Single-particle spectroscopy

Whispers of absorption

The combination of whispering-gallery-mode sensing with photothermal absorption spectroscopy promises significant advances in single-molecule identification.

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Recent decades have seen an explosion of interest in the detection and spectroscopy of single molecules. Traditional experimental techniques perform measurements on large collections of molecules such that only average properties can be determined. By contrast, through the analysis of individual molecules - be they fluorescent dyes, polymer chains, proteins or even single ions - complex physical, chemical and biological systems can be studied in minute detail. The vastly richer knowledge offered by such single-molecule techniques has had far reaching implications in fields as diverse as quantum optics, biophysics and analytic chemistry. For example, observations of DNA transcription, antigen recognition and protein unfolding at the level of individual molecules have provided great insight into the complex mechanisms governing life1.

Most early single-molecule experiments monitored light emitted from fluorescent molecules. Though powerful and still widespread, these studies typically require labeling of the target molecule with suitable dye molecules to generate a detectable signal. Labeling unfortunately comes at a cost: it not only requires a complicated modification protocol to incorporate the fluorophore into the target molecule, but it can also critically affect molecular function2, which is itself often the subject of research interest. To overcome these issues, a number of label-free detection methods have been developed3. Writing in *Nature Photonics*, Heylman and collaborators now report the detection and spectroscopic characterisation of metallic nanoparticles with a novel technique that achieves very high detection sensitivity without the need for fluorescent labels[4].

A leading label-free detection method is based on optical microcavities, the simplest example being that of two highly reflecting planar interfaces. In such cavities, light is ‘trapped’ within the intermediate space between the interfaces through multiple reflections. Similarly, electromagnetic waves can be confined to closed circular paths, known as whispering gallery modes (WGMs), within spherical, toroidal or other rotationally symmetric resonators. The refractive index of a WGM resonator is larger than that of the surrounding medium, such that light propagates around the inner surface of the resonator through successive total internal reflections. As a result of light interference after each cavity round trip, only specific wavelengths are significantly confined, that is, resonant with the cavity. The resonance wavelengths are dictated by the refractive index of both the cavity and its host medium; the latter dependence derives from the evanescent wave generated in the host medium upon total internal reflection. When a particle of interest adheres to the resonator surface, it modifies the effective refractive index experienced by the WGM, which in turn induces a measurable shift in the WGM resonance frequency: this is the basic transduction mechanism used in many WGM sensors[5].

Albeit extremely sensitive, WGM sensors lack the ability to discriminate between different types of particles. This functionality is a crucial requirement for the analysis of heterogeneous samples such as blood, and in applications such as environmental monitoring or clinical diagnostics where discrimination between toxins or pathogens and otherwise harmless molecules is of paramount importance. Fluorescence-based techniques can avoid this issue by building a chemical specificity into the labeling stage, whereas the surface of WGM sensors can be chemically modified so that only desired target molecules can bind. Nevertheless, both strategies are limited in the range and

number of particles that can be identified in any given measurement. Enter spectroscopy, which is a powerful route to molecular identification since molecules possess complex and unique spectral fingerprints. Surface-enhanced Raman spectroscopy, for example, is well-known in plasmonics and can be realized on a WGM platform thanks to the strong surface fields associated with WGMs[6]. The alternative approach explored by Heylman and co-workers demonstrates that a WGM microtoroidal resonator can be used as an absorption spectrometer. Combining the principles of photothermal spectroscopy and WGM sensing, the authors have acquired absorption spectra of metallic nanoparticles with high detection sensitivity, thus taking the first step towards label-free single-molecule identification on a WGM platform.

The photothermal effect is a phenomenon whereby the energy of light absorbed by a material or molecule is dissipated as heat, causing a corresponding temperature increase ΔT in the material. As a consequence, the refractive index of the medium is also modified according to $\Delta n = \alpha \Delta T$, where α is the thermo-optic coefficient. Heylman and collaborators use a tunable pump laser spanning wavelengths between 1280 and 1370 nm, which they tightly focus onto rod-like gold nanoparticles adsorbed to the surface of small silica toroidal resonators of major radius ~23 μm and characterized by optical quality factors up to 10^7 and mode volumes ~300 μm^3 (see Figure 1a). As a result, conduction electrons in a metallic nanorod are excited into oscillation. By choosing the dimensions of the nanorod correctly (250 nm in length and 25 nm in diameter in this case), the natural frequency of these oscillations - or localised plasmons - can be matched to that of the pump laser such that the oscillations are driven resonantly and the amount of energy absorbed by the material increases. Ohmic losses, however, cause the energy stored in the localised plasmons to be dissipated as heat, warming up the nanorod. Through thermal conduction, the heat produced is also transferred to the nearby resonator: this in turn implies that its temperature, refractive index and ultimately its resonance frequency change (see Figure 1b). If the power of the pump laser varies periodically, the thermal signal corresponding to the induced WGM resonance shift also oscillates at a given modulation frequency (here ~2 or 4 kHz). A lock-in amplifier operating at this frequency allows the team to monitor the variations of the WGM resonance while mitigating noise to ensure sensitive measurements. The sensitive read-out of the toroid resonance frequency itself is based on an additional probe laser, which is actively locked to a WGM resonance in a feedback loop with the Pound-Drever-Hall technique. Locking is realised through phase modulation of the probe laser at a frequency of 200 MHz, and the transmitted photocurrent, after mixing with a known oscillatory reference signal, provides an error signal for the loop that is proportional to the derivative of the WGM resonance lineshape. This error signal makes it possible to tune the probe laser back into resonance with the toroid frequency, and at the same time it acts as a direct measure of any WGM frequency shift that occurs. The authors stress that it is this double modulation scheme that is responsible for detection sensitivity well beyond current state of the art WGM sensors. Indeed, Heylman and co-workers experimentally determine the limit of detection for their system – an important metric quantifying the smallest measurable WGM resonance shift. To do so they systematically reduce the power of the pump beam, decreasing the absolute energy absorbed by the plasmonic nanoparticle, and find that they can measure a resonance frequency shift around 84 Hz, which is equivalent to a change in the resonator's temperature of about 100 nK or to a thermal power close to 10^{-11} W. This impressive detection limit is approximately three orders of magnitude below the thermal power emitted by a single dye molecule7. More accurate quantitative spectral measurements would require an extension of the present thermal model to include additional effects such as the expansion of the resonator and temperature changes in the host medium. Nonetheless, the current results suggest that application of the team's technique to single molecules, as opposed to plasmonic particles, might not be too far off.

