

Single-cell detection by cavity ring-down spectroscopy

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The implementation of cavity ring-down spectroscopy in an optical fiber resonator extends the viability of this highly sensitive technique for label-free detection of biological species. By chemically treating the surface of discrete tapered sensing regions along the length of a physically extended optical fiber resonator, we show single-cell sensitivity arising from optical scattering of the evanescent field surrounding the fiber. The observed detection limits, based on a minimum detectable scattering cross section on the order of $10 \mu\text{m}^2$, suggest a broad range of new applications in a simple, inexpensive device for real-time cavity ring-down biosensing. © 2004 American Institute of Physics. [DOI: 10.1063/1.1819520]

Rapid detection of micro-organisms with both high specificity and single-cell sensitivity is of broad interest in a variety of fields, including molecular biology, medicine, national security, and environmental monitoring.¹ Several techniques address this need, including mass-sensitive mechanical metrology² and fluorescence spectroscopy,³ but these involve expensive equipment or time-consuming sample preparation. Cavity ring-down (CRD) spectroscopy, which is most commonly used in the detection of trace concentrations of absorbing molecules, offers a real-time response in a simple, compact, less expensive arrangement that requires negligible sample preparation.⁴ In this Letter, we demonstrate that, using a versatile yet practical device, label-free single-cell detection of biological agents is possible by adapting an optical fiber CRD resonator.

The CRD technique has been widely adopted for molecular spectroscopy applications ranging from the monitoring of disperse atmospheric species to the spectral resolution of forbidden overtone transitions in small molecules.⁵ CRD is typically implemented in an optical resonator formed by two highly reflective mirrors and derives its high characteristic sensitivity from the measurement of the resonator's decay rate. This rate, which is directly proportional to its internal optical losses, including those due to molecular absorption and scattering, is insensitive to common sources of noise, such as laser intensity fluctuations and interference from external absorbers, allowing quantitative detection of trace concentrations.⁶

CRD has recently been shown to be an effective method for direct measurement of the loss in an optical fiber resonator, allowing its application in highly scattering matrices that are otherwise difficult to sample.⁷ Such a device, which can be constructed from inexpensive telecommunications components, is responsive to observables not accessible in a traditional CRD arrangement, such as bending attenuation,⁸ mechanical strain,⁹ refractive index changes,¹⁰ and evanescent field loss.^{7,8,10} Furthermore, optical fiber CRD eliminates the need for direct line of sight along the resonator, suggesting a range of new applications, including standoff detection or distributed monitoring over a large physical area.

The sensitivity of optical fiber CRD to a variety of parameters is augmented by incorporating a biconically tapered fiber segment in the resonator, increasing the extent of the evanescent modes in the fiber.⁷ The resulting spectroscopic enhancement, detailed in Ref. 7, arises from a taper's susceptibility to perturbations in the guided electro-magnetic mode. Formed by heating an optical fiber to its thermal softening point and mechanically drawing along its transverse axis, a biconical taper adiabatically transforms the core mode of a single mode fiber into a cladding-dominated mode containing an evanescent portion that will interact with external species. This cladding mode is converted back into the propagating core mode at the other end of the taper, but the transition is readily disturbed by either physical discontinuities in the taper or interactions with external species, including particulate scattering or molecular absorption. In a taper with low intrinsic loss, necessary when included in a CRD arrangement, the evanescent field decays radially over a distance comparable to the wavelength of the excitation light, restricting the sampling depth to the near field.

The rapid spatial decay of the evanescent field, although a limitation for large bulk sampling, can be exploited by coating the taper to allow only a chosen target analyte close enough to interact with the field. A range of chemical coatings that are readily attached to the SiO_2 surface of an optical fiber have been developed for the binding of specific targets, either by mimicking natural receptors or by exploiting other chemical interactions.^{11,12} These coatings are more commonly used in conjunction with a fluorescently labeled analyte or chromophore, but the selectivity they provide can replace the need for spectroscopic labeling, leading to simpler and faster sampling. This additional selectivity also permits the interrogation of nonabsorptive properties that are more sensitive to the presence of a target analyte.

Optical scattering is one such property that is particularly suited to the detection of cells with a fiber-optic sensor, as the high relative optical density of the nucleus in most cells leads to efficient scattering.¹³⁻¹⁵ In addition to its dependence on the nuclear refractive index, the scattering loss caused by a cell varies with the cellular size and shape, the presence of other high index of refraction organelles, and the orientation of the cell in the electromagnetic field. In a tapered optical fiber sensor, the effective scattering loss of a cell also varies with both its position along the length of the taper and its distance from the taper surface, which each can

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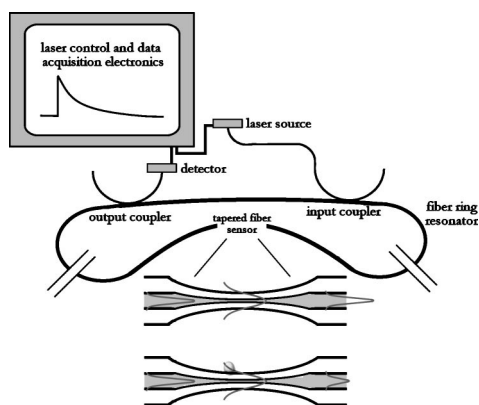


FIG. 1. Schematic diagram of the fiber optic cavity ring-down apparatus, not drawn to scale.

be controlled by physical design of the taper and chemical engineering of its coating.

