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Optical Trapping and Gram-Type Differentiation of Living Bacteria in 2D Hollow Photonic Crystal Cavities

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ABSTRACT

Thanks to their small footprint and to their ability to manipulate objects using low powers, optofluidic systems, based on the integration of photonic structures with microfluidic layers, were shown to be promising tools for biological analysis. Here, we report on the optical trapping and Gram-type differentiation of seven types of living bacteria in 2D hollow photonic crystal cavities. Photonic crystals on silicon and a polydimethylsiloxane (PDMS) frame were respectively processed using standard and soft lithography techniques. A solution of diluted bacteria in de-ionized water was injected in the PDMS frame, letting the bacteria move in unconstrained Brownian motion close to the hollow defect. Excitation of the resonant cavity with a tuneable laser permitted to record multiple trapping events. The analysis of the membrane-dependent resonance frequency shift due to the electromagnetic field-bacteria coupling allowed for the Gram-type differentiation of bacteria in a fast, label-free, and non-destructive way.

Keywords: photonic crystals, microfluidic, bacteria, optical trapping.

1. INTRODUCTION

The analysis of biological entities, such as bacteria and viruses, is of crucial interest in numerous biological and bio-medical applications. Often, the first step towards bacteria identification is the classification of the cells under study in two groups encompassing Gram positive or Gram negative types. These two classes mainly depend on the structural properties of bacteria cell wall containing different amount of peptidoglycan. The most widely used technique to perform such classification is Gram-staining, developed in 1884. Despite being a relatively fast method, it is destructive and makes use of cancerogenic dyes. Here we propose an approach based on optical trapping that allows for fast, label free and not destructive determination of the Gram-type of the studied bacteria.

Since their initial report [1], optical tweezers have become the instrument of choice to trap and manipulate a large variety of objects in the nanometre to micrometre scale range. In particular, during the last decade, optofluidic systems based on the integration of microfluidic layers with optical resonators arose as suitable structures for biological analysis. Indeed, thanks to their small footprint and especially to their ability to trap objects with low powers [2], biological entities are caught beneath their damage threshold. The trapping of biomolecules [3,4], viruses [5] and bacteria [6] was reported. Moreover, the resonant nature of the optical cavities enables for the simultaneous acquisition of information on the trapped object such as size, refractive index and morphology, thanks to the feedback effect induced by the trapped specimen on the trapping field itself [7]. Optical trapping and distinction of three different types of living bacteria were recently shown in 1D photonic crystal (PhC) cavities [8].

Here, we present our results concerning the study of seven types of living bacteria. We were able to trap them all, with the help of an optical cavity consisting in a large hole in a 2D silicon photonic crystal membrane. In addition, thanks to the feedback effect induced by the trapped specimen on the optical field, Gram type classification is performed successfully.

2. BACTERIA TYPES

The bacteria under study, shown in Fig. 1, are the following ones: *Escherichia coli* (Ec), *Yersinia ruckeri* (Yr), *Pseudomonas putida* (Pp), *Neisseria dicca* (Nd), *Staphylococcus Epidermidis* (Se), *Bacillus subtilis* (Bs) and *Listeria innocua* (Li). They were chosen because of the differences in morphology and in Gram type. Gram-staining is a technique widely used in bacteriology that allows for the classification of bacteria in two main families (Gram positive and Gram negative) depending on the structural properties of the cell wall. *E. coli*, *Y. ruckeri*, *P. putid*a and *N. sicca* belong to Gram negative family; while *S. epidermidis*, *B. subtilis* and

L. innocua are classified as Gram positives. Regarding the morphology, *N. sicca* and *S. epidermidis* are classified as cocci: they have an oval shape and they aggregate in diplococci resulting in a 1×2 µm size. The other bacteria under analysis, on the other hand, are classified as bacilli and they are rod shaped with different sizes ranging from 1×2 µm to 1×7 µm. Furthermore, Ec and Bs have multiple flagella (peritrichous) attached to their cell membrane granting them high motility. Similarly, Li and Pp have respectively two (amphitrichous) and one (monotrichous) flagella on their membrane.

