Super-resolution Optical Imaging

Using Microsphere Nanoscopy

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Abstract

Standard optical microscopes cannot resolve images below 200 nm within the visible wavelengths due to optical diffraction limit. This Thesis reports an investigation into super-resolution imaging beyond the optical diffraction limit by microsphere optical nano-scopy (MONS) and submerged microsphere optical nano-scopy (SMON). The effect of microsphere size, material and the liquid type as well as light illumination conditions and focal plane positions on imaging resolution and magnification have been studied for imaging both biological (viruses and cells) and non-biological (Blu-ray disk patterns and nano-pores of anodised aluminium oxide) samples. In particular, sub-surface imaging of nano-structures (data-recorded Blu-ray) that cannot even be seen by a scanning electron microscope (SEM) has been demonstrated using the SMON technique. Adenoviruses of 75 nm in size have been observed with white light optical microscopy for the first time. High refractive index microsphere materials such as BaTiO$_3$ (refractive index $n = 1.9$) and TiO$_2$-BaO-ZnO (refractive index $n = 2.2$) were investigated for the first time for the imaging. The super-resolution imaging of sub-diffraction-limited objects is strongly influenced by the relationship between the far-field propagating wave and the near-field evanescent waves.

The diffraction limit free evanescent waves are the key to achieving super-resolution imaging. This work shows that the MONS and SMON techniques can generate super-resolution through converting evanescent waves into propagating wave. The optical interactions with the microspheres were simulated using special software.
(DSIMie) and finite different in time domain numerical analysis software (CST Microwave Studio). The optical field structures are observed in the near-field of a microsphere. The photonic nanojets waist and the distance between single dielectric microsphere and maximum intensity position were calculated. The theoretical modelling was calculated for comparisons with experimental measurements in order to develop and discover super-resolution potential.
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Dedication

I would like to

dedicate this thesis

to my beloved father

Hwa-young Lee
1 Introduction

1.1 Research motivation and rationale

The resolution of conventional optical microscopy is limited by Abbe’s diffraction limit. This limitation is approximately 200 nm in the visible spectrum due to loss of evanescent waves in the far-field [1]. Super-resolution can be achieved by the enhancement or conversion of evanescent waves because the evanescent waves brings sub-diffraction-limited image of an object [2-6]. The perfect superlens was reported to break the diffraction limit by using negative index medium in order to restore evanescent waves [7].

The near-field superlens resolves sub-diffraction-limited imaging with a negative index metamaterial through enhanced evanescent waves [8, 9]. The far-field optical superlens (FSL) was experimentally demonstrated to use the nano-scale silver structured negative refractive index metamaterial [10-13], but their light source was UV or X-ray, and the magnification is approximately one. The conventional optical microscope cannot work with FSL in the visible wavelength because the resolution of images are outside the optical diffraction limit [14]. The hyperlens allows one to observe magnified sub-diffraction-limited objects due to converting evanescent waves into propagating waves [15]. The hyperlens was experimentally demonstrated using structured and curved metamaterial with silver and titanium oxide layers at the visible frequency [16], 36 brass fins at acoustic waves [17], gold multilayer photonic metamaterial at the visible frequency [14], and half-cylindrical 16 layers of gold and aluminium oxide on a quartz at the 365 nm wavelength [18].
The microsphere optical nano-scropy (MONS) technique was demonstrated in 2011 by the researchers at The University of Manchester using fused silica microsphere of 4.74 μm diameter coupled with a conventional optical microscope to have demonstrated a record 50 nm optical resolution with white light illumination [19]. As evanescent waves do not have any energy in the spatial direction so evanescent waves can be lost in the interaction of positive refractive index such as a conventional optical lens [6]. However, in MONS, the evanescent waves can be converted to propagating waves through non-linear oscillation and frustrated total internal reflection. The mechanism of super-resolution in MONS is related to the complex combination between optical resonances near and within the microsphere and photonic nano-jet effect [20].

In medical science, it is desirable to see viruses (typically 5 nm – 150 nm) and nano-structures of cells (such as nuclear pores: 50 - 150 nm) and their interactions optically. These are possible with electron microscopes. However, these biological samples will have to be dried and treated for use in vacuum conditions for imaging. Therefore, they are not suitable for imaging live biological samples. Fluorescent optical microscopy allows the imaging of biological nanostructures beyond the optical diffraction limit. However, they are intrusive, as fluorescent particles need to be injected into the object material unless the materials emit fluorescent lights directly. The technique can only see certain parts of the biological materials that emit the lights. Therefore, it is desirable to have an optical imaging technique that can see viruses without fluorescence.
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The previous work on the MONS technique was demonstrated in air, thus its suitability for the super-resolution imaging of biological materials such as viruses and cells in the aqueous conditions were not known. In addition, the MONS technique has been shown to see nanostructures on the surfaces of an object only. It has not been demonstrated before that super-resolution optical imaging could be used for sub-surface structures. Both applications are desirable for advancing the medical science and the electronic and optical engineering.

1.2 Aim and objectives

1.2.1 Aim

This project aims to advance the microsphere optical nano-scopy (MONS) and submerged microsphere optical nano-scopy (SMON) imaging technique to demonstrate super-resolution imaging of biological materials in aqueous conditions and super-resolution imaging of sub-surface nanostructures beyond the resolution of conventional optical microscopy and better understanding of the super-resolution microsphere imaging mechanisms through modelling and experiments.

1.2.2 Objectives

- To use computer simulations to explore and understand the mechanisms and characteristics of microsphere interactions with optical light, investigating the effects of incident light wavelength, microsphere sizes, type and surrounding media.
• To investigate experimentally the effect of microsphere size, refractive index, surrounding media, optical microscope objective lens conditions and focal image position on the image resolution and magnification. This study involves various microsphere materials including silica, polystyrene, BaTiO3 and TiO2-BaO-ZnO of 5 µm to 150 µm in diameter in air, water, biological solution and optical immersion oil.

• To investigate super-resolution imaging of sub-surface nanostructures. Imbedded nanostructures such as recorded Blu-ray disk cannot be seen by a conventional optical microscope as these structures (typically 150 nm) are beyond the optical diffraction limit and cannot be seen by an electron microscope either as the structures are sub-surface.

• To demonstrate the feasibility of super-resolution imaging of adenovirus using the SMON technique. In this work, adenovirus of 75 nm in size were to be imaged using the SMON technique in comparison with transmission electron microscopy (TEM) and scanning electron microscope (SEM) images.

1.3 Thesis outline
A literature review of super-resolution imaging techniques and the underlying principles is given in Chapter 2. The history of microsphere optical nano-scopv (MONS) and the metamaterial-based superlenses is presented. The photonic nanojet is reviewed including the backscattering enhancement effect. The purpose of the
literature review is to identify the state of the art in super-resolution imaging techniques and the knowledge gap existed.

Chapter 3 describes the methodology and equipment used in the research. It includes the optical and target materials, characterisation facilities, the modelling approach and modelling tools used, and the experimental procedures. Moreover, the principles of optical super-resolution imaging and the analytical equipment used are included.

Chapter 4 reports research on the effect of media in SMON technique. A 100 µm diameter barium titanate (BaTiO$_3$) glass microsphere was used in three different media such as water, 40% sugar solution and microscope immersion oil with visible wavelength illumination. The super-resolution imaging performance is discussed.

Chapter 5 reports research results on the understanding of effect of microsphere sizes in MONS technique. Polystyrene microspheres of 30 µm, 50 µm and 100 µm in diameter were used in air with the reflection light illumination mode coupled with a standard optical microscope. The mechanism of MONS technique is proposed by consideration of the transformation of near-field evanescent waves to far-field propagating waves.

Chapter 6 presents research on super-resolution imaging of sub-surface nanostructures using SMON technique. A 60 µm diameter TiO$_2$-BaO-ZnO glass microsphere with a refractive index of 2.2. This was used in water to image sub-surface data recorded Blu-ray structures with the reflection illumination mode. The
simulation near-field Poynting vector fields and the photonic nanojet effects are used to explain the experimental phenomena.

Chapter 7 presents a theoretical modelling of the optical interactions with microspheres. Optical field structures are observed at near-field of a microsphere of various refractive indexes in vacuum and water using Mie theory. The waist of the photonic nanojet is found to be beyond the optical diffraction limit without significant diffractions by the microsphere.

Chapter 8 presents the application of SMON technique for the super-resolution imaging of viruses and cells including 75 nm adenoviruses using 100 μm diameter BaTiO₃ glass microspheres. The super-resolution images of SMON technique are compared with images of a scanning electron microscope (SEM), scanning transmission optical microscope (TEM) and a florescence optical microscope. Adenoviruses have been observed for the first time with a white light optical microscope.

Chapter 9 gives the general conclusions and recommendations for the future. The conclusions summarise the key contributions to the optical imaging science and technology by this research. The recommendations include the proposed future research needs.
2 Literature Review

2.1 Introduction
This chapter introduces the mechanism and background of super-resolution image techniques. The principles of super-resolution microscopy, photonic nanojets and simulation techniques are also reviewed. Through the literature review, the state of the art and knowledge gaps in super-resolution imaging are identified.

2.2 Microsphere optical nano-scropy
Super-resolution imaging was obtained by combining an optically transparent microsphere with a standard optical microscope. The refractive index of a microsphere and medium can affect the imaging resolution and magnification. The object lens of a standard optical microscope can influence the contrast and sharpness of imaging. The microsphere optical nano-scropy was first reported using 2 μm to 9 μm diameter fused silica microspheres to have demonstrated 50 nm resolution in air and 8 times magnification with a standard optical microscope [19]. Microsphere super-resolution imaging was also demonstrated by semi-emersion in ethanol using a 3 μm fused silica microsphere [21] and in isopropyl alcohol using barium titanate glass microspheres [22].

2.2.1 Fused silica microsphere in medium of air
The MONS technique is based on the magnification and near-field diffraction free images to the far-field. The magnified optical image is observed by the standard optical microscope. The focal image positions are normally below the target surface.
Figure 2.1 Schematic of MONS technique with a microsphere illuminated using a halogen light source. A standard optical microscope was combined at transmitted light mode [19].

The super-resolution imaging using the MONS technique was compared with SEM images in Figure 2.2. The Figure 2.2 (a), (b) transmitted and Figure 2.2 (c), (d) refractive imaging modes were applied in experiments of ON view. The super-resolution image of Figure 2.2 (a) was obtained with the focal position 2.5 μm below the target surface. Approximately 4.17 times magnification was obtained. The 8 times magnification of the super-resolution imaging was obtained as shown in Figure 2.2 (b). The 50 nm pores of anodised aluminium oxide (AAO) sample were clearly observed with the microsphere optical imaging. The high magnification may be the effect of enhanced plasmonic fields due to the gold coating of the dielectric nanostructured AAO. The 120 nm and 180 nm lines of the Blu-ray disc was clearly
observed as shown in Figure 2.2 (c), and the 90 nm diameter of a star shape nanostructure was observed in Figure 2.2 (d).
Figure 2.2 Optical super-resolution imaging examples using the MONS technique; (a) SEM image of 360 nm lines and 130 nm spaces, (ON – Optical Nanoscope) Super-resolution image of MONS technique and about 3 times magnification; (b) SEM image of AAO sample gold coated, (ON) The super-resolution image captured by diameter \( d = 4.74 \mu m\) fused silica microspheres. The 50 nm diameter structures are clearly observed with 8 times magnification in the view of the microsphere as 400 nm structures; (c) SEM image of Blu-ray disc structures. The one pitch was approximately 300 nm with line widths around 100-120 nm, (ON) Super-resolution image at reflective halogen light mode; (d) SEM image of the star shape nanostructure that is produced on GeSbTe thin film of DVD disk, (ON) Super-resolution image at the reflective halogen light mode. The 90 nm diameter corner of the star shape is clearly observed in the view of the microsphere. The scale bar of SEM and ON (optical microscope view) is 500 nm and 5 \( \mu m\), respectively [19].
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Figure 2.3 Super-resolution strength of a microsphere with different $n_1$ refractive index and $q$ size parameter. The inset table indicates $q = 0 - 300$ at $n_1 = 1.46$ [19].

The super-resolution strength is defined by Rayleigh diffraction limit with optical focal spot radius for microspheres of different refractive indexes for different size parameters as shown in Figure 2.3. The super-resolution image can be achieved by a narrow window of the photonic nanojets [23]. The narrow window is generated by the parameter of $n_1$ refractive index of the microsphere and $q$ size parameter defined as $q = \frac{2\pi a}{\lambda}$ followed by Mie scattering theory [24]. Super-resolution can be obtained when $q < 70$ at $n_1 = 1.46$. This implies that, a fused silica microsphere achieves super-resolution imaging up to 9 μm in diameter at the $\lambda = 400$ nm wavelength in air. It was confirmed by experiments that 10 μm and 50 μm diameter fused silica microsphere could not achieve the super-resolution imaging but the 3 μm
diameter fused silica microsphere had super-resolution imaging. When the microsphere refractive index is $n_{f} = 1.80$, up to 250 $\mu$m diameter microsphere could be leading to super-resolution imaging. The super-resolution strength may be decreased when the microsphere refractive index exceeds 1.80.

### 2.2.2 Fused silica microsphere in medium of ethanol

The super-resolution imaging using fused silica microsphere was compared with SEM image in Figure 2.4. The periodic lines of Blu-ray disc was examined for super-resolution imaging, and the 3 $\mu$m diameter fused silica microsphere was half-immersed in ethanol liquid in the reflective light illumination mode. Up to 2.7 times magnification was obtained in the ethanol liquid condition. This magnification is smaller than that under the air condition but the super-resolution image of contrast and clearness is improved.
Figure 2.4 Comparison of SEM image and super-resolution images using microspheres in ethanol; (a) SEM image of a Blu-ray disc, (b) the view of a standard optical microscope without microspheres, (c) images with 3 μm diameter fused silica microspheres in air, (d) with 3 μm diameter fused silica microspheres semi-embedded in ethanol liquid [21].

2.2.3 Barium titanate glass microsphere in medium of isopropyl alcohol

Figure 2.5 shows the SEM images of 120 nm diameter gold nanoparticles with 300 nm spaces between nanoparticles, 30 nm heights on the fused silica substrate, and the nano-patterns of a Blu-ray disc.

Figure 2.5 SEM images of (a) gold nanoparticle sample and (b) Blu-ray disc [22].

The super-resolution imaging was achieved by the barium titanate glass microspheres immersed in isopropyl alcohol through a standard optical microscope as shown in Figure 2.6. In the gold nanoparticle sample, the 120 nm resolution of
gold nanoparticles was resolved. The lines of the Blu-ray disc were clearly observed through the view of the microsphere.

Figure 2.6 Super-resolution imaging by barium titanate glass microsphere. The images of barium titanate glass microsphere immersed in isopropyl alcohol on (a) the gold nanoparticle is shown in the focus of (a) the microsphere and (b) the gold nanoparticle substrate. The super-resolution images of the Blu-ray disc is presented to the focus of (c) the microsphere and (d) the Blu-ray disc substrate [22].
2.3 Superlens

The superlens was first proposed by Pendry known as ‘perfect lens’. The superlens can enhance and restore the evanescent waves of sub-diffraction-limit objects via a negative refractive index medium.

\[ n = -1 \]

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Figure 2.7 An illustration of a conventional optical lens and negative refractive index medium; (a) propagating waves through a conventional lens, (b) The loss of evanescent waves and the transmission of propagating waves, (c) progress of propagating waves in a negative refractive index medium, (d) progress and restoration of propagating and evanescent waves in a negative index medium [25].

The conventional lens based on positive-index materials follows the Abbe diffraction limit, and cannot restore the subwavelength objects due to the loss of evanescent waves beyond the near-field (within a distance of the light wavelength to the optical...
component) as shown in the Figure 2.7 (a), (b). The super-lens can restore the evanescent waves through the negative refractive index medium. The light is bent by the negative refractive index medium with a negative angle of refraction inside the material [7]. The light from a point source can be reversed back to the point as shown in Figure 2.7 (c), (d). The negative refractive index medium supports the compensation of decay in the optical path so that propagating and evanescent waves are completely united at the image plane. Standard optical microscopes cannot observe sub-wavelength features of objects because the evanescent waves that include the information of sub-diffraction-limit objects cannot be transmitted to the object lens. Only propagating waves that include the diffraction-limited features of the object can be delivered to the objects lens. The superlens can overcome such limited resolution of standard optical microscopes.

The optical superlenses are compared with conventional optical lens in Figure 2.8. The information of the object can be captured by both the propagating and evanescent waves when the light arrives at the object. Simultaneously, the propagating wave delivers above sub-diffraction-limit information of the object to the far-field, and the evanescent waves delivers diffraction-limit-free information to the near-field. However, the conventional optical lens cannot collect such information at the near-field so the sub-diffraction-limited information is lost as shown in Figure 2.8 (a). The evanescent waves can be enhanced by the superlens with a negative refractive index medium in the near-field as shown in Figure 2.8 (b). The evanescent waves can be deliver to the far-field by coupling into the propagating wave through coupled elements of the superlens in Figure 2.8 (c). In Figure 2.8 (d),
the hyperlens can transfer the evanescent waves into the propagating wave so the sub-diffraction-limited information can be obtained as magnified images in the far-field.

Figure 2.8 A comparison of conventional optical lens and various types of optical super-lenses. Blue and red coloured lines are the propagating and the evanescent waves, respectively; (a) conventional optical lens, (b) optical near-field superlens, (c) optical far-field superlens, (d) hyperlens [6].
2.3.1 Optical near-field superlens

The optical near-field superlens is fabricated with metallic structures much smaller than the optical wavelength on a transparent dielectric material, which form the magnetic and electric fields, and reduced p-polarised waves (TM mode) [7]. Silver is most commonly chosen for the optical near-field superlens because it has the lowest loss factor in the optical range. The optical near-field superlens has two limitations. Firstly, it can be applied only to the near-field with the gap between object and the optical imaging element at sub-wavelength. Secondly, the loss of the optical near-field superlens can cause poor resolution [25].

![Optical near-field superlens diagram](image)

**Figure 2.9** (a) the schematic of the near-field superlens experiment, 50 nm thick chrome is embedded to the object on quartz, 35 nm thick silver and 40 nm thick PMMA spacer (b) original image of ‘NANO’ with 40 nm line-width of, (c) an AFM image of the “NANO” with a near-field superlens on photoresist (PR), (d) an AFM image on photoresist when PMMA is filled up to replace the 35 nm thick silver under the same conditions. Scale bar of (b) to (d) is 2µm. [13].
Figure 2.9 (a) shows the schematic of the silver near-field superlens with chrome object on quartz. The object is embedded by focused ion beam (FIB) lithography with 40 nm thick chrome on quartz. The thickness of the silver near-field superlens is 35 nm and a 40 nm thick PMMA spacer is located between the chrome object and the silver near-field superlens. Photoresist (PR) is placed on top of the surface above the silver near-field superlens. The after-processing object image is captured on the photoresist through the silver near-field superlens. The comparison of the original object mask and the after-processing image by the superlens is shown in Figure 2.9 (b) and (c). The ‘NANO’ is clearly observed in both Figure 2.9 (b) and (c). When the 75 nm thick PMMA is applied instead of the 35 nm thick silver coating the imaging result is shown in Figure 2.9 (d). The line in Figure 2.9 (d) ‘NANO’ image is much wider compared to others because the sub-wavelength components can be regarded as isolated line paths, and the larger Fourier components are lost so it did not reach the imaging plane. Only the smaller Fourier components arrive on the imaging path [13].

