MD, **a-2**, DA = 1°, **a-3**, DA = 6°, and **a-4**,
 12 DA = 12° in the illumination path. FFT plots of the interferogram with **b-1**, no SD/MD,

b-2, DA = 1°, **b-3**, DA = 6°, and **b-4**, DA = 12°. Tomographic phase maps with **c-1**, no SD/MD, **c-2**, DA = 1°, **c-3**, DA = 6°, and **c-4**, DA = 12°. **d**, Sectioned 2D phase map of the microbead under various illuminations.

4.1.3 Fourier domain analysis of speckle suppression

We conducted more detailed analysis of the optical diffuser effect in the spatial frequency domain (k-space). Three representative parameters have been investigated; (1) the background speckle noise, (2) the linewidth of the QPI carrier frequency, and (3) the Signal-to-noise ratio (SNR), (See Fig. 4.3a). The spatial frequency from 60 to 93 mm⁻¹ was selected for the background speckle noise analysis, and the spatial frequency from 35 to 60 mm⁻¹ around the QPI carrier frequency was selected for the linewidth of QPI carrier frequency and SNR analysis. Figure 4.3b shows the background speckle noise has been suppressed from -7.94 dB (for no SD/MD) to -9.30 dB (for SD/MD, DA = 12°). The suppression level is found to increase with DA. Therefore, the Fourier domain analysis shows the same trend as we see in the suppressed background noise in the interferograms in Fig. 4.2a. The linewidth of carrier frequency determines the attainable lateral resolution. The linewidth of the broad pedestal decreased from 5.36 mm⁻¹ (for no SD/MD) to 4.65 mm⁻¹ (for SD/MD, DA = 12°) (See Figs. 4.3c, 4.3d, and 4.3g) and the linewidth of the narrow coherent peak decreased from 0.349 mm⁻¹ (for no SD/MD) to 0.320 mm⁻¹ (for SD/MD, DA = 12°) (See Figs. 4.3c, 4.3e, and 4.3h). The reduced amount of 0.029 mm⁻¹ in the narrow coherence peak, however, is ~0.5% of the broad pedestal peak. This number may not be statistically significant, but it can be used as a good reference for the future experiments. The QPI carrier frequency in FFT domain which contain desirable information should not overlap with the zero frequency (DC

1 peak). Hence, the narrow linewidth of this QPI carrier frequency will allow the
 2 larger crop size around QPI carrier frequency which can increase the spatial
 3 resolution in the reconstructed phase map. This suggests that the narrow linewidth
 4 is contributing to the reduction of speckle background noise. The SNR was
 5 maintained within 2.1% of its original value ($\text{SNR} = 7$ for no SD/MD) without a
 6 significant change under different DAs (See Fig. 4.3i). This implies that the
 7 optical diffuser improves the raw interferograms, provides higher lateral
 8 resolutions and causes negligible SNR degradation to the QPI. The fringe
 9 visibility also remained in a useable range for the phase reconstruction.

11 **Figure 4.3** Spectral analysis of the speckle reduction in terms of the background noise,
 12 carrier linewidth, and signal-to-noise ratio. **a**, Schematic model for the analysis. **b**, FFT
 13 line profile of the interferogram under various illuminations. Dashed rectangle shows the
 14 region of interest. **c**, FFT line profiles of QPI carriers for broad and narrow peaks. The
 15 inset shows the magnified view of the narrow coherent peak. **d**, FFT line profile of the

1 broad pedestal in linear scale. **e**, FFT line profile of the narrow peak in linear scale. **f**,
2 Background noise level comparison. **g**, Broad pedestal linewidth comparison. **h**, Narrow
3 coherent peak linewidth comparison. **i**, SNR comparison.

4 **4.1.4 Quantitative analysis of background speckle suppression**

5 For the further analysis of the background speckle noise suppression, the QPI
6 interferograms without a sample were recorded under the same illumination
7 conditions as shown in Figs 4.4a-1, 4.4a-2, 4.4a-3, and 4.4a-4. The speckle noise
8 is suppressed when using an optical diffuser which can be clearly seen in the
9 cleaner interferogram. The larger the diffusion angle (DA) , the cleaner the
10 observed interferograms are. Similarly, in the Fourier domain, the larger DAs
11 also, provide cleaner backgrounds spectrum, as shown in Figs. 4.4b-1, 4.4b-2,
12 4.4b-3, and 4.4b-4. The standard deviation of the spatial phase noise decreases
13 from 0.12 rad (≈ 170 nm) (for no SD/MD) to 0.05 rad (≈ 70 nm) (for SD/MD,
14 $DA = 12^\circ$), as shown in Figs. 4.4c-1, 4.4c-2, 4.4c-3, and 4.4c-4. In the analytical
15 procedure, the cropped spectral range around the carrier frequency are considered
16 to be the filter of background speckle noise so that the QPI will be insensitive to
17 this noise. This result confirmed that the optical diffuser can suppress the
18 background speckle noise (by 2.4 times) in the QPI.

Figure 4.4 Comparison of background spatial phase noise in various illumination with no sample in the FOV. **a1-a4**, interferograms of the background area obtained using the QPI setup for various illumination. **b1-b4**, FFT plot of the interferogram for various illuminations. **c1-c4**, 2D spatial phase map of the background obtained using the QPI setup for various illuminations. Various illuminations: 1) No SD/MD, 2) SD/MD, DA = 1°, 3) SD/MD, DA = 6°, and 4) SD/MD, DA = 12°.

4.1.5 Speckle suppression in biological images

To demonstrate that our technique can be applied for biomedical applications, we imaged a human breast cancer cell (MCF-7) as shown in Fig. 4.5. MCF-7 breast cancer cells were imaged using the QPI system with the same conditions; without optical diffuser and with three diffusion angles (DA) of the optical diffusers (SD/MD, DA = 1°, 6°, and 12°). The recorded interferograms of all condition are shown in Figs. 4.5a-1, 4.5a-2, 4.5a-3, and 4.5a-4. As a result, the interferogram of MCF-7 gets clearer when the optical diffuser is used. In addition, the cleanest interferogram are created by the optical diffuser with diffusion angle of 12°

1 (SD/MD, $DA = 12^\circ$). The reconstruction phase maps are also presented here for
2 each condition, as shown in Figs. 4.5b-1, 4.5b-2, 4.5b-3, and 4.5b-4. Specific part
3 of cell is selected to have a close-look at the consequence of speckle noise
4 reduction as shown in Figs. 4.5c-1, 4.5c-2, 4.5c-3, and 4.5c-4. The higher DA of
5 optical diffuser provides a clearer and sharper edge of the cell. Likewise, small
6 granular objects inside the cell can be clearly visualized. This reveals the
7 improvement of the visibility by suppressing the background speckle noise.
8 Again, higher DAs lead to observed detail of the cell. As a consequence, this
9 result offers the potential application of speckle noise suppression via optical
10 diffuser for biological applications, such as morphology studies, motile behavior
11 and cell interaction.

12
13 **Figure 4.5** Interferograms and topographic phase maps of the biological cells obtained
14 by a speckle-suppressed QPI. **a**, QPI images of MCF-7 breast cancer cell at various
15 illuminations. **b**, Phase maps of the cancer cell. **c**, Magnified tomographic phase maps
16 showing the higher resolution with the high DA.

