# Optical fiber-based fluorescent viscosity sensor

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Molecular rotors are a unique group of viscosity-sensitive fluorescent probes. Several recent studies have shown their applicability as nonmechanical fluid viscosity sensors, particularly in biofluids containing proteins. To date, molecular rotors have had to be dissolved in the fluid for the measurement to be taken. We now show that molecular rotors may be covalently bound to a fiber-optic tip without loss of viscosity sensitivity. The optical fiber itself may be used as a light guide for emission light (external illumination of the tip) as well as for both emission and excitation light. Covalently bound molecular rotors exhibit a viscosity-dependent intensity increase similar to molecular rotors in solution. An optical fiber-based fluorescent viscosity sensor may be used in real-time measurement applications ranging from biomedical applications to the food industry. © 2006 Optical Society of America

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Optical fiber-based biosensors have received increased attention as diagnostic tools for a variety of applications (see, e.g., Ref. 1 for a review). By virtue of their light weight, small size, low cost, immunity to electromagnetic interference, and their ability to transmit light over the length of the fiber with minimum energy loss, optical fibers provide an ideal platform for the development of new microscale sensing devices.[2–4] Inspired by these properties, we sought to develop an optical fiber-based sensor that could report changes in fluid viscosity in a quantifiable manner. Given the importance of variation of fluid viscosity to cell signaling modulation (see, e.g., Refs. 5 and 6), disease pathogenesis,[7,8] and even the food industry (e.g., the influence of viscosity on taste[9]), we envisioned that a fiber-based viscosity sensor would find several applications in the fields of analytical and biomedical sciences.

In general, optical fiber-based sensors contain a chemically modified area, usually at the fiber tip, that can interact with the target analyte. This interaction leads to changes of the light that is transmitted by the fiber, ultimately allowing measurements of the changes that occur at the microenvironment of the tip. Our design was based on creating a sensing layer at the fiber tip by covalently attaching a viscosity-sensitive fluorescent dye. The latter was selected from a family of environment-sensitive dyes, referred to as molecular rotors,[10] whose fluorescent quantum yield $\phi_F$ depending on solvent viscosity $\eta$ is described by the Förster–Hoffmann equation [Eq. (1)][11]:

$$\log \phi_F = C + x \log \eta. \quad (1)$$

Molecular rotors are characterized by two competing de-excitation pathways: intermolecular rotation and fluorescence emission. Intramolecular rotation is reduced in viscous solvents,[12] therefore the molecule exhibits an increased quantum yield. We hypothesized that covalent attachment of a molecular rotor to the fiber tip would not result in loss of its viscosity sensitivity, despite the tethering of one side of the molecule to a solid surface. Moreover, we envisioned that excitation of this dye either through the fiber or via an external light source would produce a viscosity-dependent fluorescence emission that could be transmitted through the fiber to the detector. To test this hypothesis, we covalently attached 9-(2-carboxy-2-cyanovinyl)-julolidine *N*-hydroxysuccinimidyl ester (CCVJ-NHS ester, **3**) to the exposed silica surface of an optical fiber to produce fiber-optic sensor **5a** (Fig. 1). Although CCVJ is known to act as a solution-based viscosity sensor, its photophysical properties after attachment to a solid surface have not yet been studied. As a control, we also attached fluorescein (FRS), a non-viscosity-sensitive dye, to an optical fiber via its NHS ester to produce sensor system **5b**. In both experiments the surface immobilization was performed using 3-aminopropyltriethoxysilane (APTES, **1**) as the linker. This strategy[13,14] is based on substitution of one or more of

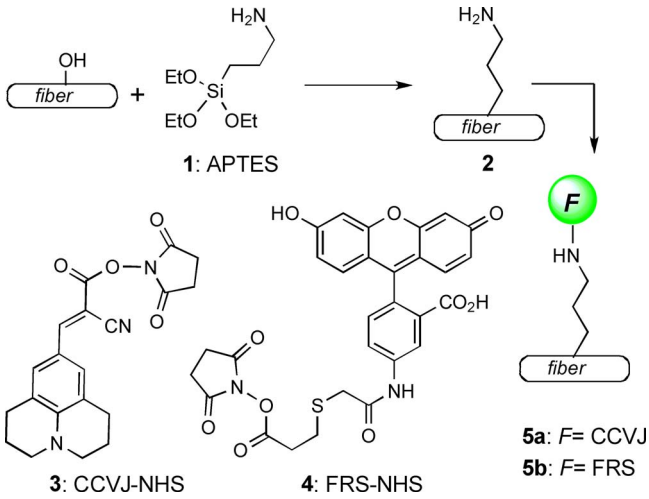


Fig. 1. (Color online) Functionalization of a fiber-optic tip with either CCVJ or fluorescein (FRS).

