

Cavity Ring-down Biosensing

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Cavity ring-down spectroscopy (CRDS) is an established technique for gases sensing that is newly emerging in the field of optical biosensing. This approach measures changes in the rate of decay of light injected into an optical resonator and relates them to optical loss along its length. This principle has recently been adapted for use in liquids, providing a highly sensitive method for quantitative biosensing applications. Such approaches, which can incorporate evanescent field sensing elements, have been demonstrated for the detection of molecular absorption in liquids, absorption of surface bound species and scattering response of individual cells. Building on the high sensitivity that CRDS brings to molecular spectroscopy instrumentation, evanescent wave CRDS represents the current state of the art holds significant potential for a range of biosensing applications.

1. Technical Concept

The interaction of light and matter can be measured in a variety of ways, including molecular fluorescence, absorption, or scattering. Of the wide range of methods that are used to quantify such interactions, each employs a measure of the change in an incident signal that is related to the presence of an analyte. For example, absorption spectroscopy detects the attenuation of light as a function of wavelength-dependent molecular absorption. This attenuation is related to the absorbance of a sample, A , which is described by Beer's Law:

$$A = \epsilon CL \quad (1)$$

where ϵ is the molecular absorption coefficient, C is the analyte concentration, and L is the length of travel of the light through the sample. Variations in concentration linearly affect absorption, resulting in attenuation of an incident beam. This simple relationship between absorbance, concentration, pathlength, and the intrinsic properties of the sample has important implications for the range of molecular spectroscopy approaches. For instance, improved absorption sensitivity can be conveniently realized by increasing the sample cell pathlength. Similarly, very low concentrations can be measured in the mid-infrared or UV spectral regions, where molecular absorption coefficients are typically the largest. Combinations of these approaches have been adapted for atmospheric monitoring of trace quantities of small molecules with a great deal of success (King, 2000; Thompson, 2002; Parkes, 2003).

1.1 Cavity Ring-down Spectroscopy

The practical limitations associated with standard absorption spectroscopy techniques can be eased by incorporating a mirrored multipass cell in place of the sample cell. When this multipass cell is formed by highly reflecting mirrors, the interaction length can be extended by a factor of several hundred. In traditional designs, such that of White (1942) and Herriott (1964), each pass of the cell was spatially resolved on the input/output

mirror, resulting in a volume that was quite large. When the light radiation is instead coupled into a stable optical cavity that refocuses the light back on itself on each pass, a very compact cell with an effective number of passes equal to $1/(1-R)$ (where R is the mirror reflectivity) can be formed. Using a cavity ring-down spectroscopy arrangement such as that shown in Figure 1, an effective pathlength of nearly 100 kilometers has been realized with a physical cell only one meter in length using a diode laser coupled into a cavity formed by mirrors with a reflectivity of 99.999% (Dudek, 2003).

In a cell with an effective pathlength of several kilometers, the resonating light trapped between the mirrors leaks out on a relatively long time scale, $\sim 3 \mu\text{s}$ for each km. By solving the differential rate equation for this resonator, the intensity of the light exiting the cell can be shown to decay as a single exponential (Lehmann, 1996)

$$I(t) = I(0) \exp\left[\frac{-tc}{L}(1 - R + \varepsilon CL)\right] = I(0) \exp[-t/\tau] \quad (2)$$

where $I(t)$ is the time dependent light intensity leaving the cavity; $I(0)$ is the initial light intensity; c the speed of light in the medium between the mirrors; L the separation between the mirrors; and τ is the decay time constant of the cavity, or “ring-down” time, defined as the amount of time it takes for the intensity to reach $1/e$ of the initial intensity.

This equation can be rearranged to express the time constant in terms of the characteristic losses of the cavity and the losses due to absorption from a sample analyte:

$$\frac{1}{\tau} = \frac{1}{\tau_0} + \varepsilon Cc \quad \frac{1}{\tau_0} = \frac{c(1-R)}{L} \quad (5)$$

in which τ_0 is the intrinsic decay time of the cavity, typically measured in the absence of the sample. Typically on the order of several microseconds long, these cavity ring-down times are entirely independent of laser intensity yet directly related to absorption. Monitoring changes in these cavity decay times is the essence of cavity ring-down spectroscopy (CRDS), allowing observation of the absorption spectrum or changes in the sample concentration.