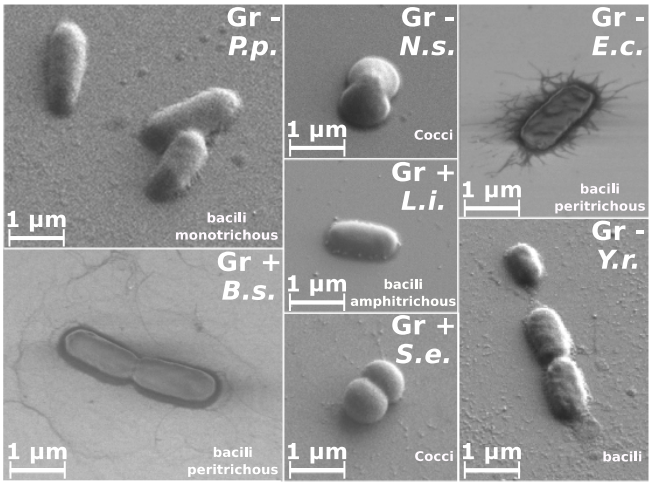

Figure 1. Scanning electron micrograph of the seven types of bacteria under study.

3. EXPERIMENTAL SETUP

The structure used for optical trapping is a hollow photonic crystal cavity fabricated on silicon-on-insulator substrate with standard electron beam lithography and inductively coupled plasma etching. The silica sacrificial layer was then removed via wet etching with a Buffered Hydrofluoric Acid (BHF) solution. The lattice holes measure 250 nm in diameter and they are hexagonally arranged with a lattice constant of 420 nm; the defect hole is 700 nm in diameter and is evanescently excited via a W1 waveguide in an end-fire setup.

To enable the transport of bacteria in the vicinity of the PhC structures, a Polydimethylsiloxane (PDMS) frame is placed on the sample. It acts as a container for a drop of a solution of the bacteria diluted in de-ionized water. Finally, a 150 µm thick glass coverslip is then attached to the PDMS and helps to avoid evaporation.

Light from a tuneable laser is injected with a polarization maintaining lensed fibre, and the transmitted power through the waveguide is collected with a microscope objective and detected by a photodiode. A visible camera placed on the top of the sample allows for imaging and visual checking of the trapping events. The device and the optical structure we developed are shown in Fig. 2. For the entire set of measurements, the same optical cavity was used, exhibiting a Q factor of 4500 in water.

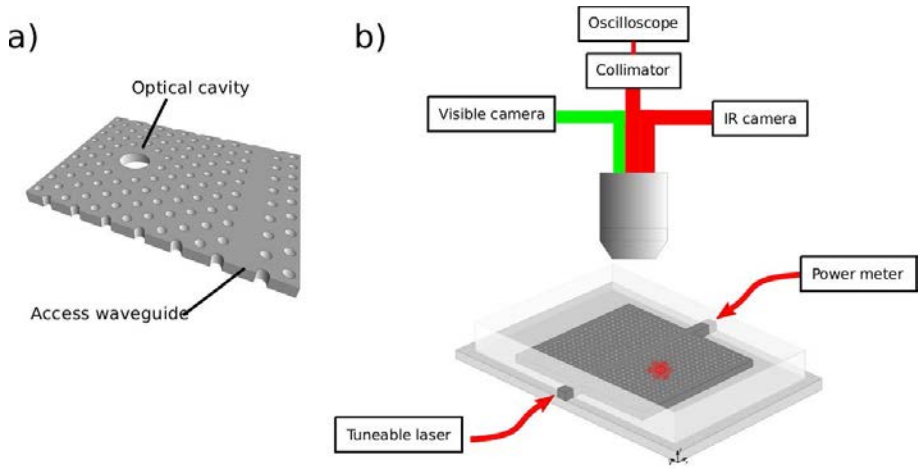

Figure 2: (a) Schematic illustration of the photonic crystal structure; (b) Illustration of the experimental setup. The injected light in the access waveguide is evanescently coupled to the hollow cavity. Transmission measurements are performed with the power meter and imaging can be performed with the visible camera.

