Chapter 3

Single-photon excitation of morphology dependent resonance

3.1 Introduction

The examination of morphology dependent resonance (MDR) has been of considerable importance to many fields in optical science. Resonant probes have advantages over conventional passive scattering probes, the most significant of which is the increased signal to noise ratio due to field enhancement localised to the immediate vicinity of the probe. The problem lies in the coupling to and from such a probe. Due to the precise nature of cavity effects and their dependence on the superposition of fields in integer multiples of size to wavelength, quite specific conditions have to be met. A probe incident with narrow linewidth continuous wave (CW) excitation has only one mode that can satisfy the cavity condition. Doping the microcavity with a fluorescent dye allows the excitation of many fluorescent resonance modes due to the broad nature of the fluorescence spectrum. Many attempts have been made to induce MDR in probes of various shapes. Various waveguides, toroids and spheres on a cantilever have been proposed and constructed. They are quite successful in inducing resonant
field enhancements and even lasing but all have limitations when it comes to probing or imaging a sample in a practical application. Scanning a resonant probe while maintaining its relative position in three-dimensions with respect to a sample and maintaining strict resonance coupling conditions to achieve a good signal to noise ratio is difficult using these methods.

Under single-photon illumination, the fluorescence excitation can be confined laterally in two-dimensions within a microsphere, which allows quantitative characterisation of the dependence of MDR peaks at varying excitation locations. The results reveal that MDR peaks have a degree of polarisation, differing between two adjacent peaks which indicates the presence of transverse electric (TE) and transverse magnetic (TM) modes. These are modes that have orthogonal states of polarisation. It is also found that the strength and polarisation of the MDR effect can be controlled by targeting specific locations within a microsphere, which provides a unique tool to gain fundamental knowledge about the microcavity.

Although the MDR effect and its subsequent applications, such as microcavity lasing, have been investigated for microparticles, microdroplets and other microobjects [22,67–69], it has been difficult in the past to quantitatively determine the features, such as the state of polarisation of MDR peaks representing TE and TM modes. Due to the difficulty of confining the excitation volume in three-dimensional space, previous experiments have been based on an averaged effect over a large excitation volume, despite the fact that the characteristics of individual MDR peaks depends on the location of the excitation spot in a microobject.

In this chapter the resonance of a fluorescent doped spherical microcavity is investigated under various coupling conditions of femtosecond pulsed single-photon excitation.
3.2 Experimental Setup

The experiment system layout is shown in Fig. 3.1. A train of linearly polarised 80 fs pulses of wavelength 870 nm (Spectra-Physics Tsunami) passes through a frequency doubler (Spectra-Physics 3980). The resulting beam of 435 nm is coupled directly into a homemade inverted scanning microscope. A high numerical aperture (NA = 1.2) water immersion objective (Olympus UplanXW60) is used to focus the pulses onto the sample. The sample consists of yellow-green fluorescence microspheres of 10 μm in diameter, which have an absorption peak close to the laser wavelength for single-photon excitation. The fluorescence emission from an excited microsphere is analysed by a high-resolution spectrograph (ARC, Δλ = 0.1 – 0.3 nm). A polarisation analyser is put in the detection path to order to investigate the polarisation nature of the fluorescence spectrum.

![Fig. 3.1: Schematic diagram of the experimental setup for single-photon excitation of MDR.](image)
3.3 Excitation localisation within a spherical microcavity

Throughout this thesis, the location of the focal point of the incident illumination relative to a spherical microcavity is defined in spherical coordinates \((r, \phi, \theta)\) originating from the centre of microcavity as pictured in Fig. 3.2. The radial distance \(r\) from the origin is normalised in terms of the cavities physical size \(a\), so that when the focal point of the incident illumination is positioned at the perimeter of the microcavity is one, i.e. \(r/a = 1\). The origin of the \(\phi\) coordinate in the azimuthal or equatorial plane is defined so that the linearly polarised incident illuminations electric-field intersects perpendicular to the cavity perimeter. Similarly, the meridian plane is defined such that \(\theta = 0\) at the equatorial plane and the incident illumination from the microscope objective passes in the direction from \(\theta = -90^\circ\) to \(\theta = 90^\circ\). The emitted fluorescence and resonance signal is collected by the same illumination objective.