2.3.2 Optical far-field superlens

The near-field superlens can only allow subwavelength resolution in the near-field. Such distance limitation can be overcome by the far-field superlens with coupling between the evanescent and the propagating waves. Using a far-field super-lens, the evanescent waves not only is enhanced but also converted into the propagating wave [6].
Figure 2.10 An illustration of the far-field superlens. (a) the arrangement of the far-field lens, subwavelength object and two main functions for imaging, (b) the configuration of the far-field superlens and detector, (c) different imaging results between the far-field superlens and diffraction-limited images at 377 nm wavelength laser beam [11].

The schematic of the far-field superlens and three curves of electromagnetic density with p-polarized incident laser beam are shown in Figure 2.10. The far-field superlens consists of subwavelength structures with silver material, which has enhancement of evanescent waves from the subwavelength object and conversion of evanescent waves to propagating waves as shown in Figure 2.10 (a). The far-field superlens is located in between the optical microscope as the detector and the specimen as shown in Figure 2.10 (b). The object with two 50 nm lines and a 50 nm space is imaged and compared between the far-field super lens and diffraction-limited image from traditional optical microscope using a 377 nm wavelength laser illumination as shown in Figure 2.10 (c). The far-field superlens can observe the
subwavelength features, as the gap is clearly distinguished with the optical microscope.

Figure 2.11 Image of the far-field superlens imaging of two 50 nm subwavelength lines and a 70 nm gap. (a) SEM image of the object, (b) Diffraction-limited image with a conventional optical microscope, (c) the far-field superlens images with s-polarization, (d) the far-field superlens with both s- and p-polarizations. The scale bars in (a), (b), (c), and (d) are 200 nm [11].

A comparison between the original object image, diffraction-limited image and the enhancement with diffraction-limited image illuminated with a 377nm wavelength laser beam is shown in Figure 2.11. The object is created by focused ion beam to 40 nm thick chromium coating on the quartz, and is observed by SEM that shows two pair of 50 nm lines and a 70 nm gap as shown in Figure 2.11 (a). Diffraction-limited image with a traditional microscope cannot detect the two subwavelength lines as shown in Figure 2.11 (b). The far-field superlens with s-polarisation also cannot resolve the two separated nano-lines because the enhancement of the evanescent waves is not sufficiently high due to the lack of surface plasmon as shown in Figure 2.11 (c). The far-field superlens with both s- and p-polarisation shows clearly the two
subwavelength lines and the gap because enough evanescent enhancements is generated through the excitation of surface plasmon at the far-field as shown in Figure 2.11 (d).

This far-field superlens technology had been proved an efficient method compared to most near-field superlens because the far-field superlens does not require point-by-point scanning. On the other hand, there are mainly two limitations. First, the object needs to be located to very close to the superlens. Second, the image is only detected with one dimension by the scattering of evanescent and propagating waves [1].

2.3.3 Hyperlens

With a standard far-field superlens, the evanescent waves is first enhanced by the surface resonance, and then it transforms into the propagating wave on the surface of the metamaterial, but the super-resolution image only remains in the final layer of the metamaterial in the near-field area [6]. In a hyperlens, the evanescent waves can be converted into magnified propagating waves.
Figure 2.12 Far-field hyperlens magnify sub-diffraction-limited object. (a) the hyperlens structure and numerical simulation of the hyperlens imaging of a sub-diffraction-limited object, (b) the super-resolution image by the hyperlens on the object of 35 nm line width and 150 nm space, (c) the image cross section with the hyperlens and without the hyperlens, (d) the super-resolution image of ‘ON’ object with 40 nm line width [18].
The far-field hyperlens has a curved structure to magnify the sub-diffraction limited images as shown in Figure 2.12. The hyperlens was made of 16 curved periodic layers of 35 nm thick Ag and Al2O3 on a quartz substrate as shown in Figure 2.12 (a). The sub-diffraction-limited ‘ON’ shape was engraved onto a 50 nm think Cr coating. The energy loss was simulated as shown in Figure 2.12 (a). The magnified super-resolution image was obtained by the hyperlens. This image is larger than the diffraction limit so a standard optical microscope can collect the image. The sub-diffraction-limited object of 35 nm line width and 150 nm space was experimentally observed by the hyperlens as shown in Figure 2.12 (b), (c). The ‘ON’ shape of 40 nm line width was also resolved by the hyperlens in Figure 2.12 (d). The magnified images were obtained by the standard optical microscope. Such optical far-field hyperlens can magnify the sub-diffraction-limited object to deliver to the far-field area.
Figure 2.13 Acoustic hyperlens of the experimental magnified image and simulation results for the dual-source object. (a) the experimental measurement of pressure in the propagated area, (b) simulation result of micro-structured elastic fins in the pressure field, (c) simulation result of medium effect for the acoustic hyperlens, (d) the magnified image of the hyperlens as red line, and without the hyperlens as blue line [17].
The magnified image of a dual-source object is achieved by the use of an acoustic hyperlens as shown in Figure 2.13. The dual-source object was experimentally measured to have an acoustic pressure applied at 6.6 kHz frequency with the input plane separation of 0.23λ. This separation was magnified to 1.85λ, and such magnified image was bigger than the acoustic wavelength. Thus, the final image can be detected in the far-field. The simulation results of elastic fins and medium for the acoustic hyperlens are shown in Figure 2.13 (b), (c). It shows that the simulation results have a good agreement with experiments, even the outer radius was 8 times bigger than the inside radius. The cross section of the magnified image with the acoustic hyperlens and the absence of the acoustic hyperlens were compared in Figure 2.13 (d). It shows that approximately 8 times magnification was obtained in the acoustic hyperlens and the two lines were not resolved without the acoustic hyperlens.

2.4 Super-resolution cellular imaging microscopy

The optical light microscopy has many advantages for the imaging of biological and cellular materials. The standard optical microscopes have a typical theoretical lateral resolution limit to around 200 nm due to Abbe’s far field diffraction limit. Recently, a number of new optical light microscopes have been developed to overcome the diffraction limit by the use of stimulated emission depletion [26-38], stochastic switching [39-41], and structured illumination [42-44]. Such super-resolution imaging techniques have been used for the imaging of molecular and subcellular behaviour. Moreover, modern labelling technologies have been developed for selective and clearer imaging of subcellular and molecular details [45-48]. These
super-resolution cellular imaging techniques have been applied to the study of the characteristics of cellular structures and the understanding of dynamic changes in heterogeneous biological tissues [49]. In this chapter, the principles and the characteristics of these super-resolution imaging techniques developed for biomedical imaging are reviewed.

### 2.4.1 Stimulated emission depletion (STED) microscopy

The stimulated emission depletion (STED) microscopy consists of an exciting laser beam and a depletion laser beam of a different wavelength as shown in Figure 2.14. The excitation beam excites the fluorophores imbedded in the biological material, often a protein, while the doughnut shaped depletion beam switches off the fluorophores to enable only the central part of the fluorophores to emit light [50]. The super-resolution imaging is obtained by STED fluorescence emission that reduces region of fluorescence signal as shown in Figure 2.14 (b). In the STED microscopy, excited beam activates fluorophores (top green layer), and depletion beam delivers molecules to the ground level stage (second red layer). The saturated depletion is obtained as the ring shape beam profile has a depletion intensity higher than the saturation level (third red layer). This combination of the depletion increases image resolution through decreasing the fluorescent spot size (bottom orange layer).
Figure 2.14 Illumination of stimulated emission depletion (STED) microscopy. (a) Two focused beams are used in order to decrease the volume of detection [49], (b) Combined beam excitation and depletion in STED microscopy [51].

The STED fluorescence emission is only generated when the depletion beam with peak intensity exceeds the saturation level in the target sample. The resolution of STED microscopy can be decided by the combination between the size of the STED fluorescence emission and inverse square root of depletion beam intensity [50]. Furthermore, the initial STED microscopy has experimentally achieved 160 nm resolution in adjacent nanocrystals [37]. In advanced STED microscopy, continuous wave (CW) has been used to resolve 29 – 60 nm resolution [36]. The size of STED fluorescence emission is physically improved to 6 nm diameter [34]. Protein localisation has been conformed through the STED microscopy [52].
Figure 2.15 CW-STED and g-STED fluorescence microscopy in (a) 40 nm yellow-green nano-sphere, (b) living cell of citrine-keratin in PtK2, (c) fixed cell of alexa fluor 488-vimentin in PtK2. Images of CW-STED and g-STED, confocal microscopy are compared in normalized intensity (right corner). The scale bar is 1 μm [53].

The gated-STED microscopy (g-STED) is an advanced technique of continuous-wave stimulated emission depletion (CW-STED) microscopy. The images of g-STED microscopy are recorded through real time processing of electronic gate or the
off-line time-correlated-counting [53]. A comparison of CW-STED and g-STED microscopy is presented in Figure 2.15. The 40 nm resolution is clearly resolved in the g-STED microscopy in Figure 2.15 (a). The images of CW-STED microscopy are more blurred because twice power is required without time-gating.

2.4.2 Photoswitchable imaging techniques (PALM and STORM)

Photoswitchable imaging techniques allow the selective imaging of only one molecule at a time from the fluorescence emission of fluorophores so individual sub-diffraction-limited features can be recorded as shown in Figure 2.16. This idea was first used in photoactivated localization microscopy (PALM) [39] and stochastic optical reconstruction microscopy (STORM) [40]. Both PALM and STROM have quite similar super-resolution imaging procedures but the main difference of the techniques is the use of different fluorescence. PALM uses genetically encoded photoswitchable protein fluorophores, while the STROM uses photoswitchable dye fluorophores [39, 54]. The photoswitchable fluorophores are applied by temporal emission control. The fluorescent state (ON) and dark state (OFF) converts these fluorophores at different wavelengths. Thus, the activated light is generated at low intensity on the target sample, and sub-diffraction-limited objects are individually imaged and localised. Then, deactivation is applied by switching to the dark state. Repeating this process (activation of imaging and deactivation of mapping for many fluorophores) allows super-resolution imaging with synchronized [39, 40, 54] or asynchronous [55] activation.
Figure 2.16 Illumination of photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM). Stochastic detection of fluorescent single particle in red stars and photobleaching and decoding of bleached single particle in white circles is based in both methods. The super-resolution imaging is obtained by the sum of image information over the acquisition time [49].
Both techniques require several conditions of fluorescent probes [51]. The probes emit a certain wavelength light in a fluorescent state but this wavelength must not emit in a dark state. The high resolution and localisation is provided when certain numbers of photons are emitted by the probes in a fluorescent state. The spontaneous activation by numerous fluorophores in diffraction-limited region should be controlled.

2.4.2.1 Photoactivated Localization Microscopy (PALM)

The method of photoactivated localization microscopy (PALM) is illustrated in Figure 2.17. All data are collaborated in a multidimensional volume as shown in Figure 2.17 Centre. The subset of photoactivatable fluorescent protein molecules are fixed within an activated cell (A and B), and most of them are bleached (C). The repeating procedure is applied many times (C and D) until inactivated sample disappears. Diffraction-limited imaging is obtained by the sum of all frames (E and F). The sub-diffraction-limited images are plotted from each molecule signal by the point-spread function of the microscope (G, right). This image is produced by the combination between expected point-spread function images (G, centre) and actual image (G, left). To repeat all the frames with point-spread function (A' to D'), super-resolution imaging is obtained (E' and F').
Figure 2.17 Schematic of photoactivated localization microscopy (PALM). The brief laser wavelength is 405 nm, and imaged at 561 nm laser wavelength until most of molecular are bleached. The scale size of F and F’ is 1 µm X 1µm, others are 4 µm X 4 µm [39].
Figure 2.18 Comparison of (a) total internal reflection fluorescence (TIRF) microscopy and (b) photoactivated localization microscopy (PALM) imaging in cryoprepared COS-7 cell presenting lysosomal transmembrane protein CD63 with the tag of PA-FP Kaede. (c) is large view image of (b). (d) is obliquely cut region of (b). (c) and (d) image can involve super-resolved image that is not obtained by TIRF [39].
The PALM is compared with TIRF (total internal reflection fluorescence) imaging of the same area in Figure 2.18. The TIRF image allows diffraction-limited imaging of membrane as shown in Figure 2.18 (a), but the associated membrane images could be obtained by PALM imaging through interaction of lysosomes or late endosomes as shown in Figure 2.18 (b) and (c). In other areas of the cryosection with oblique cutting to the lysosome, distribution of CD63 (protein coding) could be observed in the membrane plane in Figure 2.18 (d).

2.4.2.2 Stochastic Optical Reconstruction Microscopy (STORM)

The photoswitched localisation process and STORM imaging of single Cy5 isolates individual fluorophores by the 633 nm and 532 nm laser excitation is shown in Figure 2.19. Firstly, the strong 633 nm laser excitation switches all fluorophores to the dark state. The fluorophores are activated optically by 532 nm laser excitation in each image frame. Then, the 633 nm laser excitation allows sub-diffraction-limited molecules light emission until their position is plotted with high accurate localisation. In each frame, only one position of the fluorophores is activated in the field of view from the rest. It can generate unoverlapping images, and allow high accuracy of the fluorophore positions. Finally, the STROM image is obtained by many frames from fluorophore locations.
Figure 2.19 Stochastic optical reconstruction microscopy (STORM) with photoswitched localisation between a dark and a fluorescent state. (a) object labelling for red fluorophores in STORM imaging. (b) STORM produce of single Cy5 plotted hundreds flames before photobleach. The 633 nm laser was used for the excitation of fluorescence to the dark state (black line), the 532 nm laser was used for returning cycle to the fluorescence state. The red and green lines present 633 nm laser and 532 nm laser, respectively [40].

The STORM imaging can resolve two and four clusters in switch positions separated by 46 nm length dsDNA in Figure 2.20. Two clusters are clearly observed in switch positions in Figure 2.20 (a). dsDNA labelled with four clusters in switch positions is
separately measured by 46 nm contour length in Figure 2.20 (b). This results show that STROM imaging of biological objects can resolve 40 nm resolution combined with activated fluorophores in resolved time manner.

Figure 2.20 Super-resolved STORM imaging of (a) two and (b) four switches with 46 nm length of dsDNA. The illumination of DNA construct is on the left, and super-resolved STORM images are presented on the right. The centre position is marked as red dots. (a) The distance of each cluster is 46 nm, 44 nm and 34 nm, respectively. (b) The distance of four clusters along the contour is 42 nm, 39 nm, and 41 nm, respectively. The scale bar is 20 nm in (a) and (b) [40].
2.4.3 Structured illumination microscopy (SIM)

Structured illumination microscopy (SIM) has achieved super-resolution imaging by using sinusoidal illumination patterns of two excitation light sources [56]. A sub-diffraction-limited image is obtained by the combination between the orientated illumination patterns and the sum of these multiple captures as shown in Figure 2.21. Each capture gains the observation of sub-diffraction-limited objects from multiple spatial orientations. All of captured frames are computationally collected by scanning due to be one super-resolution image of sub-diffraction-limited object. Moreover, 100 nm resolution is achieved by additional modulation of orientated illumination patterns [43]. To improve the resolution, the negative illumination pattern was developed with saturated fluorophore response [33, 57].

The image resolution of SIM is compared with standard and confocal microscopy in imaging 121 nm diameter fluorescent microspheres as shown in Figure 2.22. The diameter of a fluorescent microsphere is observed as 290 nm in standard optical microscopy, 210 nm in confocal microscopy, and 130 nm in SIM. Through the result, SIM can observe sub-diffraction-limited object without blurring and distortion. The different resolutions between SIM and confocal microscope cause the strength of signal level to fall in the observable area [43]. Stronger signal can decrease detecting resolution and lead to the loss of image resolution in larger observable area.
Figure 2.21 Illumination of structured illumination microscopy (SIM). Cellular sample (solid blue line) is observed by different multiple spatial orientations. All of multiple images are summed up to resolve sub-diffraction-limited objects (solid grey line) [49].
Three-dimensional super-resolution images of nuclear lamina, DNA and NPC epitopes are compared between confocal laser scanning microscopy (CLSM) with and without deconvolution, and three-dimensional structured illumination microscopy (3D-SIM) as shown in Figure 2.23. Overlapping fluorescent signals can be observed in CLSM and CLSM with deconvolution but the image of 3D-SIM is clearly observed to show nuclear lamina and NPC in Figure 2.23 (a). Moreover, CLSM cannot observe nuclear pores but 3D-SIM observes separated NPC from irregular nuclear lamina network in Figure 2.23 (b). In the comparison of staining with an antibody, around 140 nm and 20 nm of the lamina is observed in NPC and Nup153 signals, respectively as shown in Figure 2.23 (c).
Figure 2.23 Comparison of (a) confocal laser scanning microscopy (CLSM), with (b) deconvolution, and three-dimensional structured illumination microscopy (3D-SIM) for imaging of nuclear lamina, DNA and nuclear pore complex (NPC) epitopes, and (c) mid section of four times magnification view. Immunostained C2C12 cells with antibodies are recognised as NPC epitopes (red illumination), against lamin B (green illumination), and combined with 4 ′,6-diamidino-2-phenylindole (DAPI) (blue illumination). The field views are (a) mid section and (b) apical section. The scar bar is 5 mm in (a) and (b), and 1 mm in box section and (c) [42].

2.5 Photonic nanojets

The photonic nanojets were first introduced by Chen et al. in 2004 [23]. It was first observed for a cylindrical micro-lens and the evidence of sub-diffraction-limited image detection through enhanced backscattering simulated using finite difference in
time domain (FDTD) [23]. The photonic nanojet was also observed in confocal microscope [58], optical data storage [59], two-photon fluorescence depletion microscopy [60], and optical forces at resonance, and off-resonance wavelengths [61]. The enhanced backscattering of photonic nanojets was experimentally proved using barium titanate microsphere at the visible wavelengths [62]. Thus, the photonic nanojet theory could be used to understand super-resolution imaging using microspheres.

2.5.1 Micro-cylinder photonic nanojets

The photonic nanojets was first reported in a dielectric micro-cylinder with several different refractive index of objects and media [23]. The detailed optical structures and the beam waist characteristics with several plane wave light illumination [63]. The photonic nanojets were obtained at the shadow-side surfaces of the objects transmitted by a plane wave. The waist of photonic nanojets is smaller than the diffraction limit without significant observation of diffraction in the near and near-external field of the object.
Figure 2.24 Photonic nanojets for refractive index of $n_1$ the dielectric micro-cylinder of diameter $d$, and $n_2$ medium at $\lambda_2$ plane illuminated wavelengths. The plane waves are generated from left to right. (a) $n_1 = 3.5$, $n_2 = 1.0$, $\lambda_2 = 500$ nm, $d = 5 \mu$m; (b) $n_1 = 2.5$, $n_2 = 1.0$, $\lambda_2 = 500$ nm, $d = 5 \mu$m; (c) $n_1 = 1.7$, $n_2 = 1.0$, $\lambda_2 = 500$ nm, $d = 5 \mu$m; (d) $n_1 = 1.5$, $n_2 = 1.0$, $\lambda_2 = 500$ nm, $d = 5 \mu$m; (e) $n_1 = 1.3$, $n_2 = 1.0$, $\lambda_2 = 500$ nm, $d = 5 \mu$m; (f) $n_1 = 1.1$, $n_2 = 1.0$, $\lambda_2 = 500$ nm, $d = 5 \mu$m.
µm; (d) \( n_1 = 2.3275, n_2 = 1.33, \lambda_2 = 300 \text{nm}, \, d = 10 \mu\text{m}; \) (e) \( n_1 = 3.5, n_2 = 2.0, \lambda_2 = 250 \text{nm}, \, d = 5 \mu\text{m}; \) (f) \( n_1 = 2.3275, n_2 = 1.33, \lambda_2 = 300 \text{nm}, \, d = 6 \mu\text{m} \) [23]

The photonic nanojet was demonstrated using the FDTD simulation of the electric field as shown in Figure 2.24. The waist of photonic nanojet is similar to optical diffraction limit as shown in Figure 2.24 (c). The waist of the photonic nanojet in Figure 2.24 (c) is in the range between 0.4\( \lambda_2 \) and 0.48\( \lambda_2 \) (where \( \lambda_2 \) is the optical wavelength of the projected light) and the photonic jet length is in the range between 1.6\( \lambda_2 \) and 2.0\( \lambda_2 \) [64]. The effects of the location the refractive index of the micro-cylinder and the surrounding media were investigated. The optical interaction with a 10 \( \mu\text{m} \) micro-cylinder produces a photonic jet waist of about 200 nm and the length of about 1000 nm as shown in Figure 2.24 (d). With a 5 \( \mu\text{m} \) micro-cylinder, the photonic nanojet shown in Figure 2.24 (e) produces a waist of about 160 nm and the length of about 400 nm. The waist and length of the photonic nanojet for a 6 \( \mu\text{m} \) micro-cylinder is approximately 200 nm and 500 nm respectively as shown in Figure 2.24 (f). It was observed that an increase in the size diameter of micro-cylinder can extend the length of the photonic nanojet, and the waist of photonic nanojet was mainly decided by the refractive index of the cylinder and the medium.

