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Ultrasensitive Q β Phage Analysis Using Fluorescence Correlation Spectroscopy on an Optofluidic Chip

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Abstract

We demonstrate detection and analysis of the Q β bacteriophage on the single virus level using an integrated optofluidic biosensor. Individual Q β phages with masses on the order of attograms were sensed and analyzed on a silicon chip in their natural liquid environment without the need for virus immobilization. The diffusion coefficient of the viruses was extracted from the fluorescence signal by means of fluorescence correlation spectroscopy (FCS) and found to be $15.90 \pm 1.50 \mu\text{m}^2/\text{s}$ in excellent agreement with previously published values. The aggregation and disintegration of the phage were also observed. Virus flow velocities determined by FCS were in the 60–300 $\mu\text{m}/\text{s}$ range. This study suggests considerable potential for an inexpensive and portable sensor capable of discrimination between viruses of different sizes.

Keywords

virus; diagnosis; ARROW; microfluidics; biosensor

1. Introduction

Viruses are small molecular machines which cause numerous infectious diseases such as AIDS, hepatitis, influenza, and yellow fever just to name a few. Nearly 20% of cancer cases may also have viral origins (Pagano et al., 2004). Many of these contagious diseases are deadly and every one of them degrades quality of life or causes disability. Humanity faces additional challenges due to animal diseases, which result in decrease of food supply. This underscores the importance of developing and introducing new diagnostic technologies into the healthcare system.

It is well known that early disease diagnosis can lead to faster and more competent treatment. Most diseases are diagnosed based on their clinical presentation. This demands substantial experience among medical professionals to recognize and interpret the symptoms and often must be followed by laboratory analysis on blood or tissue samples. Conventional analytical

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techniques include cell cultures (Leland and Ginocchio, 2007), hemagglutination assays (Vyas and Shulman, 1970), detection of virus-specific antibodies and viral antigens in blood, bodily fluids, and tissues (Hashida et al., 1997), detection of viral DNA or RNA by polymerase chain reaction (PCR) (Payungporn et al., 2006). The results of this analysis can substantially aid diagnosis in terms of accuracy, but most of them have a long turn around time on the order of days or even weeks (Zitterkopf et al., 2006; Marshall et al., 2001) due to slow cell growth or necessary amplification steps. Therefore, new diagnostic tests, which can give rapid, specific, and reliable answers about the type of infection in the shortest period of time requiring small analyte volumes, low agent concentrations, single or few molecule sensitivity, miniature dimensions and low cost of the equipment as well as very basic personnel training are essential.

One of the possible solutions to implement all of these qualities is a mass-fabricated integrated chip or integrated biosensor. Most such biosensors use electrical, biological, mechanical, and optical techniques or combinations thereof. Biological virus sensors (Liu et al., 2006) are rarely standalone solutions, but are merely incorporated as the recognition agent into other types of sensors. Electrical sensors have shown considerable progress over the years (Dastagir et al., 2007; Patolsky et al., 2004; Zheng et al., 2005; Dekker, 2007). Single viruses (Patolsky et al., 2004), single proteins (Zheng et al., 2005) and even DNA molecules down to tens of base pairs (Dekker, 2007) have been detected. A prominent example of mechanical sensors is an oscillating micro- or nanocantilever (Ilic et al., 2004). Among integrated optical sensors, evanescent waveguides (Ligler and Taitt, 2008; Ymeti et al., 2007) and DNA microarrays (Chiu et al., 2008) are well-established techniques. Unfortunately, most of these devices require immobilization of the molecule and can only tell whether the molecule is present in the test solution or not. That is acceptable for a simple diagnostic test, but lacks the capability to see molecules in action, for example, finding their diffusion coefficient, flow velocity, observing molecular interactions as well as distinguishing between different types of molecules simultaneously in solution. These additional analytical capabilities are highly beneficial for research applications, offering higher flexibility, mobility and increased outreach (Craighead, 2006).