A typical fluorescence spectrum from the microcavity under single-photon excitation is shown in Fig. 3.3. In order to quantify the strength of MDR spectral features from the fluorescence background the measurable quantity, visibility \((V)\) defined in Eq. 3.1 is introduced:

\[
V = \frac{(I_{\text{peak}} - I_{\text{background}})}{(I_{\text{peak}} + I_{\text{background}})},
\]

where \(I_{\text{peak}}\) and \(I_{\text{background}}\) are the intensities of a MDR peak and background fluorescence respectively.

In order to characterise the polarisation nature of a resonant MDR feature the degree of polarisation \((\gamma)\) (Eq. 3.2) is introduced:
Fig. 3.2: Excitation locations within a microsphere: (a-c) in the radial direction: (a) $r = 0$, (b) $r = 0.5a$, and (c) $r = a$; (I–III) in the meridian plane: (I) $\theta = -90^\circ$, (II) $\theta = 0^\circ$ and (III) $\theta = 90^\circ$; (i–iii) in the equatorial plane (i) $\phi = 0^\circ$, (ii) $\phi = 90^\circ$ and (iii) $\phi = 180^\circ$. 
where $I_{\alpha=\text{max}}$ and $I_{\alpha=90^\circ}$ represent to the maximum intensity of a MDR peak and the intensity of the peak when the angle of the analyser ($\alpha$) is rotated by $90^\circ$.

### 3.4 Spatial dependence of morphology dependent resonance under single-photon excitation

An archetype fluorescence spectrum measured under the experimental arrangement (Fig. 3.1) is shown in Fig. 3.3. The fluorescence emission at specific wavelengths is enhanced due to the microcavity effect. The cavity quality factor $Q$ (see Eq. 2.5), which can be estimated from the elastic-scattering line-width based on Lorenz-Mie theory [14], is approximately $773 \pm 140$ for this measurement.
A multimode resonances structure is evident from the MDR spectrum in Fig. 3.3. The complex quasi-periodic spectra and unequal mode spacing arise from both material and cavity dispersion. This multimodal behavior is manifested in the fact that the radial distribution of MDR modes is frequency dependent [60]. Higher frequency (short wavelength) modes propagate along paths that are slightly closer to the surface than those of lower frequency (longer wavelength) modes. Thus higher frequency modes travel in trajectories of a slightly larger radius and slightly longer optical path lengths (see Fig. 2.1).

The tight focus of a high numerical aperture objective gives strong fluorescence and MDR signal across the entire fluorescence spectrum with low average power, in the order of $nW$ to $\mu W$. The same property of the incident illumination also means that fluorescence can be generated by each ray throughout the entire focal volume as it interacts with and refracts through the dye doped microcavity. Single-photon excitation has a large excitation volume and significant fluorescence is generated from the out-of-focus regions. The fluorescence resulting from the out-of-focus region as illustrated in Fig. 2.14 adds to the fluorescence background and detracts from the signal coupling into MDR. If the incident average power is too high photo-bleaching and damage to the fluorescent microcavity can result.

The fluorescence excited in a microsphere is a combination of the bulk material fluorescence response and that of the microspherical cavity mode profile. The overlap of particular fluorescence wavelengths with the spectral mode profile of the microcavity at specific cavity conditions leads to the modulation of the fluorescence spectral emission. The resultant MDR phenomenon is evident in the distinct peaks of the fluorescence spectra. The MDR response is dependent on the coupling of the fluorescence excitation to the cavity mode profile. The location of fluorescence excitation determines the portion of wavelengths coupled to MDR cavity modes.