The sensitivity of CRDS arises in large part because of the low optical loss of cavities formed from modern optics. In addition, the cavity ring-down time constant is independent of fluctuations in the input light intensity removing any dependence on laser source intensity, meaning that power fluctuations and external light contributions no longer factor into signal-to-noise considerations. Similarly, cavity ring-down times rely only on losses intrinsic to the cavity, relaxing unwanted contributions from external absorbers, such as in the air path. In practice, the long interaction distances that accompany long decay times produce the very high sensitivity that is characteristic of this technique. For example, if the per pass loss of the sample matches that of the cavity, which can be as low a few parts per million, then the rate of cavity decay will be doubled. Because changes in the decay time on the order of 0.01-1% are easily detectable, a loss on the order of 10^{-3} of that of the cavity itself can be routinely measured.

1.2 Evanescent Wave Cavity Ring-down Spectroscopy

The development of CRDS sensing has traditionally been limited to relatively clean gas samples because of the significant loss caused by interfering absorbers or particulate contaminants. Such factors have also limited the adaptation of CRDS for many biosensing applications, which are typically applied to complex aqueous mixtures. The incorporation of evanescent wave techniques with CRDS technology (Pipino, 1997, Tarsa, 2004a) provides a versatile platform from which to approach biosensing applications. By taking advantage of the evanescent wave formed at a total internal reflection interface, optical waveguides can be used to sample complex mixtures with minimal background contributions.

Evanescent wave sensors rely on the field that exists beyond a total internal reflection interface, Figure 2. This field, which extends perpendicular to the reflection plane, decays exponentially over a distance, z , comparable to the wavelength of the propagating light:

$$E(z) = E_0 \exp \left[\frac{-2\pi n_1(\lambda)z}{\lambda} \sqrt{\sin^2 \theta - \left(\frac{n_2(\lambda)}{n_1(\lambda)} \right)^2} \right] \quad (6)$$

where E_0 is the incident field, λ is the wavelength of the propagating light, θ is the angle of reflection, n_1 is the wavelength dependent refractive index of the propagation medium, and n_2 is the wavelength dependent refractive index of the external medium. While the evanescent field propagates no power into the external medium, the evanescent field can lead to absorption or scattering that is reflected in an intensity loss in the reflected light. Quantification of such power changes can be related to external interactions, including molecular absorption or particulate scattering, using standard spectroscopic instrumentation. Furthermore, sensitivity to these changes can be manipulated through customization of the reflection angle, the wavelength of the propagating light, and the relative refractive indices of the sensor material and the surrounding environment.

Along with this flexibility in sensor design, adoption of evanescent wave techniques extends the range of sampling conditions accessible to spectroscopic monitoring. In many cases, this extension is accompanied by a loss in sensitivity that results from the shallow penetration depth of evanescent sensors. Decreased sensitivity can be a benefit, however, when sensing is approached in otherwise lossy media. For example, the susceptibility of traditional CRDS to optical loss previously limited its adaptation to liquid environments. With the incorporation of evanescent sensing elements in a CRDS resonator, the effects of background absorption and interfacial scattering are sufficiently minimized.

The first combination of CRDS with evanescent wave techniques was realized through the use of a monolithic prism resonator (Pipino, 1997). This device demonstrated the ability to probe thin surface coatings adsorbed onto the surfaces of a total internal reflection interface. In this proof-of-principle device, measurement just beyond the surface of this interface is also accessible, providing sensitivity to biological samples or

other immobilized species. Furthermore, variation of prism angle or excitation wavelength allows control over penetration depth, providing an adjustable parameter that can be customized to the sensing environment. A limitation of this original design is that the evanescent field is limited to a few isolated reflections (typically four) on each round trip. Recently, very high Q Whispering gallery mode resonators have been developed (Grudinin, 2006), where the light circulates around a cylindrical shaped post with continuous exposure to the surrounding medium, greatly increasing the interaction with the analyte.