One way to enrich functionality of the sensors is to use the optofluidic paradigm (Psaltis et al., 2006) by combining liquids and optics in the same physical volume. Surface plasmon resonance (SPR) sensing with nanohole arrays is a good illustration of this approach (Sinton et al., 2008). Optofluidics has also been successfully employed for detection of very low concentrations of unlabeled viruses in micro-ring resonators by monitoring the refractive index change using whispering gallery modes, which travel in the circular wall of the micro-tube (Zhu et al., 2008).

A fully planar integrated optofluidic approach would be another step forward. We have previously demonstrated detection of single fluorescent dyes (Yin et al., 2006) and labeled liposomes (Yin et al., 2007) using anti-resonant reflecting optical waveguides (ARROWs). These hollow-core waveguides can encapsulate liquids and light in the same volume, providing the most intensive interaction between light and matter with planar excitation and detection.

Here, we demonstrate detection and fluorescence correlation spectroscopy (FCS) (Magde et al., 1972) analysis of single bacteriophage Q β on a planar optofluidic chip without the need for particle immobilization. Bacteriophages were selected as a model virus, because they are harmless to humans, have been used as an alternative to antibiotics (Merril et al., 2003), and are important in the food fermentation industry (Abedon, 2005).

2. Materials and Methods

2.1. Q β phage labeling procedure

Phage Q β belongs to the Leviviridae RNA virus family and is one of the smallest bacteriophages with a diameter of only 26 nm (Golmohammadi et al., 1996). The K16H phage Q β capsid has approximately 520 potential sites for covalent bonding of fluorescent dye. For this proof-of-principle experiment direct non-specific labeling of the phage was chosen.

The labeling procedure was as follows: 50 μ l of phage Q β with protein concentration of 12.68 mg/ml in phosphate buffer (pH 7) were added to 150 μ l of 0.1M bicarbonate buffer (pH 8.5). An 8 μ l stock solution of Alexa647 dye at concentration of 25 mM was prepared in dimethylformamide (DMF). Combining dye and phage solutions resulted in final effective concentrations of phages and dye of 1.14 μ M and 0.96 mM, respectively. Therefore, the ratio of dye to phage during the labeling process was about 850, sufficient to cover all potential binding sites on the phage. After initial mixing, the solution was incubated for 20 minutes under constant stirring in a vortex mixer. Labeled phage and dye were separated through a filtration column with the gel cut-off of 130 kDa (Ultrogel ACA44, France).

2.2. ARROW-based biosensor and experimental setup

The ARROW biosensor (Fig. 1, A), consists of several functional parts. The most important one is the hollow-core waveguide, which confines light and liquid in the same volume using a multilayer structure of alternating silicon nitride (SiN) and silicon dioxide (SiO₂) on a silicon substrate. The thickness of each layer is tailored in a way that the anti-resonance condition for the transverse wavevector and the mode condition are satisfied simultaneously (Yin et al., 2004). The details of the fabrication method based on sacrificial layer technology have been published previously (Hawkins and Schmidt, 2008).

The whole chip can be conceptually divided in three parts. The first is an all-optical region, which is represented by solid-core ARROW waveguides that interface the hollow channels with the edges of the chip. The second component is a microfluidic region, which consists of hollow-core ARROW waveguide arms (Fig. 1, A; the parts of the S-shaped hollow-core connected to the reservoirs) and is utilized for transferring biomolecules to the analytical section of the chip. The third region starts at the transition between optical and fluidic guides in the horizontal section of the hollow-core and therefore is called optofluidic. This is the main part of the chip and although possessing the same structure as the microfluidic arms carries light as well. This light is emitted by the molecules, which respond to the excitation by the solid-core ARROWs. Importantly, the excitation volume is defined by the perpendicular intersection of solid- and liquid-core waveguides. This simple layout has the advantage of defining a very small excitation volume (~ 100 fL), providing low background noise and hence facilitating single molecule detection (Yin et al., 2006). Moreover, all beams stay in the same plane without the need for out-of-plane excitation by a microscope, which is the key for biosensor miniaturization. Molecular species of interest are introduced through the fluidic reservoirs (10 μ l volume each). This geometry is easily scalable to many intersecting waveguides, which could carry different molecules and have a wide range of excitation wavelengths (Schmidt et al., 2005).