We adapted an optical fiber resonator initially designed for liquid absorption spectroscopy, shown in Fig. 1, to determine both the responsiveness and detection limits of CRD sensing of cellular scattering. Fitted with a custom biconical taper 10 mm long with a minimum waist diameter of $25\ \mu\text{m}$, the resonator was otherwise constructed of common telecommunications components, including standard single-mode fiber as a propagation medium and tap couplers in place of the mirrors found in a traditional CRD device. The resonator was excited with continuous wave radiation from a distributed feedback laser centered at 1520 nm, chosen at the lower spectral range of the fiber's transmission window because of the rapid increase in light-scattering efficiency with decreasing wavelength. Although even greater improvement in light-scattering efficiency is attainable at visible wavelengths, single-mode optical fiber technology, necessary in a resonator excited by continuous wave radiation, is not yet suited for CRD sensing at shorter wavelengths because of the high intrinsic attenuation that arises from internal scattering. In addition to such excessive bulk loss in the visible region, which precludes the long path lengths necessary for CRD operation, the extension of the evanescent field into the sampling medium is directly related to the wavelength of the guided light, requiring a balance of taper design and light source choice for sufficient field accessibility. Operation of the CRD resonator at 1520 nm avoids the high intrinsic loss and limited evanescent field penetration that would be problematic with visible excitation and likely compromise the sensitivity advantages gained with the CRD method.

A biconical taper, spliced between the input and output couplers in the CRD system, was coated with a polypeptide, poly-*D*-lysine, in order to concentrate the target cells in the localized evanescent field. This polypeptide, commonly used as a culture plate coating for anchoring cells, was chosen because of its optical transparency and strong binding affinity for cellular membrane proteins.¹⁶ We employed adherent mammalian cancer cells as our target analyte, in particular the National Cancer Institute MCF-7 breast cancer cell line, because of the large size of the cells, approximately $10\ \mu\text{m}$ in diameter, and high melanin content, adding to their scattering efficiency. The large cellular size limits their sticking efficiency to the highly curved surface at the taper waist, although sufficient cell coverage was observed to both sides of the waist region. The interaction between the cellular

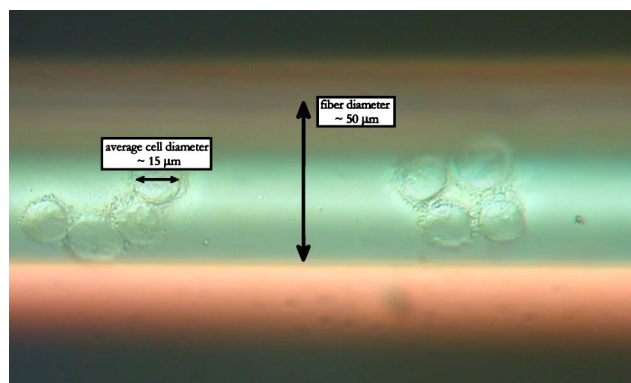


FIG. 2. Image of adsorbed cells on the surface of the fiber taper.

membrane and the poly-*D*-lysine-coated fiber, although strong, is conveniently reversed by addition of trypsin, an enzyme that cleaves the polymeric lysine-lysine bond, allowing straightforward detachment of adhered cells for rapid reconstitution of the coated fiber surface.

The coated taper was shown to be unaffected optically by both the application of poly-*D*-lysine coating and the trypsination process. A comparison of optical decay times before and after the treatments showed no statistical deviation from the measured "empty cavity" ring-down time, defined as the inherent ring-down time of the resonator arising from its intrinsic losses, measured for this resonator to be of $73.4(8)\ \mu\text{s}$. In addition, no statistical change was seen when cells were introduced to the system in the absence of a chemical coating. However, application of the cells to the coated taper indicated both the strong expected adhesion to poly-*D*-lysine, shown in Fig. 2, and a significant effect on the optical loss of the ring-down system. The scattering effect of the adhered cells was further enhanced by measuring the system loss after removal of the surrounding solution, resulting in a higher difference in refractive index. This sampling technique, in which the cell growth medium was withdrawn and the taper dried with compressed air prior to data acquisition, allowed simultaneous determination of the ring-down time and manual inspection on a compound microscope.

