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Detecting Single Bacterial Cells through Optical Resonances in Microdroplets

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Abstract

Detection of single bacterial cells through optical resonances has been demonstrated in microdroplets. The setup enables high throughput non-specific detection of single E. Coli cells without any labeling. The cells inside the microdroplet have a direct effect on the morphology dependent resonances that are supported by Rhodamine 6G fluorescence; modification of the resonances arises from changes due to scattering and changes in the local refractive index. The change in the optical resonance spectrum can be observed at low concentrations where each microdroplet contains no more than one cell.

Keywords

biosensor; Morphology Dependent Resonances (MDRs); microdroplet; E. Coli

Optical resonances in microcavities have recently been used in biosensing applications.¹⁻⁷ These studies have shown that the resonances, often called Whispering Gallery Modes (WGMs) or Morphology Dependent Resonances (MDRs), can be used for biomolecule detection. Vollmer⁴ et al. demonstrated protein detection by the shift of the resonant spectrum in a silica microsphere through the adsorption of proteins on to the microsphere, inducing a change in its optical properties and thereby offering a sensitive detection scheme.

The effect of inclusions in a microcavity is of great interest from both the theoretical and experimental perspective. The size and number of inclusions will have a direct impact on the MDRs inside the cavity through scattering and local refractive index changes. The radial position of the inclusion and its orientation with respect to the illumination source is also important. Experimental work has shown a variety of effects of inclusions in liquid microcavities.⁸⁻¹⁴

In this paper, we describe the detection of single bacterial cell inclusions through the suppression of optical resonances in liquid microcavities. The experimental setup consists of a droplet generator, a laser, a spectrometer and a CCD camera (Fig. 1). The droplet generator is a customized commercial piezo inkjet printhead (Epson, Japan). Only one of the nozzles is used to eject droplets with approximately 20 micron diameter. The microdroplets are optically pumped by the second harmonic (532 nm) of a Q-switched Nd:YAG laser (Spectra Physics, Mountain View, CA). The beam (intensity, 0.24 MW/cm²) is focused to a 50 micron spot. The fluorescent signal from the microdroplets is collected by a long working distance Plano Apochromat lens (Mitutoyo, Japan) and an objective lens. The collected emission light is focused onto a 150 mm focal-length, dual-grating, imaging spectrometer (Acton Research Corporation, Acton, MA). This spectrometer is connected to a TEK 512 ×

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512D front-illuminated thermoelectrically-cooled CCD camera (Princeton Instruments, Trenton, NJ). The scattered laser light collected by the collection lenses is filtered out by a holographic notch filter (Kaiser Optical Systems, Ann Arbor, MI) at 532 nm.

The camera, the laser and the droplet generator are synchronized by a function generator and a delay generator (DG535 SRS, Sunnyvale, CA,). With the help of synchronization, it is possible to probe every microdroplet ejected by the droplet generator. The throughput is limited by the speed of the camera and the data processing time of the controller. The maximum effective rate is limited to 10 Hz with the system resulting in data acquisition every 100 ms. By changing the delay time, the microdroplet can be interrogated between 0-50 ms after its ejection.

Incubated and washed cell cultures (E-Coli KB and Streptococcus) were diluted in Phosphate Buffer Saline (PBS) buffer containing 10 μ M Rhodamine 6G. The cells were checked under a microscope for viability to assure that most of the cells were alive during the experimental run. The cell-Rhodamine 6G solution was used immediately after its preparation to avoid any absorption/adsorption of fluorescent molecules by the cells which would reduce the concentration of free Rhodamine 6G in the solution. The cell concentration was checked in a 96-well plate reader with absorptivity measurements at 600 nm. Assigning an optical density of 1 at 600 nm (Maniatis¹⁵ et al.) to a concentration of 10^8 cells/mL, we concluded that the cell cultures prepared had on the order of 10^9 cells/mL. The volume of a 20 micron-diameter droplet is approximately 4.19 picoliters (pL). Therefore, with a cell concentration of 10^9 cells/mL and an average droplet diameter of 20 microns, every microdroplet contained 0.3 cells on average. This means that, on average, approximately three in every ten microdroplets should yield an observation of a single cell.

The MDR spectrum of 10 μ M Rhodamine 6G in PBS solution (without cells) exhibits intense maxima at the first order modes of the microdroplet cavity (Fig. 2). When cells are added to the solution, the suppression of the MDR peak intensities are observed in the microdroplet spectrum. The effect of cell inclusions in the microdroplet is similar to quenching or a reduction in the Q factor of the cavity. This quenching effect can be due to two effects: the local refractive index can change due to the presence of a cell inside the microcavity; the cells can also lead to scattering losses within the cavity. Although scattering can increase the mode visibility by increasing the photon leakage from the microcavity, it can also induce a reduction in Q factor due to an asymmetry in refractive index profile of the optical cavity, resulting in a reduction in MDR peak intensities. From the spectrum, one can observe that most of the first order modes are suppressed. Meanwhile, some of the higher order modes at the blue end of the spectrum have higher intensities compared to the original spectrum. This is mainly because of the leakage of the high order modes due to scattering. Usually, the modes with higher radial mode-order number (l) have low intensities compared to the first order modes and tend to disappear first when the cavity medium has a quenching or absorbing component. These results, in general, confirm previous experimental and theoretical studies on the effect of inclusions in microdroplets.¹⁰⁻¹⁴ In addition, there is no obvious spectral shift associated with the inclusion of cells into the optical cavity. The MDR peak locations remain unchanged.