The photonic nanojet of a dielectric micro-cylinder was demonstrated in a magnetic field \( \mathbf{H} \) with a normal incident plane wave as shown in Figure 2.25 where \( n \) is the ratio of both refractive indexes of micro-cylinder and the medium, and \( a \) is radius of the micro-cylinder. The unit of spatial frequency is \( k \), and the distance of the wave vector in surrounding area is \( 1/k \).
The field of photonic nanojet can be limited in the tangential direction and is increased in the radial direction for a micro-cylinder with a large radius. The size of photonic nanojet dimension is reduced when the radius of the micro-cylinder is decreased. The photonic nanojet is not observed if the incident light wavelength exceeds the radius of the micro-cylinder as shown in Figure 2.25 (d).
2.5.2 Microsphere photonic nanojets

The microsphere photonic nanojet was first discovered for several different sizes of the microsphere at 400 nm incident light plane wavelength by Li et al [65]. It was also demonstrated with three-dimensional polarization responses by analysis of electric field [66] and several different refractive indexes of microspheres [67]. The sub-diffraction limit photonic nanojets were observed by the size of the waist of photonic nanojet. The effects of the size and the refractive index of the microsphere were investigated.

Figure 2.26 Photonic nanojets produced by microspheres with various diameters $d$, refractive indexes $n_1$ and media $n_2$ at an incident plane wavelength $\lambda_2$ and $x$ polarisation; (a) $d = 1 \mu m$, $n_1 = 1.59$, $n_2 = 1.0$, $\lambda_2 = 400$ nm; (b) $d = 2 \mu m$, $n_1 = 1.59$, $n_2 = 1.0$, $\lambda_2 = 400$ nm; (c) $d = 1 \mu m$, $n_1 = 1.59$, $n_2 = 1.0$, $\lambda_2 = 600$ nm; (d) $d = 2 \mu m$, $n_1 = 1.59$, $n_2 = 1.0$, $\lambda_2 = 600$ nm.
\( n_2 = 1.0, \lambda_2 = 400 \text{ nm}; \) (c) \( d = 3.5 \mu m, n_1 = 1.59, n_2 = 1.0, \lambda_2 = 400 \text{ nm}; \) (d) \( d = 8 \mu m, n_1 = 1.59, n_2 = 1.0, \lambda_2 = 400 \text{ nm} \) [65] 

The distribution of photonic nanojets for the \( n_1 = 1.59 \) dielectric microsphere at \( \lambda_2 = 400 \text{ nm} \) incident plane wavelength with \( x \) polarisation and \( z \) propagating direction are shown in Figure 2.26. For microspheres with a diameter less than \( d = 4 \mu m \), the photonic nanojets are generated from the shadow side of the microsphere in contact with the microsphere surface as shown in Figure 2.26 (a), (b), (c). As the diameter of microsphere increases beyond 4 \( \mu m \), the position of photonic nanojet moves away from microsphere surface as shown in Figure 2.26 (d). The waist of photonic nanojet that is defined as the full-width-half-max (FWHM) and the maximum intensity is also increased. The waist of photonic nanojet is 130 nm with the microsphere with a diameter of \( d = 1 \mu m \), 150 nm for \( d = 2 \mu m \), 190 nm for \( d = 3.5 \mu m \), and 210 nm at \( d = 8 \mu m \). These are beyond diffraction limit for the 400 nm incident plane.
Figure 2.27 Three-dimensional photonic nanojet of a microsphere with $R = 5\lambda^2$ and $n_2 = 1.63$ with a plain incident wave. Two orthogonal planes are displayed [66].

Three-dimensional microsphere photonic nanojets are observed with the sphere radius of $R = 5\lambda^2$ and the microsphere refractive index of $n_2 = 1.63$ illuminated with a plain wave incident light as shown in Figure 2.27. The photonic nanojet is generated in as a magnified electromagnetic field following the $z$ direction. The photonic nanojet is not cylindrical symmetric as shown in Figure 2.27.

Figure 2.28 FWHM of electric field for a microsphere with $R = 5\lambda^2$ and $n_2 = 1.30$ in with a plane wave incident light [66].
In Figure 2.28, the photonic nanojet waist is smaller than the optical diffraction limit when the refractive index is $n_2 = 1.30$ with a microsphere of $R = 5\lambda_2$. The focal position is just outside of the microsphere. The distance between the photonic nanojet and the microsphere was about one wavelength. The sub-diffraction-limit photonic nanojet was obtained but the low refractive index of the microsphere cannot generate high intensity.

![Graph](image)

Figure 2.29 FWHM of electrical field generated with the microspheres with $R = 2\lambda_2$ and $5\lambda_2$ with different refractive indexes [66].

The FWHMs were examined for two microsphere sizes of $R = 2\lambda_2$ and $5\lambda_2$ with the light travelling along the $z$ direction as shown in Figure 2.29. For the large microsphere, the sub-diffraction-limit photonic nanojet was obtained when the
refractive index of the microsphere is increased from 1.6 to 2. The distance between the microsphere and photonic nanojet can be increased when the diameter of the microsphere is increased and the intensity is decreased.

(a) Homogeneous polystyrene, $n_2 = 1.59$

(b) Homogeneous silica, $n_2 = 1.43$.

(c) Graded-index dielectric, maximum index $n_2 = 2$ at the centre decreasing linearly to $n_2 = 1$ at the surface; grading realized with 100 concentric shells each 10 nm thick.
(d) As in (c), but grading realized with ten 100-nm-thick shells.

(e) As in (c), but grading realized with five 200-nm-thick shells.

Figure 2.30 Photonic nanojets in electric field for \( d = 2 \mu m \) diameter microspheres of different material and structures at 400 nm incident plane wavelength [67].

The distributions of photonic nanojets are displayed in Figure 2.30 for different microsphere materials and structures using FDTD simulation method in electric field at 400 nm of incident plane wave in Figure 2.30. Each distribution was obtained within the 4 \( \mu m \) vertical boundary and 8 \( \mu m \) of horizontal boundary in the \( xz \) plain. Figure 2.30 (a), (b) demonstrates the effect of homogenous microspheres of two different materials. Figure 2.30 (c), (d), (e) demonstrates the effect of graded-index core-shell structured microspheres with three different thickness of shells. The distribution of 100 nm and 200 nm thick cell as shown in Figure 2.30 (d), (e) is quite
similar to that of 10 nm thick shell as shown in Figure 2.30 (c). Thus, the photonic nanojet may not be influenced by the graded-index microsphere with concentric shells. However, such a core-shell structure may extend the length of photonic nanojet.

Figure 2.31 Distribution of vector directional arrow of Poynting vector fields with the FDTD simulation method in the xz plane. (a) homogeneous polystyrene microsphere, (b) 100 concentric shells with 10 nm thick graded-index microsphere [67].
The distribution of homogeneous polystyrene microsphere and the 100 concentric shells with 10 nm thick of graded-index microsphere was generated with Poynting vector field in the three-dimensional electromagnetic field as shown in Figure 2.31. The distribution corresponds to Figure 2.31 (a), (c), respectively. Comparing between the homogeneous microsphere and the graded-index microsphere in Poynting vector field, the shadow size of the graded-index microsphere can extend the nanojet further away from the sphere.

2.5.3 Backscattering enhancement

The photonic nanojet can be enhanced by the presence of nanoparticles in the areas of the nanojet by backscattering enhancement [64]. The backscattering enhancement was found in a microsphere [62, 65] and a micro-cylinder [23]. Such backscattering enhancement of photonic nanojet can be affected by the size and position of the nanoparticles at visible spectrum [65].

2.5.3.1 Micro-cylinder

The backscattering enhancement effect was obtained with FDTD simulation method as shown in Figure 2.32. Two different sizes of $s = 5$ nm and $s = 10$ nm isolated nanoparticles applied in the cross section of scattering of ±10° backscatter. The backscattering of each isolated nanoparticle can enhance the magnitude of differential parts of the cross section. The backscattering enhancement factor was obtained as shown in Figure 2.32 (c). It demonstrates that the photonic nanojet can be generated with a large size micro-cylinder, and large isolated nanoparticles can be detected in backscattering cross section.
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(a)

(b)

(c)

Differential Cross Section (m)

Scattering angle (degrees)

10^{-6}

10^{-7}

10^{-8}

10^{-9}

10^{-10}

10^{-11}

10^{-12}

10^{-13}

170 175 180 185 190

change due to insertion of a 5-nm nanoparticle

isolated 5-nm nanoparticle

change due to insertion of a 10-nm nanoparticle

isolated 10-nm nanoparticle

Backscattering enhancement factor

Side dimension s of nanoparticle (nm)

10^{0}

10^{1}

10^{2}

10^{3}

10^{4}

10^{5}

10^{6}

10^{7}

(1.25 nm)

(5 nm)

(10 nm)

(15 nm)

(20 nm)

(40 nm)

(60 nm)
Figure 2.32 Illumination of backscattering enhancement for photonic nanojets by the presence of nanoparticles using the FDTD simulation method for dielectric micro-cylinders of \( n_1 = 2.3275, n_2 = 1.33, \lambda_2 = 300 \text{ nm}, d = 6 \mu\text{m} \). The refractive index of dielectric nanoparticle is \( n_1 = 1.50 \), and side dimension is \( s \) embedded in the centre of photonic nanojet on the surface of the dielectric micro-cylinder. The cross section of scattering has \( \pm10^\circ \) of backscatter for the (a) \( s = 5 \text{ nm} \), (b) \( s = 10 \text{ nm} \) dielectric nanoparticles, and the absolute values are changed simultaneously. (c) backscattering enhancement factor by side dimension \( s \) of dielectric nanoparticles [23].

2.5.3.2 Microsphere

The enhancement factor, \( E \), is defined as [65],

\[
E \equiv \delta \text{I} / \text{I}_v = (\text{I}_{\mu+v} - \text{I}_\mu) / \text{I}_v
\]  

(1)

The scattering intensity \( I_{\mu+v} \), microsphere backscattering \( I_\mu \), and nanoparticle backscattering \( I_v \), were calculated. The change of backscattering intensity is measured when the photonic nanojet penetrates and contacts the gold nanoparticles. The evidence of enhanced backscattering by photonic nanojet is demonstrated by backscattering enhancement and normalized backscattering intensity perturbation as shown in Figure 2.33. The photonic nanojet is generated by the \( d = 3.5 \mu\text{m} \) diameter dielectric microsphere of \( n_1 = 1.59 \) refractive index in \( n_2 = 1.0 \) vacuum medium at \( \lambda_2 = 400 \text{ nm} \) incident plane wavelength. The gold nanoparticles were considered with a reflective index of \( n_1 = 1.47 - j1.95 \) [68]. The sizes of the gold nanoparticles are
between 2 nm and 60 nm. The gold nanoparticles were located in the centre and 240 nm away from the edge of the microsphere. In Figure 2.33 (a), the photonic nanojet can significantly increase the backscattering intensity of the gold nanoparticles. In Figure 2.33 (b), the normalized backscattering intensity perturbation was generated by the enhancement of backscattering.

![Graph](image)

*Figure 2.33 Illumination of (a) backscattering enhancement and (b) normalized backscattering intensity perturbation in electric field [65].*
The normalized backscattering intensity perturbation is defined as [65],

\[ \Delta I_N \equiv \frac{\delta I}{I_\mu} = \frac{(I_{\mu+v} - I_\mu)}{I_\mu} \]  

(2)

The perturbation signal \( \delta I \) can be generated by the gold nanoparticles in the photonic nanojet, and is used to calculate the background of isolated dielectric microsphere \( I_\mu \).

Thus, normalizing of \( \delta I \) from \( I_\mu \) can be directly related to detecting of this perturbation. Moreover, this perturbation of the gold nanoparticles can be obtained by enhanced backscattering intensity through the microsphere photonic nanojets. The normalized backscattering intensity enhancement \( \delta I \) is defined as below [65],

\[ \delta I \equiv \frac{\Delta I}{I_\mu} = \frac{I_{\mu+v} - I_\mu}{I_\mu} \]  

(3)

The backscattering intensity of the isolated BaTiO\(_3\) microsphere is \( I_\mu \), and such backscattering enhancement with 60 nm diameter gold nanoparticles is \( I_{\mu+v} \). These values are calculated from GMM. The range of \( 0 < \delta I < 1 \) can be detected but it may be not strong, and the range of \( \delta I > 1 \) is significant backscattering enhancement. \( \delta I \) is significantly affected by wavelengths, and a strong \( \delta I \) can be obtained when the backscattering intensity of the isolated microsphere is relatively small and no resonances.
2.5.4 Experimental observation of photonic nanojet

The photonic nanojet was experimentally observed with dielectric polystyrene microspheres at 520 nm incident plane wavelength as shown in Figure 2.34. A scanning confocal microscope was used in the diffraction-limited observation of the detection pinhole. The three-dimensional scanning was applied around the dielectric polystyrene microsphere. The microsphere of refractive index $n_1 = 1.60$ was placed on a glass substrate.

Figure 2.34 Schematic of experimental setup for the photonic nanojet. Three-dimensional scan is applied in the act of focusing and scanning [58].
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Figure 2.35 Observation and analysis of the photonic nanojet of $d = 5 \mu m$ polystyrene microsphere in air. The (b) red and (c) blue dots are generated by the deconvolution of collection of an efficiency function. The position of the microsphere is indicated by the white circle. (a) experimental observation of photonic nanojet with the optical view, (b) Best focal position at horizontal axis, (c) intensity distribution at vertical axis, (d) Full width at half maximum (FWHM) of the photonic nanojet; the dash values are from numerical simulation in air $n$, and the green dots are generated by the combination between two-dimensional scanning and the deconvolution of collection efficiency function [58].
The photonic nanojet for $d = 5 \, \mu m$ polystyrene microsphere was experimentally observed as shown in Figure 2.35. The raw data of the photonic nanojet was corrected by the deconvolution of collection efficiency function. The optical view of the photonic nanojet is presented in Figure 2.35 (a). The focusing effect of horizontal view at focal position was generated in Figure 2.35 (b). The results of the horizontal focusing effect are quite similar to a Gaussian shape as expected in the simulations [63, 65, 66]. The intensity profile of the vertical view was plotted along the axis of the photonic nanojet. The intensity enhancement was normalized by the measurement of incident intensity in the homogeneous surrounding region. With $d = 5 \, \mu m$ polystyrene microsphere, FWHM was calculated 320 nm below the microsphere that is the focal position. The diffraction limited FWHM was observed over the distance of 1.5 $\mu m$ from the edge of the microsphere.
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Figure 2.36 Experimental observation of the photonic nanojet of \( d = 3 \, \mu m \) polystyrene microsphere in air. (a) a raw image of collection efficiency function and (b) measured intensity with the horizontal view, (c) central intensity enhancement of vertical view, and (d) FWHM of the photonic nanojet [58].

The raw image of collection efficiency function was used to measure the photonic nanojet of \( d = 3 \, \mu m \) polystyrene microsphere as shown in Figure 2.36. The FWHMs of \( d = 3 \, \mu m \) and 5 \( \mu m \) polystyrene microspheres are approximately 270 nm and 320 nm, respectively. The FWHM of \( d = 3 \, \mu m \) polystyrene microsphere is about 15\%
smaller than \( d = 5 \) \( \mu \)m polystyrene microsphere. However, the intensity enhancement of \( d = 3 \) \( \mu \)m polystyrene microsphere was weaker than \( d = 5 \) \( \mu \)m polystyrene microsphere.

![Image](image_url)

**Figure 2.37** The observation of the photonic nanojet for \( d = 1 \) \( \mu \)m polystyrene microsphere in air. (a) experimental observation by collection efficiency function, (b) horizontal intensity view, (c) intensity enhancement alone z dimension [58].

The observation of the \( d = 1 \) \( \mu \)m polystyrene microsphere photonic nanojet was by the raw image of collection efficiency function as shown in Figure 2.37. The focusing effect and intensity was not strong so the clear shadow effect was not observed, and the distance effect of FWHM can be not measured. The FWHM was
approximately 300 nm that is wider than \( d = 3 \mu m \) polystyrene microsphere. Thus, the microsphere photonic nanojet can be optimised above 3 \( \mu m \) diameter of polystyrene microsphere.

### 2.5.5 Experimental confirmation of backscattering enhancement

The backscattering enhancement of the microsphere photonic nanojet was first experimentally confirmed by a 4.4 \( \mu m \) diameter BaTiO\(_3\) microsphere in medium of the polydimethylsiloxane (PDMS) at visible wavelengths [62]. The backscattering of 50 and 100 nm diameter of gold nanoparticles was enhanced when the gold nanoparticles were located within the photonic nanojet. The backscattering is significantly affected by the wavelength of lighting system and the numerical aperture of the image detection. The experimental results of the backscattering enhancement well agreed with numerical calculations of Mie theory.

![Figure 2.38 Experimental detection of the backscattering enhancement of microsphere photonic nanojet [62].](image)
The detection system of the backscattering enhancement was presented with the 4.4 μm diameter BaTiO₃ microsphere and 50 and 100 nm gold nanoparticles in Figure 2.38. The detection system was developed by a Fourier transformation between the microsphere and the nanoparticles in the scattering field. The incident light propagates through the microsphere and nanoparticles along the z axis. The scattered field that is collected by an objective lens over the azimuthal angle is calculated. The numerical aperture of the objective lens can decide the collection cone. The diverging scattered fields can be converted in a spherical coordinate, and then collected by the objective lens.

Figure 2.39 Images of normalized maximum intensity observed with (a) experimental work and (b) simulation results [62].

The comparison of experiment and simulation images in air is shown in Figure 2.39. The normalized maximum intensity of two 4.3 μm diameter polystyrene microspheres was observed in the object lens of NA = 0.6 at 400 nm – 700 nm wavelengths. These images show a good agreement of normalized maximum
intensity between experiments and simulations. The photonic nanojet can be from the area of the maximum intensity.

Figure 2.40 Backscattering intensity enhancement of the 4.4 μm diameter BaTiO3 microsphere in medium of PDMA from (a) generalised multisphere Mie theory and (b) experimental measurement at the object lens of NA = 0.12 [62].
The backscattering intensity of the microsphere photonic nanojet was compared between generalised multisphere Mie theory (GMM) [69] and experimental measurement in Figure 2.40. The backscattering was measured by the 4.4 μm BaTiO₃ microsphere of refractive index ₙ₁ = 2.1 immersed in PDMA of refractive index ₙ₂ = 1.41 at between plane incident wavelengths. In Figure 2.40 (a), the backscattering intensity was compared between the isolated microsphere and the microsphere with the 60 nm gold nanoparticle where located within the photonic nanojet. The refractive index of gold is wavelength-dependent and obtained from [68]. In Figure 2.40 (b), the backscattering intensity was experimentally obtained by the 4.4 μm diameter BaTiO₃ microsphere in PDMA medium associated with 100 nm diameter gold nanoparticles in the object lens of NA = 0.12. The experimental backscattering intensity was quite similar to the numerical calculation of GMM as shown in Figure 2.40 (a). Moreover, the peak value is smaller than numerical results.
The backscattering intensity was measured for difference gold nanoparticle sizes located in the photonic nanojet zone at $\lambda_1 = 400$ nm incident plane wavelength and $d = 3.5$ μm polystyrene microsphere as shown in Figure 2.41. Although, the backscattering intensity of raw gold nanoparticles is bigger than GMM calculation and the experiments, the 50 nm gold nanoparticle could be detected at visible spectrum through the backscattering enhancement from microsphere photonic nanojet because of backscattering intensity enhancement $0 < \delta I < I$. For gold nanoparticles above 80 nm in size, such backscattering can be significantly generated.

