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CONF-980117--**Ultrasensitive Detection of Cell Lysing in an Microfabricated Semiconductor Laser Cavity**

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RECEIVED**MAR 09 1998****OSTI****ABSTRACT**

In this paper we report investigations of semiconductor laser microcavities for use in detecting changes of human blood cells during lysing. By studying the spectra before and during mixing of blood fluids with de-ionized water, we are able to quantify the cell shape and concentration of hemoglobin in real time during the dynamical process of lysing. We find that the spectra can detect subtle changes that are orders of magnitude smaller than can be observed by standard optical microscopy. Such sensitivity in observing cell structural changes has implications for measuring cell fragility, monitoring apoptotic events in real time, development of photosensitizers for photodynamic therapy, and in-vitro cell micromanipulation techniques.

1. INTRODUCTION

Microfabricated electro-optical-mechanical systems¹⁻⁵ are expected to play an important role in future biomedical technologies. Semiconductor photonic materials and devices are attractive components of such systems because of their ability to generate, transmit, modulate, and detect light.⁶ In this paper we report investigations of semiconductor laser microcavities for use in detecting subtle changes of human blood cells during lysing. In particular, we are able to quantify the concentration of hemoglobin in real time during the dynamical process of lysing.

The method of intracavity laser spectroscopy has been previously described in detail.⁷⁻¹¹ The technique involves forming a laser microcavity between two reflecting surfaces. Fluids comprising cells or molecular species can be injected into the intracavity space to modify the resonant frequencies of the laser cavity. Light emitted from the cavity can be detected with a spectral analyzer to measure the resulting frequency changes. The technique provides coherent light images as well as spectra and has several critical advantages over conventional cell analysis methods,¹²⁻¹³ including higher speed, lower cost, higher sensitivity, and microelectronic compatibility. A schematic of the laser and apparatus to operate and read its output is shown in Fig. 1.

In previous experiments, the laser has shown potential to probe the human immune system⁷ (caliper cell and nucleus dimensions of lymphocytes), characterize genetic abnormalities⁸ (quantify sickled and normal erythrocyte shapes), and distinguish normal and cancerous cells in tumors.¹⁰ With further developed microfluidics, it may be useful for pharmaceutical research of high-speed drug testing of living cells, finding rare cells in large populations, characterizing apoptosis in real time, or development of dyes for photodynamic therapy.¹⁴

A technical challenge in realizing these goals is the development of methods to inject, mix, and remove fluids from the intracavity region. It is the purpose of this paper to present results of the first experiments on intracavity fluid injection and mixing and application to lysing of red blood cells. The experiments demonstrate the requisite features of a useful microfluidic device for cell research. Namely, that cells can be injected into the microcavity, that a separate fluid can be injected to modify the cells, that fluid mixing occurs, and that the cell structural changes can be sensitively observed by recording the cavity emission spectrum as a function of time.

2. MICROFABRICATION

The micro device has been described previously.¹⁵ Briefly, one surface is the top of a semiconductor wafer comprising a laser gain region atop a multilayer reflector. The other surface is a dielectric multilayer atop a glass substrate. The glass surface is micromachined to define surfaces or channels for transporting fluids. In the first case, a wicking microbridge is formed by machining two slots into the glass surface and contacting it to a cleaved semiconductor rectangle as shown in Fig. 1. The dimensions of the rectangle are about 5 mm and are less than the glass slots. Thus, the glass surface between the slots is exposed outside the contact area of the semiconductor. The optical contact between the glass and semiconductor

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surface leaves a small air gap that can be controlled by pressure from 0 to several micrometers. The gap is set by bonding the wafer along its perimeter to the glass surface. The gap dimension of the resulting assembly is then assessed by viewing the bridge region through the glass substrate.

