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Final Report on LDRD Project: Semiconductor Surface-Emitting Microcavity Laser Spectroscopy for Analysis of Biological Cells and Microstructures

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Abstract

This article discusses a new intracavity laser technique that uses living or fixed cells as an integral part of the laser. The cells are placed on a GaAs based semiconductor wafer comprising one half of a vertical cavity surface-emitting laser. After placement, the cells are covered with a dielectric mirror to close the laser cavity. When photo-pumped with an external laser, this hybrid laser emits coherent light images and spectra that depend sensitively on the cell size, shape, and dielectric properties. The light spectra can be used to identify different cell types and distinguish normal and abnormal cells. The laser can be used to study single cells in real time as a cell-biology lab-on-a-chip, or to study large populations of cells by scanning the pump laser at high speed. The laser is well-suited to be integrated with other micro-optical or micro-fluidic components to lead to micro-optical-mechanical systems for analysis of fluids, particulates, and biological cells.

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Semiconductor Surface-Emitting Microcavity Laser Spectroscopy for Analysis of Biological Cells and Microstructures

I. Introduction

In the early 1980s we succeeded in producing high quality quantum wells and mirrors using strained-layer epitaxy.[1] In 1986 we achieved one of the first demonstrations of surface-emitting laser action in a photopumped all-epitaxial high-Q resonator with quantum wells and nonabsorbing mirrors.[2] A cross section of such a laser is revealed in the micrograph of Fig. 1. It comprises three basic sections: upper and lower mirrors surrounding a quantum well active region. We have continued to push semiconductor microtechnology forward with external structuring of the laser mirrors and cavity to yield desired properties. An example is etching of vertical cavity surface emitting lasers into mesa structures.[3] Structuring within the cavity has also been used to enhance laser performance. The quantum wells in vertical cavity surface emitting lasers have been engineered such that gain regions coincide with field anti-nodes to enhance lasing efficiency.[4] Passive intracavity regions which do not provide gain may also be structured. Honeycomb lattices have been added to assist in the confinement of light lateral to the wafer plane.[5]

In parallel with our work in the basic technology of semiconductor surface emitting lasers, we have discovered and investigated a revolutionary new way this technology can be used to characterize the properties of biological cells as well as other micron sized structures. The method employs semiconductor surface emitting microcavity lasers with a single biological cell or other microstructure acting as an internal component of the laser. Until our investigations, the ease of introducing fluids or small objects into a laser microcavity by extending it with glass optics had been overlooked. A cell actually aids the light-generating process, so the emitted laser beam is impressed with information about the cell. This new "biological microcavity laser," or "biocavity laser," so named because the cell is an integral part of the laser resonator, provides the basis for new biomedical analyses of cell structure. This includes both living and fixed cells from humans, animals, or plants. And, the technique doesn't require the customary chemical staining procedure to render a cell's structure visible. Further, the cells can be connected in tissues, so long as the tissue is thinned to monolayer dimensions to fit within the laser cavity. Thus, this intracavity laser technique has potential uses for a novel kind of microelectronic cytometry and histopathology based on coherent light spectra and images of cells and intracellular structures. It could advance cell microbiology, immunology, DNA fragment sizing, and genetic sequencing as well as provide a new clinical technique for early diagnosis of disease. Beyond these applications it

may also have uses for high speed analyses of liquids, gases, and particulates for environmental monitoring or for ultra sensitive detection of molecules.

So far, we have studied simple cells as well as dielectric microstructures to determine the ability of the laser to detect their basic properties, including abnormalities in the case of cells. In preliminary experiments, the laser has shown potential to probe the human immune system (caliper cell and nucleus dimensions of lymphocytes), characterize genetic disorders (quantify sickled and normal red blood cell shapes), distinguish cancerous and normal cells from tumors, and determine the size of small (few micron) dielectric particles. The results of these studies indicate that the laser technique holds great promise for ultrahigh speed analysis of large populations of cells and micron size objects, accurate characterization of cell size and shape, and sensitive probing of subtle changes in cell structure and chemistry.