4.2 FCR-QPI for high-visibility, high-speed, spectroscopic phase measurement with a high phase stability

4.2.1 3D coherence cloud of the frequency comb

Using our measurement data (See **Methods Sec. 3.2.6**), we conducted 3D surface fitting using polynomial functions and extracted 2D plots of the cloud with the axis of the spatio-temporal coherence and speckle contrast (See Figs. 4.6a to 4.6d). Figures 4.6a-1 and a-2 shows a 3D spatio-temporal coherence cloud with three axes, the spatial coherence (V), temporal coherence (L_c), and speckle contrast (C), and its 2D projection, realized by our coherence control. The spatial coherence was quantified here by the visibility, V of the interferogram. The temporal coherence was quantified by the coherence length, L_c . The speckle level was calculated via the contrast, C , which is calculated by $C = SD_I / \langle I \rangle$, where SD_I is the standard deviation of the overall intensity, and $\langle I \rangle$ is the averaged intensity for each background image (See **Methods Sec. 3.2.6**). For a better light source for various biomedical imaging application, it has been difficult to fulfill the spatiotemporal tunability requirements with a single light source; however, we successfully filled this gap by the use of a frequency comb and the coherence control unit (laser speckle reducers and band-pass filters). For comparison, we have summarized state-of-the-art light sources according to their spatio-temporal coherence and plot along with our spatiotemporal coherence cloud (See Figs. 4.6b, 4.6c, and 4.6d). Continuous-wave lasers exhibit high coherence leading to extremely high speckle contrast which hinders the sensitive phase imaging. Super-luminescent light emitting diodes (sLED), super-luminescent laser diodes (SLD), and random lasers exhibit high spatial coherence and moderate speckle

1 contrast ($C \leq 0.5$), which is still not low enough for highly sensitive phase
2 imaging. Light emitting diode (LED) has a low spatial coherence hence low
3 speckle noise but it also exhibits low visibility interference patterns leading to an
4 unsuccessful phase reconstruction. The micro-LED (mLED) has higher spatial
5 coherence than the LED; however, it is not high enough to form high-visibility
6 interferograms. These precedent light sources occupy specific spots in the
7 coherence cloud and each owns its strength for a specific imaging application.
8 The optimization of temporal and spatial coherences of the illumination is
9 possible to form the best image quality. A single frequency comb, instead, could
10 cover a wider 3D space of the coherence cloud as shown by the purple dots in
11 Figs. 4.6a to 4.6d; the tunable ranges for temporal coherence is from 8.1 to 56.3
12 μm , spatial coherence is from 0.63 to 0.98 V, and the speckle contrast is from
13 0.29 to 0.91. By simply tuning the spatio-temporal coherences and speckle levels
14 of a frequency comb, one can improve the quality of the QPI interferograms for
15 their applications.

Figure 4.6 Spatial coherence, temporal coherence, and speckle contrast provided by this study and prior light sources. **a-1**, 3D coherence cloud of the frequency comb with three axes: the temporal coherence (L_c), spatial coherence (V), and speckle contrast (C) and **a-2**, its 2D projection. For comparison, state-of-the-art light sources are displayed together at three cross-sectional views: **b**, Speckle contrast vs. temporal coherence, **c**, Speckle contrast vs. spatial coherence, **d**, Temporal coherence vs. spatial coherence.

4.2.2 The effect of LSR on the illumination beam in time- and radio-frequency (RF) spectral domains

Figure 4.7a shows near-infrared supercontinuum of the frequency comb covering a broad spectral range of 620-980 nm (spectral bandwidth ≈ 360 nm). To control the temporal coherence, we installed the band-pass filters (BPF) with various spectral bandwidths ($\lambda_{\text{center}} = 750$ nm, BW = 40, 25, 10 nm) in the illumination path (See Figs. 4.7b-1, 4.7b-2, and 4.7b-3). To control the spatial coherence hence

1 to suppress the speckle, we placed laser speckle reducers (LSR) after the BPFs.
2 The effect of the LSRs was characterized in both, the time and radio-frequency
3 (RF) domains, (See Figs. 4.7c, 4.7d, 4.7e, and 4.7f). Three illumination
4 conditions were considered (i) a frequency comb without LSR (See Fig. 4.7c),
5 (ii) a continuous-wave laser diode with LSR (See Fig. 4.7d), and (iii) a frequency
6 comb with LSR (See Fig. 4.7e). For the first illumination condition (i.e.,
7 frequency comb without LSR), the ultra-short pulses from the frequency comb
8 were directly delivered to a highly sensitive avalanche photodetector (APD
9 C5658, Hamamatsu) through our system with the absence of the sample. A
10 periodical signal, which was monitored by an oscilloscope, showed a pulse
11 spacing of 4 ns in Fig. 4.7c-1. This corresponds to the repetition rate of 250 MHz,
12 which was detected by a spectrum analyser (See Fig. 2c-2). Figure 2c-3 provides
13 a finer resolution of the 250 MHz RF linewidth (with a 20-kHz span and a 10-Hz
14 RBW) with a signal-to-noise ratio (SNR) of 70 dB. For the second illumination
15 (i.e., laser diode with LSR), light from the continuous-wave laser diode was
16 focused onto the activated LSR. LSR was composed of micro-optic diffusers
17 embedded in a dielectric polymer. As the LSR is activated, micro-particles were
18 oscillating (~ 300 Hz) and scattered the incoming light. The scattered light was
19 delivered through our system and detected by an amplified Si photodetector
20 (PDA36A-EC, Thorlabs). Figure 4.7d-1 shows the oscillating signal of the LSR
21 in time domain with a period of 3.37 ms. Due to the lower frequency limit of the
22 RF spectrum analyser, we characterized the signal in the frequency domain using
23 Fast Fourier Transform (FFT) (See Fig. 4.7d-2). Figure 4.7d-2 shows the
24 fundamental frequency of the oscillation (297 Hz) and harmonic frequencies of
25 the LSR harmonics, by definition, are integer multiple of the fundamental

1 frequency (See Fig. 4.7d-3). For the final illumination condition (i.e. frequency
2 comb with LSR), the ultra-short pulses from the frequency comb were focused
3 onto the activated LSR then the scattered light was detected by an APD for time-
4 domain analysis. In the time domain, the output signal showed variations in
5 amplitude at the constant period of the frequency comb's pulses (i.e. 4 ns) (See
6 Fig. 4.7e-1). It suggests that this amplitude variation came from the dynamic
7 signal of the oscillating LSR adds to the signal of the frequency comb. This was
8 supported by the RF frequency spectrum, which showed the harmonic
9 frequencies from the LSR (with the spacing of 297 Hz) appearing within the
10 original frequency from the frequency comb (at 250 MHz) (See Figs. 4.7e-2 and
11 4.7e-3). The temporal amplitude variation also appeared in the frequency peaks
12 of the frequency comb in the frequency domain as shown in Fig. 4.7f. This
13 demonstration reveals that there is an effect of LSR added to the signal from the
14 frequency comb in time and RF spectral domains which caused the speckle
15 suppression.

Figure 4.7 The effect of LSR on the illumination beam in time- and radio-frequency (RF) spectral domains. **a**, Spectrally broadened frequency comb. **b**, Band-pass-filtered spectrum with different spectral bandwidths of **b-1**, 40 nm, **b-2**, 25 nm, and **b-3**, 10 nm. **c, d, e**, Time-domain and RF-domain analysis of coherence-controlled frequency comb. Time-domain signals of **c-1**, frequency comb without LSR, **d-1**, continuous-wave (CW) laser diode (LD) with LSR, and **e-1**, frequency comb with LSR. **c-2, d-2, e-2**, Frequency-domain signals of **c-2, c-3**, frequency comb's repetition rate and harmonics, **d-2, d-3**, CW laser diode with LSR, and **e-2, e-3**, frequency comb with LSR. **f**, Temporal amplitude variation in the frequency domain of the frequency comb with LSR.