3. MICROFLUIDICS IN A CAVITY

3.1 Microcapillarity

When a droplet of liquid is applied to the air gap in the microbridge, the fluid front is wicked into the cavity with an effective velocity

$$v \approx (L/4h) (\tau/\eta) \cos\alpha \quad (1)$$

where L is the cavity thickness, η is the viscosity, τ is the surface tension, and α is the contact angle, and h is the fluid penetration into the cavity. The prefactor is a penetration aspect ratio of the cavity and has an average value of 10^{-3} . The natural velocity $\tau/\eta = 7 \times 10^3$ cm/s and $\cos\alpha$ is 0.1 to 0.9. For 1 cm penetration, Eqn 1 predicts velocities the order of several cm/s and independent of the bridge width w . Experimental measurements were in agreement with this prediction. The observed velocity of water at 3 mm (halfway across the bridge) into a dry cavity was near 2 mm/s and was independent of bridge widths of 200, 700, and 1000 microns. The velocity increased with cavity thickness and decreased with penetration as predicted by Eqn 1.¹⁵

If the droplet is applied to a liquid-filled microbridge, the motion is more complicated to explain. The entering liquid can wet the solid surfaces if it has a greater affinity than the existing liquid. However, our experiments with microspheres show that the liquid preferentially wets the existing liquid on the edges of the bridge where the velocity is not restricted by the small cavity spacing. The liquid can then move laterally into the cavity by microcapillary, diffusive or convective forces.

3.2 Interfluid diffusion

With purely diffusional motion, the molecules will penetrate the cavity with a length $L = \sqrt{Dt}$ where D is the diffusion coefficient and t is time. Thus the diffusion front will exhibit a square root dependence on time. To estimate the magnitude of diffusion, we can scale the well-known diffusion coefficient of oxygen in water by using the molecular weight M of the diffusing molecule in the equation $D = 2 \times 10^{-5} \text{ cm}^2/\text{s} \sqrt{32/M}$. According to this result, in 10 seconds the diffusion lengths of methanol, ethanol, propanol, and rhodamine molecules are 140, 130, 120, and 35 μm , respectively.

These estimated diffusion rates of travel (~ 0.01 mm/s) are much slower than the microcapillary velocity of water into a dry cavity (2mm/s) or most of the observed velocities of fluids injected into the bridge described below near (0.1mm/s). Thus, we conclude that convection, not diffusion, is the primary means of mixing the injected fluid with water on the microbridge.

3.3 Microfluidic injection experiments

Experiments were performed by prewetting the microbridge with water and injecting a Rhodamine dye predissolved in water, soapy water, ethanol, methanol, and propanol. The movement of the dye molecules was followed by imaging the microbridge with a laser scanning confocal microscope. Images of the fluorescing dye in ethanol injected into the water are shown in Fig. 2 at several times between $t=0$ and 53 seconds. The dye molecules move from the left where they are injected to the right as they are drawn across the bridge by capillary and diffusive action. The cup shape of the dye's leading edge suggests that the injected fluid moves initially along the bridge edges and then move laterally into the cavity. Similar images were recorded with the other solvents. The results are summarized by plotting the position of the dye's leading edge against time in Fig. 3. These data show that the velocity is nearly constant across the bridge, and that it varies by almost two orders of magnitude for the different solvents. Parameters for the various solvents are listed in Table I.

Table I. Solvent Parameters

Solvent	η (centipoise)	τ (dyne/cm ²)	τ/η (cm/s)	vapor press RT (torr)
water	1.00	72	7200	19.0
ethanol	1.18	22	1800	60.5
propanol	2.15	24	1100	19.4

We assume that the solvated dye molecule moves with the solvent as it mixes with the water on the microbridge. From the natural velocity listed in Table I, it is apparent that water should advance more quickly than ethanol into the bridge. Further, soap/water should enhance the speed by improving the wetting of the surfaces. All of these trends are observed in the data of Fig. 3. However, it is very surprising that the propanol solution moves as quickly as observed. This is probably due to an enhanced wetting of the surfaces by propanol.