4. RESULTS

For every type of bacteria, a drop of solution was inserted in the PDMS frame, allowing for the bacteria to move in unconstrained Brownian motion and eventually get trapped while passing in the vicinity of the excited resonant cavity. After the experiment, the PDMS frame was emptied and the sample rinsed with de-ionized water, to avoid bacteria contamination in subsequent measurements. The same routine was performed for the seven types of bacteria.

Optical trapping experiments are performed as follows. Light from a tuneable source at the resonance frequency is injected in the input waveguide: the evanescent coupling between the W1 waveguide and the optical cavity results in a maximum of the field confined in the resonant cavity and consequently in a minimum of the detected power through the waveguide, as shown in Fig. 3(a) – black curve. When a bacterium is trapped, a variation in the refractive index overlapping with the confined field occurs. Note that the cavity dimension is too small to entirely trap the bacterium. The optical fields effectively overlap with the cell membrane which has the signature of the bacterium Gram type. This leads to a redshift in the resonance wavelength that can be detected by an augmentation in the transmitted power, *e.g.* Fig. 3(a) – green curve. This shift is specimen-dependent as it depends on the physical and morphological characteristics of the trapped object. This is illustrated in Fig. 3(b-c) where the transmission recorded during a trapping event (i) together with the corresponding histogram (ii) are shown for B. subtilis (Gram +) and N. sicca (Gram –) respectively. The histogram mean (μ) and standard deviation (σ) are shown and testify of the bacteria-dependent transmitted signal that can be used to separate the two Gram families. To take advantage of this feature, at least 9 trapping events were recorded for each type of bacteria. This gives a significant data set that we used to reveal the Gram type of each bacterium under study.

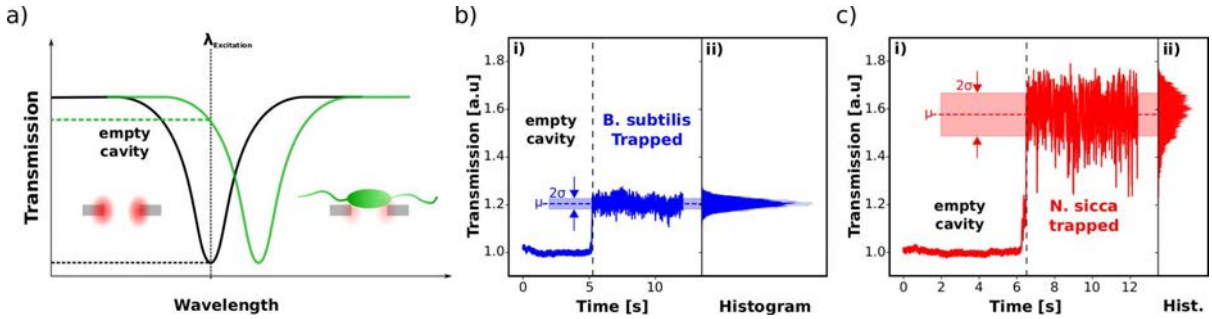

Figure 3: (a) Illustration of the resonance frequency shift induced by a trapped bacterium; (b-c) Examples of transmitted power signal recorded during a trapping event of B. subtilis and N. sicca (i) together with the corresponding histogram (ii).

To do so, we used two complementary approaches. First, as a consequence of the bacteria-dependent shift in the transmission signal, we performed a simple statistical data analysis. It consisted in computing the different moments of the histogram generated by the trapping signal and study their correlation. Unsurprisingly, the first two moments give the most insights. As shown in Fig. 4(a), the standard deviation is plotted in function of the mean for each recorded signal (dots). The corresponding ellipsoids centres are displayed as crosses and their coordinates are the mean of both parameters *i.e.* $<\mu>$, $<\sigma>$. Their minor and major axes are defined by $2\sigma_\mu$ and $2\sigma_\sigma$ and their orientation is given by the slope of a first order linear fit. The striking feature is emphasized by the grey shaded area around a mean of 1.4. It clearly shows that the data gathered allows us to separate Gram positives and negatives bacteria according to their frequency shift response. Another peculiar feature can be stressed. Taking into account σ leads to a partial differentiation of bacteria types within a given Gram family. Indeed, the ellipsoids area represent around 70% of the distribution. Therefore, we have around 70% probability to discriminate one of the Gram positive bacterium from the rest of its family. For the negative class, this probability is lower due to overlap between ellipsoids. Finally, the third centred reduced moment has systematically shown to be negative. That is, all histograms display longer tails towards small transmission shifts. This is due to the fact that the frequency shift is bounded.