4.2.3 The effect of coherence tunability on an interferogram

To demonstrate that our coherence control can provide high-phase sensitivity and high visibility interferograms, we illuminated a transparent polystyrene microbead with various spatio-temporal coherences of the frequency comb by tuning the bandwidths (BW) of the band-pass filters (BPF) and diffusion angles

1 (DA) of the laser speckle reducers (LSR). The resulting phase reconstructions at
 2 various spatio-temporal coherences of illumination are shown in Figs. 4.8a to
 3 4.8d. The temporal coherence, L_c was tuned from 8.1 μm (no BPF) to 56.3 μm
 4 (BPF, BW = 10 nm), while the spatial coherence, V was tuned from 0.98 (BPF,
 5 BW = 10 nm, no LSR) to 0.63 (no BPF, LSR, DA = 12°). Phase reconstruction
 6 results were also compared with those acquired with a continuous-wave (CW)
 7 laser diode with a temporal coherence length of ~20 mm as shown in Fig. 4.8e.
 8 Severe phase discontinuity was found when using frequency comb without the
 9 band-pass filter (See Fig. 4.8a). Typically, the interference pattern is only
 10 observed when the path length or time difference between two beams (reference
 11 and sample beams) matched within the coherence length of the light. Therefore,
 12 for light sources with short coherence length (i.e., a frequency comb without
 13 band-pass filter), the interference patterns are visible in a smaller field compared
 14 to long coherence length (i.e., a frequency comb with band-pass filter having
 15 narrower bandwidth). When the frequency comb was used to illuminate the
 16 polystyrene microbead without the band-pass filters, the interference patterns
 17 occupied a small area (i.e., microbead's area) rather than covered a full field-of-
 18 view. Therefore, the phase retrieval cannot be successfully performed over full
 19 field-of-view; hence the phase discontinuity in Fig. 4.8a. The successful phase
 20 reconstructions were found in the frequency comb when using the band-pass
 21 filters (See Figs. 4.8b to 4.8d) and in the CW laser diode (See Fig. 4.8e) because
 22 the longer temporal coherence length (L_c) in those illuminations enhances high-
 23 visibility of the interferograms for the successful phase reconstruction in a wider
 24 field-of-view. Besides, the spatial coherence was controlled by the laser speckle
 25 reducer to improve the quality of the topographic phase map by reducing the

1 background speckle noise. To quantify the background speckle noise, we
2 calculated the standard deviation (SD) of the background area in the topographic
3 phase map of all illuminations (See Figs. 4.8a to 4.8e). Standard deviation was
4 found to be lowest ($SD = 0.189$) in the frequency comb with band-pass filter
5 (bandwidth of 10 nm), and with laser speckle reducer (diffusion angle of 12°) as
6 shown in Fig. 4.8d. In the CW laser diode, the lowest standard deviation was
7 found to be 0.204 when using the laser speckle reducer with diffusion angle of 6°
8 (See Fig. 4.8e).

9 **Figure 4.8** Coherence tunability analyzed by using an interferogram (without the
10 simulated speckles). Phase reconstruction of polystyrene beads illuminated by **a, b, c, d**,
11 a coherence-controlled frequency comb and **e**, a continuous-wave (CW) laser diode
12 without the use of scattering film.

14 In order to mimic the cells in solutions as in lab-on-a-chip devices or in our
15 body, a scattering film (polycarbonate film, $\rho = 1.2 \text{ g/cm}^3$) was installed before
16 the polystyrene microbeads ($D = 10 \pm 0.66 \mu\text{m}$, manufactured by SPI supplies)

1 before illuminated by various spatio-temporal coherence of the frequency comb.
2 Figures 4.9a to 4.9e show the resulting topographic phase maps of the microbead
3 at various spatial and temporal coherences of illuminations. Phase reconstruction
4 results were also compared with those acquired with a continuous-wave (CW)
5 laser diode. Firstly, without the temporal coherence control (i.e., frequency comb
6 without band-pass filter), the low temporal coherence of a frequency comb made
7 the interferogram not successfully formed over the full field-of-view as shown in
8 Figs. 4.9a and 4.9b. Secondly, without the spatial coherence control, the coherent
9 speckle caused background phase distortion, so the phase reconstruction could
10 not be successful; the non-natural phase discontinuity was clearly observed in
11 first two rows, Figs. 4.9a to 4.9d. With a continuous-wave laser diode, coherent
12 speckle is even more pronounced, so the phase profile cannot be reconstructed
13 even with the spatial coherence control, as shown in Fig. 4.9e. The best coherence
14 regime for this case study was temporal coherence length (L_c) of 56.3 and spatial
15 coherence (V) of 0.73. The higher L_c promotes higher visibility in interferograms
16 but induces higher level coherent speckles; these coherent speckles were
17 effectively suppressed in our FCR-QPI by using laser speckle reducer (V of 0.73)
18 without a significant degradation of the temporal coherence. The physical 3D
19 information of the polystyrene bead was successfully extracted from the
20 interferogram in this best coherence regime, which is the critical advantage of
21 QPI over conventional 2D imaging techniques. The calculated height of the bead
22 is 9.9 μm (RI of the microbead: 1.579; RI of the immersion oil: 1.493 at $\lambda = 750$
23 nm) which matches well to the specification diameter of the microbeads with a
24 1% error (the variation of the microbead's size is 0.66 μm (in standard deviation))
25 Our FCR-QPI could control the coherence regime so as to tailor the best quality

- 1 interferogram over a large field-of-view for successful phase reconstruction even
 2 under a highly scattering medium.

3
 4 **Figure 4.9** Coherence tunability analyzed by using an interferogram (with the simulated
 5 speckles). Topographic phase maps of PS beads illuminated by **a, b, c, d**, a coherence-
 6 controlled frequency comb and **e**, a continuous-wave (CW) laser diode with the use of
 7 scattering film.

8 4.2.4 Multi-wavelength QPI

9 In this study, the multi-wavelength QPI scheme was employed to monitor the
 10 changes in cellular morphology, cellular volume, and intracellular RI. three
 11 wavelengths were filtered from a frequency comb ($\lambda_{\text{centre}} = 700, 750, \text{ and } 800 \text{ nm}$)
 12 and used to illuminate MCF-7 human breast cancer cells (See Fig. 4.10a). The
 13 optical phase delay (OPD (unit: radian)) at each wavelength can be described by
 14 $\Delta\phi(x,y;\lambda) = (2\pi/\lambda) \cdot \Delta n(\lambda) \cdot h(x,y)$, where Δn is the RI difference between the cell
 15 and culture media, and h is the cellular physical height. Time-lapse

1 interferograms and reconstructed topographic phase maps at $\lambda = 700$ nm (See
 2 Figs. 4.10b-1 to 4.10b-5), at $\lambda = 750$ nm (See Figs. 4.10c-1 to 4.10c-5), and at λ
 3 $= 800$ nm (See Figs. 4.10d-1 to 4.10d-5) show the change in morphology and
 4 OPD of a MCF-7 cell within 9 hours. At early stages, the cell exhibited a
 5 polygonal shape. After an hour of observation, the cell began to shrink and
 6 adopted a round shape. This phenomenon of cell shrinkage happens from the
 7 absence of certain survival environmental factors and was reported to be detected
 8 at the early stage of oncosis, accidental cellular death [182, 183]. The damaged
 9 cells fail to maintain the required ion concentrations, so that the extracellular
 10 fluids enter the cell. This ultimately causes the cellular volume to increase (See
 11 **Appendix E**). After a 7-hour observation, cell membrane releases the
 12 intracellular contents, which results in the OPD to decrease at a 9-hours of
 13 observation (See Figs. 4.10b-5, 4.10c-5, and 4d-5). These alterations of the
 14 cellular morphology and OPD show a good agreement with the results by K.
 15 Creath *et al.* [184]. We also analysed our coherence-control method for higher-
 16 sensitivity cellular imaging. Without the use of LSR in the illumination path, high
 17 speckle noise appeared in the raw interferograms which leads to a discontinuity
 18 in the topographic phase maps (Fig. 4.10e-1 to Fig. 4.10e-5). The cellular
 19 refractive index correlation to the intracellular mass and concentration was
 20 evaluated as refractive index ratio (RI ratio). We calculated the ratio between two
 21 refractive indices measured at different wavelengths (700 and 800 nm) (See
 22 **Methods Sec. 3.2.3** for details). Time-lapse RI ratio maps are shown along with
 23 cellular phase maps in Fig. 4.10g-1 to Fig. 4.10g-6 and Fig. 4.10f-1 to Fig. 4.10f-
 24 6. The RI ratio inside the cell varied between 0.92-1 which is higher than the RI
 25 ratio of surrounding medium (0.8 - 0.9). These results agree well with the

1 previous report [31]. In addition, the results show that the RI ratio of the cell
2 decreased over time (from 0 to 9 h). Since the RI of cell is mostly represented by
3 the cellular organelles or biochemical components (i.e., protein); the decreasing
4 of the RI together with the morphology disruption can be evidence of cellular
5 death. To provide more quantitative analysis, the optical volume and the
6 integrated RI ratio were calculated by integrating the optical path difference
7 (optical volume, unit: μm^3) and RI ratio over the cell. Figure 4.10h shows that the
8 integrated cellular RI ratio decreases drastically at early 20 minutes, whereas the
9 optical volume increases in the meantime; the optical volume increase is made
10 by the cellular swelling; the RI ratio decrease is due to the intracellular protein
11 dilution and damages in cellular organelles. After the initial RI ratio decrease, the
12 RI ratio continues to decrease by 77.3 % over 9 hours. These results show that
13 the RI ratio can be used to monitor cellular degradation without prior knowledge
14 of cellular RIs or dispersion curves. Multiple wavelengths of multiple CW lasers
15 are commonly used for spectroscopic phase measurements; however, they suffer
16 from high speckle noise [106]. The frequency comb which enables spectroscopic
17 phase measurement within a single light source with speckle suppression can be
18 utilized for highly-sensitive cellular analysis. Frequency combs provide better
19 measurement precision/stability than CW laser in a long-term measurement
20 (refers to **Sec. 4.2.6.**).