The fluorescence studies were performed on a custom-built optical setup (Fig. 1). A HeNe laser is coupled into single mode fiber, which is in turn butt-coupled into the solid core. The chip position can be adjusted with home-made piezomanipulators. Light emitted by molecules is collected by an objective lens (NA 0.85, Newport, USA) and separated from the background using a fluorescence filter (XF3030, Omega Optical, USA). After being coupled into a multi-mode fiber, signal photons arrive at a single photon avalanche photodiode detector (SPAD)

(SPCM-AQR-14-FC, Perkin Elmer Optoelectronics, Canada). The photons are recorded using a time-correlated single photon counting PCI-board (Time Harp 200, PRT 400 router; Picoquant, Germany) and commercial software (Timeharp, Picoquant, Germany) in time-tagged time-resolved (TTTR) collection mode (Zander et al., 2002). For the electrokinetic control a generic power supply was used to apply voltage in the range of 0-70 V across the liquid channel through Ag/AgCl electrodes.

2.3. Experimental procedure

Prior to an experiment the chip is cleaned in the Nanostrip (Cyantek, CA, USA) for about one hour to remove any contamination and render the surface of the waveguide hydrophilic. Nanostrip is exchanged with nanopure water for two hours. After the sample has dried, fluidic reservoirs are glued in place with epoxy.

During the experiment the hollow core waveguide is filled with clean buffer solution (bicarbonate buffer, pH 8.5) and 0.1% Triton X-100 to reduce unspecific binding of particles to the waveguide walls. The background count is recorded for at least 15 minutes to ensure no fluorescent contamination is present. Next, the buffer solution with the particles of interest is pipetted into one of the reservoirs and fluorescence signal from particles that enter the excitation volume via diffusion, pressure, or electrokinetic movement is recorded.

We observed a definite correlation between polarity of the voltage and resulting fluorescence signal. Based on the knowledge of the surface charge of the phage and SiN wall at the buffer pH (8.5), we conclude that the flow was dominated by electroosmosis.

2.4. Fluorescence correlation spectroscopy and ARROW FCS model

Fluorescence correlation spectroscopy (FCS) is an ingenious technique which allows for extraction of a wide range of dynamic characteristics of the molecules by analyzing the minute fluctuations of their emitted fluorescence signal (Magde et al., 1972). This differs from the more obvious and conventional methods where the intensity of the signal is monitored to surpass some threshold level. FCS works best for ultra-low concentrations of molecules, because individual fluctuations become more apparent. FCS and its derivatives have been employed for measuring wide range of biological parameters and processes (Zander et al., 2002). Viruses have been previously detected and analyzed with FCS on non-integrated platforms (Hirschfeld et al., 1977; Lacroix-Gueu et al., 2005; Toivola et al., 2002).

The uncorrected fluorescence correlation function is defined as follows:

$$H(\tau) = \frac{\langle \delta F(t) \cdot \delta F(t+\tau) \rangle}{\langle F(t) \rangle^2}, \quad (1)$$

where $\delta F(t)$ and $\delta F(t+\tau)$ are measured fluctuations in the fluorescence intensity at times t and $t+\tau$ respectively and $\langle F(t) \rangle^2$ is a squared time average of the fluorescence signal used as a normalization factor. Equation (1) can be further specified by linking the parameters of the setup and molecules to the autocorrelation function. We have developed an ARROW FCS model which takes into account all of the parameters influencing excitation-detection volume and such molecular properties as diffusion coefficient, flow velocity, and local concentration (Yin et al., 2007; Rudenko et al., 2008). The resulting expression for the fluorescence autocorrelation function is:

$$H(\tau) = \frac{F_c(\tau)}{\pi^{3/2} w_{xc} w_y w_z \langle C \rangle} \left[1 + \frac{4D\tau}{w_{xc}^2} \right]^{-\frac{1}{2}} \left[1 + \frac{4D\tau}{w_y^2} \right]^{-\frac{1}{2}} \left[1 + \frac{4D\tau}{w_z^2} \right]^{-\frac{1}{2}} e^{\frac{-(V_z\tau)^2}{w_z^2 + 4D\tau}}, \quad (2)$$

with w_{xc} being the spot size of the collection efficiency function in x-direction; w_y , w_z - spot sizes of the excitation beam in y- and z-direction, respectively; $\langle C \rangle$ - local concentration of particles; D — diffusion coefficient of molecules; V_z — flow velocity in z-direction; τ — lag time (see Fig. 2). $F_c(\tau)$ is defined as follows:

$$F_c(\tau) = \frac{\left((1+r^2)^2 + 2r^2 e^{-\frac{4w_{xc}^2 w_0^2 D\tau}{w_{xc}^2 + 4D\tau}} \cos(2w_0 L) + 2r^2 e^{-w_{xc}^2 w_0^2} + 4r(1+r^2) \cos(w_0 L) e^{-\frac{w_{xc}^2 w_0^2 (w_{xc}^2 + 8D\tau)}{4(w_{xc}^2 + 4D\tau)}} \right)}{\left(1 + r^2 + 2r e^{-\frac{w_{xc}^2 w_0^2}{2}} \cos(w_0 L) \right)^2} \quad (3)$$

with $w_0 = \frac{4\pi n_c}{\lambda}$ and L — length of the waveguide cavity in x-direction.

$F_c(\tau)$ takes into account standing wave phenomena due to the finite reflectivity r of the dielectric ARROW walls (Rudenko et al., 2008). It is important to note that high transmission from the solid core into the liquid core and back into the solid core at the interface is one of the design priorities. Therefore, with $r \approx 0$, $F_c(\tau)$ simply equals unity for all lag times τ . It is also worth to take notice of the similarity between the ARROW FCS and the standard confocal FCS models (Zander et al., 2002).

Non-zero background counts can be accounted for by a modified fluorescence autocorrelation function given by (Koppel, 1974):

$$G(\tau) = \frac{(\langle F(t) \rangle + \langle B(t) \rangle)^2}{\langle F(t) \rangle^2} H(\tau), \quad (4)$$

where $F(t)$ is a fluorescence signal, $B(t)$ — background, $H(\tau)$ — uncorrected autocorrelation function, $G(\tau)$ — corrected autocorrelation function.

2.5. Data analysis

The resulting recorded signal is imported into custom-written software and numerically correlated by applying a symmetric biased estimator of the *fluorescence* autocorrelation coefficients at lag $\tau = k\Delta t$ (Saffarian and Elson, 2003) to the discrete time series treated as realization of a stationary process of photon counts:

$$\tilde{G}(k) = (N - k) \frac{\sum_{t=m}^{N-k+m} x_t x_{t+k}}{\sum_{t=k+m}^{N+m} x_t \sum_{t=m}^{N-k+m} x_t} - 1 \quad (5)$$

Here N is the number of time bins in the specified part of the signal, x_t — the time series of the photon counts per bin, m — starting point in the sequence to be autocorrelated, Δt is the temporal resolution of the discrete signal.

The produced series of autocorrelation coefficients represents a numerically computed *fluorescence* autocorrelation function, which can be fitted using the ARROW FCS model. The fitting parameters are: diffusion coefficient D , flow velocity in z-direction V_z , local particle concentration $\langle C \rangle$ and level of the autocorrelation function at long lag times ($G(\tau \rightarrow \infty)$) — B . Fixed parameters such as $w_{xc} = 4.68 \mu\text{m}$, $w_y = 1.82 \mu\text{m}$, $w_z = 5.95 \mu\text{m}$ were obtained from simulations of the waveguide structure using commercial software package (FimmWave, Photon Design, UK).

The goodness of the fit was judged using chi-square test, Aderson-Darling test and residual autocorrelation (Straume and Johnson, 1992).

