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Real-time optical detection of single human and bacterial viruses based on dark-field interferometry

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ABSTRACT

The rapid and sensitive detection and characterization of human viruses and bacteriophage is extremely important in a variety of fields, such as medical diagnostics, immunology and vaccine research, and environmental contamination and quality control. We introduce an optical detection scheme for real-time and label-free detection of human viruses and bacteriophage as small as ~24 nm in radius. Combining the advantages of heterodyne interferometry and dark-field microscopy, this label-free method enables us to detect and characterize various biological nanoparticles with unsurpassed sensitivity and selectivity. We demonstrate the high sensitivity and precision of the method by analyzing a mixture containing HIV virus and bacteriophage. The method also resolves the distribution of small nano-impurities (~20–30 nm) in clinically relevant virus samples.

1. Introduction

Nanoparticles play a significant role in various fields such as biomedical imaging and diagnostics (Choi et al., 2007; Huang et al., 2007), process control in semiconductor manufacturing (Wali et al., 2009), environmental monitoring and climate change (Ramanathan and Carmichael, 2008; Morawska, 2010). Inhalation of ultrafine particulates in air has been shown to have adverse effects, such as inflammation of lungs or pulmonary and cardiovascular diseases (Oberdörster, 2000; Somers et al., 2004). Nano-sized biological agents and pathogens such as viruses are known to be responsible for a wide variety of human diseases such as flu, AIDS and herpes, and have been used as biowarfare agents (Krug, 2003; Anderson et al., 2006).

It has become increasingly important to rapidly and accurately quantify viruses. Accurate quantification of the presence of human viruses such as HIV, herpes or influenza in blood samples is essential for clinical diagnosis and also for vaccine development. It is also highly important to be able to distinguish between different kinds of viruses present in a sample. For example, a single patient may be infected with multiple viral pathogens such as HIV and HCV, and it is important to identify and also quantify both kinds of viruses in order to treat the patient.

Water contamination control is another application, where detecting and quantifying nanoscale contaminants such as bacteriophages is important (Salter et al., 2010; Santiago-Rodríguez et al., 2010).

Most of the existing virus particle quantification techniques either suffer from significant technical glitches or are extremely time and cost consuming. For example, the Quantitative Electron Microscopy (QEM) technique (Tsai et al., 1996; Chuan et al., 2007), which counts polystyrene beads constructed to presumably contain a certain number of HIV-1 particles, assumes that the number of beads per virus particle is constant, a fact that cannot be experimentally confirmed given the low-resolution of electron microscopy for small particles such as viruses. The Image Enhanced Microscopy (IEM) technique counts virus particles labeled with fluorescent dyes (Dimitrov et al., 1993; Hübner et al., 2009), but the dye-labeling efficiency could not be experimentally confirmed, and hence quantification is unreliable. The quantitative-PCR method for counting viral RNA genome copy numbers is also popular, but it only indirectly determines the number of the viral particles, and does not actually count them (Hockett et al., 1999; Engelmann

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et al., 2008). The plaque titer method (Dulbecco and Vogt, 1954; Cromeans et al., 2008), on the other hand, can only be used to quantify viral particles that cause visible cell-damage. At present there does not exist any virus quantification method available to biologists which can quickly and reliably detect, quantify and characterize virus particles with single particle sensitivity.

Recently there have been several studies focused on developing sensitive optical or electrical techniques for label-free viral biosensing. Electrical sensors have been demonstrated to be able to detect single viruses in solution (Patolsky et al., 2004; Fraikin et al., 2011), but they suffer from the drawback that they are extremely sensitive to changes in ionic strengths of the media (Stern et al., 2007). Optical techniques based on sensing discrete resonance shifts in whispering gallery mode (WGM) microcavities due to binding of single virus particles have been developed (Vollmer and Arnold, 2008; Vollmer et al., 2008; Zhu et al., 2009), but they cannot be used to distinguish between viruses of different sizes present in a heterogeneous mixture. Other optical sensing platforms such as those based on nanoplasmonics (Yanik et al., 2010) or interferometry (Ymeti et al., 2007; Daaboul et al., 2010) have been developed; but while some of them are time-consuming and unconducive to real-time sample characterization, others rely on extensive surface preparation steps or availability of specific antibodies for the target viruses in a sample. A single method which can quickly and accurately quantify levels of different viruses present in clinically relevant samples without additional sample preparation steps, has remained elusive for practical implementation.