Figure 4.10 Phase-coherent multi-wavelength QPI for monitoring intracellular degradation. **a**, Experimental configuration. Time series of interferograms and the reconstructed topographic phase maps of a MCF-7 breast cancer cell illuminated by three wavelengths of the frequency comb with LSR; the data from **b1-b5**, 700 nm, **c1-c5**, 750 nm, and **d1-d5**, 800 nm. **e**, the data from 800 nm without LSR. Dynamic changes in cellular morphology and optical phase delay were measured over 9 hours. **f1-f6**, Time series of phase maps of the cell at 750 nm, and **g1-g6**, RI ratio between RIs at two wavelengths (700 nm and 800 nm). **h**, Integrated optical path difference (optical volume) and RI ratio over time.

4.2.5 Dynamic QPI

High-speed dynamic motions of micro-objects; an optical fibre and microspheres are measured here up to 16.9 kHz as shown in Fig. 4.11. A single-mode optical fibre has a higher refractive index of core ($n_{\text{core}} = 1.452$; $D_{\text{core}} = 8.2 \mu\text{m}$) and a lower refractive index of cladding ($n_{\text{cladding}} = 1.447$; $D_{\text{cladding}} = 125 \mu\text{m}$). The difference of the refractive index will create the difference of OPD at different positions (core, cladding-core, and cladding). (See Fig. 4.11a). When a PZT modulates the lateral position of an optical fibre by $\sim 10 \mu\text{m}$ in a background medium ($n_{\text{background}} = 1.443$), the OPD varies at the modulation frequency ($f = 500 \text{ Hz}$) (See Fig. 4.11a-I). When the central position of the PZT modulation was shifted, the RIs of the core, cladding, and background medium showed different phase modulation amplitudes as shown in Figs. 4.11a-I to 4.11a-V; these amplitudes match well with the theoretical RI values with a 6.4% error (See **Appendix D** for more details). At the core position, the modulation frequency was doubled with a lower modulation amplitude, which is because the PZT modulation depth is larger than the optical fibre's core diameter. To demonstrate the high-speed measurement capability, PZT's modulation frequency was tuned up to 4 kHz. Figure 4.11b-I shows the measured phase modulation amplitudes at different frequencies (from 1 Hz to 4 kHz), which clearly shows the typical resonance spectrum of a PZT; the first resonance peak of PZT was located at 370 Hz. Figure 4.11b-II and 4.11b-III show the phase modulation at 200 Hz in the time- and frequency-domains. Harmonic components are observed at the integer multiples of 200 Hz, which is because of imperfect sinusoidal modulation. Due to the PZT's mechanical limitation, the highest phase-modulation signal was tested at 4 kHz which shows higher harmonics up to 12 kHz (See Figs. 4.11b-IV

1 and 4.11b-V). Even higher frequency motions of micro-particles up to 16.9 kHz
2 were measured as shown in Fig. 4.11c. Micro-spheres ($D = 10 \mu\text{m}$) were bonded
3 onto the top surface of a dielectric elastomer actuator (DEA) having four
4 electrodes around the circumference. With an appropriate oscillation, the micro-
5 spheres circulate around the DEA's centre with a frequency of $\sim 297 \text{ Hz}$. The
6 phase modulation of several oscillating micro-spheres was detected as shown in
7 Fig. 4.11c-I. By performing FFT, we could observe many high order harmonics
8 of 297 Hz up to 16.9 kHz. (See Fig. 4.11c-II).

9

10 **Figure 4.11** FCR-QPI for monitoring high-speed dynamic motions. Sinusoidal position
11 modulation by PZT results in the lateral movement of the optical fibre. Phase modulation
12 measured at different centre positions: when laser beam is on **aI-1**, cladding (left), **aI-2**,
13 cladding-core boundary (left), **aI-3**, core, **aI-4**, cladding-core boundary (right), and **aI-5**,
Results and discussion

cladding (right). **b-I**, Dynamic phase motion of an optical fiber (from 1 Hz to 4 kHz) Time- and frequency-domain analysis at 200 Hz and 4 kHz: **b-II**, 200-Hz time-domain, **b-III**, 200-Hz frequency-domain, **b-IV** 4-kHz time-domain, and **b-V**, 4 kHz frequency-domain. **c**, Dynamic phase motions of rotating micro-spheres: **c-I**, time-domain signal, and **c-II**, frequency-domain signal.

4.2.6 Phase stability measurement

Phase stability-enhanced-measurement precision of FCR-QPI when using different light sources; a continuous-wave laser diode (LD), a femtosecond pulse laser without frequency stabilization (FS) and a frequency comb (FC) was quantitatively analysed. We first analysed the phase stability using the interferograms obtaining by using the CW LD and FC. Figures 4.12a-1 and 4.12b-1 display the magnified view at background area of the interferograms obtained using FC and CW LD, respectively. A single pixel of the time-lapse interferogram was chosen to analyse the phase noise or phase instability as shown in Figs. 4.12a-2 (for FC) and 4.12b-2 (for LD). The standard deviations (*SD*) of the phase noise were calculated as 0.058 for the FC and 0.551 for the LD. The higher *SD* in LD shows the higher noise occurring in the long-term measurement. We also characterized the phase noise in the frequency domain by performing FFT. As a result, the spectral power densities of LD and FC are shown in Figs. 4.12a-3 (for FC) and 4.12b-3 (for LD). The average background noise of the FC (-9.15 dB) was lower than the LD (-7.34 dB). This lower background noise in the FC enhances the SNR in the phase image. Additionally, the phase noise was analysed using Allan deviations as shown in Fig. 4.12c. Four illumination conditions were studied; FC illuminated onto the sample (FC Mea.) and without the sample (FC Ref.), LD illuminated onto the sample (LD Mea.), and without

1 the sample (LD Ref.). Allan deviations for the FC and the LD decreased over
2 averaging time of 2 s, then began to increase afterward over averaging time of
3 100 s. Allan deviations of the LD and the FC without the sample (LD Ref. and
4 FC Ref.) decreased to approach their minimum value of 13.7×10^{-3} (for LD) at
5 averaging time of 5 s, and 5.19×10^{-3} (for FC) at averaging time of 10 s. It then
6 began to increase afterward and reached 55.4×10^{-3} (for LD) and 14.14×10^{-3} (For
7 FC) over an averaging time of 100 s. The lower Allan deviation of the FC will
8 promote higher phase stability in a long-term measurement.