3. Results and Discussion

3.1. Single fluorescent beads as a virus model

We first turn to initial experiments, in which fluorescently labeled polystyrene spheres (dark red Flouospheres and Tetraspec, Invitrogen; 40 and 100 nm nominal diameter), diluted in nanopure water, were used as a virus model. The chosen bead size range covers typical virus dimensions. The concentration of fluorescent spheres, calculated based on simple dilution, was practically equal, namely, 32 pM for Ø40 nm spheres and 30 pM for Ø100 nm. The time traces and corresponding correlation functions are shown in Fig. 3.

The background was uncorrelated with an average count of 400/s. Smaller nanospheres produced an average count of 1100/s with peak values up to 4500/s. Larger nanospheres exhibited higher peak (13000/s) and average signals (2500/s), since they have more implanted dye molecules. The full length (250s) of the obtained signals was numerically autocorrelated at a lag discretization time of 0.5 ms and fitted with the ARROW FCS model (Eq. 2). FCS analysis of the fluorescent signal revealed diffusion coefficients for Ø100 nm and Ø40 nm beads of $6.80 \pm 0.15 \mu\text{m}^2/\text{s}$ and $16.40 \pm 0.25 \mu\text{m}^2/\text{s}$, respectively. This agrees well with estimates based on the Stokes-Einstein relation at low Reynolds numbers for spherical particles:

$D = \frac{k_b}{6\pi\eta r}$ (k_b : Boltzmann constant, η : temperature dependent dynamic viscosity of the fluid, r : radius of the particle) $D_{SE100} = 4.90 \mu\text{m}^2/\text{s}$ and $D_{SE40} = 13.50 \mu\text{m}^2/\text{s}$, correspondingly. The slight overestimation of the diffusion coefficient can be due to the unknown exact size distribution of the beads. The concentration of the beads was extracted to be 1-3 beads on average in the excitation volume ($0.3 \leq G(0) \leq 1$) in excellent agreement with concentrations estimated based on dilution from manufacturer-specified values.

When the individual time traces were added and analyzed with FCS, a single diffusion coefficient of $8.20 \pm 0.15 \mu\text{m}^2/\text{s}$ and a two-fold concentration increase were extracted. This averaging phenomenon is well known (Meseth et al., 1999). The extracted parameters become a weighted average based on the relative concentration and brightness of species in the solution. Since the concentration of both types of beads was similar and the brightness was larger for big beads, the calculated FCS curve is skewed towards large bead FCS, which is clearly reflected in the extracted parameters.

This experiment establishes the high sensitivity of ARROW-based sensor down to picomolar concentrations and the ability to detect and distinguish fluorescent objects in the size range of viruses.

3.2 Virus detection with single particle resolution on a chip

Compared to the polystyrene bead, a phage is a substantially more complicated particle due to possible protein interactions with the walls of the waveguide, sensitivity to the buffer conditions, aggregation, and disintegration.

Following the experimental procedure with the phage Q β , background (not shown) and phage fluorescent signals (Fig. 4, A, bottom) were obtained. The signal was numerically autocorrelated at a lag discretization time of 0.5 ms over 300 s and fitted with the ARROW FCS model. The resulting diffusion coefficient was $D = 31.75 \pm 0.95 \mu\text{m}^2/\text{s}$, the flow velocity in z-direction was $V_z = 253 \mu\text{m}/\text{s}$ and the local concentration of the particles was $\langle C \rangle = 3.5 \text{ pM}$ with an excitation volume of about 300 fl. This concentration on average corresponds to single particles residing in the excitation volume at a time. Therefore, most of the spikes in the signal can be attributed to individual particles traversing the excitation volume. The diffusion coefficient is another important quantity which provides means for the type identification of the detected particle. The value of $32 \mu\text{m}^2/\text{s}$ is higher than previously published for the Q β phage (Giddings et al., 1977; Camerini-Otero et al., 1974). Since the phage capsid has a spherical shape (Golmohammadi et al., 1996), the Stokes-Einstein relation can be also employed to estimate the diffusion coefficient. Using this equation and the diameter of the phage capsid, we get $D = 18.7 \mu\text{m}^2/\text{s}$. This discrepancy can be understood by careful examination of the fluorescence signal which is composed of populations of peaks with substantially different heights. This is similar to the case of different bead sizes discussed above and suggests a heterogeneous mixture of particles. To verify this hypothesis, the signals were separated by setting a threshold of 70 counts/10ms as shown in Fig. 4 A center and top, respectively. While not being exact, the threshold value was chosen at a discontinuity of the peak count histogram that showed a large number of low intensity peaks and a separate group of high intensity peaks.