4. RED BLOOD CELL STRUCTURE AND OSMOTIC LYSIS

4.1 Red blood cell structure

The normal red blood cell is a biconcave disk about 7 μm in diameter and 2 μm thick. The plasma membrane is a trilaminar layer comprising hydrophilic outer (glycolipid/glycoprotein) and inner (protein) regions and a central hydrophobic layer (protein, cholesterol, and phospholipid). This semipermeable lipid bilayer membrane is highly elastic and is supported by a cytoskeleton mesh and intracellular filaments for integrity. The membrane pliability is critical not only for cell survival as it travels through the microvasculature, but also for its function of oxygen delivery. Decreased cellular deformability and attendant shape changes have been recognized as distinguishing features of a number of congenital or hereditary hemolytic anemias leading to decreased cell survival. Many factors can contribute to loss in deformability, including loss of Adenine Triphosphate (ATP), accumulation of calcium or cholesterol.¹⁶

4.2 Osmotic lysis of red blood cells

An important feature of red blood cells is osmosis and membrane permeability, the ability of membranes to selectively transport fluids. The cells are freely permeable to water and anions like Cl^- and HCO_3^- but nearly impermeable to cations like Na^+ and K^+ , so the cell maintain volumes and homeostasis. Integral membrane pumps driven by ATP transport Na^+ out and K^+ into the cell, respectively.

If red blood cells are placed in an isotonic (therefore isosmotic) solution, they will be in osmotic equilibrium and the cells will neither swell and burst (lyse) nor shrink (crenate). However if the cell membrane is permeable to the solute and initially there is a decreasing concentration gradient from outside to inside the cell, solute will diffuse into the cell and add to the solute already present there. Eventually the osmotic potential of the cytosol will be greater than the osmotic potential outside and the bathing solution will have become hypotonic. The natural consequence is for osmosis to occur, for water to diffuse into the cell, producing lysis. In lysing red blood cells, the biconcave shape changes to spherical and the membrane ruptures. The time it takes for lysis to occur is an indirect function of permeation rate. The physical rupture of the bilipid membrane under tension has been analyzed theoretically and found to be a thermally activated process.^{17,18} The measurements here are taken at room temperature and would underestimate the actual lysis at 37 C in the body.

In the following sections we present a novel method for measuring the influx of water into red blood cells and subsequent rupture by using intracavity laser spectroscopy. The technique uses the decrease in cell refractive index due to dilution of hemoglobin in the cell. Microscopic observation of cells under a coverslip requires tens of seconds to reveal structural changes due to lysis. Using the microcavity, refractive changes due to the influx of water can be observed within tens of milliseconds.

The lysis of red blood cell by osmosis, or osmotic fragility, is frequently used to characterize the integrity of the cell

membrane.¹⁶ These tests are performed to detect a variety of detrimental red blood cell conditions such as hereditary spherocytosis and thalassemia. Hereditary spherocytosis is a relatively common disorder characterized by red blood cells which are intrinsically defective because of their sphere-like shape. These cells have increased osmotic fragility. Red blood cells from people with thalassemia show some cells with increased osmotic fragility, but a larger fraction with decreased fragility. Another test is used to confirm the diagnosis of PNH (paroxysmal nocturnal hemoglobinuria). Definitive diagnosis of PNH depends on a having a positive acidified serum test (Ham test). In acidified serum, complement is activated by the alternate pathway; it binds to red blood cells, and lyses the abnormal PNH cells which are unusually susceptible to complement.

5. MICROCAVITY SPECTRA AND RELATION TO CELL STRUCTURE

Cells inside the microcavity serve as optical waveguides to confine light generated in the resonator by the semiconductor. The waveguiding effect is due to slight differences in the dielectric constants between various cell components and the surrounding fluids. The laser operates at resonant frequencies established by the dielectric properties of the cells. By using a high resolution spectrometer, these lasing frequencies can be resolved into narrow spectral peaks. The spacing and intensity distribution between peaks provides a unique spectral signature for each different cell as shown in Fig. 4 for red blood cells.