Secondly, seen as a signal processing problem, the L^2 cross distances between transmitted powers of all bacteria types were computed. Since no temporal reference is set for the various trapping events, we defined this measure between two signals f and g by

$$d(f,g) = \min_{0\le\tau\le T} \sqrt{\int_0^T dt|f(t) - g(t-\tau)|^2}. \quad (1)$$

The results for a set of eight representative measurements are shown in Fig. 4(b). The height of a bar is the computed distance between the two corresponding x and y bacteria types. Interestingly, we clearly see that the two different Gram families cluster together. Moreover, no crossover is present: all Gram positive types are close

to each other meanwhile being far from all Gram negative ones, and vis-versa. This second analysis thus confirms the conclusions obtained with the statistical approach.

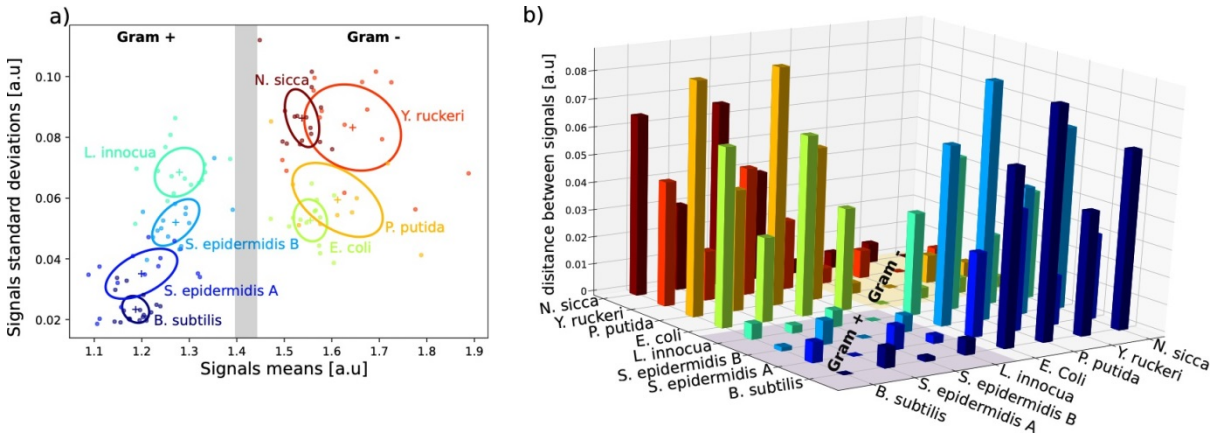

Figure 4: (a) The standard deviations σ of all recorded trapping events are plotted in function of the corresponding mean μ. Respective ellipsoids are centred in the centre of mass with coordinates $<\mu>$, $<\sigma>$. Their minor and major axes are defined as $2\sigma_\mu$ and $2\sigma_\sigma$ and their orientation is given by a first order linear; (b) For each couple of bacteria types (x and y axes) the distance Eq. (1) is computed and reported on the z-axis.

Further improvements will be carried out to be able to access single bacteria features like size or motility. Due to an averaging effect those properties cannot be seen in the present data. The reason is that multiple bacteria got trapped simultaneously as verified with the visible camera. Thanks to the scalability of electromagnetism we are optimistic in achieving single bacteria identification.

5. CONCLUSIONS

In conclusion, we report on the optical trapping in a 2D silicon hollow photonic crystal cavity as a non-destructive characterization method on single cells. This technique is illustrated here on seven species of living bacteria, featuring different morphologies, motilities, and Gram staining properties. We showed that Gram-type could unambiguously be determined on this set of species, in a fast and label-free way. Moreover, it is conceivable to extend this method to Gram-variable and Gram-indeterminate bacteria. Further effort will be put on trapping single bacterium at a time. We expect these single trapping events to give detailed information on the membrane properties, morphologies and motilities.

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