When fitted individually, the population of small peaks exhibited a diffusion coefficient of $43.85 \pm 1.50 \mu\text{m}^2/\text{s}$ while the higher peak intensity population showed a diffusion coefficient of $15.90 \pm 1.50 \mu\text{m}^2/\text{s}$. This means that the solution, indeed, consisted of a mixture of different particles. Larger particles with lower diffusion coefficient had higher emitted intensities, while smaller particles had lower intensities and diffused faster. Judging by the proximity of these numbers to the diffusion coefficients of the phage ($14\text{--}19 \mu\text{m}^2/\text{s}$) and its building blocks — pentamers and hexamers ($85\text{--}90 \mu\text{m}^2/\text{s}$) — as well as based on (Meseth et al., 1999) we conclude that a partial disassembly of the phage occurred and, therefore, a wide distribution of the diffusion coefficients had been observed. The value for the larger components that correspond to complete phages is indeed in excellent agreement with the literature. When the whole signal is correlated, the diffusion coefficient is a weighted average of the individual diffusion coefficients of constituting particles. Contamination from free unbound Alexa 647 dye molecules could easily be ruled out due to the much larger diffusion coefficient of $250 \mu\text{m}^2/\text{s}$ (Yin et al., 2007).

An important advantage of the ARROW biosensor is that the collection efficiency at the wall of the waveguide is close to zero due to the vanishing field amplitude of the ARROW waveguide modes at these points. Therefore, the collected signal does not include any contribution from phages stuck to the wall (if any). This differs from more conventional evanescent waveguide sensors, where the signal is derived from the molecule immobilized on the wall.

The partial disassembly of the phages can be due to the addition of DMF with a dye at about 4% by volume to the phage solution during the labeling process and also because of the traces of Triton-X 100 left on the walls of the waveguide during background recording. These agents have been reported to affect stability of the phage envelope, albeit at somewhat higher

concentrations and shorter incubation times (Etten et al., 1976; Olofsson et al., 1998). The partial phage disassembly was confirmed by size-exclusion fast protein chromatography (FPLC).

Different heights of the peaks within the “sub-signals” can be explained by several effects, including a distribution within non-uniform excitation volume and non-uniform labeling. Other subpopulations can also exist, but can not be resolved by our analysis.

These results demonstrate the excellent sensitivity of our device on the single virus level. The achieved sensitivity is approaching detection of single fluorescent particles and is better or comparable to other integrated techniques such as electrical nanowire arrays (Patolsky et al., 2004), nanoelectromechanical cantilevers (Ilic et al., 2004; Gupta et al., 2004), evanescent waveguide sensors (Donaldson et al., 2004; Lee and Thompson, 1996; Martinez et al., 2005), electrical microfluidic chips (Liu et al., 2007) and capacitance-based sensing (Balasubramanian et al., 2005).

The main advantages of our planar optofluidic platform compared to these techniques is the intersection geometry that allows for fully planar optical detection, the small lithographically defined excitation volumes, and the absence of an immobilization requirement. These imply excellent potential for research applications in vitro and in vivo as well easier integration of opto- and microfluidic components.