5.1 Longitudinal optical modes in microcavity

When no cell is present, the cavity resonance frequencies will be established by the cavity length L and the dielectric constant of the fluid in the cavity. The longitudinal mode wavelengths are

$$\lambda_l = \xi / (\ell - \phi / 2\pi) \quad (2)$$

where ℓ is the longitudinal mode index and ϕ is the sum of mirror phases and $\xi = 2\sum \ell_i n_i$ is the roundtrip optical pathlength in the cavity comprising a sum of lengths ℓ_i of index n_i , (typically a fluid region and a semiconductor gain region). The longitudinal mode separation or free spectral range is given by

$$\Delta\lambda_l / \lambda_l = -(\lambda_l / \xi) [1 + \lambda_l \xi' / \xi + \lambda_l^2 \phi' / 2\pi\xi] \quad (3)$$

where primes denote derivatives with respect to wavelength. For a fluid of index n_1 , Eqn 2 can be rewritten as the bare-fluid mode wavelength $\lambda_l = 2L^* n_1 / \ell$, where L^* is an effective length of the cavity.

5.2 Transverse optical modes in the microcavity

To first order, a cell in the cavity can be modeled by a disk of index n_2 surrounded by a fluid of index n_1 . This problem is similar to the solution of optical modes in a dielectric waveguide¹⁹ and has been discussed previously.⁸ In this case, the cell will perturb each bare-fluid mode of Eq (1) by adding a series of modes at longer wavelengths λ_{lmn} where the indices ℓ , m , and n correspond to the axial, radial, and azimuthal solutions to the wave equation for light confined by the cell in the cavity.

The fundamental transverse mode has the longest wavelength separation from the bare-fluid mode λ_l of the cavity and is given to good approximation by

$$\Delta\lambda_{lmn} = \Delta\lambda_l - \lambda_l^3 \times_{00}^2 / 2\pi^2 n_2^2 d^2 \quad (4)$$

where d is the cell diameter and the first term arises from the dielectric shift $\Delta\lambda_l = \Delta\xi / (\ell - \phi / 2\pi)$, where $\xi = 2\sum \ell_i n_i$, due to the change in index from the fluid (blood plasma) to that of the cell. The second term arises from the lateral confinement of light by the cell, and its calculated value for red blood cells is 1.8 nm. The calculated value for platelets is about 9 nm. Experimentally it is observed that $\Delta\lambda_{lmn} \approx 20$ nm for red blood cells, so the primary cause of the change is the index shift.

The solutions for the fundamental and higher order transverse modes are solutions to the wave equation and are given by the characteristic equations

$$X J_{m\pm 1}(X)/J_m(X) = \pm Y K_{m\pm 1}(Y)/K_m(Y) \quad (5)$$

and

$$X^2 + Y^2 = V^2 \quad (6)$$

where J and K are Bessel and modified Bessel functions, respectively, and $X = kd$ and $Y = \gamma d$ are wavevector parameters of the cell and surrounding regions, respectively. The optic parameter $V = (2\pi a/\lambda_1)(n_2^2 - n_1^2)^{1/2}$ where a is the cell radius and the later factor is the numerical aperture of the cell.

Each mode corresponds to an eigenfrequency of the resonator. The free space spectral wavelengths are given by

$$\lambda = 2\pi n_1 / [(X_{mn}/d)^2 + (\ell\pi/L^*)^2]^{1/2} \quad (7)$$

where X_{mn} is the n^{th} root of Eqn 5. The set of roots includes the fundamental mode of Eqn 4 which is derived from Eqn 7 in the limit $(\ell\pi/L^*) \gg X_{mn}/d$, and shorter wavelength modes up to a limit set by $X_{mn} \leq V$. At this cutoff condition, Eqn 7 becomes

$$\lambda_{\min} = 2\pi n_1 / [(2\pi/\lambda_1)^2 (n_2^2 - n_1^2) + (\ell\pi/L^*)^2]^{1/2} \quad (8)$$

but $\ell\pi/L^* = 2\pi n_1 / \lambda_1$, so that $\lambda_{\min} = \lambda_1$.