The fluorescent spectra from different spatial locations of incident excitation within the microcavity indicate that different coupling of MDR is possible. The ratio of MDR
peaks to background fluorescence changes with excitation position. For example when the focal position is varied in the radial direction (Figs. 3.4a-c, $0 \leq r \leq a$, $\theta = 0^\circ$, $\phi = 0^\circ$), an enhancement of the MDR effect is observed; this is intuitive as the rays in close proximity to the boundary have increased probability interacting at glancing angles (Fig. 3.4c, $r = a$, $\theta = 0^\circ$, $\phi = 0^\circ$).

When the focal spot is repositioned in the meridian plane ($r = a$, $-90^\circ \leq \theta \leq 90^\circ$ and $\phi = 0^\circ$) only small variation in the MDR peaks are observed, as shown in Fig. 3.5. It is expected that the glancing coupling at $\theta = 0^\circ$ be stronger than that at $\theta = 90^\circ$.

If the excitation focus is repositioned in the equatorial plane (Figs. 3.6i-iii, $0 \leq \phi \leq 180^\circ$, $r = a$, $\theta = 0^\circ$). The polarisation direction of the linearly polarised incident beam changes with respect to the cavity boundary, resulting in changed coupling conditions into MDR modes. This indicates the presence of differently polarised modes within the MDR spectrum.

Placing a polarisation analyser in the detection path enables further exploration of the polarisation nature of MDR modes. The focal position is fixed at $r = a$, $\theta = 0^\circ$, $\phi = 0^\circ$ and the visibility of two adjacent cavity modes, 513.7 nm and 517.3 nm measured for all angles of the polarisation analyser ($\alpha$). Rotating the polarisation analyser by $90^\circ$ the MDR peak at 513.7 nm becomes less pronounced compared with that at 517.3 nm (Fig. 3.7). Rotating the polarisation analyser by a further $90^\circ$ results in the reemergence of peak 513.7 nm compared with that at 517.3 nm. The phenomena that two adjacent peaks have orthogonal polarisation states indicate characteristic TE and TM oscillating cavity modes, as seen in the variation of the visibility in Fig. 3.8.

To quantify the changes in peak strengths illustrated above the average visibility of multiple MDR peaks in the radial direction, meridian and azimuthal planes is illustrated in Fig. 3.9. In the radial direction ($0 \leq r \leq a$, $\theta = 0^\circ$, $\phi = 0^\circ$), an increase in the average visibility of 0.08 from the centre of the cavity and the perimeter.
Fig. 3.4: Single-photon MDR spectra as a function of excitation spots in the radial direction ($r$). (a) $r = 0$, (b) $r = 0.5a$, and (c) $r = a; \theta = 0^\circ$ and $\phi = 0^\circ$. 
Fig. 3.5: Single-photon MDR spectra as a function of excitation spots in the meridian plane ($\theta$). (I) $\theta = -90^\circ$, (II) $\theta = 0^\circ$ and (III) $\theta = 90^\circ$; $r = a$ and $\phi = 0^\circ$. 
Fig. 3.6: Single-photon MDR spectra as a function of excitation spots in the equatorial plane ($\phi$). (i) $\phi = 0^\circ$, (ii) $\phi = 90^\circ$ and (iii) $\phi = 180^\circ$; $r = a$ and $\theta = 0^\circ$. 

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Fig. 3.7: Polarisation dependence of fluorescence spectra: (a) without an analyser; (b) analyser angle $\alpha = 0^\circ$ and (c) analyser angle $\alpha = 90^\circ$. MDR peaks at wavelengths 513.7 nm (dashed) and 517.3 nm (solid), respectively.
is evident as shown in Fig. 3.9a. It is intuitive that the localisation of a focal spot at the perimeter leads to improved coupling of the resonance rays due to the glancing angles of incidence with respect to the boundary.

The dependence of the average MDR visibility on the excitation positions in the meridian plane ($-90^\circ \leq \theta \leq 90^\circ$, $r = a$, $\phi = 0^\circ$) is shown in Fig. 3.9b. The MDR effect is enhanced when the incident illumination is focused at the equatorial plane of the microcavity, i.e. when $\theta = 0^\circ$. It is due to more rays that are coupled into the cavity satisfy the total internal reflection condition if the focal spot is at the equator of a sphere. The break in symmetry between the two hemispheres can be ascribed to the spherical aberration induced by focusing through the sphere when $\theta = 90^\circ$ accentuated by the large focal volume and the inherently poor localisation of single-photon excitation in the propagation direction.