Figure 3 shows ten consecutive spectra from the same cell detection experiment. From the estimate that three out of ten microdroplets should contain a single bacterial cell, we expect that approximately three out of ten spectra should indicate the presence of a cell, on average. Through the spectra, it can be seen that in two out of ten microdroplets, the MDR peak intensities have dropped dramatically. For low cell concentrations, the number of cells in a single microdroplet should follow Poisson statistics, and it is possible to have more than one cell in each microdroplet when the resonance suppression is observed. With an average

concentration of 0.3 cells per droplet, the probability of detecting $n=1,2,3$ cells would be 0.222, 0.033 and 0.003 respectively, suggesting that multiple cell events are relatively rare at this concentration, and that the observations derive from the presence of single cells.

Single cell detection through MDR spectra was confirmed by the observation of diffraction patterns through the interaction between the microdroplet and the laser. The images of the diffraction patterns from the microdroplets containing a single streptococcus cell are shown in Figure 3. Whenever a single cell event is detected through the spectrum, a secondary, dimmer, diffraction pattern is observed on top of the primary diffraction pattern. The primary diffraction pattern is due to laser scattering from the microdroplet. The secondary diffraction pattern is due to laser scattering from the round cell in the microdroplet. The secondary diffraction pattern appears at different locations on the primary one, since it depends on the location of the cell which is random within the microdroplet. The diffraction pattern data can also be used to rule out the presence of multiple cells in a microdroplet, because multiple cells would exhibit multiple secondary diffraction patterns superimposed on the primary diffraction pattern.

We also examined the difference between the signal from the *E. Coli* culture and the *Streptococcus* culture to check the effect of inclusion morphology on MDR spectra. *E. Coli* is rod-shaped and *Streptococcus* is round-shaped. Both types of cells are approximately 1-2 microns in size. The comparison of the MDR spectra from both experiments does not suggest an obvious difference to distinguish between the shapes of the cells inside the microdroplet. However, there is an indication that cell morphology can play a role in altering the shot-to-shot differences in MDR spectra. The round-shaped cells lead to a constant reduction when the resonance suppression is observed – surprisingly, the rod-shaped cells show a variation in the spectra instead of a constant reduction in the intensity of MDRs. The variation of intensity reduction arises from the random orientation of the rod cells with respect to the location of the MDRs inside the spherical microcavity. Observations of this sort can be used as an indication of cell morphology.

The location of the cell inside the microdroplet plays an essential role in determining the changes observed in the MDR spectrum. The cell, as an inclusion, will affect the MDRs if it is located near the droplet rim where the resonances are intense. If the cell is located near the center of the droplet, it will not affect the MDR spectra; therefore it will count as a “miss” in the detection process. In addition, the scattering effect of the inclusion will increase, if it is located at one of the two focal points of the microdroplet (due to lensing effect of the droplet on the incoming laser beam).

Furthermore, we checked the effect of cell viability on the MDR spectra. To do this, the cell cultures were exposed to UV light for ten minutes. The cell viability was checked under microscope. After UV exposure, the cell motility was reduced by more than 90%. When these cells were used for the experiment, the results showed no difference between the live cells and the dead ones. This is due to the fact that the cell membrane remains intact for a while after the exposure. In particular, *E. Coli* cells have a cell wall in addition to the membrane that would maintain the same cell shape after lysis.

The detection of single bacterial cells in microdroplets has been demonstrated using optical resonances. At cell concentrations as low as one per microdroplet; the MDR peaks have been suppressed. The mechanism is mainly due to the local refractive index change and scattering caused by the cells. Absorption/adsorption of fluorescent molecules might have contributed further to the signal reduction. The detection system is non-specific; any inclusion that would cause a change in the optical characteristics of the microcavity would change the nature of the MDR peaks. Specificity would require an upstream bio-recognition

event that might take the form of an immuno-reaction. The advantage of the system is the omission of the labeling procedure for the bio-particulates to be detected. The fluorescent marker is simply added to the final solution without any specific binding protocols to label the cells to be detected. The effect of the cell morphology and viability on the MDR peaks was also studied. Cell morphology affected the variability of the spectra from droplet to droplet as rod-like cells rotated randomly inside the droplets. Cell viability did not affect the spectra. Further analysis of the data focused on the mode visibility and lasing thresholds might help distinguish different cell shapes and cell viability, especially when combined with additional light scattering information.

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Fig. 1.
Experimental setup for optical resonance experiments in microdroplets.

Fig. 2. The suppression of MDR peaks when the E-Coli cells are added to the fluorescent solution. Here the sample solution consists of 10 μM Rhodamine 6G in PBS with E Coli cells ($\sim 10^9$ cells/mL). The sample size in a 20 micron droplet is about 4.2 pL.

Fig. 3.

Ten consecutive spectra from the cell detection experiments in microdroplets. Here, a single cell presence is detected in two droplets in a total of ten droplets observed through the suppression of MDR peaks (spectra numbers two and nine counting from front to back). A single cell event is confirmed through the observation of a secondary, dimmer, diffraction pattern (due to cell presence) that is superimposed on the primary diffraction pattern caused by the microdroplet alone.