Optical detection of nanoscale biological agents (such as viruses) using light scattering is difficult due to their low scattering cross-section and low index contrast to the surrounding medium. Light scattering from a homogeneous sphere has a rigorous solution, as derived by Mie (1908). Particles much smaller than the wavelength of the excitation light can be described by a dipolar polarizability α. The polarizability is given by

$$\alpha = 4\pi\varepsilon_o R^3 \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}, \quad (1)$$

where R is the particle radius, and ε_p and ε_m are the dielectric permittivities of the particle and the surrounding medium, respectively. An incident oscillating electric field $\mathbf{E}_{\mathrm{exc}}$ induces a dipole $\mathbf{p}$ in the particle according to $\mathbf{p} = \alpha\mathbf{E}_{exc}$ (Bohren and Huffmann, 1983). The induced dipole radiates (i.e. scatters) a secondary electric field $\mathbf{E}_s \propto \alpha\,\mathbf{E}_{exc}$. Evidently, α defines the scattering and absorption efficiencies and bears information on both particle size (R) and composition (ε_p), and hence provides an important fingerprint in nanoparticle characterization. In nanoparticle detection techniques such as dynamic light scattering (Berne and Pecora, 2000) or flow cytometry (Givan, 2001), which probe the intensity of the scattered light $I \propto |\mathbf{E}_s|^2$, the detector signal scales with $|\alpha|^2 \propto R^6$. The strong size dependence makes it extremely difficult to detect small particles such as viruses based on standard light scattering. Virus detection approaches based on flow cytometry rely upon fluorescent labeling of segments of the viral genome (Brussaard et al., 2000; Ferris et al., 2002; Stoffel et al., 2005), and hence are not label-free. In addition, no quantitative information can be obtained about the size of the virus particles (Porter et al., 1997). On the other hand, interferometric detection (Lindfors et al., 2004; Batchelder and Taubenblatt, 1991; Batchelder et al., 1991; Plakhotnik and Palm, 2001) exhibits a weaker size dependence and therefore provides significantly better signal-to-noise for small particles. For interferometric detection, the detector signal is proportional to the amplitude of the scattered light $|\mathbf{E}_s|$, and hence scales with $\alpha \propto R^3$. Interferometric detection can provide *single* particle sensitivity and has the potential for real-time detection (Ignatovich and Novotny, 2006; Mitra et al., 2010; Person et al., 2011; Deutsch et al., 2010).

In real-time interferometric nanoparticle monitoring, particles typically are made to traverse a stationary laser focus, and the scattered field from a single particle is combined with a reference field and recorded interferometrically with a photodetector. In this article we introduce a new technique, which combines heterodyne interferometry with dark-field microscopy (Braslavsky et al., 2001). The dark-field approach prevents any background light from reaching the detector in the absence of a particle at the laser focus, and hence improve detection sensitivity by reducing the background noise. Using heterodyne interferometry it is possible to effectively decouple the amplitude and phase of the detector signal and hence improve detection accuracy. Using this combined approach, we demonstrate a sensitivity superior to other interferometric techniques, and can clearly differentiate between single biological nanoparticles (phage and viruses) in a mixture. Such high sensitivity and resolution enables us to detect even impurities in virus samples.

2. Materials and methods

2.1. Dark-field heterodyne interferometric detection

Fig. 1 illustrates the basic concept of the detection scheme (refer to Supplementary Section 1 for a detailed description). Structured illumination is used to create converging annular illumination at the focal plane of high-NA objective, where particles such as viruses traverse the illumination spot inside a glass nanofluidic channel (see Section 2.2). Such configuration allows to separate the light back-scattered by the nanoparticle from the portion of the incident light specularly reflected by the channel interfaces, i.e. the background light. Eliminating the background lowers the noise floor in the detector signal and therefore results in high detection sensitivity.

Without dark-field detection, such as for interferometric detection strategies which employ a 'bright-field' scheme where a collimated gaussian beam is tightly focused to illuminate a particle, the signal S recorded by the photodetector shown in Fig. 1 would be

$$S \propto E_s E_r\, e^{i[\Delta\omega t + \Delta\phi_{sr}]} + E_s E_b\, e^{i\Delta\phi_{sb}} + E_b E_r e^{i[\Delta\omega t + \Delta\phi_{rb}]}, \quad (2)$$

where $\Delta\phi_{rb}$ is the phase difference between E_b and the frequency-shifted reference field E_r. $\Delta\phi_{sr}$ is the phase difference between the field scattered E_s and the reference field E_r, and $\Delta\omega$ is the heterodyne detuning frequency, that is, the frequency difference between E_r and E_s. Note that since the last term in Eq. (2) does not depend on the scattered field E_s, it does not contain any information about the particle, but only increases the noise floor and thus reduces the method's sensitivity. Because lock-in detection uses $\Delta\omega$ as a reference frequency, the third term cannot be eliminated, unlike the second term. Usually a differential detection strategy is employed where a split detector or a balanced detector is used to eliminate the third term (Mitra et al., 2010). However, such a strategy relies on ideal detector alignment and a perfectly stable system. In practice, an interferometric system is not sufficiently stable to completely eliminate the contribution of E_b in reduction of detection sensitivity, unless E_b is eliminated as good as possible. In the present approach, E_b is suppressed by means of dark-field detection, which eliminates the need for differential detection, ideal detector alignment, and beam stability to eliminate background.

With dark-field detection, the terms containing E_b in Eq. (2) are eliminated and only the first term survives. The detector signal S hence becomes

$$S \propto E_s E_r\, e^{i[\Delta\omega t + \Delta\phi_{sr}]}. \quad (3)$$