The total number of modes, including 2 polarizations and 2 helicities, is given by $M_t \approx 4V^2/\pi^2 + 2$. For typical red blood cells with 7 μm diameter (in typical experiment with whole blood diluted in 50:50 in isotonic solution) index $n_2 = 1.390$ and $n_1 = 1.335$, at 850 nm, $V = 10$, so about 40 total modes are present. Experimentally, it is found that modes of different polarization and helicity are nearly or exactly degenerate, respectively. Thus, the number of distinctly observable modes is $M_{ob} \approx V^2/\pi^2 \approx 10$ for red blood cells. For blood platelets of 3 μm diameter and $n_2 = 1.37$, $V \approx 3.4$ and $M_{ob} \approx 1$. For white blood cells of diameter 12 μm and $n_2 = 1.37$, $V \approx 14$ and $M_{ob} \approx 20$. Experimentally we find observable modes of 7-9, 1-2, and 15-30 for red, platelet, and white cells, respectively. Fig. 4 shows about 8 modes for a red blood cell. Thus, the disk model is reasonably accurate for describing the transverse modes in cells.

4.3 Determination of intracellular hemoglobin concentration using microcavity spectroscopy

A single cell has about 10^9 hemoglobin molecules. The protein hemoglobin constitutes 95% of the red blood cells dry weight. In normal hydrated cells, the concentration of hemoglobin is in the range 32 to 36 g/dL. In anemic individuals these level dip to half these values or even less in severe cases. In the microcavity, the waveguiding property of the cell is a function of the refractive indices of the cell and the surrounding fluid. The former is primarily determined by the hemoglobin concentration and the later is that of blood plasma (basically water with some proteins and electrolytes). Barer²⁰ has shown a linear relationship between the index and the molecular concentration as

$$n = n_0 + \alpha C \quad (9)$$

where n_0 is the index of the solvent, α the specific refractive increment of the molecule, and C the concentration in grams per 100 ml. Literature values²⁰ for hemoglobin are 0.00193 at 586nm. Thus, a normal red blood cell concentration of 34 g/dL gives an index of $n_0 + 0.0656$. The values of n_0 for blood plasma and water at 850 nm are 1.342 and 1.330, respectively.

From Eqn. 9 we can determine the mode spectral shift $\Delta\lambda_m$ of a microcavity loaded with a red blood cell as $\Delta\lambda_m/\lambda_m = \Delta n_1/(n_1 + \ell_2 n_2/\ell_1)$ where $\Delta n_1 = \alpha C$ is the change in cell index, and the term in brackets is a spatial weighting of the optical intensity due to the relative thickness of the cell ℓ_1 and an internal cavity length ℓ_2 of index n_2 . Typically $\ell_1 = 2.5 \mu\text{m}$, $\ell_2 = 1.4 \mu\text{m}$, $n_2 = 3.1$, so $\Delta\lambda_m/\lambda_m = 0.021$ and $\Delta\lambda_m = 18 \text{ nm}$. This is near the value of 20 nm typically observed for normal red blood cells in experiments. Fig. 4 shows a shift of 19.3 nm. Alternately, the hemoglobin concentration can be determined from the mode shift as $C = \Delta n_1/\alpha$ in g/dL.

6. SPECTRA AND IMAGES OF RED BLOOD CELL LYSING

6.1 Experimental conditions

Whole blood samples were diluted with phosphate buffered salt solution and fed into the microbridge laser cavity by a micropipette. The blood fluid wicked into the microcavity and a significant fraction of the cells became attached to the surfaces of the glass or semiconductor. In this stationary condition, de-ionized water was wicked into the microbridge using a fused silica microcapillary described above. The rate of water transfer into the cavity was characterized in separate dye injection experiments described earlier in this paper. Typical times for water capillarity/diffusion across the 5 mm bridge were about 50 seconds depending on the conditions of the microcavity surfaces.