Although the demonstration carried out by Heylman and co-workers is restricted to plasmonic absorbers, it exemplifies the possibilities that absorption spectroscopy can afford. Use of plasmonic nanoparticles has attracted attention in WGM sensing, but only as a means to enhance the local field

and thus detect ever-smaller particles[5,9]. Moving beyond this paradigm, the authors have experimentally studied the physical interaction between very narrow WGM resonances and relatively broad plasmonic resonances for the first time. To support their experimental observations, Heylman and colleagues use a coupled-oscillator model to describe the WGM-nanoparticle interaction. Their theoretical analysis shows that the large difference in the widths of the WGM and plasmonic resonances implies that the interference between WGM and plasmonic modes produces a characteristic asymmetric spectral lineshape (see Figure 1c) known as a Fano resonance8. More specifically, the spectral profile of this Fano resonance depends on the difference between the WGM and plasmonic resonance frequencies because the latter dictates the relative phase between the modes. As toroidal resonators support many WGMs at different wavelengths, each of which interacts with the localised plasmon, one should expect a dense 'forest' of Fano resonances to appear. Indeed, the existence of a set of Fano resonances was experimentally observed by Heylman and collaborators, whose data further indicates that the asymmetry of the Fano resonances varies across the measured absorption spectrum, supporting the coupled-oscillator model that was adopted in this and previous theoretical studies. Although the present experiments are restricted to a wavelength range of ~100 nm, the fundamental limitation on the bandwidth is given by the transparency window of the resonator material.

Single-molecule detection has proven pivotal in many branches of science. The work of Heylman and colleagues highlights the potential analytic capabilities of single-molecule WGM measurements, which allow for highly sensitive spectral analysis of individual unlabeled molecules. This capability can be expected to facilitate the development of on-chip single-molecule identification technology for clinical, environmental and several other applications. Therefore, these whispers of absorption are just the beginning of a promising scientific conversation.

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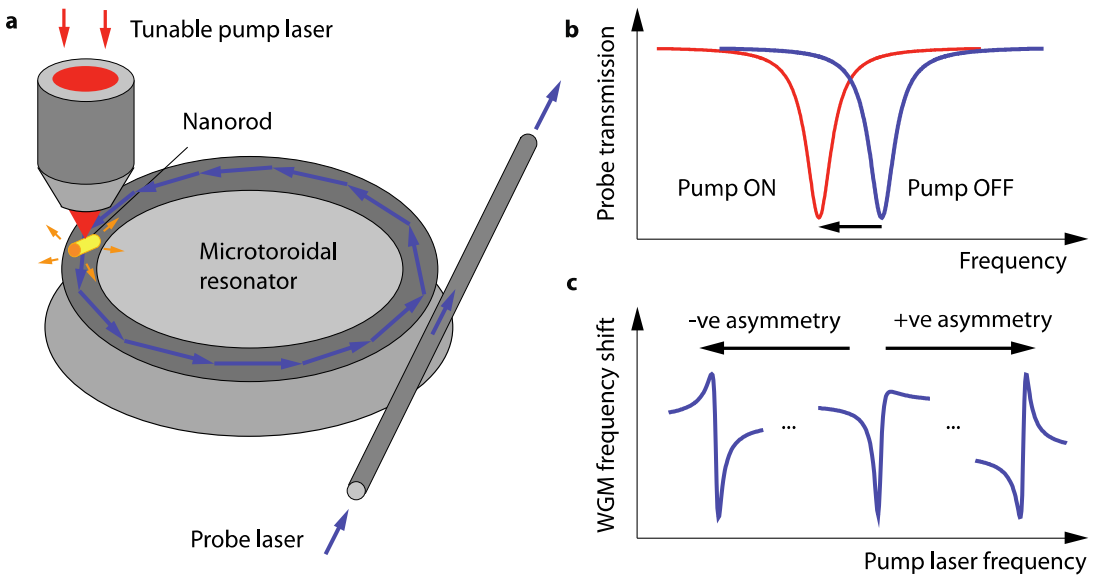


Figure 1: Absorption spectroscopy of plasmonic nanoparticles using a microtoroidal whispering gallery mode (WGM) resonator. **a** Using a pump-probe beam arrangement, a plasmonic nanorod is resonantly illuminated causing the nearby microcavity to heat up. **b** Due to the photothermal effect, the temperature change in the resonator causes its resonant frequency to shift. **c** The measurement of the WGM frequency shift as a function of the pump laser frequency gives access to an absorption spectrum that exhibits several Fano resonances originating from WGM-nanoparticle interactions. These Fano resonances are characterised by different asymmetries in distinct spectral regions.