4. Conclusion

Viruses were detected and controlled on a compact optofluidic chip with single particle resolution. Diffusion coefficient ($15.90 \pm 1.50 \mu\text{m}^2/\text{s}$), flow velocities (60-300 $\mu\text{m}/\text{s}$) and concentration (3.5 pM) of viruses were extracted from time-dependent fluorescence data using fluorescence correlation spectroscopy. The possibility to distinguish single phages from their structural parts, agglomerates, or mixtures thereof on an integrated chip was demonstrated.

Our approach can be straightforwardly extended to simultaneous detection of several virus types. Those could be differentiated spectrally (by having different dyes attached) or by their diffusion coefficient (Hirschfeld et al., 1977) and emitted intensity. The achieved sensitivity and level of discrimination between different molecules compares favorably with similar types of integrated sensors. This proof-of-principle experiment for optofluidic virus detection can be extended to virus-specific detection methods based on antibody labeling or molecular beacons. In real world research applications the sample composition can be unknown and, therefore, more careful purification could be required. In clinical settings, the characteristics of the target virus are known, which can allow for performing rapid pathogen identification in conjunction with dye-labeled antibodies or molecular beacons to meet the specificity requirement. Integration of filters (Schmidt et al., 2005), detectors, and silicon-based electronics with supplemental microfluidic infrastructure is feasible and can enable ARROW biosensor to become a fully developed lab-on-a-chip. These results are promising for a new type of sensitive virus detector based on integrated optofluidics, especially because of planarity and use of fiber-optics, and the potential for elimination of an amplification step.

Additional work on automating the analysis and performing more trials with different viruses and their mixtures will help implement this ultrasensitive optical analysis in viable commercial devices.

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Figure 1. Optofluidic chip and experimental setup schematic

(A) Optofluidic chip and experimental setup schematic with a cartoon of a labeled phage; (B) Solid-core ARROW waveguide facet with an overlaid mode; (C) Liquid-core waveguide facet with an overlaid mode; (D) Photograph of the assembled ARROW biosensor. An intersection geometry is employed, where solid- and liquid core waveguides cross at 90° angle creating ~ 100 fl excitation-detection volume commensurate with single molecule detection due to low background noise. The chip is fully self-contained having microfluidic reservoirs and channels, optofluidic and standard optical waveguides.

Figure 2. Cross sectional view of the ARROW waveguide liquid-solid core interface

Cross section of the solid-liquid core ARROW waveguide intersection with an excitation beam and molecule under study. The multilayer structure of the waveguide confines light inside the hollow-core, which is filled with a buffer, containing molecules. w_{ye} - beam spot size in y-direction, w_z - beam spot size in z-direction, w_{xc} , w_{yc} — collection efficiency in x- and y-directions, respectively. P-axis — collection efficiency in [a.u.].

Figure 3. Fluorescence correlation spectroscopy of nanoscale polystyrene beads

(A) Comparison of signals from fluorescent beads. Top: Ø100 nm, center: Ø40 nm, bottom: buffer background count. The traces are offset vertically for clarity. Photon counts are in normalized units; (B) Normalized FCS (points) and fit (lines) for Ø40 nm, Ø100 nm, and a mixture of beads. Ø40 nm beads: blue dots, and red line; Ø100 nm beads: black dots and orange line; mixture: grey dots and green line. Clear difference in the FCS signal can be noticed between beads of smaller and larger sizes. The FCS of the mixture is skewed to the large beads FCS because of their higher brightness and the fact that concentrations of both bead sizes were practically equal (1-3 beads in the excitation volume). At present image magnification the size of the errorbars is on the order of the point.

Figure 4. Fluorescence correlation spectroscopy of Q β bacteriophage

(A) Bottom: original phage signal; center: high intensity signal, top: low intensity signal. Traces are offset for clarity; (B) Fit of the separated phage signal. High intensity peaks: grey dots (data), green line (fit); low intensity peaks: blue dots (data), red line (fit); original mixed signal: black dots (data), orange line (fit). Due to the higher density of the low intensity peaks population, fits and data for the mixed signal are skewed towards low intensity peaks values. At present image magnification the size of the errorbars for some points is only slightly larger than the point itself.