Semiconductor technology has many desirable attributes for cell and microstructure analysis. Semiconductor devices can be fabricated with nanometer to millimeter dimensions, comparable to the sizes of cells and their components. Semiconductor microlasers, diffraction optics, and optical detectors can be integrated onto a single wafer. The result is a microlaser analysis lab on a chip that is sensitive, very fast, and compatible with parallel processing for high throughput. This report summarizes the results of our research over the past three years on microcavity surface emitting laser techniques for analysis and characterization of biological cells and micro-opto-mechanical systems

II. Optical and Waveguide Properties of Cells

The biomedical use of lasers in studying cells has expanded in recent years. Gas lasers are routinely used for sorting cell types,[6] characterizing cell physical properties,[7] and studying cell biochemistry by laser scanning confocal microscopy.[8] Further, lasers are used to micromanipulate cells and organelles by optical trapping.[9,10] Microsurgery at the cellular level can also be performed with short wavelength lasers.[11] Such laser applications take advantage of specific light-cell interactions such as light scattering, light induced fluorescence, refractance, or absorbance to extract cell information or modify the state of the cell. But in all these cases, the cells are located outside the laser resonator and the laser light is weakly confined in the cells, since they serve only as passive scatterers or absorbers.

In contrast, the new intracavity laser technique confines intense laser light inside the cell. The cells serve as optical waveguides (or lens elements) to confine (or focus) light generated by a semiconductor laser in a Fabry-Perot resonator. The waveguiding effect is due to slight differences in the dieletric constants between various cell components and surrounding fluids. These

variations arise from different protein or DNA/protein concentrations in the cytoplasm, nucleus or organelles.

Cells are remarkably transparent within the "therapeutic window" of the optical spectrum from 700 to 1000 nm. Most commercially important semiconductors, like gallium arsenide, emit in the middle of the cell transparency window, between 800 and 900 nm. Thus, laser emission from semiconductors can probe cells without altering or damaging them due to absorption. Because of their high transparency and refractive index difference from that of surrounding fluids, cells actually aid and trigger laser action in a microcavity. The laser light corresponds to electromagnetic modes defined by the cell structure and depends sensitively on the size and shape of the cells. The result is lasing images of cells that arise because the laser operates at resonant frequencies established by their dielectric properties. A high resolution spectrometer can resolve these lasing frequencies into narrow spectral peaks whose spacing and intensity distribution provide unique spectral signatures for different cells.

Red blood cells provide a good illustration of optical properties, such as geometry, refractive index, absorption and surface roughness, that are important to how cells behave as waveguides in microcavities. Optical micrographs of blood smears with normal and abnormal (sickled) cells are shown in Fig. 2. The normal red blood cell has a toroidal shape defined by a cytoskeletal network of spectrin.[12-14] The toroid diameter is about 7 μm and its thickness about 2 μm. The cell's refractive index is determined primarily by its internal hemoglobin/spectrin complex, whose index as a function of wavelength is shown in Fig. 3a. The refractive indices of water, blool plasma and nucleic acid are also shown. The cell index is about 2% higher than that of the surrounding blood plasma.[15,16] with the refractive index of hemoglobin ~ 1.40 compared to ~ 1.35 for blood plasma over a wide range of wavelengths.[16] Surprisingly, the refractive indices of hemoglobin and spectrin are almost perfectly matched.[16] The optical absorption of the cell is also determined by its hemoglobin and water constituents. The absorption coefficient is low at optical wavelengths from 700 to 1000 nm (see Fig. 3b) and less than 10^{-2} cm^{-1} near 850 nm.[17] The surface roughness of red blood cells is small (less than 50 nm) and typical of other cells. Thus, biological cells do not appreciably scatter semiconductor laser emission.

To help illustrate the waveguiding of light by cells, we have recorded ordinary near infrared transmittance photomicrographs of unstained red blood cells dried in air. The photomicrograph shown in Fig. 2b reveals that cells are highly transparent at these wavelengths. Also, the effects of light confinement can be seen. The image shows a bright ring defined by the refractive index contrast between the red blood cell (high index) and

surrounding air (low index). The same optical focusing effects, though with less contrast, are present in cells in their hydrated physiologic state.