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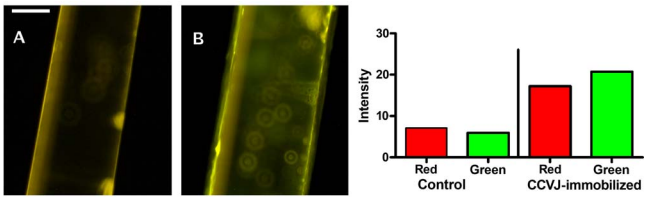


Fig. 2. (Color online) Epifluorescent image of the fiber tip sections where the cladding was removed. Both fibers were illuminated at the distal end with a 440 nm laser. Image A shows a fiber without any immobilized dye (control). The emitted light is caused by fiber autofluorescence. Image B shows a fiber with immobilized CCVJ (**5a**). Stronger green emission intensity, on average 20% higher than that of the control fiber, is clearly visible. The scale bar is 150 $\mu$m.

the triethoxysilyl groups with the hydroxyl groups on the glass surface, followed by coupling of the terminal amino groups with the succinimidyl ester of the fluorescent dye (Fig. 1).

The fluorescence detection system was based on a Spex FluoroMax-3 (Jobin–Yvon). Light was coupled into a 600 $\mu$m core fiber (Ocean Optics) through a dichroic mirror with a 475 nm cutoff wavelength (Chroma) and a collimating SMA connector (Thorlabs). One end of the fiber was mechanically stripped from the polymeric coating over 15 mm and lowered into 48% hydrofluoric acid at a speed of 22 mm/h; the cladding was etched away and a tapered tip of 10 mm length and 2° taper angle was formed by slow immersion. The tapered region was also the area of surface immobilization. Tapering of the fiber increases the light-capturing ability,[15] and the 600 $\mu$m core diameter was chosen to provide sufficient immobilization surface. With this arrangement, excitation light was sent down the fiber to the tip. Any light collected by the fiber tip was sent back into the fluorophotometer, separated from the excitation light by the dichroic mirror and reflected into the detector channel. Successful immobilization of CCVJ and FRS on the fiber tip was tested with epifluorescent microscopy and spectroscopy. Figure 2 shows microscope images (Olympus IX-70 with a 10× objective) of a fiber with immobilized CCVJ compared with a control fiber without dye. Quantitative intensity measurements revealed 20% higher green emission that can be attributed to the CCVJ dye. The emission spectra of the immobilized dyes exhibit a relatively complex pattern of peaks as shown in Fig. 3. The main emission peaks of the dyes, CCVJ and FRS, are clearly recognizable at 505 and 530 nm, respectively (Fig. 3). Furthermore, Fig. 3 shows that the emission intensity of immobilized CCVJ increased in fluids of higher viscosity, a change not observed with FRS.

The viscosity sensitivity of the immobilized molecular rotors was tested by immersing the tips in ethylene glycol–methanol mixtures with final concentrations of 0%, 5%, 10%, 15%, and 20% methanol in ethylene glycol (Table 1). The lowest solvent viscosity was chosen to approximate blood viscosity (5–6 mPa s). For each solvent, the fluorescence emission spectra were acquired using the fluorophotometer with a custom fiber attachment in one of two modes: internal illumination, where excitation light ($\lambda_{ex}$=440 nm) generated by the fluorophotometer was sent down the fiber, and external illumination, where a 440 nm, 3 mW laser (CrystaLaser) was used in conjunction with a 10× beam expander (Thorlabs) to illuminate the tip directly.

Spectrum scans were acquired at a 5 mm slit width from 475 nm (the cutoff wavelength of the dichroic mirror) to 600 nm. One scan took about 2 min. Before each scan, the tip was cleaned with ethanol and air dried. In each spectrum, the maximum intensity $I_M$ was recorded and related to the solvent viscosity through Eq. (2), which governs the intensity–viscosity relationship of molecular rotors under the assumption that emission intensity is linearly related to the fluorophore quantum yield.[16,17] An indicator of sensitivity is the exponent $x$ in Eq. (2), which is close to 0.6 with molecular rotors in free solution. In Eq. (2), $C'$ is a constant reflecting background light, rotor density, and temperature:

$$I_M = C' \eta^x. \quad (2)$$

Examination of the viscosity sensitivity of the fiber-bound dyes under internal illumination is shown in Fig. 4. In methanol–ethylene glycol mixtures and when using the fluorophotometer to provide excitation light, immobilized CCVJ showed a clear increase of peak intensity with increasing viscosity with an exponent of $x$=0.23 (correlation coefficient $R^2$=0.89). The intensity–viscosity relationship was markedly weaker for FRS ($x$=0.04, $R^2$=0.54) as shown in Fig. 4. The recording of viscosity by the fiber-bound dyes under external illumination is shown in Fig. 5. The viscosity–intensity relationship became markedly stronger when the external laser illumination for

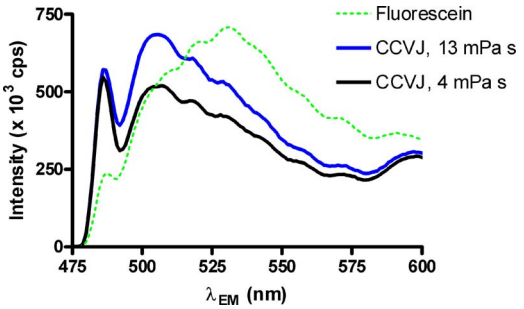


Fig. 3. (Color online) Emission spectra of immobilized CCVJ (**5a**) and FRS (**5b**) when excited through the fiber at 440 nm. CCVJ emission intensity increases in fluids of higher viscosity.