*Figure 2.41 Backscattering intensity enhancement by gold nanoparticles, GMM calculation, and experiment [62].*
2.6 Summary and identification of knowledge gap

It is clear from the literature review that significant work has been reported on super-resolution imaging including microsphere optical nano-scopy, superlens and hyperlenses combined with standard optical microscopy. Photonic nanojets and back scattering enhancement have provided evidence of special optical properties around the microspheres and micro-cylinders. In photonic nanojets, the jet size increases with the sphere size and the refractive indexes of microspheres and micro-cylinders affects focal position and width of the photonic nanojet. The following knowledge gaps have been identified,

- There has been no theory explaining how evanescent waves convert to propagating waves in microsphere nano-imaging.

- The effect of sphere size and media on microsphere has been not studied systematically.

- There has been no application of the use of microsphere nano-scopy for nano-imaging biological materials such as viruses.

- There has been no reported work on super-resolution imaging of sub-surface nanostructures using microsphere nano-scopy.

These gaps will be addressed within this thesis.
3 Methodology and Equipments

3.1 Introduction

This chapter presents the methodology used in this research. The experimental equipment and simulation tools are described including their operating principles and essential characteristics. Several parameters of the MONS and SMON techniques that can affect the resolution and optical magnification are also discussed. This chapter also describes types of materials and modelling tools used to understand the mechanism of MONS and SMON techniques.

3.2 Materials

3.2.1 Microsphere

The microsphere is made of synthetic or natural materials. Various types of microspheres are currently used in research institutes and industry. The microspheres can be classified based on the material and structure types. The material type includes glass, ceramic and polymer. The structure types include single solid material, multiple materials, and hollow microspheres. In the MONS technique, the evanescent waves that include sub-diffraction-limited information can be transferred into the propagating wave. The converted propagating wave can be observed through the objective lens of a standard optical microscope. The refractive index of a microsphere can influence the magnification and resolution of the target images. Above $n_1 = 1.8$ refractive index, the super-resolution imaging of MONS is not generated in air [19]. Furthermore, the size of a microsphere can be related to the strength of super-resolution imaging. If the size is too big, the optical resonance
appears around the microsphere, which disturbs super-resolution imaging [62]. Thus, the use of microspheres for imaging needs to consider factors including refractive index and sphere size. These can influence the final spatial resolution and magnification of the MONS and SMON imaging technique.

### 3.2.2 Blu-ray disc

The Blu-ray disc is an optical storage device for high-definition recoding or the storage of digital data. The dimension of a Blu-ray disc is 12 cm diameter that is the same as the DVD and CD discs. The single-side and dual-layer Blu-ray disc can record 25 GB and 50 GB digital data, respectively. The Blu-ray has three data storage types such as BD-ROM (Read-Only-Memory), BD-R (Write-Once-Only), and BD-RE (Rewritable). The structure of Blu-ray discs for single-side and dual-layer are shown in Figure 3.1. The single-side Blu-ray disc only allows one to record one side of the recording surface so the single-side Blu-ray disc has L0 recording layer but the dual-layer Blu-ray disc consist of L0 recording, 25 µm space layer, and L1 recording layer. The 100 µm thick cover layer is the same in both the single-side and dual-layer Blu-ray discs.
In SMON technique, both blank and data-recorded single-side Blu-ray discs were experimentally reported in this thesis. The 100 µm thick cover layer was removed by a shallow cut from the edge followed by peeling. The dielectric film was not removed by chemical etching during experiments for the data recorded Blu-ray disc so the recording layer was not exposed on the surface of the Blu-ray disc to allow sub-surface imaging.

Figure 3.1 Blu-ray structure for the single-side, dual-layer [70]
3.3 Characterisation equipments

3.3.1 Scanning electron microscope (SEM)

Scanning electron microscope (SEM) is widely used for the observation of nano-and micro-metre scale structures and materials. The image of an SEM is generated by scanning of a focused electron beam. The detector in the SEM collects the scattered electrons from the target sample. The resolution of SEM can be below 1 nm but the environmental condition of SEM can influence this resolution. Typically, a metallic sample is used and a vacuum condition is required. For examining dielectric and wet samples, SEM is not ideal. The detection modes of SEM consist of secondary electrons, characteristic X-rays, cathodoluminescence, specimen current, back-scattered electrons, and transmitted electrons. The SEM from Hitachi High Technologies (S-3400N) was used in this work. The sub-diffraction-limit object was observed by the SEM in order to compare with the super-resolution imaging of MONS technique. The conducting and unconducting samples used secondary electron and back scattered electron mode, respectively. Moreover, the dielectric sample was sputtered by a thin layer of gold or platinum coating for increasing electric conduction.

3.3.2 Optical microscope

Two standard optical microscopes were used. These are Leica DM2500M upright microscope and Axiovision upright fluorescence microscope. The optical images were captured by a digital CCD camera and stored in digital format. The resolution is restricted by the optical diffraction limit depending on the wavelength of light source and the optical properties of the objective lens. The Leica DM2500M upright
microscope was used in most of the experiments. Axiovision upright fluorescence microscope was also used for the fluorescent imaging of a control sample for biological targets.

3.3.3 Sputtering machine

The sputtering machine coats the target with various metallic materials. The coating thickness is controlled with nano-meter resolution. When the machine is operated, gas is introduced in the tube, and then free ions and electrons are moved to opposite electrodes. At the same time, current is generated. After that, the electrons interact with gas atoms by ionisation when the voltage is raised. The deposition and luminous glow occur when the breakdown potential of the voltage is exceeded in the tube. In the experiments, the sputtering machine of Emitech K575x was used for thin metal coating on the target surface. The sub-diffraction-limited objects were coated by gold or platinum for the ease of observation using the SEM and for increasing plasmonic effect to have extra magnification and resolution.

3.4 Laser technology and devices

3.4.1 Nanosecond laser

Laserline Laserval Violino was used for labelling the test samples. The laser device is based on Nd:YVO₄ at 532 nm wavelength. A green colour beam is generated with the maximum power of 7 W at 7 ns pulse duration and frequency range between 1 and 30 kHz. The laser beam is controlled by the two dimensional stage of Galvo head. The beam profile of the laser machine is Gaussian intensity distribution. The focused laser beam diameter is approximately 55 μm circular spot.
3.5 Simulation tools

3.5.1 CST Microwave Studio

CST microwave studio is the one of most well recognised numerical simulation software. Many researchers have used it for modelling optical interactions with materials. The modelling is based on finite integration technique (FIT) and capable of variable simulations through many tools. It supports structural three- and two-dimension analysis so that electromagnetic and density fields are analysed in time or frequency domain.

The finite integration technique (FIT) numerically involves calculations on electromagnetic field in frequency and time domain associated with spatial separated actions. FIT was initially developed by Thomas Weiland in 1977. The basic concept of FIT is the Maxwell equations in sets of integral methods. It has a remarkable advantage for optical applications due to the support of full area of electromagnetic fields in time and frequency domains and optical properties such as scattering, reflective and transmission, and oblique optical incident source. Furthermore, modelling structures and boundary conditions can be flexibly applied in geometry. Various types of profiles such as dispersion, anisotropy and non-linearity are involved in material properties. CST have many tools inside, especially, CST microwave studio suite was used to simulate microsphere electrical field distribution because it can solve high frequency problems accurately and easily associate with multi-physics problems such as electric, magnetic, current and their density fields. Moreover, many mathematical or physical equations can be incorporated in the fields,
and the structural of modelling can be calculated with individual parameters in the parameter sweep.

### 3.5.1.1 Comparison to 64bits and 32bit windows

The CST simulations on various time solver parameters and mesh size are compared for Windows 32bits and 64bits operation systems. The 200 nm unit cell structure was set and calculated at 500 nm incident wavelengths with first direct solver mode (low memory) and frequency domain with S-parameters. Interactions between the time parameters and several different mesh volumes are shown in Table 3.1.

**Table 3.1 Dependence of time parameters on various mesh size with 32bits and 64bits Window operation systems**

<table>
<thead>
<tr>
<th>Step per wavelength</th>
<th>Number of tetrahedrons</th>
<th>Mesh time (s)</th>
<th>Solver setup (s)</th>
<th>Solver time (s)</th>
<th>Total time (s)</th>
<th>Rate (32/64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>32</td>
<td>64</td>
<td>32</td>
<td>64</td>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>8,135</td>
<td>8,073</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>57,649</td>
<td>57,541</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>199,111</td>
<td>198,933</td>
<td>15</td>
<td>10</td>
<td>130</td>
<td>31</td>
</tr>
<tr>
<td>20</td>
<td>449,112</td>
<td>448,381</td>
<td>28</td>
<td>19</td>
<td>597</td>
<td>154</td>
</tr>
</tbody>
</table>

The first direct mode is suggested to have 10 steps per wavelength. Four different steps per wavelength are observed with time parameters in the 32bit and 64bit Windows operation system. The number of meshes in the 32bit is quite close to the
64bits; the meshes of the 64bits are little smaller and the difference between two mesh volumes do not seem to affect the computing time in each solver modes. Generally, time parameters depend on mesh size, which is generated by the unit cell size and step per wavelength. At 5 steps per wavelength, it is not so different but 64bits OS is about 20% faster in the suggested mode. In the fine mesh volumes, 32bit OS is about 3.6 times slower, especially, solver setup time is approximately 4 times faster in 64bit Windows operation system, otherwise, other time parameters are obviously similar as up to two times. Thus, the 64bits Windows operation system is preferred and recommended that the simulation is located in the high mesh volumes.

3.5.2 DSIMie simulation software

Electromagnetic waves can involve interactions of macroscopic and mesoscopic objects with the incident light source. The two sizes of objects are quite in common because they are consisted of a large number of atoms such as bulk materials. However, in mesoscopic system, a more accurate approach of Maxwell equations is required because the object size is similar to the wavelength. The DSIMie was developed by Zengbo Wang and Boris Luk'yanchuk using the Fortran computer language. The DSIMie is based on an analytical infinite series in fast calculation of optical properties for single microsphere at near-field distribution. Incident light source, micro size of spheres, refractive index of single microsphere and medium can be applied in the software. The focal position and intensity are calculated by Mie solution to Maxwell's equations in this thesis.
3.6 Experimental procedures

3.6.1 Location of the microsphere
The location of the microsphere is important for the effective imaging and measurement of magnification and resolution using the MONS technique. Microspheres immersed in deionised water are spread on the target surface with a syringe. If the microsphere is required to move to a specific location, the microsphere is carefully moved by a plastic tweezers with the aid of a low magnification optical microscope before they are completely dried on the target surface. The low magnification objective lens of the optical microscope allows long working distance so the microsphere can be easily controlled by the tweezers.

3.6.2 Light source of optical microscope
The direction of light source can affect magnification and resolution in MONS technique. The reflective, transmitted, dual light conditions are considered in this work. In upright microscopes, the reflective light condition is from up to down. The reflective light of the target surface is collected by the objective lens. The transmitted light condition is from down to up. The light transmits through the target and reaches the objective lens but only transparent sample is possible for imaging in this mode. The dual light condition is to use both reflective and transmitted light conditions at the same time.

3.6.3 Immersion liquid
The immersion liquid can enhance the resolution and contrast due to gathering more information of super-resolution imaging between the microsphere and the object lens.
Higher refractive index of immersion liquid can allow gathering more information but the magnification is decreased. Thus, the balance between resolution and magnification is required during the experiments.
4 Effect of Surrounding Media on Microsphere Optical Imaging

Summary

The resolution of an optical microscope is restricted by the diffraction limit, which is approximately 200 nm for a white light source. This research reports that sub-diffraction-limited objects can be resolved in immersion liquids using a submerged microsphere optical nano-scopy (SMON) technique. The image magnification and resolution were experimentally obtained. It is shown that a 100 µm diameter barium titanate (BaTiO₃) glass microsphere combined with a standard optical microscope can image sub-diffraction-limited objects with a halogen light in three different media: water, 40% sugar solution and microscope immersion oil. The super-resolution effect and imaging performance are discussed for immersion liquid types.
4.1 Introduction

Conventional optical microscopy has a theoretical limitation in optical resolution at approximately 200 nm in the visible spectrum because of the loss of evanescent waves in the far-field [1]. Imaging technologies with a sub-diffraction-limited spatial resolution are essential to nano-science and biomedical science. One of the methods to overcome the optical diffraction limit is the use of metamaterial based perfect superlenses operating in the near field [7]. The near-field evanescent waves were transferred to the far field by combining the propagating wave with the evanescent waves at the image plane through the metamaterial optical superlens (FSL) [8, 10-13]. However, the light source was UV or X-ray, and the magnification was approximately 1. This limitation prevents the use of the technology in combination with conventional optical microscopes because the resolution of the imaged objects is smaller than the diffraction limit of standard optical microscopes [14]. To overcome this limitation, Liu et al. demonstrated that sub-diffraction-limited objects could be magnified and observed by a conventional optical microscope with a magnifying optical hyperlens based on a curved (e.g. hypobolic) metamaterial lens, which can convert a evanescent waves into a propagating wave [18]. Nano-scale spherical lens were used to resolve objects beyond the diffraction limit in the near-field [71].

The super-oscillatory lens discovered recently achieved the far-field super-resolution by using an optical mask [72]. Super-resolution and magnified virtual imaging was first demonstrated by Wang et al. using fused silica microspheres to image gold-coated anodic aluminum oxide (AAO) membrane with a conventional optical
microscope with a record resolution of 50 nm [19]. Super-resolution imaging was also realized by fused silica microspheres with semi-immersing liquid of ethanol and microscope immersion oil [21, 73] and a barium titanate glass microsphere immersed in isopropyl alcohol [22]. This research is focus on the effect of different liquid immersion media (water, 40% sugar solution and Leica microscope immersion oil) through the optical imaging performance of a BaTiO$_3$ glass microsphere coupled with a standard optical microscope.

*Figure 4.1 Schematic of SMON technique with a microsphere and a standard optical microscope at the reflective mode.*
4.2 Methods

4.2.1 Limitation of optical resolution

The limitation of optical resolution is expressed by the Rayleigh criterion, which includes diffraction-limited resolution \(d\), for an optical lens with a numerical aperture \((NA)\) at a wavelength \(\lambda_0\), such as,

\[
d = 0.61 \times \frac{\lambda_0}{NA}
\]  

(1)

Based on the equation, the resolution of standard optical microscopes can be limited to approximately 200 nm at visible wavelengths. Based on this, the minimum resolution of the x50 objective lens (NA: 0.75) is 317 nm – 570 nm at visible wavelengths between 390 nm and 700 nm [74]. Furthermore, the high NA lens over 1.0 may be not suitable to apply the MONS technique with big microspheres because the working distance of the lens can be smaller than the microsphere size. Accordingly, the microsphere makes contact with the lens so the focal image position can be not approached below the target substrate.

4.2.2 Preparation of materials

The Verbatim blu-ray disc BD-R 25GB was examined as the sub-diffraction-limit object. The soft protect film was ejected by a shallow cut in the blu-ray discs. Chemical etching was not applied in the blu-ray discs.
4.2.3 Experimental setup

The microspheres were spread on the backside of the blu-ray disc with liquid drops. The observation of super-resolution was explored by the view of the microsphere. The standard optical microscope (Leica DM 2500M) was used in combination with 100 µm BaTiO$_3$ glass microspheres immersed in water, 40% sugar solution, and Leica microscope immersion oil as shown in Figure 4.1. The refractive indexes of BaTiO$_3$ glass, water, 40% sugar solution, and Leica microscope immersion oil are 1.90, 1.330, 1.399 [75] and 1.518, respectively. The halogen light was projected to the target at the reflective mode. The field of view was adjusted to the size of the microsphere diameter. The focal plane of the optical microscope was explored at various distances from target surface in order to understand its effect on the image contrast and the resolution. The effects of a 100 µm BaTiO$_3$ glass microsphere immersed in different media were examined for the imaging of a blu-ray disc.

4.3 Results and discussion

4.3.1 Comparison of SEM and SMON images

The blue-ray disc lines were magnified by the 100 µm BaTiO$_3$ microsphere in three different media as shown in Figure 4.2. The magnified blue-ray disc regular lines were clearly observed in three different media with the microsphere. Without the microsphere, these lines cannot be observed. The different media generated different magnifications. The magnifications in water, 40% sugar solution, and the Leica microscope immersion oil were experimentally observed to be 3.3-3.8 times, 2.5-3.0 times and 2.0-2.5 times respectively. The 120 nm and 180 nm periodic lines of the blu-ray disk were clearly magnified in water and 40% sugar solution in Figure 4.2 (b),
(c) but the ratio of the periodic lines was not held in the Leica microscope immersion oil in Figure 4.2 (d). It may cause that the magnified image is smaller than the minimum resolution of the objective lens of the optical microscope, which can be limited by numerical aperture of the object lens.

Figure 4.2 Super-resolution and magnified blu-ray disc images obtained using the SMON method in different liquid media. (a) Scanning electron microscope (SEM) image of a Blu-ray disc with a line width of 120 nm and a spacing of 180 nm.
Magnified images through the optical microscope were observed (b) in water, (c) in 40% sugar solution, and (d) in microscope immersion oil using a 100 µm diameter BaTiO₃ microsphere.

4.3.2 Magnification and image focal position in different media

The relative refractive index, image focal positions and magnifications of three different media are shown in Table 4.1. The relative refractive index is defined as \( n_2 = n_2 / n_1 \), as a function of the refractive index parameter \( n_1 \) for a medium and \( n_2 \) for a microsphere. The magnification and resolution can be affected by the relative refractive index between a BaTiO₃ glass microsphere and an immersion liquid. Higher relative refractive index can increase magnification and it will enable better resolution. This is true until \( n = 1.8 \) [19]. To use immersed liquid, the image resolution and magnification can be decided by the relative refractive index between a microsphere and immersed liquid rather than the refractive index of a microsphere. The immersed liquid can realize super-resolution over \( n = 1.8 \) of a microsphere.