The average visibility as a function of azimuthal angles for excitation locations in the equatorial plane ($0 \leq \phi \leq 180^\circ$, $\theta = 0^\circ$, $r = a$) is shown in Fig. 3.9c. It is found that a periodic variation appears in the visibility due to the excitation position. When the polarisation of the incident light is fixed, the strength of the MDR peak can be
controlled by the focal position. This occurs due to the fact that the polarisation state of the incident beam with respect to the boundary of the microcavity changes with different $\phi$ values. If the excitation beam is linearly polarised in the $x$-direction (as indicated in Fig. 3.2), the polarisation direction is perpendicular to the boundary of the sphere for excitation locations $i$ and $iii$, and becomes parallel for excitation location $ii$. This periodic variation of the MDR visibility within the azimuthal plane demonstrates the different coupling of the incident field to the resonant modes of the cavity which is indicative of TE and TM mode behaviors.

The average degree of polarisation ($\gamma$) in the radial direction ($0 \leq r \leq a$, $\theta = 0^\circ$, $\phi = 0^\circ$) increases as the strength of the resonances increase as given in Fig. 3.10a. This increase indicates that the polarisation preservation of cavity modes increases with their visibility. The average degree of polarisation given in Fig. 3.10b shows little variation with the focal localisation in the meridian plane ($-90^\circ \leq \theta \leq 90^\circ$, $r = a$, $\phi = 0^\circ$). This can be largely attributed to the poor axial confinement of the randomly polarised fluorescence generated throughout the entirety of the focal volume and its poor coupling to MDR modes. The low efficiency of fluorescence coupling to MDR corresponds to poor mode polarisation preservation as there is little difference between it and the background fluorescence. Only in the equatorial plane ($0^\circ \leq \phi \leq 180^\circ$, $\theta = 0^\circ$, $r = a$), where the separation between excitation positions can become large, is there a distinguishable variation in the degree of polarisation of MDR modes from the background (Fig. 3.10c). This is due to the polarisation direction of the resonances shifting with the excitation from perpendicular to parallel with respect to the microcavity boundary and negligible overlap of the focal volumes at $\phi = 0^\circ$ and $\phi = 90^\circ$, respectively.
Fig. 3.9: The average visibility (a-c) of MDR peaks as a function of excitation spots in the radial direction ($r$), the meridian plane ($\theta$) and the equatorial plane ($\phi$), respectively.
Fig. 3.10: The average degree of polarisation (a-c) of MDR peaks as a function of excitation spots in the radial direction \((r)\), the meridian plane \((\theta)\) and the equatorial plane \((\phi)\), respectively.
3.5 Fluorescence lifetime of a spherical microcavity

In order to quantify the microcavity phenomena under single-photon excitation the spectral and temporal properties need to be examined. The spectral variation of the MDR visibility and degree of polarisation with respect to the localisation of the incident excitation within a microcavity demonstrated in Section 3.4 shows significant cavity enhancement. The impact of the variation in fluorescence coupling to MDR cavity modes and its effect on the temporal properties of the microcavity fluorescence lifetime are examined in this section.

The experimental arrangement for the measurement of the microcavity fluorescence lifetime under single-photon excitation is an adaptation of that in Section 3.2 with the addition of an ultrafast intensified CCD (ICCD) camera (LaVision), as shown in Fig. 3.11. The resolution of the ICCD camera is 200 ps and its repetition rate is tuned to synchronise with the repetition rate of the laser, which is 82 MHz.