9 For higher update rates, Allan deviations at various averaging times for the
10 following three light sources: a CW LD, a femtosecond pulse laser without
11 frequency stabilization (FS), and a frequency comb (FC) with full frequency
12 stabilization were analysed as shown in Fig 4.12d. Light from those light sources
13 was delivered to our FCR-QPI setup; then the signal was recorded by a PD and
14 oscilloscope (Rigol, DSA6102) for the period of 700 s. Allan deviations of the
15 LD, FS, and FC decreased until the averaging interval of 1 s, then it began to
16 increase for the longer averaging intervals due to the environmental drift (See
17 Fig. 4.12d). The lowest value of Allan deviation (2.39×10^{-3}) was found in the FC
18 (FC Ref.) at the averaging time of 10 s which is better than the lowest value of
19 Allan deviations of FS (3.39×10^{-3} , 10-s averaging) and LD (4.17×10^{-3} , 10-s
20 averaging). For the longer averaging intervals, the Allan deviation for FC slowly
21 increased until it was 6.26×10^{-3} (for Ref.) at 100 s. Meanwhile, for FS and LD,
22 the Allan deviations dramatically increased to 8.41×10^{-3} (for Ref.) and 44.10×10^{-3}
23 (for Ref.) at 100 s, respectively. This result indicates that FC provides long-term
24 phase stability comparing to FS and LD due to its direct stabilization to a Rb
25 atomic clock. Depending on the dynamic motion of interest, our technique can

1 provide the update rates up to 10 MHz by using a high-speed PD; the ultimate
2 speed is set by the photodetector's bandwidth. The green filled regime in Figs.
3 4.12c and 4.12d are a newly enabled stability area by our FCR-QPI. The Allan
4 deviations in Fig. 4.12d are slightly different from the previous results as shown
5 in Fig. 4.12c. because of the different environmental perturbations. However, the
6 results from both detectors show good promises for utilizing the frequency comb
7 for measurement.

8
9 **Figure 4.12** Analysis of coherent speckle in an interferogram and temporal phase
10 instability. **a-1, b-1**, Magnified view of the background interferograms imaged by the
11 frequency comb (FC) and CW laser diode (LD). **a-2, b-2**, Time trace of phase instability
12 of the FC and LD obtained from a time-series interferogram. **a-3, b-3**, Spectral power
13 density of the interferogram imaged by the FC and LD. **c**, Allan deviation of phase
14 instability in an interferogram of FC and LD with the sample (Mea.) and without the
15 sample (Ref.). **d**, Allan deviation of phase instability at high update rate of frequency

1 comb (FC), femtosecond laser (FS) and CW laser diode (LD) with the sample (Mea.)
2 and without the sample (Ref.).

3 **4.3 Measurement of heart rate and blood flow in** 4 **zebrafish by using FCR-QPI**

5 **4.3.1 Imaging of red blood cells**

6 We have demonstrated that FCR-QPI enables non-invasive, high-speed
7 measurement of zebrafish's RBCs flow with high spatial and temporal resolution.
8 Since the understanding of zebrafish's cardiovascular function will help scientists
9 to explore potential cardiovascular drugs and therapies, which could be used in
10 humans. Here, we conducted a further study of zebrafish's blood flow in various
11 fish ages using our FCR-QPI technique. In addition, we also studied the dynamic
12 motions of zebrafish's heart. Two detectors, a CCD camera and a high-speed PD
13 were used in this study. A CCD camera was used to capture an interferogram of
14 the RBC flow at 48 hpf as shown in Fig. 4.13a. Figure 4.13a shows the process
15 to obtain a reconstructed phase map of RBC consisting of FFT, cropping and
16 filtering, inverse FFT, and phase unwrapping. The resulting topographic phase
17 map was magnified to show the optical phase delay (OPD, unit:rad) of the RBC.
18 Time-series phase maps of the RBCs flow in a blood vessel were analysed from
19 time-series interferograms as shown in Fig. 4.13b. Different OPD of RBC in each
20 frame results from non-uniform layers of the blood vessel at each position that
21 the RBC passed. This result indicates that FCR-QPI provides good-quality
22 interferogram which could enable us to monitor the dynamic motions even at a
23 cellular level.

Figure 4.13 QPI interferogram and topographic phase maps of RBC flow. **a**, Process to reconstruct a topographic phase map of RBC showing its optical phase delay (OPD). **b**, Time-series phase maps showing RBC flow in the blood vessel.

4.3.2 Dynamic motion of zebrafish's heart

FCR-QPI was employed to monitor the heart development and measure the heart rates of the zebrafish. A CCD camera was used to obtain the morphology of the zebrafish's heart, whereas the dynamic motions of the heart were measured by a high-speed photodetector. Figures 4.14a and 4.14b show the phase images of a heart tube at 24 hpf and a chambered heart at 48 hpf (pink-drawing area). Figure 4.14c-I shows the phase modulation signal originating from the heart contraction and relaxation at 24 hpf. By performing the FFT, the frequency of the signal is obtained as 1.56 Hz which corresponds to the heart rate of 93 bpm (beats-per-minute) (See Fig. 4.14c-II). The signals of heartbeat in the zebrafish at 48 hpf

1 were measured at different positions; atrium (A), atrioventricular (AV) canal, and
2 ventricle (V) (See Figs. 4.14d-1, 4.14d-2, and 4.14d-3). Time-span of the signal
3 at the ventricle is larger than the atrium because of the larger volume of the
4 ventricle. At the AV canal, both signals from the atrium and ventricle were
5 combined and detected. We measured the heart rates in various fish ages as shown
6 in Fig. 4.14e. At an early stage of development, heart rate increased from 94 bpm
7 (24 hpf) to 173 bpm (50 hpf). Then, it began to decrease until it was 122 bpm at
8 56 hpf. Noteworthy, the rate of heart not only depends on the ages but also the
9 surrounding temperatures. We performed this experiment at the temperature of
10 28°C. The study of heart motions can be used to evaluate the abnormality of heart
11 rhythm which known as cardiac arrhythmia.

12
13 **Figure 4.14** FCR-QPI for measuring the dynamic motions of zebrafish's heart. Phase
14 image of zebrafish's heart at **a**, 24 hpf and **b**, 48 hpf. **c**, Periodic signals of heart beat at
15 24 hpf analysed in **c-I**, time, and **c-II**, frequency domains. Periodic signals of heart beat

1 at 48 hpf measured at **d-1**, atrium, **d-2**, A-V canal, and **d-3**, ventricle. **e**, Heart rate
2 measured at various fish ages

3 **4.3.3 Blood flow measurement**

4 The dynamic flow of RBCs in the blood vessel was monitored in real time using
5 a CCD camera and a high-speed PD. At 24 hpf, blood flows in two main vessels;
6 dorsal aorta (DA) and postcardinal vein (PCV). In this stage, RBCs flow
7 individually inside the vessel as shown in schematic Fig. 4.15a which enables the
8 measuring of individual RBC. Figures 4.15b-1 and 4.15b-2 show bright-field and
9 phase images of the DA and PCV located at the middle trunk of the zebrafish.
10 Figure 4.15c-I shows the phase change of RBC monitored at a high-speed
11 photodetector made by RBCs passing through our point of interest. A phase
12 profile of individual RBC was retrieved as shown in Fig. 4.15c-II. Blood flow in
13 the zebrafish's vessel at this stage can be quantified as RBC velocity. The RBCs'
14 mean diameter estimated from the CCD's image was $\sim 10.9 \mu\text{m}$. Blood flow
15 velocity or RBC velocity was calculated by knowing the distance of RBCs travel
16 in a single frame of the CCD camera. Blood flow velocity increased with the
17 zebrafish development as shown in Figs. 4.15d and 4.15e. Figures 4.15e-I and
18 4.15e-II show mean RBC velocity in the DA and PCV at 24, 26 and 28 hpf. It
19 shows that the velocity was higher in the older fish ($192.5 \mu\text{m/s}$ for 24 hpf, 320.4
20 $\mu\text{m/s}$ for 26 hpf, and $608.8 \mu\text{m/s}$ for 28 hpf). The velocity of RBC flow in the DA
21 was higher than in the PCV. The maximum blood flow velocities in the DA and
22 PCV were also measured and shown similar trends (See Figs 4.15f-I and 4.15f-
23 II).

Figure 4.15 Blood flow development in zebrafish from 24 to 28 hpf. **a**, Schematics of the measurement of RBC flow in a blood vessel. **b-1**, Bright-field and **b-2**, phase images of DA and PCV located at the middle trunk. **c-I**, Dynamic phase motions of RBCs flow in DA. **c-II**, A phase profile of a single RBC. Mean flow velocities in **d-I**, DA, and **d-II**, PCV, and **e**, maximum flow velocities in **f-I**, DA and **f-II**, PCV at 24, 26, and 28 hpf.