When the image plane position was close to the target surface, the optical magnification was decreased. On the other hand, when the image plane position was far below from the target surface, the magnification was increased. However, not all positions had good contrast of the images. The clearest and best contrast positions were generally observed close to centre of the image focal planes.
Table 4.1 The experimentally determined image magnifications and imaging plane positions in different media

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>40% Sugar Solution</th>
<th>Leica microscope Immersion Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refractive index</td>
<td>1.33</td>
<td>1.399</td>
<td>1.518</td>
</tr>
<tr>
<td>Relative Refractive index with BaTiO₃</td>
<td>1.429</td>
<td>1.357</td>
<td>1.252</td>
</tr>
<tr>
<td>Magnification</td>
<td>3.3 - 3.8</td>
<td>2.5 - 3.0</td>
<td>2.0 - 2.5</td>
</tr>
<tr>
<td>One pitch size</td>
<td>1000 nm</td>
<td>850 nm</td>
<td>700 nm</td>
</tr>
<tr>
<td>Groove sizes</td>
<td>600 µm / 400 µm</td>
<td>500 µm / 350 µm</td>
<td>450 µm / 250 µm</td>
</tr>
<tr>
<td>Focal image position</td>
<td>100 µm below</td>
<td>62 µm below</td>
<td>25 µm below</td>
</tr>
<tr>
<td>Focal image range</td>
<td>70 µm - 150 µm</td>
<td>42 µm - 87 µm</td>
<td>15 µm - 45 µm</td>
</tr>
</tbody>
</table>

4.3.3 Specular highlight

Specular highlight was observed as colour rings at the focal image ranges in the three immersion liquids as shown in Figure 4.3. The colour rings were generated by specular reflection of the halogen light, and strongly affected by the relative refractive index between a BaTiO₃ glass microsphere and the immersion liquid [76]. The size of colour rings was expanded when the relative refractive index was higher. The centre of blue colour ring had better contrast and resolution than other colour rings. In dry condition without liquid immersion, the super-resolution and magnification were not realized because the windows of the super-foci can be increased, moreover, the refractive index of BaTiO₃ glass microsphere exceeds the theoretical maximum refractive index of super-resolution as up to \( n = 1.8 \) [19].
Super-resolution optical imaging using microsphere nanoscopy

Figure 4.3 Different sizes of specular highlight and magnified Blu-ray disc images in different media. (a) The diffraction-limited image of a Blu-ray disc was observed by the standard optical microscope in water without the microsphere. The optical magnified Blu-ray disc images were obtained with a 100 µm BaTiO$_3$ microsphere in (b) water, (c) 40% sugar solution, and (d) Leica microscope immersion oil.
4.3.4  Mie theory with Poynting vector field

The magnification and super-resolution of SMON can be understood by Mie theory [20]. The 100 µm BaTiO$_3$ microsphere with a parallel incoming plane wavelength of 600 nm is generated in three different media as shown in Figure 4.4. The simulation was carried out using the Mie theory to examine the directional streamlines and electric intensity in the Poynting vector field. The microsphere can generate the photonic nanojet, and the width of the photonic nanojet is tighter than the diffraction limit without significant optical diffraction [23]. This optical path could be reversed in reflective mode of a standard optical microscope so the super-resolution can be observed. The maximum intensity positions (MIP) can affect super-resolution imaging. When the MIP is close to the microsphere, resolution and magnification might be increased. It may cause the sufficient energy to focus on the phonic nanojet. Thus, the width of the photonic nanojet might be minimized. The MIP is affected by relative refractive index of a microsphere and an immersion liquid. The higher relative refractive index can increase the intensity and simultaneously reduce the distance between the microsphere and MIP. The distance between the microsphere and the MIP is 27.35 µm, 36.75 µm, and 62.90 µm in water, 40% sugar solution, and Leica microscope immersion oil, respectively. Moreover, the distance may affect contrast and resolution in the area of specular highlight.
Figure 4.4 Simulation of Mie theory in the field of Poynting vector. The 100 µm BaTiO₃ microsphere responses to incident light in three different media: (a) water,
(b) 40% sugar solution, and (c) Leica microscope immersion oil. (d) Intensity distribution in the xz plane in water (blue), 40% sugar solution (green), and in Leica microscope immersion oil (red).

4.4 Conclusions

Sub-diffraction-limit imaging using a large BaTiO$_3$ sphere coupled with a standard optical microscope has been demonstrated in water, 40% sugar solution, and Leica microscope immersion oil. The optical magnification was approximately 3.3 times, 2.8 times, 2.3 times in water, 40% sugar solution, and Leica microscope immersion oil, respectively. The magnified images were observed when the optical microscope was focused below the target. The focal image positions can be affected by the types of immersion liquids. In water and 40% sugar solution, the magnified super-resolution image was clearly observed but the image distortion was observed in Leica microscope oil because of the limitation of the objective lens. Colour rings were observed due to specular reflection through reflective halogen light, and the size of colour rings can be affected by the relative refractive index between a microsphere and an immersion liquid. The super-resolution imaging can be explained by the photonic nanojet with the reversed optical path. The MONS can develop imaging of sub-diffraction-limited feature in liquid conditions combined with standard optical microscopy. Biology and dielectric samples required liquid conditions can be directly observed without chemical processing, fluorescing, vacuum, and metal coating. Such potential can encourage understanding mechanism and interaction through high resolution and easy processing.
5 Effect of Sphere Size on Optical Imaging

Summary

The microsphere optical nano-scropy (MONS) technique recently demonstrated the capability to break the optical diffraction limit with a microsphere size of 2 - 9 µm fused silica. This follow-on research demonstrates that larger polystyrene microspheres of 30 µm, 50 µm and 100 µm in diameters can overcome the diffraction limit in optical imaging. The sub-diffraction features of a blu-ray disc and gold nano-patterned quartz were experimentally observed in air by coupling the microspheres with a standard optical microscope at the reflection light illumination mode. About 6 - 8 times magnification was achieved using the MONS. The mechanism of the MONS was theoretically explained by considering the transformation of near-field evanescent waves into far-field propagating waves. The super-resolution imaging was demonstrated by experiments and theoretical simulations.
5.1 Introduction

The spatial resolution of conventional optical microscopy is restricted by the diffraction limit, which prevents the imaging of sub-wavelength objects with dimensions less than 200 nm. Super-resolution imaging can be achieved by the capture of near-field evanescent waves which are diffraction-unlimited [6]. The perfect superlens is well known to overcome the Abbe’s diffraction limit by using a negative index medium which can restore evanescent waves [7]. The near-field superlens resolves sub-diffraction-limited imaging using a negative index metamaterial [8, 9]. With the plane metamaterial superlenses, the evanescent waves is coupled with the propagating wave in the far-field without magnifications [1, 11]. By the introduction of hyperlenses using metamaterials, curved lens geometry such as hyperbolic observe magnified sub-diffraction-limited objects by converting the evanescent waves into a propagating wave [15]. The hyperlens was experimentally demonstrated at visible wavelengths using nine pairs of silver and titanium oxide metamaterial layers and gold multilayer photonic crystals [14, 16], thirty-six brass fins for the acoustic wave [17], and half-cylindrical 16 layers of gold and aluminium oxide on a quartz at 365 nm wavelength [18]. Super-resolution imaging by the use of the microsphere optical nano-scropy (MONS) technique was demonstrated using a fused silica microsphere of 4.74 μm diameter combined with a conventional optical microscope to resolve 50 nm objects with a white light source [19].
Figure 5.1 Schematic of the MONS technique for super-resolution imaging. The MONS consists of a microsphere, and a standard optical microscope at the reflective mode illuminated with a halogen light source.

5.2 Theory and Experimental Procedure

5.2.1 Theory of MONS technique

The MONS technique can increase the resolution beyond the Abbe limit by transforming evanescent waves to propagating waves. The mechanism can be related to Helmholtz equation [77], in a homogeneous medium:

\[ k_0^2 = k_x^2 + k_y^2 + k_z^2 \]  \hspace{1cm} (1)
where \( k_0 = (2\pi n)/\lambda \), \( n \) is the refractive index, and \( k_x, k_y, k_z \) are the wavevector components. The evanescent waves can be obtained when,

\[
(k_x^2 + k_y^2) > k_0^2
\]  

(2)

\( k_z \) becomes imaginary as a decay of the wave in the \( z \) direction. The resolution is improved when the components of the wavevector \( \vec{k} \) in the \( x \) and \( y \) planes are increased, and the value of \( \lambda \) is decreased. As a result, the evanescent waves are decayed simultaneously. However, the MONS can solve such limitation of evanescent waves by transforming evanescent waves into propagating waves. In the MONS, the electromagnetic (EM) field \( E(x,y) \) is adjusted by the imaging plane beyond a microsphere. It is transformed by,

\[
E'(x,y) = E(x,y)G(x,y)
\]  

(3)

where the transformation functions \( G(x,y) \) and \( E'(x,y) \) can be calculated by geometric optics and imaging plane of a microsphere. \( G(x,y) \) represents the conversion of the imaging plane from the objects plane to a special plane beyond a microsphere. The super-resolution effect can be explained by the analysis of the spatial wavevector coordinates. The \( G(x,y) \) can be expressed by Fourier transformed into the wavevector spatial coordinates as [78]

\[
G(k_x, k_y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} G(x,y) \exp[-i(k_xx + k_yy)] \, dx \, dy
\]  

(4)

The Fourier amplitude \( A(k_x,k_y) \) is determined by the Fourier inverse of \( E(x,y) \), which is given by
\[ A(k_x, k_y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} E(x, y) \exp[-i(k_x x + k_y y)] \, dx \, dy \]  

(5)

where \( k_x, k_y \) are the wavevector in the \( x \) and \( y \) directions. The \( E'(x,y) \), \( G(x,y) \) and \( E(x,y) \) are given by equations (3), (4) and (5) respectively. \( E'(k_x,k_y) \) can be obtained by,

\[ E'(k_x,k_y) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} A(k'_x, k'_y) G(k_x - k'_x, k_y - k'_y) \, dk'_x \, dk'_y \]  

(6)

The convolution of the spatial wavevector transfer functions \( G(k_x,k_y) \) and \( A(k_x,k_y) \) can cause the spreading of the spatial wavevector. Thus, the evanescent waves in the object plane can be transformed by the convolution to the propagating wave. The spreading of the wavevector could be significantly affected by \( G(k_x,k_y) \) with very wide \((k_x,k_y)\) coordinations so that very narrow detectors such as a microsphere can capture sub-diffraction features.

Plane electromagnetic field incident perpendicular to a flat and smooth metallic boundary plane is refractive as a homogeneous reflective plane wave. In the MONS, the object plane is curved at certain points where the object has a concave and convex surface. From the convex surface, the reflective EM beam is diverging and thus decreasing the EM field near such points, while from the concave surface, the reflective EM is converging and thus increasing the EM field intensity near these points. The reflective EM field near the object plane has variable EM field intensity from one to one correspondence of the object curvature properties. A similar effect
occurs for the transmitted EM field through the object plane where the convex and concave surface affects the EM field distribution near the object plane. The concave surface leads to convergence of the transmitted EM beam while the convex surface leads to beam divergence. Such effects lead to EM imaging of the object including their fine structure that can be much smaller than a wavelength. Thus, the super-resolution imaging is achieved by such EM waves collected by the microsphere that absorb evanescent waves and transform them into propagating waves.

5.2.2 Experimental detail

A small microsphere can limit the view of imaging in a standard optical microscope so the whole view of an object can be not obtainable or the entire view of image can be disturbed by the edge of a microsphere. To improve this, larger polystyrene microspheres (up to 100 µm diameter) were studied in the present investigation. The effect of sphere size was examined by both experiments and numerical simulations using Mie theory.

5.3 Results and discussion

5.3.1 SEM image

In this experiment, 30 µm, 50 µm and 100 µm diameter polystyrene (PS) microspheres were combined with a standard optical microscope (Leica DM 2500M) with a x50 NA:0.75 objective lens in air, and reflective illumination with a halogen light source as shown in Figure 5.1. The refractive index of the PS microsphere is approximately 1.59 in the visible spectrum. The sub-diffraction-limed objects used in the experiment were a blu-ray disc and gold nano-patterned quartz. The blu-ray disc
has periodic lines of 120 nm spacing (dark colour) and 180 nm convex objects (bright colour) in Figure 5.2 (a). The gold nano-patterned quartz includes 150 nm diameter spots (bright colour) and 600 nm spacing (dark colour) in Figure 5.2 (b). The microspheres were spread on the target surface through water drops, and were left to dry. Then the optical microscope was used for observation of targets through the microspheres. The optical imaging of the three different microsphere sizes were recorded and compared with the SEM images. The focal image position of the optical microscope was varied to obtain the sharpest images in each case.

Figure 5.2 Scanning electron microscope (SEM) images of (a) blu-ray disc and (b) gold nano-patterns on the quartz. The blu-ray disc consists of 180 nm lines and 120 nm spacing. The gold nano-patterns on the quartz have 150 nm diameter spots with 600 nm spacing.
5.3.2 Experimental observation of polystyrene microspheres

The minimum resolution \((d)\) of the optical microscope with the x50 with NA:0.75 objective lens can be calculated with the known NA and wavelength \((\lambda_0)\), as \(d = 0.61 \times (\lambda_0 / \text{NA})\). The minimum resolution of the optical microscope is around 300 nm at the visible spectrum. The sub-wavelength objects of the blu-ray disc and the gold nano-patterned quartz were experimentally observed by the 30 µm, 50 µm and 100 µm PS microspheres in air as shown in Figure 5.3. These diffraction-limited objects could be not observed without the microspheres in the optical microscope.

(a)  
(b)  
(c)  
(d)
Figure 5.3 Magnified optical images with 30 µm, 50 µm, and 100 µm diameter PS microspheres in air. The blu-ray disc was resolved with (a) 30 µm, (b) 50 µm, and (c) 100 µm PS microspheres when the optical microscope was focused 100 µm, 120 µm, and 300 µm below the target surface, respectively. The gold nano-patterned quartz was observed with the (d) 30 µm, (e) 50 µm, and (f) 100 µm PS microspheres with 60 µm, 140 µm and 200 µm below the target surface, respectively.

5.3.3 Magnification and focal image position

The focal image positions and magnifications for the three different sizes of the PS microspheres are shown in Tables 5.1. Five pitches were examined to generate the average value of one pitch. The one pitch includes the width of one convex line and space for the blu-ray disc, and width of one spot and space for the gold nano-patterned quartz.
Table 5.1 Experimental magnifications and focal image positions of the blu-ray disc and the gold nano-patterns on the quartz

<table>
<thead>
<tr>
<th></th>
<th>Blu-ray disc</th>
<th>Gold nano-patterns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 µm</td>
<td>50 µm</td>
</tr>
<tr>
<td>Magnification</td>
<td>6.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Focal image position</td>
<td>99 µm</td>
<td>120 µm</td>
</tr>
</tbody>
</table>

In SEM calibration, the width of one pitch was 300 nm for the blu-ray disc and 750 nm for the gold nano-patterned quartz. For the blu-ray disc imaging, the sharpest and the best contrast images were observed when the optical microscope was focused on 99 µm, 120 µm and 300 µm below the target surface for the 30 µm, 50 µm, and 100 µm PS microspheres, respectively. The ratio of convex (light colour) and spacing (dark colour) surface were kept in the PS microspheres. The black spot was observed in the centre of the 30 µm microsphere imaging. The 30 µm microsphere might not support sufficient energy to cover the magnified area in the blu-ray disc so the diffraction-limited feature cannot well reach the optical microscope passed by the 30 µm microsphere. For the 30 µm and 50 µm microspheres, overlapped magnified images were observed by the interference between neighbouring microspheres. It can disturb the super-resolution imaging. However, the 100 µm microsphere can avoid this interference effect because the view window of the microsphere is larger than the view window of the optical microscope so that the interference cannot reach neighbouring microspheres. In the gold nano-patterned quartz, the sharpest images were observed at 60 µm, 140 µm and 200 µm below the target surface for the 30 µm, 50 µm and 100 µm PS microspheres, respectively. The magnified images of the
gold-coated spots were unusually expanded, hence, the ratio of the spot and space was not stable compared with the SEM image. It may cause strong plasmon effect of the gold-coated surface and contribute to distortion. As a result, the surface of the gold-coated spots can be covered by plasmon so the expanded spots were observed in the optical microscope. Moreover, the black spot was not observed in the gold nano-patterned quartz because the only region of the gold nano-patterns delivers to the microsphere and the transparent quartz substrate does not disturb the imaging at the same time. Hence, essential energy of imaging can be obtained in the surface of the gold nano-patterned quartz. Otherwise, the near-field information of the blu-ray substrate delivers to the microsphere so the sub-diffraction-limited feature of the blu-ray can be disturbed by the imaging of the substrate. Therefore, the focal image position and this range can be affected by the substrate conditions. It was also experimentally confirmed that the field of view (FoV) and aperture in the optical microscope can significantly affect image resolution but the magnification was not affected. The value 1 of FoV and the smallest aperture was optimum to give the clearest images. These values only allow limited light conditions for imaging so it can prevent unwanted diffusion or scattering from the light source.
Figure 5.4 Magnification factor analysis of PS microspheres in the blu-ray disc. α is defined as focal image position below the target substrate divided by the diameter of PS microsphere. The circle spots are sampling positions for super-resolution imaging. The red solid line, green dot line and blue dash line are for 30 µm, 50 µm and 100 µm PS microspheres, respectively.

The comparison of the magnification and the focal image positions in the blu-ray disc for 30 µm, 50 µm and 100 µm PS microspheres is shown in Figure 5.4. In experiments, the super-resolution imaging is lost in out of range graphs. The magnification is increased when the focal image position is far from the target substrate but the resolution and contrast of image was rapidly decreased. The image resolution and contrast is good at 50 µm and 100 µm PS microspheres but poor at 30 µm PS microspheres because the black spot is continuously observed to disturb
imaging. The magnification can depend on the size of the PS microsphere. The 30 µm, 50 µm and 100 µm magnification at $\alpha = 3.0$ is approximately 6.5 times, 7.5 times and 8.0 times, respectively. The good resolution for focal image positions is 100 µm – 140 µm for 50 µm PS microspheres and 260 µm – 300 µm for 100 µm PS microspheres. It should be noted that, as the evanescent waves does not have a diffraction limit, the imaging resolution is very much dependent on how much the images can be magnified to the diffraction limit for the standard optical microscope and pick of the converted images. For this particular optical microscope with diffraction limit of 300 nm, about 7 times of the microsphere magnification would allow the optical microscope to observe 43 nm (300 nm / 7 times magnification) in an ideal situation if there is no spherical aberration of the objective lens.

5.3.3.1 Blu-ray disc

Image resolution and focal image positions of 100 µm PS microspheres is compared in the blu-ray disc as shown in Figure 5.5. The clear super-resolution images are obtained below 260 µm – 300 µm focal image position in Figure 5.5 (c), (d), (e). The distorted images are observed in Figure 5.5 (a), (b), and the super-resolution imaging can begin to be lost by blurring in Figure 5.5 (f). The magnification is increased when the focal image position is deeper but the super-resolution can be lost below 320 µm. The loss of resolution may cause the focal position of the object lens to be misaligned to the image position of the microsphere.
Figure 5.5 Focal image positions in magnified optical images of the blu-ray disc. The images are obtained with 100 µm PS microspheres below (a) 220 µm, (b) 240 µm, (c) 260 µm, (d) 280 µm, (e) 300 µm, and (f) 320 µm positions, respectively. The scale bar is 5 µm.

5.3.4 Mie theory with Poynting vector field

The Poynting vector streamlines and the highest intensity positions of 30 µm, 50 µm and 100 µm diameter PS microspheres were simulated at 600 nm visible wavelength using Mie theory calculation as shown in Figure 5.6. For the PS microspheres, the relationship between Poynting vector streamlines and the maximum intensity can provide the procedure of super-resolution imaging in the MONS. The scattering and diffusion of the microspheres is not observed during Poynting vector streamlines in 30 µm, 50 µm and 100 µm diameter PS microspheres so EM waves over the
Super-resolution optical imaging using microsphere nanoscopy

Microspheres may not interrupt imaging. It means sufficient energy of imaging can be provided for the microspheres, and then this energy can directly be delivered to the object lens. Thus, the high intensity energy can involve more information of sub-diffraction-limited features from transforming evanescent waves into propagating waves through the microsphere. Moreover, during light tracing of the microsphere at the near-field, the resonance effect is also enhanced by the conversion of the evanescent waves to the propagating wave [15] and this enhanced effect can encourage the energy of imaging.
Figure 5.6 Simulation of Poynting vector field and the intensity positions at 600 nm wavelength. The Poynting vector field of (a) 30 µm, (b) 50 µm, and (c) 100 µm PS microsphere is simulated, and the colour difference indicates the intensity change. The red and blue colours have high and low intensity, respectively. Focal positions of (d) 30 µm (red), 50 µm (blue), and 100 µm (purple) PS microspheres are calculated in a two-dimensional field in the xz plain. The highest intensity positions are 17.75 µm, 30.27 µm, and 62.34 µm, respectively. Intensity energy was recorded in the z plain at x = 0. Incident light source transmits from top to down.