6.2 Images

The cells were observed to lyse after the injection of water. Sequential images of the lysing cells were recorded by both video microscopy and laser scanning confocal microscopy. In the video images, the cell boundaries appear as dark rings around a light background. Within about 20 seconds, the cell boundaries lose contrast and the cells disappear. The confocal microscope images shown in Fig. 5 also show this loss of contrast, but only after the image intensity has reversed from bright to dark. Apparently the laser scanning image is sensitive to the phase of the reflection due to the shorter wavelength and monochromaticity of the probing source. Changes from the initial state of high contrast to the final state of near invisibility occur in about 20 seconds as in the video image. The start of the lysing process was observed to vary from seconds to several minutes after water injection, depending on the location of the cell relative to the injection point.

6.3 Intracavity spectra

Typical spectra for red blood cells lysing in the cavity are displayed in Fig. 6. Initially, the spectrum is static and comprises a peak due to the bare-fluid mode and series of cell modes at longer wavelengths. As described previously, the cell mode spectrum exhibits 3 basic features, each related to the cell structure and composition. First, the displacement of the longest wavelength mode from the fluid mode represents the index difference between the fluid (diluted blood plasma) the cell (principally a hemoglobin solution). This difference increases with hemoglobin concentration. Second, the relative spacing of the modes is representative of the size of the cell. Large mode spacings corresponds to small cell diameter. Finally, the envelope of modal intensities is representative of the cell shape. Cells with high biconcavity have spectra with significant intensity in the first and higher overtones. Spherical cells have spectra with dominant fundamental modal intensity.

Under lysing, the spectra undergo dramatic changes that last for 10 to 20 seconds. There are four salient features that implore description and explanation. Initially, the right-most peak shifts continuously to shorter wavelengths. The shifting peak is not a mode hopping to shorter wavelengths. As the peak moves, there is no residual intensity at wavelengths longer than that of the peak. The shift represents a true displacement of a low order mode (either the fundamental or the first overtone). Secondly, the intensity of that peak increases two- or three-fold during the shift. Thirdly, during the shift the shorter wavelength peaks appear to remain stationary with little change in position or intensity. Finally, the shift continues until that peak merges with the bare fluid mode.

The peak shift is plotted against time in Fig. 7. The initial shift of the peak is 1 nm/s for the first several seconds, then more rapidly 3 nm/s to bring it to within a few nanometers of the bare-fluid mode. After this, the final merging requires more time to complete. In most cells the merging is complete in 5 to 10 seconds, leaving only a single bare-fluid mode in the spectra. Visual inspection of the cell under this condition reveals only a faint, nearly invisible image of the original cell with little change in diameter. The resolution of peak shift is about 0.01 nm. At initial times, this corresponds to 10 ms time resolution. Thus, the microcavity spectra are several orders of magnitude more sensitive to cell structure changes than standard optical microscopy.

6.4 Interpretation of cell lysing spectra

A simple interpretation of the above observations is as follows. The shift of the low order mode arises from the decrease in refractive index of the cell. This can be explained by a dilution of the cell fluids (primarily hemoglobin) by the uptake of water. This is consistent with the fact that the number of modes decreases with time. This can happen only if the cell

radius or refractive index decreases. Neither do the higher order spectral modes spacings increase nor do recorded images show changes in the cell diameter. The cell diameter does not decrease. Thus, the refractive index of the cell is decreasing. Before lysing, the cell has a biconcave structure and exhibits a dominant first overtone mode in the spectra. During water uptake, the cell becomes more spherical and the fundamental mode dominates and begins shifting. Eventually, the whole cell is diluted and the fundamental and higher overtones all collapse into the bare fluid mode.