Microscopic visual inspection of the coated taper also provided an accurate method for the determination of the number of adhered cells and a quantitative comparison with the optical ring-down time, shown in Fig. 3. This comparison is well described by a linear fit with $R^2=0.93$, from which the theoretical sensitivity can be calculated. Within the fit region, the standard error in the system was measured to be $0.044\ \mu\text{s}$ over 200 ring-down transients, which in principle can be detected in less than 0.2 s of real time. This corresponds to a signal-to-noise ratio of 5 for the detection of a single mammalian cancer cell, based on the measured change in ring-down time of $0.23\ \mu\text{s}/\text{cell}$.

The high sensitivity of the tapered fiber sensor is complemented by a relatively long linear range extending from 0–150 cells. A plot of ring-down time as a function of adhered cells becomes asymptotic to a fixed maximum loss resulting from the limited percentage of propagating power contained in the evanescent field. The upper limit is also affected by the additional noise inherent in measuring many cells, as the effect of nonuniformity in the cell distribution and the resulting reformation of the disturbed field over the taper length becomes more apparent. While the linear range

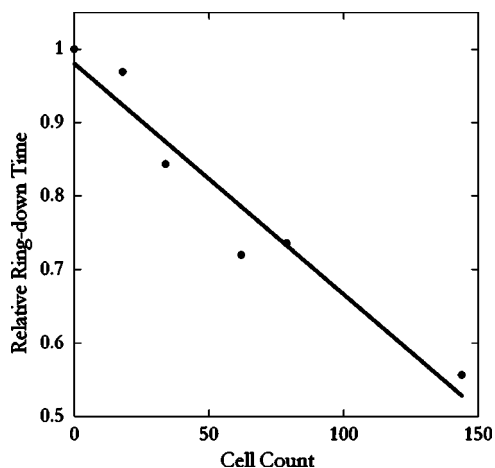


FIG. 3. Relationship of cavity ring-down decay time, normalized to the ring-down time with a clean fiber surface, to the number of cells adhered to the tapered fiber surface ($R^2=0.93$).

could be likely extended by use of longer tapers, the practical application of a cell sensor favors low concentrations, as higher cell populations become visible to the naked eye and are quantifiable by straightforward optical density measurement.

The measured subcellular sensitivity implies that smaller species with a lower scattering cross section can be detected with this optical fiber CRD system. To estimate the limits of the sensitivity with different species, we applied a simple mathematical model based on the Fresnel reflection at a cylindrical interface to approximate the power scattered by a sphere in the evanescent field near the tapered region.¹⁷ This model employs a simple ray approximation for the propagating field, an accurate representation of the propagating field because of the multimode fiber structure near the taper waist. Our calculated results, based on the minimum detectable loss measured in the system, show a sensitivity to a scattering coefficient of 20 cm^{-1} , or a total scattering cross section of $10 \mu\text{m}^2$ for a particle the same size as the mammalian sample cells. These values are of the same magnitude as those reported for a variety of other relevant species, including certain marine microbial particles and various bacterial spores.^{18,19}

Despite the low detection limits calculated and measured for the optical fiber CRD device, improvements in selectivity that are normally found in spectral fingerprinting can be achieved with careful molecular design of the taper coating. We employed a poly-*D*-lysine coating because of its compatibility with both the fiber surface and the target cells, but the nature of the attraction also led to contamination of the coated taper surface with cellular debris. This resulted in an unacceptable source of error for experiments sampling non-sterile cell solutions, which experience higher rates of cellular necrosis, although a coating specifically designed for our analyte would improve the system response in such a heterogeneous matrix. A variety of such biorecognition coatings has been designed to attract and bind biological agents while excluding contaminants, and their development remains an active field of research.^{11,12,20}

The diverse coating technology that has already been used with optical fiber sensors is complemented by the high sensitivity of CRD sensing in a fiber-optic resonator. We

have demonstrated the limits of this sensitivity in a simple, inexpensive, and versatile device, showing single-cell detection for a variety of species adsorbed to the fiber surface. By implementing molecular recognition technology to enhance the selectivity of the system, it is possible to exploit the near field sampling boundary of a tapered fiber sensor, allowing highly sensitive, real-time detection of unlabeled species by measurement of optical scattering. In a CRD device, which we have shown to be highly responsive to optical scattering, the incorporation of a biconically tapered fiber sensor in a resonator constructed from common telecommunications components leads to a practical biosensor that can be expanded for environmental monitoring over several kilometers or reduced for quantitative measurement of cellular populations in a laboratory culture dish. While the sensitivity of this device reflects the currently available technology, advances in molecular recognition chemistry and optical fiber engineering will further improve both selectivity and detection limits to permit single species biosensing of different bacterial spores or even viral particles.

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