The super-resolution imaging of the microsphere can be expected by the focal position and the maximum intensity. The focal position is decided by the relationship between the size and refractive index of dielectric materials [23, 79]. The high refractive index of dielectric materials can increase the maximum intensity but the focal position may locate inside of the dielectric materials. In Figure 5.7, the inside focus from the high refractive index of the microsphere can create scatterings around the microsphere so the imaging of transforming evanescent waves into propagating waves may be disturbed by such optical interruptions. Furthermore, from the super-resolution strength, the super-resolution imaging of the microsphere in air can be restricted by the refractive index \( n > 2.0 \), or the over 10 µm diameter of the microsphere with the refractive index \( n = 1.46 \) [19]. For increasing maximum intensity, the curvature of the microsphere can generate higher maximum intensity than the solid immersion lens. For example, The maximum intensity of a fused silica microsphere \( (I_{\text{max}} = 107) \) is higher than the same diameter of the solid immersion lens \( (I_{\text{max}} = 90) \) [19].
Figure 5.7 Poynting vector streamlines of the high refractive index microspheres at 600 nm wavelength. 100 µm diameter microsphere with the refractive index (a) \( n = 2.2 \) and (b) \( n = 2.6 \) is simulated. The highest intensity positions are (a) 38.40 µm and (b) 29.12 µm.

5.4 Conclusions

The MONS can deliver the magnified image of sub-diffraction structures in the reflective EM radiation. In the MONS, a microsphere acts as a very narrow detector that absorbs evanescent waves and transforms evanescent waves into propagating waves. Large microspheres (above 30 µm) can also achieve super-resolution imaging with large view windows in air without immersion liquids. In the 30 µm, 50 µm and 100 µm diameter PS microspheres, the magnification and resolution is affected by the focal image position and the size of the microsphere. The super-resolution was experimentally observed when the optical microscope was focused below twice to triple diameters of the microspheres from the target surface in the blu-ray disc and the gold nano-patterned quartz. Approximately, 6 - 8 times magnification was achieved to resolve sub-diffraction features using the optical microscope.
6 Super-resolution of Imaging of Sub-surface Nano Structures

Summary

The work reports the super-resolution imaging of sub-surface nanostructures, beyond the optical diffraction limit by submerged microsphere optical nano-scoppy (SMON) technique. TiO$_2$-BaO-ZnO glass microspheres of 60 µm diameter immersed in water coupled with a standard optical microscope allows the observation of sub-diffraction-limit sub-surface structures at the reflection light mode. About 5 - 6 times additional optical magnifications were experimentally observed through the microspheres. The SMON technique was also theoretically examining the electrical field Poynting vectors and photonic nanojets. The near-field optical characteristics of the microsphere that involve optical field structures were found which would support the proposed conversion of the evanescent waves into propagating waves as the imaging mechanism. The photonic nanojet of the microsphere through the water medium can penetrate through the thin transparent dielectric film to allow the imaging of sub-wavelength and sub-surface nanostructures to be achieved.
6.1 Introduction

The optical resolution of a standard optical microscope is limited by the diffraction limit of the object lens and light illumination. Such limitation of the optical resolution is about half of the light wavelength. The evanescent waves carry the information of sub-diffraction-limit objects. However, they remain in the close proximity (with the light wavelength) of the optical component, thus cannot reach the far-field for practical observation using optical microscopes. The object lens of a standard optical microscope is operated at a far-field working distance from the target material. As a result, the standard optical microscopy cannot capture sub-diffraction-limit structures.

To overcome the far field diffraction limit of optical resolution, a perfect superlens was theoretically introduced by negative index medium that restores evanescent waves [7]. The metamaterial optical superlens (FSL) resolves sub-diffraction-limited objects by the conversion of enhanced near field evanescent waves into far-field propagating waves [8, 10-13]. However, the magnification of such FSL was approximately 1. This magnification does not allow them to be coupled with a standard optical microscope [14]. The optical hyperlens can overcome this limit by adapting curved metamaterial optical lenses that can magnify the near field evanescent waves during the transformation to far field propagating waves [18, 80, 81]. The microsphere optical nano-scropy also demonstrated super-resolution imaging by magnifying and transferring the near field evanescent waves to propagating waves in combination with a standard optical microscope through a fused silica dielectric microsphere in air [19], with semi-immersing liquid [21, 73], and the use of barium
titanate glass microspheres in isopropyl alcohol [22]. Optical super-resolution imaging was also obtained by detection of the evanescent field below transparent surface layers in scattering-type scanning near-field optical microscopy (s-SNOM) [82], and scanning tunneling optical microscopy [83].

In the work, the super-resolution imaging of sub-surface nano-structures submerged microsphere optical nano-scropy (SMON) technique is reported using TiO$_2$-BaO-ZnO glass microspheres of 60 µm diameter immersed in water. The image resolution and magnification are compared with the SEM images of nanostructures in data-recorded and blank Blu-ray discs. The mechanism of the sub-surface optical super-resolution imaging is described using photonic nanojet and the characteristic of near-field Poynting vectors.

### 6.2 Methods

#### 6.2.1 Preparation of materials

The optical data-recorded and blank blu-ray discs were used to examine the super-resolution imaging of SMON technique. The soft protect film of the blu-ray discs was removed by a shallow cut from the edge. The blu-ray discs were not chemically etched or sputtered during experiments so the thin dielectric film remained on the recordable layer.

#### 6.2.2 Experiment setup

For experiments, the microspheres were spread on the surface of the blu-ray discs with water drops. The standard optical microscope (LEICA DM 2500M) was for
observation of super-resolution imaging through the view of the microsphere. Two different object lenses were operated at reflective mode of halogen light in the standard optical microscope. To examine optical transparent super-resolution by SMON technique, \( d = 60 \, \mu m \) diameter TiO\(_2\)-BaO-ZnO glass microsphere of refractive index \( n_1 = 2.2 \) was immersed in water of refractive index \( n_2 = 1.33 \). The focal image position was determined from the focal position of the blu-ray substrate. It was calculated by a \( 1 \, \mu m \) scale at \( z \) dimension of the object lens. The magnification and image resolution was considered for each focal image position.

### 6.2.3 Simulation setup

In the theoretical simulations, the \( d = 60 \, \mu m \) diameter TiO\(_2\)-BaO-ZnO glass microsphere was applied a form of Poynting vector flow in the electromagnetic field. The incident plane wavelengths of 400 nm, 500 nm, 600 nm, and 700 nm emitted the dielectric microsphere along the \( z \) coordination, and two-dimensional distribution was considered in the \( xz \) plane. The energy flux was obtained by directional vector arrows and the colour distribution of electric intensity. The super-resolution imaging of SMON technique may be determined by the photonic nanojet of a dielectric microsphere and optical resonances of near-field transformations. The photonic nanojet could be evidence of super-resolution imaging induced by enhanced backscattering [23, 58, 64]. The width of photonic nanojets was measured and compared with diffraction limit. The optical near-field transformations can be observed in Poynting vector analysis of a dielectric microsphere in the electromagnetic energy [84]. The relationship between optical near-field
transformations and a dielectric microsphere can provide the transformation of the near-field evanescent waves and the far-field propagating waves [79].

### 6.3 Results and discussion

#### 6.3.1 Observation of optical transparent super-resolution

The super-resolution optical images captured using the SMON technique was compared with the SEM images in Figure 6.1. The $d = 60 \, \mu m$ diameter, $n_1 = 2.2$ TiO$_2$-BaO-ZnO glass microspheres were spread on both data-recorded and blank Blu-ray discs immersed in water. The optical super-resolution of sub-surface nanostructures was examined in the data-recorded blu-ray disc. The magnification, focal image position, and image resolution was determined in the blank blu-ray disc. The observation of super-resolution was done at the same position of Figure 6.1 (c), (e) and Figure 6.1 (d), (f), respectively. The SEM image in Figure 6.1 (a) shows the data-recorded Blu-ray disc surface. The irregular data spots were not observed using the SEM because a thin dielectric film coats the recording layer. The SEM observation of the blank Blu-ray disc shows periodic lines of about 300 nm pitches with 120 nm (dark colour) and 180 nm (bright colour) lines. The optical images using the SMON technique are shown in Figure 6.1 (c)-(f) with object lenses of $l_1 = x50$ NA:0.75 and $l_2 = x100$ NA:0.85. The location of irregular data spots was identically the same in the optical images using both object lenses. The best resolution and contrast was observed in the centre and near the boundary of blue colour area. The imaging was distorted in the boundary of the microsphere view, and it was more clearly observed with the object lens of $l_1 = x50$ NA:0.75.
Figure 6.1 Comparison of scanning electron microscope (SEM) and optically magnified optical super-resolution images of data-recorded and blank Blu-ray discs.

The Blu-ray disc nanostructures are observed with the $d = 60 \mu m$ TiO$_2$-BaO-ZnO glass microsphere combined with $l_1 = x50 \text{ NA:0.75}$ and $l_2 = x100 \text{ NA:0.85}$ object lenses of a standard optical microscope at a focal plane position $130 \mu m$ below the Blu-ray disc top surface. (a) the SEM imaging of a data-recorded Blu-ray disc
surface, (b) SEM image of a blank Blu-ray disc, (c) an optical image of the data recorded Blu-ray disc using the microsphere combined with an $l_1 = \times50 \text{ NA:0.75}$ objective lens, (d) an optical image of a blank Blu-ray disc with the microsphere coupled with an $l_1 = \times50 \text{ NA:0.75}$, (e) an optical SMON image of data recorded Blu-ray disc with an $l_2 = \times100 \text{ NA:0.85}$ object lens, (f) an optical SMON image of blank Blu-ray disc with an $l_2 = \times100 \text{ NA:0.85}$ objective lens.

The comparison between magnification and resolution in SMON technique is shown in Figure 6.2. The finest resolution and contrast was observed below 130 um focal image position in Figure 6.2 (c). When the optical image position was placed at a distance beyond 140 µm below the target surface, the magnification was increased. However, the irregular data spots were not observed and only periodic lines were observed in Figure 6.2 (d), (e). The super-resolution imaging was lost below 160 µm focal image position in Figure 6.2 (f). The loss of resolution may be due to the near-field interaction in the dielectric film. The thin dielectric film is transparent for the near-field of the microsphere. Thus, weak near-field interaction is generated by low absorption of the thin dielectric film [82]. Such interaction is collected by the microsphere, and transformed to propagating wave [77]. As a result, the optical super-resolution imaging can be captured by the object lens through the view of the microsphere.
Figure 6.2 Comparison of focal image positions in magnified optical super-resolution images of data-recorded Blu-ray disc. The images are observed with the $d = 60 \mu m$ TiO$_2$-BaO-ZnO glass microsphere combined with $l_2 = x100$ NA:0.85 object lenses below (a) 110 $\mu m$, (b) 120 $\mu m$, (c) 130 $\mu m$, (d) 140 $\mu m$, (e) 150 $\mu m$, and (f) 160 $\mu m$ positions, respectively. The scale bar is 5 $\mu m$.

6.3.2 Experimental calibration

The optical magnification was measured by the periodic lines of blank blu-ray disc at different focal positions below the target surface for both object lenses of $l_1 = x50$ NA:0.75 and $l_2 = x100$ NA:0.85 as shown in Table 6.1. The one pitch width value was measured by the average of five pitches, and the magnification was calculated by comparing with the SEM image of the blank Blu-ray disc. The magnification was slightly higher in the object lens of $l_2 = x100$ NA:0.85 but the image contrast and resolution was slightly decreased. The clearest and best contrast image was observed
at the focal image positions between 125 \( \mu \text{m} \) – 135 \( \mu \text{m} \) below the top surface of the Blu-ray disc. At focal locations shorter than 125 \( \mu \text{m} \), the magnification and contrast were both decreased. At optical focal positions beyond 135 \( \mu \text{m} \) below the target surface, the magnification was increased, but the contrast and resolution were reduced. The focal image position can determine the best resolution position and the magnification.

Table 6.1 Experimental calibration of optical super-resolution imaging for the blank Blu-ray disc. The focal image positions and magnifications were measured with the object lenses of (a) \( l_1 = x50 \text{ NA:0.75} \) and (b) \( l_2 = x100 \text{ NA:0.85} \).

<table>
<thead>
<tr>
<th>Focal image position (( \mu \text{m} ))</th>
<th>(a) ( d = 60 \mu \text{m}, n_1 = 2.20, n_2 = 1.33, l_1 = x50 \text{ NA:0.75} )</th>
<th>(b) ( d = 60 \mu \text{m}, n_1 = 2.20, n_2 = 1.33, l_2 = x100 \text{ NA:0.85} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>One pitch (nm)</td>
<td>Magnification</td>
<td>One pitch (nm)</td>
</tr>
<tr>
<td>120</td>
<td>1460</td>
<td>4.87</td>
</tr>
<tr>
<td>125</td>
<td>1500</td>
<td>5.00</td>
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<tr>
<td>130</td>
<td>1600</td>
<td>5.33</td>
</tr>
<tr>
<td>135</td>
<td>1660</td>
<td>5.53</td>
</tr>
<tr>
<td>140</td>
<td>1720</td>
<td>5.73</td>
</tr>
</tbody>
</table>

6.3.3 **Directional Poynting vector flow with electric intensity**

The directional Poynting vector distribution at electric field intensity indicated by the colours for the \( d = 60 \mu \text{m} \) TiO\(_2\)-BaO-ZnO glass microsphere is shown in Figure 6.3. A high intensity field is indicated with a red colour and a low intensity field is indicated with a blue colour. The refractive indexes of the microsphere and immersion liquid are \( n_1 = 2.20 \) and \( n_2 = 1.33 \), respectively. The incident light plane wavelengths are \( \lambda = 400 \text{ nm}, 500 \text{ nm}, 600 \text{ nm}, \) and 700 \( \text{nm} \) from top to down. The
near-field transformations that include optical field structures are obviously observed in the lower part of the microsphere with low electric field intensities without significant optical diffractions.

Figure 6.3 Simulation of Poynting vectors flow for $d = 60 \, \mu\text{m}$ TiO$_2$-BaO-ZnO glass microspheres immersed in water. Incident plane waves of $\lambda = (a) \, 400 \, \text{nm}$, (b) $500 \, \text{nm}$, (c) $600 \, \text{nm}$, and (d) $700 \, \text{nm}$ wavelength from top to down.
The interference of the evanescent waves and the propagating wave can create the near-field transformations [85]. This interferences are observed at the incident plane wavelengths of $\lambda = 400$ nm, 500 nm, 600 nm, and 700 nm. The microsphere can enhance this interference through the near-field resonance effect [15]. Thus, this near-field transformation can be observed which demonstrates the conversion of evanescent waves into propagating waves. The information of sub-diffraction-limit objects is carried through the process of transformation to the far field propagating waves. As a result, the sub-diffraction-limit images can be restored by the SMON technique.

6.3.4 FWHM and DMI

The full width half maximum (FWHM) and distance for the maximum intensity (DMI below the sphere bottom surface) for the $d = 60$ µm and $n_1 = 2.20$ TiO$_2$-BaO-ZnO glass microspheres is calculated by the simulation results of electric intensity at 400 nm to 700 nm incident plane waves immersed in $n_2 = 1.33$ water as shown in Table 6.2. The FWHM divided by the incident plane wavelength are compared with the DMIs. The $\lambda_{1-4}$ visible spectrum is determined by the average FWHM of wavelengths between $\lambda_1 = 400$ nm and $\lambda_4 = 700$ nm. DMIs are slightly extended when the FWHM is divided by an incident plane wave is increased. The width of photonic nanojet is equal to the FWHM of the intensity [64]. The width of the photonic nanojet for the visible incident plane wave is $0.63 \lambda_{1-4}$ that is similar to the diffraction limit. Moreover, the length of photonic nanojet could be longer than the thin dielectric film in the Blu-ray disc so that the photonic nanojet can penetrate the thin dielectric film and reach the data layer. Such photonic nanojet may provide the
optical super-resolution imaging based on the enhanced backscattering principle within the visible spectrum when the area of the photonic nanojet reaches the sub-diffraction-limit object [23].

Table 6.2 FWHM and DMI for $d = 60 \, \mu m$ TiO$_2$-BaO-ZnO glass microspheres immersed in water

<table>
<thead>
<tr>
<th>$d = 60 , \mu m$, $n_1 = 2.20$, $n_2 = 1.33$</th>
<th>$\lambda_1 = 400$ nm<img src="https://via.placeholder.com/15" alt="" /></th>
<th>$\lambda_2 = 500$ nm<img src="https://via.placeholder.com/15" alt="" /></th>
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<th>$\lambda_4 = 700$ nm<img src="https://via.placeholder.com/15" alt="" /></th>
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<td>FWHM (nm)</td>
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<td>344.36</td>
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<tr>
<td>DMI (nm)</td>
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<tr>
<td></td>
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<td>5213</td>
<td>4867</td>
<td>5059</td>
<td>5194</td>
</tr>
</tbody>
</table>

### 6.4 Conclusions

It has been shown that the SMON technique can achieve super-resolution imaging of sub-surface nanostructures beyond the diffraction limit. The TiO$_2$-BaO-ZnO glass microspheres immersed in water allows the observation of the nanostructures on the data-recorded Blu-ray discs through a standard optical microscope. The irregular spots and periodic lines were observed in the data-recorded and blank Blu-ray discs. Approximately 5 - 6 times magnification was obtained using microscope object lenses of $l_1 = x50$ NA:0.75 and $l_2 = x100$ NA:0.85. The finest super-resolution imaging was observed in the centre of the microsphere, and with the image focal position of 125 $\mu m$ – 135 $\mu m$ below the target surface. Optical filed structures were observed in the microsphere through simulations without significant optical diffraction. Such near-field transformations can provide the conversion of evanescent...
waves into propagating waves so the sub-diffraction-limit details can be restored by the SMON. The width of photonic nanojet is similar to diffraction limit as $0.63 \lambda_{\text{1-4}}$ at visible spectrum. The photonic nanojet could penetrate the thin dielectric film and arrive at the recordable layer in the Blu-ray disc beneath it. Thus, sub-surface super-resolution imaging can be achieved. The technique may support potential applications for imaging sub-diffraction-limit objects of dielectric materials and sub-surface biology imaging.
7 Microsphere Photonic Nanojets and Optical Resonances

Summary

This work reports on microsphere photonic nanojets by using the Mie theory to analyse the behaviour of the Poynting vector fields at incident visible wavelengths. Optical field structures of optical whirlpools and rhombuses are observed when incident plane waves transmit 5 µm and 30 µm dielectric microspheres of various refractive indexes from \( n = 1.4 \) to 2.2 embedded in vacuum and water media. The waist of the photonic nanojets and the distance between the microsphere and maximum electric intensity position determines the potential of super-resolution imaging. These parameters vary with refractive indexes and dielectric microsphere sizes.
7.1 Introduction

The spatial optical resolution of an imaging system is affected by the quality and configuration of optics and more importantly by the diffraction limit. To resolve sub-diffraction-limited objects, metamaterial based super-lenses have been used to restore evanescent waves [7]. Microspheres have been successfully used for nanofabrication [86, 87] and for super-resolution imaging [19]. The photonic nanojet can also approach sub-diffraction-limited detection enhanced by backscattering of light by dielectric micro-particles [64]. The backscattering enhancement induced by the photonic nanojets was experimentally demonstrated using a barium titanate microsphere at visible wavelengths [62]. Dielectric microcylinders were also shown to be able to generate nano-scaled photonic jet, and it can enhance backscattering of light in the visible spectrum [23]. Such enhancements can overcome the optical diffraction limit without significant optical diffractions.