The implementation of prismatic elements for evanescent wave CRDS later led to the incorporation of other waveguides in CRDS arrangements. For example, adaptation of optical fiber cavities for evanescent wave CRDS led to the first practical liquid sensing and eventually the first CRDS biosensing of individual cells (Tarsa, 2004b). Different approaches for optical fiber CRDS circumvent scattering effects that contribute unacceptably high levels of optical loss in traditional mirror-based arrangements. An example, shown schematically in Figure 3, contains the entire resonant structure in a length of single mode fiber, exposing discrete sections of fiber for distributed sensing applications. When employed for single cell sensing, the cavity ring-down time is measured and related to the number of adhered cells by an expression similar to (5):

$$\frac{1}{\tau} = \frac{1}{\tau_0} + nQc \quad (7)$$

where n is the number of scattering cells, and Q is a calibration constant related to the sensitivity of the sensing element. With this apparatus, biological sensing becomes accessible through particle scattering of the evanescent field surrounding the fiber. Because molecular absorption in the surrounding matrix can be minimized through design of the fiber sensing regions, background effects can be significantly reduced without compromising sensitivity to biological scattering.

The variety of CRDS arrangements, ranging from traditional mirror based cavities to fiber optic resonators, offers significant latitude in experimental design and sensing implementation. Experimental arrangements for localized quantification or distributed sensing can be realized, each with different benefits and limitations. By adapting these approaches for bio-sensing applications, CRDS holds significant potential for rapid detection of a range of biological specimens with both high selectivity and sensitivity. Currently, over a hundred papers per year are being published that utilize CRDS or closely related techniques, and rapid development of the method and its applications, including to biosensing applications, can be expected to continue.

2. History

The first CRDS devices were designed to measure low levels of optical loss in highly reflective mirror coatings (Anderson, 1984). By coupling a pulsed laser into a cavity formed by these mirrors, it was possible to measure changes in reflectivity on the order of parts per million. It was soon realized that the cavity could also be filled with an absorbing analyte, and absorptive loss could be measured at similar levels (O'Keefe, 1988). This initial advance was applied to different systems of weakly absorbing gaseous

absorbers, including vibrational absorption of several energy quanta, or fundamental absorption of very low concentrations. The high specificity of molecular gas phase spectroscopy complemented these approaches, yielding a spectral fingerprint in addition to quantitative information about concentration.

Despite the initial success of these first experiments, it was soon discovered that substantial improvements could be realized by replacing the pulsed laser sources with compact continuous wave semiconductor lasers (Romanini, 1997). The high stability of single mode diode lasers allowed the selective excitation of the fundamental cavity mode, reducing contributions higher order modes, which have different decay rates. Combined with beam conditioning optics, this mode matching approach was shown to improve signal-to-noise by a factor of ~10-100 (Dudek, 2003). In addition to substantial improvements in sensitivity, the implementation of continuous wave diode lasers led to the first commercial CRDS instruments (Tiger Optics, 2000). These devices, which can be operated at room temperature with minimal power requirements, were first used for sensitive monitoring of semiconductor process gases. However, an adaptable CRDS platform was soon expanded for health related measurements, such as real-time breath analysis (Dahnke, 2001), and homeland security based sensing, including explosive gas detection (Usachev, 2001). In addition, CRDS has been used to make precise measurement of isotopic ratios of CO₂ (Crosson 2002) and methane (Dahnke, 2001b) which have biological and environmental applications.

While most CRDS experiments have been done in the gas phase, some measurements on liquids have been reported. (Xu, 2002) used an absorption cell inside an optical cavity that was tilted to Brewster's angle to greatly reduce reflection losses. Snyder (2003) extended this design to simultaneously realize Brewster's angle for both the glass-air and glass-water interfaces and demonstrated that such a cell can be used for HPLC detection of compounds labeled with strongly absorbing dyes. This work was recently extended by Bechetel (2005) with significantly improved sensitivity. Hallock (2002) showed that low loss cavities could be constructed with the dielectric mirrors directly in contact with the solvent and absorption of solvated species detected. Similarly, Bahnev (2005) built a miniaturized CRDS cell for HPLC detection, although one important limitation of these measurements is that typical liquid phase electronic absorption bands are wider than the high reflective band of available low loss, dielectric mirrors. This does not limit sensitivity, as the sample can be flushed through the cell, but it does limit selectivity as spectral shape of the absorption cannot be used to establish the absorbing species