At 48 hpf, the chambered heart creates a pulsatile blood flow with an increase in the number and blood flow velocity as shown in Fig 4.16a. Figures 4.16b-I and 4.16c-I show phase modulation of the pulsatile blood flows in DA and PCV of 48 hpf zebrafish, respectively. The groups of RBC flow with an average time interval of 0.452 s in the DA. There are several RBCs in a magnified blood pulse as shown in Figs. 4.16b-II and 4.16c-II. In the DA, the signals of the blood pulses were compressed and no presence of the RBC peaks between each

1 blood pulse. In contrast, the blood pulses in PCV were extended and had the RBC
2 peaks between each blood pulse. To conduct more quantitative analysis, number
3 of RBCs in a single blood pulse and blood pulse ratio were quantified as shown
4 in schematic Fig. 4.16d. The number of RBCs was simply realized by counting
5 the number of the RBC peaks, while the pulse ratio was obtained by dividing the
6 amplitude of Gaussian envelope of the signal with the amplitude of baseline
7 (signal where there is no RBC). The average number of RBCs in one pulse and
8 average pulse ratio were measured at the middle trunk (DA and PCV) and tail
9 vessels (CA and CV). Caudal artery (CA) and caudal vein (CV) are designated
10 name of the DA and PCV located at the tail portion. The number of RBCs and
11 pulse ratio were highest in the DA ($n = 16$, pulse ratio = 28.5) and lowest in the
12 PCV ($n = 10$, pulse ratio = 5.5) as shown in Figs. 4.16e and 4.16f. The blood
13 pressure will decrease with increasing traveling distance from the heart (e.g.,
14 blood travels with the shortest distance to the dorsal aorta, so it has the highest
15 blood pressure) [188]. With higher blood pressure (represented by the pulse
16 ratio), the number of RBCs passing through our point of interest will be also
17 higher. This result shows that FCR-QPI provides a quantitative assessment of the
18 high-speed RBCs flow in a developing zebrafish. Moreover, the measurement
19 precision at a cellular level can be achieved by our FCR-QPI without the use of
20 any contrast agents or multiple preparation steps.

Figure 4.16 Blood flow measurement in zebrafish at 48 hpf. **a**, Schematics of the RBC flow in blood vessel at 48 hpf. Phase modulation of pulsatile RBCs flow in **b-I**, DA, and **c-I**, PCV. Individual RBC-induced phase modulations within a single heartbeat of **b-II**, DA, and **c-II**, PCV. **d**, Schematics for quantifying the number of RBCs and pulse ratio **e**, Average number of RBC, and **f**, pulse ratio measured in DA, CA, CV, and PCV.

Chapter 5

Conclusions and recommendations

3

4

5.1 Conclusions

5.1.1 Speckle suppression for phase-sensitive QPI

7 In this study, we employed a rotational optical diffuser to suppress the speckle
8 noise of a coherent illumination in off-axis common-path QPI. Continuous-wave
9 laser diode ($\lambda = 638$ nm) was used as a coherence laser source. The QPI unit was
10 comprised of Wollaston prism, half-wave plate ($\lambda/2$), and linear polarizer which
11 could offer high-temporal stability and high-acquisition rate. The speckle was
12 reduced by controlling the spatial coherence of the illumination using the optical
13 diffuser with various diffusion angles ($DA = 1^\circ$, 6° , and 12°). The results indicate
14 that the speckle noise in the background is reduced by 1.36 dB when using the
15 optical diffuser in the illumination path. The lateral resolution was improved by
16 the decreased linewidth of the QPI carrier frequency which was narrowed down
17 to 0.029 mm^{-1} (to 0.71 mm^{-1} for the broad pedestal). Speckle reduction promoted
18 the clean interferogram while maintained phase information ($\sim 4\%$ phase
19 variation). This technique demonstrated the feasibility of study biological
20 specimen. To conclude, we proposed an effective and compact speckle reduction

1 method which provides highly-sensitive, high-speed, and speckle-suppressed
2 coherent QPI for material-science, biological and biomedical applications.

3 **5.1.2 FCR-QPI for high-visibility, high-speed, spectroscopic** 4 **phase measurement with a high phase stability**

5 In this study, we utilized a frequency comb as a light source in quantitative phase
6 imaging, which was named here as the frequency-comb-referenced quantitative
7 phase imaging (FCR-QPI). The Michelson interferometer was configured to
8 produce the interference fringes using the frequency comb. We demonstrated that
9 the frequency comb of a femtosecond pulse laser can provide a wide coherence
10 tunability for higher sensitivity and higher visibility to QPI's interferograms for
11 the successful phase reconstruction (temporal coherence: 8.1 to 56.3 μm , spatial
12 coherence: 0.63 to 0.98 V , and the speckle contrast: 0.29 to 0.91). The temporal
13 and spatial coherence of the frequency comb were tuned by using the band-pass
14 filters with various bandwidths (BWs = 40, 25, and 10 nm) and the laser speckle
15 reducers with various diffusion angles (DAs = 1° , 6° , and 12°). The multiple
16 wavelengths filtered out from the frequency comb (λ = 700, 750, and 800 nm)
17 enables the spectroscopic phase measurement which decouples the cellular
18 refractive index and physical height to monitor the cellular swelling and protein
19 degradation during the cellular death via the optical volume and refractive index
20 ratio. The refractive index ratio at two wavelengths (700 nm and 800 nm)
21 decreased during the cellular death by 77.3% of its original value over 9 hours.
22 In addition, the high-speed dynamic motions of transparent micro-objects are
23 measured up to 16.9 kHz. FCR-QPI which exhibits high phase stability (2.39×10^{-3}
24 ³ at 10-s averaging time) will provide higher sensitivity to QPI. FCR-QPI offers

1 wide coherence tunability, high-speed, spectroscopic phase measurement with
2 high-phase stability, so it will open various QPI imaging regimes in a broad range
3 of biomedical applications

4 **5.1.3 Measurement of heart rate and blood flow in zebrafish by** 5 **using FCR-QPI**

6 In this study, we demonstrated that the frequency-comb-referenced quantitative
7 phase imaging (FCR-QPI) can be used to measure heart rate and red blood cells
8 flow in the zebrafish embryo. FCR-QPI offers high temporal resolutions and real-
9 time monitoring of high-speed individual red blood cell flow (an average time
10 interval of 0.452 s) even in a blood pulse of the zebrafish at 48 hours-post-
11 fertilization. Due to its non-invasive and high-speed measurement, FCR-QPI can
12 be employed as a tool for study high-speed motions in biological samples as well
13 as in micro-physical and micro-mechanical samples.

14 **5.2 Recommendations**

15 1) Frequency-comb-referenced quantitative phase imaging (FCR-QPI) technique
16 can be demonstrated for measuring fast dynamic motions in various micro-
17 sample (up to MHz). We have demonstrated that FCR-QPI can measure the high-
18 speed oscillating motions of the optical fibre at 16 kHz by using a high-speed
19 photodetector. We would like to suggest our technique for various applications
20 such as high-speed flow cytometry.

21 2) Frequency-comb-referenced quantitative phase imaging (FCR-QPI) can be
22 used to study ocular vasculature development in zebrafish embryos. At an

1 embryonic stage, zebrafish's eyes are optically transparent which allows the
2 accessibility of ocular vessels such as optic artery and optic vein. Two main
3 ocular vasculature systems in the zebrafish embryo are found in human: hyaloid
4 vascular system and ciliary vascular system. The development of zebrafish's
5 ocular vessels is rapid. For example, hyaloid vasculature formation can be found
6 within 2-hours timescale and can be observed in the zebrafish at 18 hours-post-
7 fertilization (hpf) until 36 hpf. FCR-QPI technique will enable non-invasive,
8 label-free observation of ocular vasculature development in zebrafish [185].