The phenomenon of the photonic nanojet was theoretically investigated in circular dielectric microcylinders [23, 63], and dielectric microspheres [65-67, 88] with incident plane waves. The diffraction effect of a dielectric microsphere array was demonstrated in far field photonic nanojets [89, 90]. The photonic nanojet was also experimentally confirmed with a polystyrene microsphere on a glass substrate with a confocal microscope [58]. The applications of the photonic nanojet have been investigated in various fields including optical data storage [59], two-photon fluorescence depletion microscopy [60], plasmonic nanodisc lithography [91], and optical forces at resonance, and off-resonance wavelengths [61].
This report studies the interactions of optical resonances and photonic nanojets that are generated by dielectric microspheres of diameters of $d = 5$ µm and 30 µm with refractive indexes of $n_1 = 1.46$ (fused silica), 1.59 (polystyrene), 1.93 (TiO$_2$-BaO-SiO$_2$ glass), 2.20 (TiO$_2$-BaO-ZnO glass) embedded in refractive indexes of $n_2 = 1.0$ (vacuum) and 1.33 (water) media at incident plane wavelengths of $\lambda = 300$ nm, 400 nm, 500 nm, 600 nm, 700 nm, 800 nm, respectively. The distance between the microspheres and the maximum electric intensity position (DMI), maximum electric intensity and full width half maximum (FWHM) were determined. The potential of super-resolution imaging is explained with the analysis of the waist of the photonic nanojet, and the optical field structures in the Poynting vectors in the near and near-external fields of a microsphere.

### 7.2 Methods

Light scattering and optical cross-section of light interacting with a microsphere is modeled using the Mie theory considering electric ($\mathbf{E}$), magnetic ($\mathbf{H}$) and Poynting vector ($\mathbf{S} = \mathbf{E} \times \mathbf{H}$) fields. Using analytical Mie theory with Poynting vectors in the electromagnetic field, it shows that not only 5 µm but also 30 µm diameter dielectric high refractive index microsphere can achieve photonic nanojet effect in the visible spectrum illuminated with incident plane waves. Previously, photonic nanojets were limited to below 10 µm small particles [23, 62, 65] because they might take advantage of producing minimized FWHM and enhanced backscattering. The Poynting vector flow was used to analyze the field distribution by the direction of propagation [85, 92] because the image distribution of Poynting vector can allow one to understand the local changes of the electromagnetic energy [84]. Thus, the
capability of super-resolution imaging might be predicted with the photonic nanojet in the near and near external fields of a microsphere.

In the simulations, when the incident plane waves propagate through a single dielectric microsphere along the $z$ coordination, the flux of electric and magnetic vectors is along the $x$ and $y$ coordinations, respectively. The two-dimensional Poynting vector flow is considered for a single microsphere embedded in a medium in the $xz$ plane. The directional energy flux density is generated by streamlines and directional vector arrows. The distribution of the Poynting vectors was obtained to show drastic transformations in the field distribution. This relationship includes several different parameters of incident plane wavelengths, microsphere sizes, and refractive indexes of a microsphere in different media, which shows optical resonances and the complicated optical field structures of near-field transformations [79, 93].

7.3 Results and discussion

7.3.1 5 µm dielectric microsphere

The behaviour of the Poynting vectors in a photonic nanojets for a $d = 5$ µm dielectric microsphere is shown in Figure 7.1. The red coloured lines indicate high electric intensity and the blue coloured lines indicate low electric intensity. Dielectric microspheres with two different refractive indexes of $n_1 = 1.46$ and $n_1 = 2.20$ are embedded in $n_2 = 1.0$ vacuum and $n_2 = 1.33$ water media with the incident light with $\lambda_2 = 400$ nm wavelength plane wave. The optical field structures of near-field transformations that contain optical whirlpools are observed in the area of low
electric intensity in the near and near-external field of the microsphere. The near-field transformations can be generated by the interference between the evanescent and the propagating waves \[85\]. In Figure 7.1. (a), the \( d = 5 \, \mu m \) dielectric microsphere of \( n_1 = 1.46 \) refractive index in \( n_2 = 1.0 \) vacuum can clearly generate the transformations of the optical whirlpools without significant diffractions in the near and near-external fields of the microsphere. Furthermore, it was experimentally confirmed to have super-resolution imaging through microsphere optical nano-scopy \[19\]. The interactions between the evanescent and the propagating waves can encourage the photonic nanojets. This interference may provide a theoretical explanation of super-resolution imaging by transforming the evanescent waves from the near to far field propagating wave. Moreover, the resonance effect can be improved in the near field of the microsphere by this interference relationship \[15\].

In Figure 7.1. (b) \( n_2 = 1.33 \) water, the focal position is located far from the microsphere, and optical whirlpools are not generated in the near field of the microsphere. The microsphere of \( n_1 = 2.20 \) refractive index in \( n_2 = 1.0 \) vacuum has the photonic nanojet focal point inside the microsphere as shown in Figure 7.1. (c). Optical interferences around the microsphere are generated so that a second focal position is observed near the top boundary of the microsphere. The second focal position can divert the energy of optical resonances so that the transformations of optical whirlpools may not be obviously generated. Moreover, the resonances surround a microsphere can decrease the backscattering enhancement of photonic nanojets \[62\]. Thus, the super-resolution imaging might be interrupted by the interference of the second focal position. In Figure 7.1. (d) \( n_2 = 1.33 \) water, the photonic nanojet is formed on the boundary of the microsphere. The optical
whirlpools are partially generated that it might not be sufficient for the near-field evanescent waves to propagate wave transformations.

Figure 7.1 Poynting vectors of light transmitting through transparent dielectric microspheres of \( d = 5 \, \mu\text{m} \) diameter and refractive index \( n_1 \) immersed in refractive index \( n_2 \) of a medium. Incident light \( \lambda_2 = 400 \, \text{nm} \) wavelength plane from top. (a) \( n_1 = 1.46, n_2 = 1.0 \); (b) \( n_1 = 1.46, n_2 = 1.33 \); (c) \( n_1 = 2.20, n_2 = 1.0 \); (d) \( n_1 = 2.20, n_2 = 1.33 \).
Figure 7.2 Poynting vector traces for plane wave light transmission through a dielectric microsphere of $d = 30 \mu m$ diameter at the $\lambda_2 = 400 \text{ nm}$ wavelength. (a) $n_1 = 1.59, n_2 = 1.0$; (b) $n_1 = 1.59, n_2 = 1.33$; (c) $n_1 = 2.20, n_2 = 1.0$; (d) $n_1 = 2.20, n_2 = 1.33$. 
7.3.2 30 µm dielectric microsphere

The Poynting vector traces of photonic nanojets of $d = 30$ µm dielectric microsphere are shown in Figure 7.2. The optical whirlpools are obviously observed in Figure 7.2. (a) $n_1 = 1.59$, $n_2 = 1.0$ and (c) $n_1 = 2.20$, $n_2 = 1.33$. It can be the result of satisfied interference between evanescent and propagating waves, so it can provide super-resolution imaging to observe sub-diffraction-limit objects. In Figure 7.2. (b) $n_1 = 1.59$, $n_2 = 1.33$ water, the focal position is far from the microsphere, and the optical whirlpools are observed in the near and near-external fields of the microsphere. It may cause insufficient near-field transformations. In Figure 7.2. (c) $n_1 = 2.20$, $n_2 = 1.0$ vacuum, the vector flow near the top of the microsphere is disturbed, and it is similar to the phenomenon of Figure 7.1. (c). It may cause the changing location of the focal position inside the microsphere.

7.3.3 Optical field structures of dielectric microsphere

Optical whirlpool and rhombus structures are generated in the field of Poynting vectors around a microsphere. Optical whirlpool structures can be found at the bottom part inside the microsphere. Two optical whirlpool structures are observed each side of the light axis inside the $d = 30$ µm diameter microsphere in Figure 7.2 (a) $n_1 = 1.59$, $n_2 = 1.0$ and (d) $n_1 = 2.20$, $n_2 = 1.33$. Optical rhombus structures are created near the microsphere boundary. The location of the optical rhombuses depends on the refractive index of the microsphere and the medium. Optical rhombuses are also observed outside of the microspheres in Figure 7.2 (b) $n_1 = 1.59$, $n_2 = 1.33$, and on the boundary in Figure 7.2 (a) $n_1 = 1.59$, $n_2 = 1.0$, and both inside and outside in Figure 7.2 (d) $n_1 = 2.20$, $n_2 = 1.33$. Such optical field structures can be clearly observed in a
bigger diameter of microsphere because of high energy field intensity. The specific
dielectric properties of the microsphere can enhance the intensity of incident waves
by optical focusing and the size of the microsphere. Furthermore, the energy flow
can be disturbed by a second focal position if the focal position is inside the
microsphere in Figure 7.2 (c) \( n_1 = 2.20, \ n_2 = 1.0 \). Such interruption may break the
optical field structures.

### 7.3.4 FWHM and DMI for 5 µm dielectric microsphere

The FWHM and DMI of \( d = 5 \) µm dielectric microsphere were determined at the
incident plane waves of \( \lambda = 300 \) nm, 400 nm, 500 nm, 600 nm, 700 nm and 800 nm
wavelengths as shown in Figure 7.3. The interactions with microspheres with
refractive indexes of \( n_1 = 1.46, 1.59, 1.93, \) and 2.20 are compared with \( n_2 = 1.0 \)
vacuum and \( n_2 = 1.33 \) water media. The size of FWHM is increased when the light
wavelength is increased. On the other hand, DMI can be slightly decreased when the
wavelength is increased. This effect can be observed clearly in \( n_2 = 1.33 \) water media
because of optical aberration. In \( n_2 = 1.0 \) vacuum, the focal positions of \( n_1 = 1.93 \) and
2.20 are located inside the microsphere. It can be expected that the near-field
transformations may not occur between evanescent and propagating waves in the
near and near-external fields of the microsphere so that it may not support super-
resolution imaging.
Figure 7.3 FWHMs and DMIs for a dielectric sphere with a $d = 5 \, \mu m$ with refractive indexes of $n_1 = 1.46$, 1.59, 1.93, and 2.20, respectively, in $n_2 = 1.0$ vacuum and $n_2 = 1.33$ water media at wavelengths ($\lambda$) between 300 nm and 800 nm. (a) and (b) $d = 5 \, \mu m$, $n_2 = 1.0$; (c) and (d) $d = 5 \, \mu m$, $n_2 = 1.33$

7.3.5 FWHM and DMI for 30 $\mu$m dielectric microsphere

In the $d = 30 \, \mu m$ dielectric microsphere, the FWHM and DMI have been computed with incident plane waves in media of $n_2 = 1.0$ vacuum and $n_2 = 1.33$ water as shown in Figure 7.4. The FWHM and DMI is increased compared with $d = 5 \, \mu m$ dielectric microsphere. By increasing the size of the microsphere, DMI can obviously come out the microsphere with refractive indexes of the $n_1 = 1.93$ and 2.20 and the activity of optical resonance can be stronger in the near and near-external field of the microsphere. As a result, clear optical whirlpools can be observed at the near horizontal plane of the microsphere.
Figure 7.4 FWHMs and DMIs of dielectric microspheres with a $d = 30 \, \mu m$ with the four different refractive indexes of the microspheres embedded in $n_2 = 1.0$ vacuum and $n_2 = 1.33$ water media at 300 nm – 800 nm wavelengths. (a) and (b) $d = 30 \, \mu m$, $n_2 = 1.0$; (c) and (d) $d = 30 \, \mu m$, $n_2 = 1.33$

7.3.6 Diffraction limit and dielectric microsphere

The FWHMs divided by the incident plane wave wavelengths ($\lambda$) for $d = 5 \, \mu m$ and $30 \, \mu m$ dielectric microsphere are shown in Table 7.1. The refractive indexes of microspores and media are compared with wavelengths between 300 nm and 800 nm. The $\lambda_{2,5}$ in the visible spectrum is calculated by the average FWHM of $\lambda_2 = 400 \, nm$ to $\lambda_5 = 700 \, nm$. The FWHM divided by incident plane wavelengths ($\lambda$) can be independent of wavelengths, as the values are quite similar in wavelengths between 300 nm and 800 nm. The values can be generally affected by the refractive indexes of the microsphere and the media. The waist of photonic nanojet is determined by the waist of FWHM intensity [64].
Table 7.1 FWHM divided by incident plane wavelengths ($\lambda$) for $d = 5 \ \mu m$ and $30 \ \mu m$ dielectric microsphere in $n_2 = 1.0$ vacuum and $n_2 = 1.33$ water media. (a) $d = 5 \ \mu m$, $n_2 = 1.0$; (b) $d = 5 \ \mu m$, $n_2 = 1.33$; (c) $d = 30 \ \mu m$, $n_2 = 1.0$; (d) $d = 30 \ \mu m$, $n_2 = 1.33$

(a) $d = 5 \ \mu m$, $n_2 = 1.0$

<table>
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<th>400 nm</th>
<th>500 nm</th>
<th>600 nm</th>
<th>700 nm</th>
<th>800 nm</th>
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<tr>
<td>$n_2$ = 1.46</td>
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(b) $d = 5 \ \mu m$, $n_2 = 1.33$

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(c) $d = 30 \ \mu m$, $n_2 = 1.0$

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(d) $d = 30 \ \mu m$, $n_2 = 1.33$

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In the $d = 5 \, \mu m$ dielectric microsphere, the waist of the photonic nanojet is similar to the diffraction limit for the $n_1 = 1.46$ and 1.59 dielectric microsphere in $n_2 = 1.0$ vacuum medium. In $d = 30 \, \mu m$ dielectric microsphere, the photonic nanojet can have similar size of the diffraction limit in the $n_1 = 2.20$ dielectric microsphere in $n_2 = 1.33$ water medium. These photonic nanojets may have the capability of super-resolution imaging due to enhanced backscattering of visible light by sub-diffraction-limit objects [23].

7.4 Conclusions

It has been shown the photonic nanojets and the Poynting vector interaction of dielectric microspheres embedded in media of vacuum and water illuminated by near-ultraviolet, visible and near-infrared plane waves. The photonic nanojet divided by incident plane wavelengths can be affected by the size, and refractive indexes of dielectric microspheres and medium rather than the incident plane wavelengths. The optical resonances including optical whirlpools and rhombuses are observed in Poynting vector fields by analytical Mie theory. For the $30 \, \mu m$ dielectric microsphere with a refractive index of $n = 2.20$ in water, and the $5 \, \mu m$ dielectric microsphere with a refractive index of $n = 1.46$ and 1.59 in vacuum, the waist of photonic nanojets is similar to the optical diffraction limit without significant optical diffractions and the second focal position. For the dielectric microspheres with a refractive indexes of $n = 2.20$ and 1.93 in vacuum, the waists of photonic nanojets are smaller than the optical diffraction limit but the optical resonances can be disturbed because the second focal position can divert the energy of optical resonances. The phenomena of the microsphere photonic nanojets may provide
potential applications for detecting of sub-diffraction features with visible light in the fields of material, biology, chemical, and medical sciences.

7.5 Chapter appendix

Following the formulae [94] used in Mie theory calculation of electromagnetic field for outside and inside sphere is expressed in incident plane wave propagation along $z$ coordination, electric vector ($\mathbf{E}$) field along $x$ coordination, and magnetic vector ($\mathbf{H}$) field along $y$ coordination. The spherical coordination $[r, \theta, \phi]$ is indicated into the incident plane wave with the sphere positions in electric and magnetic field,

$$E_r = e^{i k_m r \cos \theta} \sin \theta \cos \phi,$$
$$H_r = \sqrt{\varepsilon_m} e^{i k_m r \cos \theta} \sin \theta \sin \phi,$$
$$E_\theta = e^{i k_m r \cos \theta} \cos \theta \cos \phi,$$
$$H_\theta = \sqrt{\varepsilon_m} e^{i k_m r \cos \theta} \cos \theta \sin \phi,$$
$$E_\phi = -e^{i k_m r \cos \theta} \sin \phi,$$
$$H_\phi = -\sqrt{\varepsilon_m} e^{i k_m r \cos \theta} \sin \phi,$$

The wave vectors for media, sphere and vacuum are $k_m = 2\pi \sqrt{\varepsilon_m / \lambda}$, $k_p = 2\pi \sqrt{\varepsilon_p / \lambda}$ and $k_0 = 2\pi / \lambda$ respectively, where $\lambda$ is the radiation wavelength. The dielectric permittivity of media and sphere is $\varepsilon_m$ and $\varepsilon_p$ respectively, and the magnetic permittivity is $\mu$. The dielectric sphere with radius $a$ is identified by the complex refractive index of media $\sqrt{\varepsilon_m} = n_m + i k_m$ and sphere $\sqrt{\varepsilon_p} = n_p + i k_p$. 

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\[ E_{r}^{(s)} = \frac{\cos \varphi}{(k_m r)^2} \sum_{n=1}^{\infty} n(n+1) e^{B_n} \zeta_n(k_m r) P_n^{(1)}(\cos \theta), \quad (7) \]

\[ E_{\theta}^{(s)} = \frac{\cos \varphi}{k_m r} \sum_{n=1}^{\infty} \left[ e^{B_n} \zeta_n(k_m r) P_n^{(1)}(\cos \theta) \sin \theta - i m B_n \zeta_n(k_m r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} \right], \quad (8) \]

\[ E_{\phi}^{(s)} = \frac{\sin \varphi}{k_m r} \sum_{n=1}^{\infty} \left[ e^{B_n} \zeta_n'(k_m r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} - i m B_n \zeta_n(k_m r) P_n^{(1)\prime}(\cos \theta) \sin \theta \right], \quad (9) \]

\[ H_{r}^{(s)} = \frac{\sqrt{\epsilon_m} \sin \varphi}{(k_m r)^2} \sum_{n=1}^{\infty} n(n+1) m B_n \zeta_n(k_m r) P_n^{(1)}(\cos \theta), \quad (10) \]

\[ H_{\theta}^{(s)} = i \frac{\sin \varphi}{k_0 r} \sum_{n=1}^{\infty} \left[ e^{B_n} \zeta_n(k_m r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} + i m B_n \zeta_n'(k_m r) P_n^{(1)\prime}(\cos \theta) \sin \theta \right], \quad (11) \]

\[ H_{\phi}^{(s)} = i \frac{\cos \varphi}{k_0 r} \sum_{n=1}^{\infty} \left[ e^{B_n} \zeta_n(k_m r) P_n^{(1)\prime}(\cos \theta) \sin \theta + i m B_n \zeta_n'(k_m r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} \right], \quad (12) \]