![Fig. 3.11: Schematic diagram of the experimental setup for single-photon fluorescence lifetime imaging.](image)

The fluorescence lifetime is acquired by the continuous acquisition of a series of images with a temporal resolution of 200 ps gated over 12.2 ns, which is the time between two successive laser pulses. From this image series, the fluorescence decay of
regions of interest can be determined. The resultant fluorescence decay curve is then fitted to an exponential decay to determine the fluorescence lifetime. An example of a fluorescence lifetime image series under single-photon excitation is shown in Fig. 3.12, where 42 images over 8.2 ns of 12.2 ns are shown for clarity. The incident laser pulse is delayed until 400 ns, shown in image panel 2 and marked by a ×. The schematic of the microcavity is also overlayed in image panel 2, its position remains constant for the entire image series.

The measured fluorescence lifetime as a function of the excitation localisation in the radial direction ($0 \leq r \leq a$, $\theta = 0^\circ$, $\phi = 0^\circ$) is illustrated in Fig. 3.13. It is noticed that the increase of fluorescence lifetime is analogous to that of the visibility measured in Fig. 3.9a, demonstrating that as the MDR effect becomes more pronounced, more energy is stored within the cavity. The fluorescence excited from the microcavity centre experiences little overlap with the microcavity mode profile compared to excitation near the perimeter of the microcavity. Hence the leakage of the fluorescence energy from the microcavity is reduced as the excitation is positioned further from the microcavity centre and coupling to MDR increases.

Fluorescence lifetime is also accentuated by the increased coupling of out-of-plane incident excitation that partially propagates within the cavity at near glancing angles to MDR modes. The ability to induce fluorescence throughout the entire excitation volume under single-photon excitation increases the signal from the out-of-focus regions regardless of the focal points localisation within the microcavity.

3.6 Summary

In this chapter the demonstration of MDR modes and their polarisation-dependent features under single-photon excitation have been presented. Frequency doubled femtosecond pulsed excitation has demonstrated an increase in the MDR visibility
Fig. 3.12: Fluorescence lifetime imaging of a microcavity under single-photon excitation at $r = a$, $\theta = 0^\circ$, $\phi = 0^\circ$. Images 1 → 42 over 8.2 ns at 200 ps exposure time. The laser pulse delayed until 400 ns is shown in image panel 2. The focal position is marked by $\times$ with respect to the dashed outline of the microcavity.
Fig. 3.13: Fluorescence lifetime of a microcavity under single-photon excitation at various focal positions in the radial direction.

when the tight lateral focus from a high numerical aperture objective is incrementally localised from within the cavity. The quality factor $Q$ under single-photon excitation has been measured to be $773 \pm 140$.

The orthogonal oscillation of adjacent mode peak strength demonstrate that the MDR are polarised. Oscillation of cavity modes in this way is illustrative of TE and TM modes. A MDR modes strength is dependent on the incident excitation location given by $r$, $\theta$ and $\phi$.

The increased average MDR visibility between the microcavity perimeter and centre in the radial direction is approximately 8%. The quality of each MDR mode is dependent on the overlap and coupling between the fluorescence spectrum and the microcavity mode profile for a given localised excitation position. The large excitation volume and poor axial localisation increase the coupling into high order modes and background fluorescence. The greatest change of the average visibility in the azimuthal ($\theta$) plane of approximately 5.0% indicates that the localisation of MDR excitation in the axial direction is quite poor compared to the equatorial plane.
The difference of the degree of polarisation between the perimeter and the centre is approximately 3.8%. The average degree of polarisation in the meridian ($\theta$) plane has a deviation of approximately 0.24% due to the poor axial localisation of incident illumination consistently contributing to randomly polarised background fluorescence through the meridian plane. However, the 6.6% deviation of the degree of polarisation in the equatorial $\phi$ plane is due to the change in coupling of MDR modes as the polarisation direction shifts from perpendicular to parallel with respect to the microcavity boundary. The fluorescence lifetime is increased by approximately 8.35% with the localisation of the incident excitation in the radial direction.

Finally, it should be pointed out that the separation of excitation and resonance wavelengths is not too large with this technique. In the next chapter, to overcome some of the aforementioned difficulties, the highly localised spatial nature of two-photon excitation is exploited so that the introduction of MDR in a microcavity can be tightly controlled. Therefore fluorescence excitation at various spots within the three-dimensional space of a microsphere can be investigated in detail.