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Appendix

A. Author publications

- Boonruangkan, J. *et al.* A frequency comb enables coherence-tailored, multi-wavelength, high-speed quantitative phase imaging with a high phase stability. (*Submitted to Nature Communications, Dec 2019*)
- Boonruangkan, J., Farrokhi, H., Kwok, S., Carney, T., & Kim, Y. J. Quantitative Phase Imaging using a Frequency Comb for Speckle-less Cellular Motion Measurement. *CLEO/Europe-EQEC Conference* (2019).
- Boonruangkan, J., Farrokhi, H., Kwok, S., Carney, T., & Kim, Y. J. Fast and sensitive quantitative phase imaging using a frequency comb. *CLEO: Science and Innovations (pp. SM2H-3): Optical Society of America* (2019).
- Boonruangkan, J., Farrokhi, H., & Kim, Y. J. Rotational diffuser for speckle reduction in quantitative phase imaging. *Conference on Lasers and Electro-Optics/Pacific Rim (p. s1147): Optical Society of America* (2017).
- Farrokhi, H., Boonruangkan, J., Chun, B.J., Rohith, T.M., Mishra, A., Toh, H.T., Yoon, H.S. & Kim, Y.J. Speckle reduction in quantitative phase imaging by generating spatially incoherent laser field at

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- Farrokhi, H., Rohith, T. M., Boonruangkan, J., Han, S., Kim, H., Kim, S. W., & Kim, Y. J. High-brightness laser imaging with tunable speckle reduction enabled by electroactive micro-optic diffusers. *Scientific Reports* **7**(1), 15318 (2017).

B. Coherence function

The fundamental concept of coherence is explained by using mutual coherence function ($\tilde{I}_{12}$) which defined by

$$\tilde{I}_{12} = \tilde{I}(P_1, P_2; \tau) = \langle u_1(t) u_2^*(t+\tau) \rangle$$

where $u_1(t) = u(P_1, t)$ and $u_2(t) = u(P_2, t)$ are complex-valued scalar of optical waves at points P_1 and P_2 in a space, τ is a relative time delay between the two interfering beams, and the angle brackets signify an infinite time average. The cross-correlation of electric field with itself, $\tilde{I}_1(0)$ is its intensity (I_1). Then, $\tilde{I}_{12}$ is thus seen to be a temporal cross-correlation function between the optical waves u_1 and u_2 . A normalized version of $\tilde{I}_{12}$ is known as the complex degree of coherence $\tilde{\gamma}_{12}(\tau)$ which defined by [17]

$$\tilde{\gamma}_{12}(\tau) = \tilde{I}_{12}(\tau) / \sqrt{(\tilde{I}_{11}(0) \tilde{I}_{22}(0))}$$

which obeys the inequality of $0 \leq \tilde{\gamma}_{12}(\tau) \leq 1$. There is a close relationship between these quantities describing the coherence of light and the cross-correlation functions of speckle amplitudes. At this point, it is able to distinguish between temporal and spatial coherence. If the P_1 and P_2 merge to the same point,

say at P_1 , then $\tilde{\gamma}_{12}(\tau) \rightarrow \tilde{\gamma}_{11}(\tau)$, which is the normalized autocorrelation function of the fields at point P_1 , and can be referred to as the complex degree of temporal coherence. By the Wiener-Khinchin theorem, $\tilde{\gamma}_{11}(\tau)$ is related to the power spectral density $g_{11}(\nu)$ of $u_1(t)$ through, $\tilde{\gamma}_{11}(\tau) = \int_{-\infty}^{+\infty} \hat{g}_{11}(\nu) e^{j2\pi\nu\tau} d\nu$, where $\hat{g}_{11}(\nu)$ is a normalized version of the power spectral density $g_{11}(\nu)$. The coherence time of the wave amplitude is conventionally defined as the equivalent width of the complex degree of temporal coherence, $\tau_c = \int_{-\infty}^{+\infty} \tilde{\gamma}_{11}(\tau) d\tau = \hat{g}(0)$. The linewidth ($\Delta\nu$) of light is the width of the power spectral density which is inversely related to the autocorrelation function and the coherence time. A light source that provided a broad spectrum has a short coherence time ($\tau_c \approx 0.66/\Delta\nu$ for Gaussian beams) which prevents the unwanted interference of various diffracted signals arriving to the same point, say P_1 , hence lowers the autocorrelation function ($\tilde{\gamma}_{11}(\tau)$) over the exposure time, and leads to low speckle noise. Whereas, a light source that provided a narrow linewidth has a long coherence time, which increases autocorrelation function of the signals and leads to high speckle contrast. If two waves of $u_1(t) = A_1(t)e^{-j2\pi\nu_0 t}$ and $u_2(t) = A_2(t)e^{-j2\pi\nu_0 t}$ with mean frequency of ν_0 and the time-varying complex envelop (i.e., $A_1(t)$ and $A_2(t)$) arrive at two separate points (i.e., P_1 and P_2) in the image plane, we have the mutual coherence function as $\tilde{\Gamma}_{12} \approx \tilde{J}_{12}e^{-j2\pi\nu_0\tau}$ and the complex degree of coherence function as $\tilde{\gamma}_{12}(\tau) \approx \tilde{\mu}_{12}e^{-j2\pi\nu_0\tau}$, where the mutual intensity ($\tilde{J}_{12}$) and the complex coherence factor ($\tilde{\mu}_{12}$) are defined by

$$\tilde{J}_{12} = \langle A_1(t)A_2^*(t) \rangle, \quad \tilde{\mu}_{12} = \tilde{J}_{12} / \sqrt{\tilde{\Gamma}_{11}(0)\tilde{\Gamma}_{22}(0)}$$

The magnitude of the complex coherence factor always lies between 0 and 1 ($0 \leq \tilde{\mu}_{12} \leq 1$). The mutual intensity $\tilde{J}_{12}$ and the complex coherence factor $\tilde{\mu}_{12}$

are functions strictly of P_1 and P_2 , and independent of τ , which describe the spatial coherence of the light.

C. Speckle suppression in various imaging systems

C.1 Speckle suppression in non-coherent bright-field laser imaging

To demonstrate the low spatial coherence of a continuous wave (CW) laser under the proposed speckle reduction method, a comparative experiment was carried out with and without the optical diffusers as well as a broadband halogen lamp source, as an ideal source for speckle-free imaging. Imaging tests were conducted in transmission mode under Kohler illumination, as shown in Fig. C.1-a. As the first step, no imaging object was installed on the object plane for the background check; thus, the light from the source passes through a highly scattering film (polycarbonate film, $\rho = 1.2 \text{ g/cm}^3$, $\text{DA} = 10^\circ$) and directly imaged on the CCD. The background speckle images taken with five illumination conditions are shown in Figs. C.1-b to C.1-f. For quantitative comparison, for each case, we calculated the speckle contrast (C) of the image and also the standard deviations (SD) of the intensity line profile. The speckle contrast (C) was calculated as $C = SD_I/I$, where SD_I is the standard deviation of the intensity, and I is the average intensity for each image. In the case of broadband Tungsten-Halogen light source, the speckle noise was imperceptible, which was quantified to the speckle contrast of 0.07 and SD of 0.01 (See Fig. C.1-b). When a high-brightness CW laser was used as the light source, serious coherent speckles were detected as shown in Fig. C.1-c, providing the C of 0.99 and SD of 0.15. To avoid the CCD saturation, the

overall intensity was intentionally attenuated. For best quality imaging, the coherent background speckle noise in laser imaging should be far suppressed so that clear images can be provided with a high brightness of the laser illumination. Figures C.1-d to C.1-f show the images under the tunable speckle reduction by introducing the optical diffusers (SD/MD) in the illumination path. The speckle contrast decreases as the DA of the diffuser increases, from 0.99 without the diffusers to 0.83, 0.57, and 0.49 with increasing diffusion angle; *SD* decreases from 0.15 to 0.12, 0.09 and 0.07 in the meantime. By controlling the particle size and DA of the optical diffusers, we were able to tailor the spatial coherence down hence the speckle contrast. In order to demonstrate the improved image quality with the speckle reduction, a 1963A US Air Force (AF) resolution test chart was imaged under the five illumination conditions as shown in Fig. C.2. The scattering film was placed on the illumination side of the AF chart to simulate the random particles in the optical microscope systems, such as biological tissues, plasmonic nanoparticles, and non-ideal optics with surface roughness or contamination. The optical configuration in Fig. C.2-a is equivalent to imaging system for the rough surfaces. Under the spatially coherent CW laser illumination, the complicated background speckle patterns overlap with the AF chart image as shown in Fig. C.2-b; even with the high brightness of CW lasers, this severe image contamination has hampered the wide use of CW laser as the light source for the optical imaging with scatters inside. With the aid of the optical diffusers, the speckle noise was far suppressed so cleaner object images could be recorded as shown in Figs. C.2-c to C.2-e; as DA increases, the quality of the images improves, which was quantified in *SD* from 0.26 to 0.11, 0.07, and 0.05. Although the image is still cleaner in the case of using the broadband halogen

lamp as shown in Fig. C.2-f, note that a CW laser provides highly bright illumination and a high temporal coherence which can be used for optical interferometry.