While these first instruments were operated in much the same way as traditional spectrometers, new generations of CRDS-based devices were soon invented. The first developments took the form of monolithic prism-based sensors, in which the traditional linear cavity was replaced by a hexagonal or octagonal prism (Pipino, 1997). By evanescently coupling light into and out of this resonant structure, CRDS operation was realized both for characterization of the prism material itself and for detection of absorbing species at the prism interfaces. Everest (2006) recently used this technique to monitor hemoglobin adsorption on silica. This implementation of evanescent wave CRDS was later extended into the development of a linear prism-based resonator that replaced the traditional coated mirror reflectors with broadband roof-angle prism retroreflectors (Engel, 1999). Instead of exploiting the evanescent wave sensing structure, this embodiment took advantage of the wide wavelength range that could be

passed through the prism reflectors, allowing laser multiplexing and multiple analyte detection in a single instrument. Other inventions exploited the evanescent wave sensing capabilities of the monolithic prism resonator. The first such approaches incorporated a prismatic element in the middle of a mirror-based resonator (Shaw, 2003). By angling the prism entrance interface at Brewster's Angle, where reflection losses are significantly reduced, this approach sampled binding events at the external interface of the cavity integrated element. The use of whispering gallery mode resonators offers the promise of highly compact and sensitive biosensors based on the CRDS principle (Grudin, 2006). Savchenkov (2006) have developed "white light" whispering gallery mode micro resonators that have very high Q values (low loss) but have essentially a continuous spectrum allowing the full sample spectrum to be measured. Furthermore, these prism based approaches do not have the wavelength limits of dielectric mirrors.

3. State of the Art

The limited sensitivity of this approach to evanescent wave CRDS, which samples at a single discrete point only once per pass, was dramatically extended by the invention of optical fiber CRDS. The first demonstration that optical fibers could be used to construct a CRDS resonator explored the effects of cavity stability and optical fiber loss in a linear resonator. By coating the ends of a length of optical fiber, von Lerber (2002) showed that a traditional CRDS resonator could be constructed using a length of fiber as a propagation medium. Similarly, Gupta (2002) showed that low loss optical resonators could be constructed using Bragg reflectors built directly into the optical fiber. These first optical fiber CRDS instruments were soon extended with two different approaches using an extended fiber ring as a resonator medium, avoiding the need for reflectors that typically limit bandwidth. In both arrangements, the physical pathlength of the resonator is greatly extended, lengthening cavity ring-down times and resulting in a practical device for measuring a wide variety of new parameters. In the earliest work, the use of a fiber taper greatly increases evanescent field exposure, providing enhanced control over sensitivity to external species. For example, the first implementation of evanescent field sensing with optical fiber CRDS was demonstrated with the resolution of 1-octyne surrounding a modified sensing region of the fiber resonator (Tarsa, 2004a). It was also shown that the device could be modified to measure tiny levels of strain on a fiber taper (Tarsa, 2004c). In an analogous design, Loock and coworkers incorporated a small microgap into the fiber ring to measure small volumes of liquid (Tong, 2003) and have recently reviewed potential applications to microliquid samples (Loock, 2006). These two approaches represented the first practical implementations of optical fiber CRDS that led directly to CRDS biosensing.

Along with these optical fiber CRDS approaches, another area of considerable recent activity with important implications for biosensing is the development of broad bandwidth CRDS. Such advances are in contrast to early CRDS experiments, which used a tunable light source to tune through an absorption spectrum or to monitor the changes in a narrow spectral window as a function of time. The incorporation of broad band sensing capabilities will enable many biosensing applications that require parallel detection across a broad spectral region.

With the ability to perform CRDS absorption measurements in liquid matrices, optical fiber CRDS relaxed previous limitations that prevented the practical measurement of

biological samples and liquids. For example, the use of evanescent wave CRDS to measure such samples was first shown by the resolution of single cells adhered to the surface of a fiber resonator, shown in Figure 4. Such single throughput measurement can be further enhanced with broadband scanning technologies, the first demonstration of which was by Engeln (1996), who showed that CRDS could be combined with a Fourier Transform Spectrometer to determine the cavity decay at each mirror spacing. Scherer (2001) further showed that a grating spectrograph could be used to disperse the light as a function of wavelength in one axis of a CCD camera and sweep the signal across the along the slit of the spectrograph with a rotating mirror to temporally disperse the cavity decay. In both of these earlier works, dye lasers with modest bandwidth were used. Ball (2003) expanded this approach with a CCD camera that allows the charge to be moved up and down the chip, allowing temporal dispersion without moving parts and allowing signal averaging on the silicon. Thompson (2007) has shown that Light Emitting Diodes can be used as broad bandwidth sources for CRDS measurements, and Fiedler (2003) has used incoherent arc lamps. Building on these approaches, Johnston (2007) is developing a white light spectrometer that combines a cavity based upon Brewster angle prisms discussed above and white light super continuum generation in a photonic crystal fiber. The later allows radiation from $\sim 500 \text{ nm} - 2 \mu\text{m}$ to be generated from a low power ($30 \mu\text{J}$ per pulse) diode pumped $1.06 \mu\text{m}$ laser.