There is no evidence in the spectra or the images to support a diametrical swelling of the cell. If this were the case, the spectral mode spacing would decrease and there would be an initial red shift of the major peak (or at least a slowing of the blueshift).

The increase in mode intensity with time may result from two effects. The first is simply that a small absorption by hemoglobin occurs at the pump laser wavelength of 532 nm. Thus as the hemoglobin concentration decreases, the pump more effectively transmits to the semiconductor. Alternately, the pump spot may be larger than the cell and more optimally match the increasing modal area as the cell index decreases.

It is important to consider the effect of laser power on the cell structure. The incident power was always kept quite low (about 1 milliwatt) to avoid heating in the cells. In fact, cells not exposed to water exhibited spectra that remained constant in time. The absence of any significant mode shifts or intensity changes indicates that the heating effects are small. Under condition of very high pump powers the merging of the cell and bare fluid modes is never completed. A sharp peak, near but distinct from the fluid mode is evident. Visual inspection under this condition reveals a markedly different image. The cell image retains high contrast but is dramatically reduced in diameter. The diameter of these "dwarf cells" are two to three times smaller than the original. The formation of these shrunken cells with intact membranes is likely due to heating.

7. CONCLUSIONS

We have presented a novel method for injecting and mixing fluids in a microcavity for the purpose of modifying its output spectrum. The method uses microcapillarity to wick fluids into a few micron cavity space already filled with another fluid. The rate of fluid injection was studied by using fluorescent dyes in various solvents of different viscosity and wetting ability. The rates of injection were on the time scale of seconds, convenient for experimental data acquisition. We used this technique of microfluidic injection to expose normal red blood cells in isotonic solution to water for the purpose of studying osmotic lysing. We found the microcavity emission spectra to be very sensitive to the subtle changes in cell shape and size and hemoglobin concentration. This method is quantitative much more sensitive than microscope imaging. The technique requires only a single cell and is therefore useful when blood samples are minute. The the method may be useful for determination of various anemic conditions of the red blood cells, namely the hemoglobin concentration and osmotic fragility of the membrane.

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Fig. 1. (Upper left figure) schematic of the microbridge formed with a semiconductor laser wafer on top and a microchannelled dielectric mirror on the bottom. A microcapillary tube (not shown) is used to inject fluid onto the bridge in the microcavity space between the semiconductor and dielectric surfaces. (Right hand figure) schematic of the optical system for reading information from the microcavity. L-lens, BS-beamsplitter, LD-laser diode, PD-photodiode (or photodiode spectrometer), BL-biological microcavity laser.

Fig. 2. Confocal laser scanning images of fluorescing Rhodamine 6G dye in ethanol injected onto the water-wetted microbridge at times 0, 12, 19, 28, 34, 41, 45, and 53 seconds after injection. The scale bar is shown at the bottom. The width of the bridge is about 900 μm .

Fig. 3. Position of the front of the injected fluorescing dye as a function of time after injection for several dye solvents. The locus of points determines a line with slope yielding the velocity of the fluid front.

Fig. 4. Static spectrum of the microcavity emission containing only blood plasma (lower trace) and a red blood cell in plasma (upper trace) at room temperature. The dominant peak at 848 nm is shifted 19.3 nm from the bare fluid mode at 829 nm. This shift is linearly proportional to the hemoglobin concentration in the cell.

Fig. 5. Confocal laser scanning images of 488 nm light reflected from a red blood cell undergoing osmotic lysis in water. The images are recorded at times 0, 7, 23, and 40 seconds after the injection of the water.

Fig. 7. Wavelength shift and corresponding hemoglobin concentration as a function of time during the osmotic lysing of a red blood cell on the microbridge.

Fig. 6. Dynamic emission spectra of the microcavity with a red blood cell in the microbridge after injection of water. The time after injection is indicated to the right of each spectrum.

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