Figure C.1 Speckle reduction by introducing optical diffusers in illumination path. **a**, Optical configuration. Scattering film was imaged with five different illuminations, by **b**, white light (WL), **c**, a CW laser, **d**, a CW laser, with a static diffuser (SD) and moving diffuser (MD) in the illumination path with a diffusion angle (DA) of 1° , **e**, a CW laser, SD/MD, DA = 6° , and **f**, a CW laser, SD/MD, DA = 12° . The intensity line profiles and standard deviation (SD) of the images are shown below. LD: laser diode, L: lens, OD: optical diffuser, SF: scattering film, O: objective lens, and CCD: charge coupled device

Figure C.2 Speckle reduction in an optical imaging. **a**, Optical configuration. 1963A test chart was imaged with a scattering film under five different illuminations, **b**, a CW laser, **c**, a CW laser, with static diffuser and moving diffuser (SD/MD) in the illumination path with a diffusion angle (DA) of 1° , **d**, a CW laser, SD/MD, DA = 6° , and **e**, a CW laser, SD/MD, DA = 12° , and **f**, white light (WL). The intensity line profiles and standard deviation (SD) of the images are shown below. LD: laser diode, L: lens, OD: optical diffuser, SF: scattering film, AF: air force test target, O: objective lens, and CCD: charge coupled device.

C.2 Speckle suppression in Young's double slit experiment

This study carefully checked whether the coherence can be still preserved even the use of optical diffuser for the speckle suppression. Young's double slit experiment was introduced here, which is a well-known method for characterizing the spatial coherence of the light sources. For the purpose, a double slit was fabricated with $10\ \mu\text{m}$ width and $50\ \mu\text{m}$ separation (See Fig. C.3-a) by the focused-ion-beam process at a 150-nm thick gold thin-film on top of a single crystal sapphire substrate. A 15-nm thin titanium layer was located between the gold and sapphire as the adhesive layer; chemical vapor deposition was used to coat the gold by titanium layers. Light from a CW laser transmitted through the

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optical diffuser and then collimated by a plano-convex lens (See Fig. C.3-b). The collimated beam was incident on the double slit and the resulting two diffracted beams propagated through the free space, which is long enough for the far-field Fraunhofer diffraction. The two diffracted beams made interference at a CCD as shown in Fig. C.3-b. The visibility of the interference pattern at the CCD provides a measure of the spatial coherence. For comparison, we first measured the spatial coherence of the broadband light source (Tungsten-Halogen light source) as the reference as shown in Fig. C.3-c. Then, the spatial coherence of a CW laser under the speckle reduction by different optical diffusers were evaluated as shown in Figs. C.3-d to C.3-g. The visibility of the interferogram shows the spatial coherence of the light source, whereas the broad envelope in the shape of sinc function is dominated by the diffraction due to the finite slit width. For quantitative description of the spatial coherence, we obtained the mutual coherence function (γ) from the interference fringes. The degree of coherence between two electric field, E_1 and E_2 , is defined as $E_1 E_2^* / I_1 I_2$, where $I_1 = |E_1|^2$, and $I_2 = |E_2|^2$. In our coherence measurement, the intensity on the two slits is equal and γ reduces to the visibility $\gamma = V = (I_{max} - I_{min}) / (I_{max} + I_{min})$, where I_{max} and I_{min} are the maximum and minimum intensities of the interference fringes. The reference visibility with the broadband light source was 0.41. Compared to this, the visibility with a coherent CW laser diode under different diffusers decreased from 0.97 to 0.94, 0.65, and 0.54 with the increased DA from 0° to 1° , 6° , and 12° . This implies that one can actively tune the spatial coherence over a wide tunable range so that the visibility can be controlled from 0.54 to 0.94 with the aid of the optical diffusers; 0.97 is the maximum visibility that can be realized with a highly coherent CW laser and 0.41 is the minimum visibility attained by

an incoherent broadband light source. Therefore, the spatial coherence of a coherent laser can be tuned to satisfy the requirements by different microscope systems, so that the background speckle noise can be efficiently suppressed while preserving the visibility of the interferograms for the quantitative phase analysis.

Figure C.3 Speckle reduction in Young's double slit experiment. **a**, SEM images of the double slit. **b**, Optical configuration. Young's double slit interferograms were recorded under five different illuminations, **c**, a white light (WL), **d**, a CW laser, **e**, a CW laser, with static diffuser and moving diffuser (SD/MD) in the illumination path with a diffusion angle (DA) of 1° , **f**, a CW laser, SD/MD, DA = 6° , and **g**, a CW laser, SD/MD, DA = 12° . The normalized intensity line profiles are shown below. LD: laser diode, OA: optical attenuator, L: lens, OD: optical diffuser, DS: double slit, and CCD: charge coupled device.

D. Simulation research of the optical phase delay in an optical fibre

To simulate the optical phase delay of an oscillating optical fibre, we first estimated the optical path length (OPL) of the light traveling through the fibre. The OPLs were separately considered at the cladding and the core of the fibre. Figure D.1 shows the schematics of the OPL when light passing through only the cladding ($OPL_{cladding}$) and the OPL when light passing through the core which also passing though the cladding ($OPL_{cladding-core-cladding}$). At any position, X of the fibre, the OPLs at cladding and core can be calculated as the following equations;

$$OPL_{cladding} = 2\sqrt{R^2 - X^2} \cdot n_{clad}, \text{ when } r < X < R$$

$$OPL_{cladding-core-cladding} = (2\sqrt{R^2 - X^2} - \sqrt{r^2 - X^2} \cdot n_{clad}) + (2\sqrt{r^2 - X^2} \cdot n_{core}), \text{ when } 0 < X < r$$

where R is cladding radius, r is core radius, n_{clad} is RI of cladding, and n_{core} is RI of core.

Figure D.1 Schematics of optical path length (OPL) at any position of the single mode optical fibre.

In our FCR-QPI setup, two interference beams carry the same information, but one beam was slight shifted by tilting one mirror before two beams will interfere at the detector. If we know the separation distance between two interference beams, the optical path difference (OPD) can be calculated from the difference of OPLs of the two beams, $\Delta OPL = OPL - OPL_{shift}$. The OPD was converted to the optical phase delay (OPD) via $OPD = 2\pi \cdot (\Delta OPL) / \lambda$, where λ = the wavelength of the frequency comb. The cosine function was then applied to OPD because the intensity of light related to the cosine function of phase, $I \propto \cos(\Delta\phi)$. To obtain the position-dependent-phase modulation (at core, cladding, and between core and cladding), we set each position at centre and scanned bidirectionally equal to the lateral displacement of the fibre ($\sim 10 \mu\text{m}$). Figure D.2 displays the simulation results comparing to our experimental results which show good agreement and has the average error of $\sim 6.415\%$. In addition, the conversion factor can be calculated from this simulation research to transform the voltage related intensity of light (V) to the phase (rad).

Figure D.2 Normalized amplitude of the optical phase delay of the optical fibre in our experimental results and simulation results at cladding, cladding-core, and core.

E. Cellular death

Oncosis is an accidental death of cells caused by environmental perturbations or external noxious stimuli. Cell undergoes oncotic process exhibits distinct morphological characteristics. Initially, external noxious stimuli induce cell membrane injury and lead to cellular and organelles swelling. After that, cell membrane becomes leaky and non-selective membrane. Lastly, the cell membrane eventually breakdowns and releases the cellular structural protein which is in a necrotic phase. For biochemical levels, oncotic cell death is induced by energy depletion. It involves mitochondria damage which leads to a reduction of ATP (Adenosine triphosphate) generation and loss of energy storage. Eventually, the DNA is damaged, and the disruption of other cellular organelles are found [182, 183].