The combination of these broadband techniques and temporal resolution approaches with CRDS technology suggests a strong future for CRDS biosensing. The high sensitivity of CRDS may bring new advances to more common biological approaches, potentially allowing researchers to watch molecular absorption in tiny samples or quantify trace components of complex biological mixtures. In addition, the incorporation of technologies already in use for optical biosensing will help drive CRDS biosensing research forward. These approaches, such as chemical surface modification, can be combined with advances in fiber optic and laser technology to advance CRDS biosensing beyond the current state of the art.

4. Potential for Improving Performance or Expanding Current Capabilities

The relatively recent adaptation of CRDS to biosensing applications leaves significant room for development, both for improved selectivity and expanded sensitivity. Because CRDS biosensing devices rely on evanescent wave techniques, advanced technology developed for traditional evanescent sensors can be readily adapted for CRDS instrumentation. Such advances in sample concentration and separation can be incorporated with CRDS sensors, providing new approaches for highly sensitive biosensors. Alternatively, intrinsic improvements in the CRDS sensors can enhance performance by exploiting rapidly advancing laser and waveguide technology. The parallel approach of improving sensor construction and adapting surface chemistry will ultimately lead to new generations of CRDS devices for real-time biosensing in a range of detection environments. The fiber loop with a small gap developed by Loock and coworkers as well as the microcavities developed by Bahnev and coworkers can likely be adopted to extend microfluidic technology, which is already highly developed for biological sensing applications.

Chemical modification of optical waveguide surfaces provides a customizable approach to improving biosensing technology that can target either sample concentration or signal

enhancement. Different surface chemistries, which incorporate considerable flexibility for measuring a variety of biological samples, can be employed based on the expected sample matrix composition, providing *in situ* separation of individual species from complex media or concentration of trace species at the sensor surface. Similarly, analyte specificity can be tuned with methods ranging from surface charge manipulation to the incorporation of surface bound monoclonal antibodies or bacterial phages. This wide range of approaches can be used to develop sensors that are specifically tailored to the sensing environment.

Highly specific surface coatings can be combined with advances in waveguide materials and laser emitters to dramatically improve CRDS biosensing. The development of new fiber materials will permit single mode transmission at shorter visible wavelengths, where optical scattering is enhanced and electronic transitions in biological samples dominate, and at longer infrared wavelengths, where vibrational transitions are strong. In addition, inexpensive laser diodes at newly accessible wavelengths can be interfaced with single mode fiber to increase the versatility of CRDS biosensing. Such approaches are complemented by broad band white light scanning CRDS, discussed above, bringing the high sensitivity of CRDS to the diverse range of biological sensing problems.

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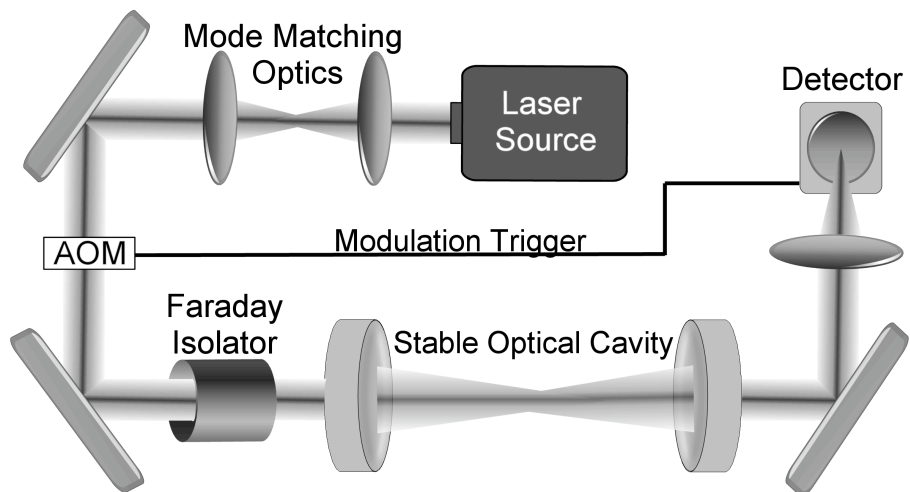