**Table 1. List of the Test Fluids (Mixtures of Methanol and Ethylene Glycol) with Their Respective Physical Properties**

| Methanol (Vol.%) | Viscosity (mPa s) | Refractive Index |
|---|---|---|
| 0 | 13.35 | 1.429 |
| 5 | 11.39 | 1.424 |
| 10 | 9.71 | 1.419 |
| 15 | 8.28 | 1.414 |
| 20 | 7.06 | 1.409 |

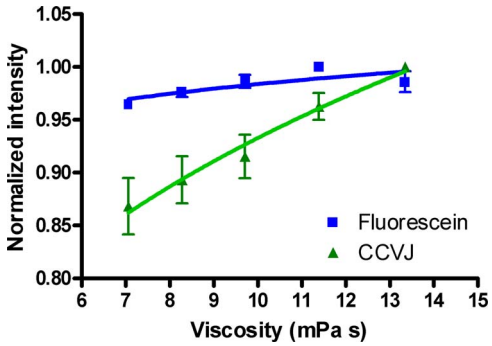


Fig. 4. (Color online) Viscosity-intensity relationship of immobilized CCVJ (**5a**) and FRS (**5b**) under internal illumination of the tip. The error bars indicate standard deviation for $n=4$ independent experiments.

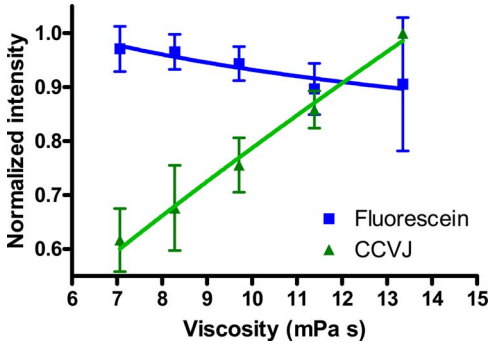


Fig. 5. (Color online) Viscosity–intensity relationship of fiber immobilized CCVJ (**5a**) and FRS (**5b**) under external laser illumination of the tip. The error bars indicate a standard deviation for $n=4$ independent experiments.

CCVJ ($x=0.78$, $R^2=0.9$) was used. There is a negative relationship between intensity and viscosity in FRS ($x=-0.13$, $R^2=0.19$); see Fig. 5.

To interpret the above results, one needs to take into consideration changes both in fluid viscosity and refractive index. A higher methanol content is associated with a decrease in both viscosity and refractive index (Table 1). Thus, the refractive-index mismatch between fiber and fluid decreases with increasing viscosity (decreasing methanol content). This mismatch has different consequences depending on whether internal or external excitation light is being used. Excitation light that arrives from within the tip has a higher probability of total internal reflection at higher refractive-index mismatch, reducing the amount of excitation light that reaches the fluorophore. Consequently, FRS peak intensity increases with decreasing refractive-index mismatch (Fig. 4). On the other hand, the tip acts as a conical lens for the external excitation light. As the refractive-index mismatch increases, more light is refracted within the tip, and more light for fluorophore excitation is available. In this case, therefore, FRS intensity decreases with decreasing refractive-index mismatch, as can be seen through the negative FRS slope in Fig. 5. Since changes of rotor emission intensity are relatively small, the impact of small refractive-index changes becomes visible. In addition, the fiber cladding (the polymeric layer surrounding the core) exhibits autofluorescence in the same wavelength range as the dyes. When intense blue excitation light travels down the fiber, additional green background light is created, which is superimposed over the dye emission. Since the background emission does not change with viscosity, the relative viscosity-related change is reduced. This leads to relatively low values of $x$ (Fig. 4) and explains the discrepancy between internal and external illumination.

In conclusion, we have demonstrated that covalent attachment of a molecular rotor to the surface of an optical fiber produces a fiber-based fluorescent viscosity sensor. The immobilized molecular rotors retain their viscosity-dependent fluorescent quantum yield, making their behavior similar to that observed for solubilized rotors, with similar sensitivity. Moreover, the fiber can accurately transmit the emitted fluorescence under both internal and external illumination with excitation light. This behavior gives rise to a new generation of solid-state fluorescence-based viscosity sensors with potential applications in remote sensing and for the measurements of viscosity without sample contamination.

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