The inside of non-magnetic sphere with index \(a\) for internal scattering field is given by

\[ E_{r}^{(a)} = \frac{\cos \varphi}{(k_p r)^2} \sum_{n=1}^{\infty} n(n+1) e^{A_n} \psi_n(k_p r) P_n^{(1)}(\cos \theta), \quad (13) \]

\[ E_{\theta}^{(a)} = \frac{\cos \varphi}{k_p r} \sum_{n=1}^{\infty} \left[ e^{A_n} \psi_n'(k_p r) P_n^{(1)\prime}(\cos \theta) \sin \theta - i m A_n \psi_n(k_p r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} \right], \quad (14) \]

\[ E_{\phi}^{(a)} = \frac{\sin \varphi}{k_p r} \sum_{n=1}^{\infty} \left[ e^{A_n} \psi_n'(k_p r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} - i m A_n \psi_n'(k_p r) P_n^{(1)\prime}(\cos \theta) \sin \theta \right], \quad (15) \]

\[ H_{r}^{(a)} = \sqrt{\epsilon_m} \sin \varphi \frac{(k_p r)^2}{\sum_{n=1}^{\infty} n(n+1) m A_n \psi_n(k_p r) P_n^{(1)}(\cos \theta), \quad (16) \]

\[ H_{\theta}^{(a)} = i \frac{\sin \varphi}{k_0 r} \sum_{n=1}^{\infty} \left[ e^{A_n} \psi_n(k_p r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} + i m A_n \psi_n'(k_p r) P_n^{(1)\prime}(\cos \theta) \sin \theta \right], \quad (17) \]

\[ H_{\phi}^{(a)} = i \frac{\cos \varphi}{k_0 r} \sum_{n=1}^{\infty} \left[ e^{A_n} \psi_n(k_p r) P_n^{(1)\prime}(\cos \theta) \sin \theta + i m A_n \psi_n'(k_p r) \frac{P_n^{(1)}(\cos \theta)}{\sin \theta} \right], \quad (18) \]
coefficients

\[ e_{B_n} = i^{n+1} \frac{2n + 1}{n(n+1)} a_n, \quad m_{B_n} = i^{n+1} \frac{2n + 1}{n(n+1)} b_n. \] (19)

\[ e_{A_n} = i^{n+1} \frac{2n + 1}{n(n+1)} c_n, \quad m_{A_n} = i^{n+1} \frac{2n + 1}{n(n+1)} d_n. \] (20)

where

\[ a_n = \frac{y \psi'_n(x) \psi_n(y) - x \psi'_n(x) \psi_n(y)} {y \xi'_n(x) \psi_n(y) - x \psi'_n(y) \xi_n(x)} \] (21)

\[ b_n = \frac{y \psi'_n(y) \psi_n(x) - x \psi'_n(y) \psi_n(x)} {y \xi'_n(y) \psi_n(x) - x \psi'_n(y) \xi_n(x)} \] (22)

\[ c_n = \frac{y \xi'_n(x) \psi'_n(y) - y \xi'_n(x) \psi_n(y)} {y \xi'_n(x) \psi'_n(y) - x \psi'_n(y) \xi'_n(x)} \] (23)

\[ d_n = \frac{y \xi'_n(x) \psi'_n(y) - y \xi'_n(x) \psi_n(y)} {y \xi'_n(x) \psi'_n(y) - x \psi'_n(y) \xi'_n(x)} \] (24)

\[ x = k_m a, \quad y = k_p a, \] (25)

\[ \zeta_n(\rho) = \rho \delta^{(1)}_{n+\frac{1}{2}}(\rho), \quad \zeta'_n(\rho) = \frac{\partial \zeta_n(\rho)}{\partial \rho}, \] (26)

\[ \psi_n(\rho) = \rho j_{n+\frac{1}{2}}(\rho), \quad \psi'_n(\rho) = \frac{\partial \psi_n(\rho)}{\partial \rho}, \] (27)

the scattering coefficients

associated Legendre function,

\[ p_n^m(\cos \theta) \equiv \frac{(1 - (\cos \theta)^2)^{m/2}}{2^n n!} \frac{d^{n+m}}{d(\cos \theta)^{n+m}} ((\cos \theta)^2 - 1)^n, \quad (m \geq 0) \]

\[ p_n^m(\cos \theta) \equiv (-1)^m \frac{(n + m)!}{(n - m)!} p_n^{-m}(\cos \theta), \quad (m < 0) \] (28)
spherical Hankel function,

\[
h_n^{(1)}(\rho) = \sqrt{\frac{\pi}{2\rho}} H_n^{(1)}(\rho),
\]

(29)

spherical Bessel function,

\[
j_n(\rho) = \sqrt{\frac{\pi}{2\rho}} j_{n+\frac{1}{2}}(\rho).
\]

(30)
8 Optical Super-Resolution of Smooth Muscle Cells and Viruses

Summary
Standard optical microscopes cannot obtain nano-size of imaging by their direct observation. Scanning electron microscopes (SEM) are generally used in the observation of nano-size structure but require vacuum and dry conditions so that live biology imaging and liquid environments cannot be supported. The fluorescence optical microscopy is well-known to observe indirect optical biological imaging on the stimulated emission from fluorescent samples but the observation is generally bigger than real size. This work suggests that such limitations can be overcome by the method for near-field optical super-resolution imaging of submerged microsphere optical nano-scopy (SMON). Optical imaging using large 100 μm diameter BaTiO₃ glass microsphere was experimentally demonstrated in water medium and compared with SEM images and fluorescent labeling images. Optical super-resolution imaging of 75 nm Adenoviruses and 80 - 120 nm nuclear pores of vascular smooth muscle cells was experimentally confirmed through SMON technique.
8.1 Introduction

The optical resolution of standard optical microscopes is limited in around 200 nm in visible wavelength illuminations. Such limitation is due to diffraction limit of an optical object lens so the direct observation of nano-size biology imaging is prevented. To overcome such limitation, transmission electron microscopy (TEM) and scanning electron microscope (SEM) are generally used to observe nano-size of biology imaging at extremely high resolution. However, these techniques are only supported in vacuum and dry condition so they do not provide live biology imaging and liquid conditions.

Fluorescence optical microscopy is well-developed recently for super-resolution imaging of bacteria, cellular tissue and viruses up to 6 nm resolution [50, 51, 95, 96]. The technique of fluorescence optical microscopy is generated by detection between stimulated emission of fluorescent sample and the excitation of a specific wavelength. To develop illumination, saturated structured illumination microscopy (SSIM) and stimulated emission depletion (STED) are simultaneously used in activate fluorescent emissions [33, 45]. Statistical optical reconstruction microscopy (STORM) and photo-activated localization microscopy (PALM) was developed due to enhance statistical observation and image resolution of fluorescent stimulated emission [39, 40]. Scanning near field optical microscopy (SNOM) can also obtain super-resolution imaging by point by point scanning of optical tip located in near-field up to 60 nm [97], but SNOM technique requires the long time processing for the full image so it may limit the observation of dynamic behaviour for biology samples.
The super-resolution imaging of negative refractive index metamaterials was demonstrated [7, 13, 98] but it was not applied to biology samples and high level of illumination attenuation can prevent the practical application of super-resolution biology imaging. The femtosecond x-ray laser was recently used in super-resolution imaging of viruses just before destruction [99]. The binary nano-structured mask was also used in the super-oscillatory lens optical microscopy for super-resolution imaging [72]. The image resolution is about 105 nm, and it can provide opaque surface with optical transparent materials. In biology super-resolution imaging, the fluorescent microscope is widely used in research area but the limited area of cellular structure only can be provided because of fluorescent illuminated light and immunestaining. Holography is one solution for probing biological images of bacteria [100] but it is an indirect process and relies on digital processing and detectors. SMON technique was first reported with optical super-resolution of 50 um through fused silica microsphere in dry condition [19].

This work introduces the SMON technique immersed in water for direct optical super-resolution imaging of 75 nm diameter adenovirus and 80 nm – 120 nm diameter smooth mussel cell nuclear pores. 100 µm diameter BaTiO₃ glass microspheres were experimentally demonstrated in water media through a standard optical microscope. Dual light of reflective and transmitted illumination was used in experiments.
8.2 Materials and methods

8.2.1 Experimental setup

The schematic of SMON technique is shown in Figure 8.1. Dielectric and high refractive index microsphere of 100 µm diameter BaTiO$_3$ glass was used, and the refractive index is around 1.90 at visible wavelengths. The BaTiO$_3$ glass microsphere was spread on the target of virus and cell, and then water drop was applied between the specimen and the microscope object lens. Dual halogen light illumination was simultaneously projected to the target.

![Figure 8.1 The schematic of experimental setup using dual light conditions.](image-url)
8.2.2 Preparation of adenovirus sample

The type 5 of replication-disabled adenoviruses was used with deletions of E1 and E3 genes. The cover glass slide was coated 5 nm gold to support the imaging of optical standard microscope and SEM. Adenovirus was diluted in distilled water, and the diluted adenovirus was spread on the coated cover glass and left to dry. One drop of 4% paraformaldehyde was used to fix adenovirus for 20 minutes.

8.2.3 Preparation of cell sample

A7r5 smooth muscle cells of rat aorta were incubated on 0.2% gelatin coated cover glass slide at 37°C and 5% CO₂ in medium of Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin/streptomycin. 4% paraformaldehyde fixed the cell sample for 20 minutes, and hemotoxylin was used to incubate the cell sample for 5 minutes. After that, the cell sample was also grown on eosin for 5 minutes and washed by water and left to dry. The haematoxylin and eosin (H&E) staining was used because of increasing contrast.

8.2.4 Preparation of TEM sample

Diluted adenoviruses were coated by carbon for TEM grid, and negative stain was used through uranyl acetate.
8.2.5 Preparation of fluorescent sample

8.2.5.1 Adenovirus immunestaining

1 µl adenovirus stock was diluted in 10 µl deionised water. 3 µl diluted viruses were smeared on gelatine-coated cover glass. The cover glass was pre-coated with about 30 nm platinum that include written numbers by 532 nm green marker laser. The adenovirus were fixed in 4% paraformaldehyde for 20 minutes and incubated with 5% foetal calf serum (FCS) in phosphate buffer saline (PBS) for 30 minutes. The sample was then probed by incubating with rabbit polyclonal anti-adenovirus Type 5 antibody (ab6982, Abcam, 1:500 dilution) for 1 hour followed by incubating with AlexaFluor488-conjugated donkey anti-rabbit secondary antibody (Molecular Probes, 1:1000 dilution). After washing with PBS and a final wash with deionised water, the sample was left to dry at room temperature.

8.2.5.2 Nuclear pore immunostaining

Rat aortic smooth muscle cells (A7r5 line) were grown on cover glasses in a 6-well plate. After 24 hours, the cells were fixed and probed with the same procedure for adenoviruses as described above except for the primary antibody used was mouse monoclonal anti-nuclear pore complex proteins antibody (Mab414, ab24609, Abcam, 1:500 dilution) and secondary antibody was AlexaFluor-594 donkey anti-mouse IgG (Molecular Probes, 1:1000 dilution).
8.3 Results and discussion

8.3.1 Experimental demonstration of SMON

The observation of anodic aluminium oxide (AAO) sample was experimentally demonstrated with the 100 μm BaTiO3 microsphere immersed in water through the standard optical microscope. Dual light condition was applied and the object lens of x 50 NA: 0.75 was used. The average size of AAO pores is approximately 50 nm as shown in Figure 8.2 (a). The super-resolution imaging of AAO pores were observed by SMON technique in Figure 8.2 (b).

Figure 8.2 Image of AAO pores in (a) SEM and (b) SMON
The metal coating on the substrate can increase the resolution, contrast and magnification if the target is nano-size. It may cause evanescent waves to increase by the surface plasmon effect. For example, the magnification of BaTiO$_3$ glass microsphere immersed in water was about 3.5 times without metal coating but 14 times magnification was obtained with gold coating.

### 8.3.2 Image of adenoviruses

The size of adenoviruses was observed in TEM as shown in Figure 8.3. The individual adenoviruses size is approximately 75 nm.

![Figure 8.3 Image of adenovirus and cluster in TEM](image)

The optical super-resolution imaging of adenovirus was demonstrated with 100 µm BaTiO$_3$ glass microspheres immersed in water through a standard optical microscope. The object lens of x100 NA:0.95 was used in an Olympus optical microscope at dual...
light conditions. The microspheres were spread on adenovirus sample, and the best image was observed at 70 μm below the focus of the target substrate. The SMON images were compared with SEM images and the same location with a close up view in Figure 8.4. The individual adenovirus was obviously observed by SMON technique.

Figure 8.4 SMON image of adenovirus; (a) comparing between adenovirus clusters and SEM image, (b) image of close up view.
Figure 8.5 Comparison of SMON imaging and fluorescent imaging of immunostaining in adenoviruses. Right side view is a close up of left side view; (a) optical image without SMON technique, (b) optical super-resolution image with SMON technique, (c) fluorescent image with SMON technique.
The imaging of adenoviruses immunostaining was compared between SMON technique at standard optical microscope and a florescent microscope in Figure 8.5. The Leica standard optical microscope (Leica DM 2500M) was used in experiments of SMON technique with the object lens of x50 NA: 0.75. The Axiovision upright florescent microscope was demonstrated for florescent images. In the standard optical microsphere without SMON technique, the adenoviruses immunostaining was not observed in Figure 8.5 (a). However, the circle single shape and clusters of the adenoviruses immunostaining were obtained with SMON technique in Figure 8.5 (b). The location of adenoviruses was confirmed with Figure 8.5 (c).

### 8.3.3 Image of A7r5 smooth muscle cells

A7r5 smooth muscle cells was deposited on the gold-coated cover slide glass, and experimentally demonstrated with a Leica optical microscope with the object lens of x100 NA: 0.85 at dual light conditions. A 100 µm diameter BaTiO₃ microsphere was spread on the target and water drop was applied between the target and the object lens. The optical imaging of smooth muscle cells was observed at the standard optical microscope without the microsphere as shown in Figure 8.6 (a), (b). The optical super-resolution imaging was observed with SMON technique. The SMON imaging of nuclear pores obtained 80 nm and 120 nm diameter. It may cause plasmon effect from gold coating so enlarged view are generated.
Figure 8.6 Super-resolution imaging of A7r5 smooth muscle cells through SMON technique. (a) imaging of the standard optical microscope with the object lens of x100 NA:0.85, (b) close up view of the same location as SMON imaging, (c) optical super-resolution imaging of the nuclear pores in A7r5 smooth muscle cells through SMON technique. Arrows point out the location of nuclear pores.
Figure 8.7 Compared Super-resolution imaging between SMON and fluoresce in A7r5 smooth muscle cells (excited red colour) and adenoviruses (excited green colour); (a) fluorescent imaging of A7r5 smooth muscle cells and adenoviruses, (b) optical image without SMON technique, (c) close up view of fluorescent imaging, (d) optical super-resolution image of SMON technique.

The comparison of fluorescent and SMON image was generated in A7r5 smooth muscle cells (excited red colour) and adenoviruses (excited green colour) in Figure 8.7. The nuclear pores of A7r5 smooth muscle cells were not observed and
adenoviruses are particularly obtained through SMON technique as shown in Figure 8.7 (d). The view size of adenoviruses was approximately 700 – 800 nm that is about 10 times bigger than real size. It may cause the plasmon effect of platinum may support extra magnification.

### 8.4 Conclusions

By the 100 µm BaTiO₃ glass microsphere immersed in water, the optical super-resolution images of SMON technique was obtained in 75 nm diameter of adenoviruses and 80 – 120 nm diameter of A7r5 smooth muscle cells at dual simultaneous lighting of reflective and transmitted illumination. The super-resolution imaging was verified and compared by the same location of SEM and fluorescent images. About 3.5 time and 14 time magnifications were obtained in AAO sample of raw substrate and thin gold-coated substrate, respectively. The metal coating may enhance evanescent waves so the magnification, resolution, and contrast may be increased in SMON technique. The SMON technique would provide the potential image area of biology imaging that include live virus, bacteria, and cell due to direct imaging process, common lighting and atmosphere conditions.


9 Conclusions and Recommendations for Future Work

9.1 Conclusions

This research has further advanced the science and technology of microsphere optical nanoscopy (MONS) and submerged microsphere optical nanoscopy (SMON). Both experimental observations and theoretical investigations have been carried out. Effects of sphere size and surrounding media have been investigated. The research has been focused on the use of larger (30 - 100 µm diameter) microspheres. The imaging of virus and cells and sub-diffraction limited sub-surface structures have been reported for the first time. The following sections provide a summary of major findings from this research.

9.1.1 Media effect of super-resolution imaging technique

Sub-diffraction-limit imaging using a large BaTiO$_3$ glass microsphere through a standard optical microscope was demonstrated in three different media of water, 40% sugar solution, and Leica microscope immersion oil. The optical magnification of a Blu-ray disc with 120 nm line width and 180 nm spacing using a 100 µm BaTiO$_3$ glass microsphere is approximately 3.3 times, 2.8 times, 2.3 times in water, 40% sugar solution, and Leica microscope immersion oil, respectively. The focal image positions can be affected by the refractive index of immersion liquids. In water and 40% sugar solution, the magnified super-resolution images were obtained but Leica
microscope oil distorted the image. Colour rings have observed around the imaging centre.

9.1.2 Size effect of microsphere size in super-resolution imaging
Larger polystyrene microspheres of 30 µm, 50 µm and 100 µm in diameters can allow super-resolution imaging in air. The Blu-ray disc (120 nm line width and 180 nm spacing) and gold nano-patterned quartz (150 nm diameter gold spot and 600 nm spacing) were experimentally observed using the MONS technique in the reflection light mode. Approximately 5 - 7 times magnification has been obtained. The mechanism of the MONS technique has been theoretically explained to involve the conversion of near-field evanescent waves to far-field propagating waves.

9.1.3 Super-resolution imaging of sub-surface nanostructures
TiO₂-BaO-ZnO glass microspheres immersed in water were used to demonstrate super-resolution imaging of the data-recorded (sub-surface nanostructures) Blu-ray disc. The irregular data spots were experimentally observed for the first time. With the objective lenses of \( l_1 = \times50 \text{ NA:0.75} \) and \( l_2 = \times100 \text{ NA:0.85} \), approximately 5 - 6 magnification was experimentally achieved. The finest super-resolution images was observed with the optical focal plane at about 125 µm - 135 µm below the Blu-ray disc surface. In the simulations, the near-field optical field structures were observed inside the microsphere.
9.1.4 Super-resolution imaging with photonic nanojets

The photonic nanojets were simulated for the various microspheres of different sizes and surrounding media using Mie theory to analyse the behaviour of the Poynting vector fields. The near-field optical field structures were observed in 5 µm and 30 µm diameter dielectric microspheres of various refractive indexes from $n = 1.4$ to $2.2$ in vacuum and water media. The waist of photonic nanojets was shown similar to the optical diffraction limit in the 30 µm dielectric microsphere with a refractive index of $n = 2.20$ in water, and the 5 µm dielectric microsphere with a refractive index of $n = 1.46$ and 1.59 in vacuum.

9.1.5 Biological super-resolution imaging

Super-resolution imaging of 75 nm diameter adenoviruses and 80 – 120 nm A7r5 smooth muscle cells was observed by the standard microscopy through a 100 µm diameter BaTiO$_3$ glass microspheres immersed in water. To improve the biological image contrast and resolution, the dual lighting condition of reflection and transmission illumination was simultaneously applied.

9.2 Recommendations for the future work

The work detailed in the thesis would open up a new research field for biological super-resolution imaging and imaging of sub-surface nanostructures for applications in electrical engineering, material science, biology, chemistry, and medical sciences.
9.1.1. Further development of super-resolution imaging techniques

Further understanding of the limit of sphere size, shape and media on the imaging resolution and magnification is needed. The imaging resolution is currently limited to 50 nm. To improve the resolution beyond this, other sphere geometry and structures could be considered. The image magnification is not uniform at different locations of spheres. An imaging processing software needs to be developed to allow the correction of different magnifications at different locations. The spread of the microspheres on the target surface is not practical for real applications. The imaging technique needs to be developed to allow the microspheres to be attached to the objective lens of the optical microscope. The distance between the microsphere and the target surface is within the optical wavelength. This limits wide applications. Far-field super-resolution imaging would be in the spotlight for nano-imaging. Such techniques may require full understanding of the super-resolution mechanisms and combination of a number of imaging techniques.

9.2.1 Improvements in photonic nanojets

In the area of the photonic nanojet, the undiffraction-limited information can be delivered to the dielectric layer, and the microsphere can collect this information at the near-field of the dielectric layer. Such optical transparent super-resolution imaging could apply the far-field super-resolution imaging within the area of the photonic nanojet. The far-field super-resolution imaging could be expected by the width and length of the photonic nanojet. Such photonic nanojets may need the understanding of relationship between refractive indexes and sizes of microspheres or micro-cylinders, and effect of media and a substrate.
References