Figure 1: Typical cavity ring-down spectroscopy arrangement. This device is based on a semiconductor laser source, which is efficiently coupled into the optical cavity through a mode matching telescope. Before the cavity, a Faraday isolator prevents feedback from the reflective elements into the laser, ensuring the wavelength stability of the system. Also before the cavity is an acousto-optic modulator (AOM), which allows the laser to be rapidly switched off when the cavity is fully excited. Upon extinguishing the excitation light, the detector measures the time for the cavity emission to reach $1/e$ of the maximum intensity, also known as the “ring-down time.” Other approaches to CRDS use similar arrangements, often with substitution of the cavity mirrors for some other low loss reflective component.

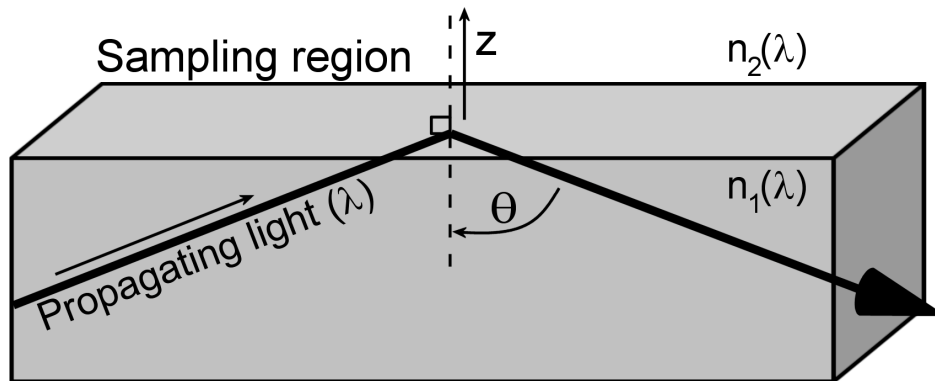


Figure 2: Evanescent wave spectroscopy. The key component of CRDS biosensing is the use of evanescent wave sensors, which allow sampling in high loss matrices that were not previously accessible. This diagram, which is drawn for a slab waveguide, shows the operating principle of evanescent wave sensing. As light hits a total internal reflection interface, a non-propagating field extends normal to the reflection surface. This field decays exponentially over approximately the wavelength of the reflecting light and can interact with samples in close proximity to the surface. This effect is exploited by CRDS biosensing to minimize background losses that are present in complex biological mixtures. Such near field sensing can be further combined with chemical treatments that localize bioanalytes to create a highly sensitive and selective biosensor.

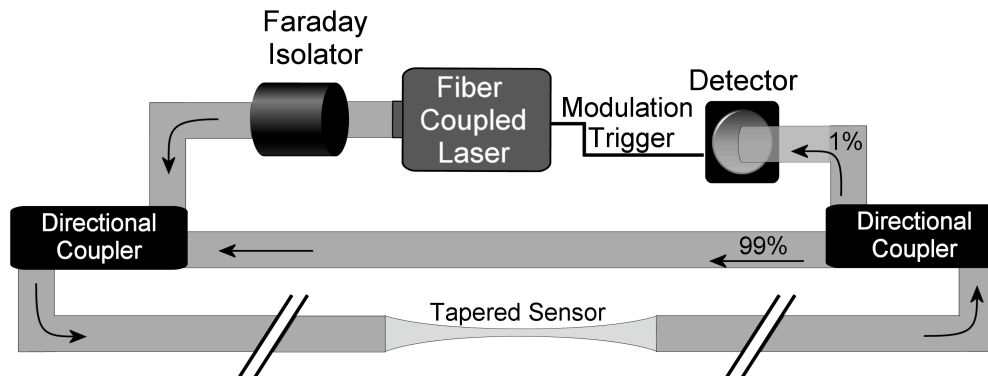


Figure 3: Evanescent Wave Cavity Ring-down Spectroscopy. This device, which is constructed from several kilometers of single mode fiber, allows CRDS sensing at the tapered sensing region while eliminating background contributions along the unmodified length of fiber. Similar to a standard CRDS arrangement, this device replaces the traditional mirrors with directional couplers and substitutes single mode fiber for free space transmission. Because single mode fiber selects the propagating mode near the laser output, mode matching optics are not necessary. In EW-CRDS, the relatively high coupling ratios of off-the-shelf tap couplers increase the per pass loss of this resonator, though the intrinsic loss of the fiber dominates cavity loss over the resonant distance. Despite the relatively high loss of these components, this approach represents the first practical application of CRDS sensing to biological systems.

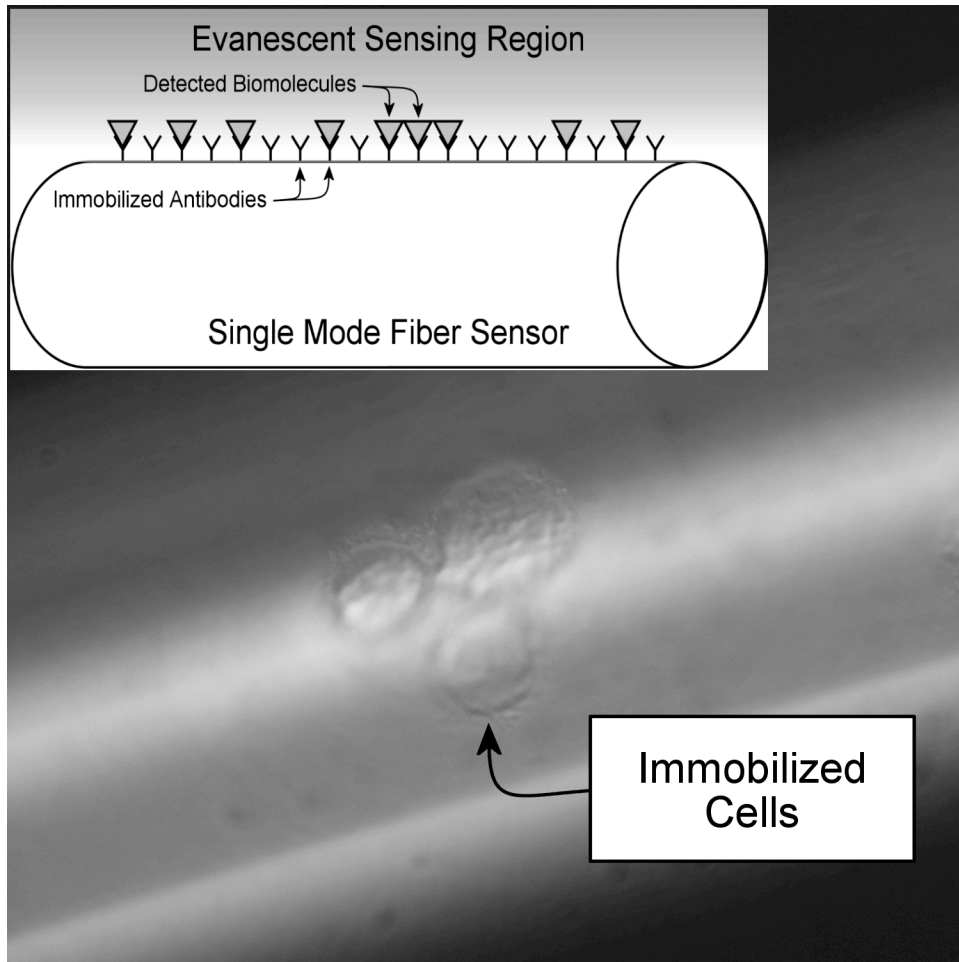


Figure 4: CRDS Biosensing. By chemically modifying the optical fiber surface in the sensing region (inset), CRDS biosensing can be realized for samples ranging from small molecule absorbers to individual cells. In this work, adapted from Tarsa (2004b), mammalian breast cancer cells were immobilized through a nonspecific poly-D-lysine coating. CRDS signals varied linearly with cell adhesion count, and sensitivity was as low as a single cell. Additional coatings can be easily incorporated into this design, allowing for example, nucleic acid consprotein from cell lysates or protein immobilization through antibody coatings.