The micrographs suggest that light is focused by the cell's convex region with high refractive index near it's circumference, and is diffused by its central concave region. This is as expected from the theory of geometrical optics for a heterogeneous medium, according to which light rays bend toward the regions of higher refractive index.[18] Thus, the entire hemoglobin-filled cell, with 2% higher index than the surrounding plasma, acts as a lens with variable focal length across its diameter. This focusing, which has the net effect of concentrating light to high index regions, is evident with only a single pass of light through the cell, as shown in Fig. 2b. Overall, red blood cells are nearly perfect optical elements (lenses) with little optical scatter (surface or internal) or optical loss.

III. Microcavity Basics

The microcavity comprises a dichroic multilayer dielectric coated glass mirror and an AlGaAs/GaAs surface-emitting semiconductor laser wafer consisting of a multilayer mirror and quantum well gain regions grown epitaxially onto a gallium arsenide substrate,[19] as indicated in Fig. 4. This semiconductor gain/mirror combination takes the convenient form of a monolithic wafer compatible with standard microelectronic lithography. The gain regions provide photons and amplification for lasing in an optical mode supported by a cell or microstructure. Micron size objects or living or fixed cells are sandwiched between the surface-emitting wafer/mirror and the dielectric mirror such that the two mirrors form a Fabry-Perot resonator, as indicated in Fig. 4. The figure also indicates how the lateral confinement of light in a cell is greatly amplified when it is located inside the Fabry-Perot resonator (two mirrors face-to-face). Light can undergo multiple paths through the cell as it reflects from the two external mirrors. Slight differences in refractive index between cell components and the surrounding media produce an effective waveguide to confine light. Light rays (arrows) are shown for paths through convex surfaces of a representative red blood cell. These light rays tend to fold onto themselves to define stable optical modes. We have utilized a ray tracing analysis[20] to verify the existence of stable modes. Light rays (leftmost arrows) are also shown passing through a concave surface of the red blood cell. These rays diverge and do not form stable optical modes.

A separate pump laser (not shown in Fig. 4) photopumps the semiconductor gain region to produce electron-hole pairs in the quantum wells.[19] After a short delay of a few picoseconds, electrons and holes spontaneously recombine to emit light near 850 nm. If the pump beam is sufficiently intense, the pair density exceeds the transparency density near

$1x10^{12}$ cm^{-2} and optical gain is developed. In this case, a spontaneously emitted photon can transit the cell, reflect from a mirror, and reenter the gain region where it can stimulate the recombination of another photon. The process is repeated until the light intensity is amplified many orders of magnitude on a time scale of picoseconds. Light passing through a cell has a higher probability of being amplified because of the waveguiding effect which helps concentrate the light. Some of the highly intensified light in the cavity leaks through the mirrors. The result is an intense, coherent light signal emitted from a region localized at the cell.

The semiconductor can be photopumped with several different laser sources ranging from continuous wave to nanosecond, picosecond and femtosecond pulsed lasers. The short pulse sources produce the highest electron-hole pair density and gain in the semiconductor. As the gain increases in magnitude, so does its spectral width. The number of lasing modes described below will increase with the width of spectral gain. The low gain case (cw laser pumping) is effective for production of carrier densities of 1 to $2x10^{12}$ cm^{-2} and spectrally narrow gain that permits only one lasing mode to oscillate. Alternatively, femtosecond pulses produce high gain conditions (carrier densities of 3 to $5x10^{12}$ cm^{-2}) where the gain is spectrally broad and permits many lasing modes to oscillate simultaneously. The low gain case produces very simple spectra, but provides information by identifying the dominant lasing mode. The more complicated multimode spectrum of the high gain case provides information about the object's size and shape.

The microcavity surface-emitting laser can be operated with or without a cell present. However, the pump power required to achieve lasing threshold in the bare cavity is much higher than with a cell-filled cavity. With a pump spot 50 to 100 microns in diameter (about 10 times the cell diameter), the cell can support a lasing mode while the larger background spot remains below lasing threshold. The background spontaneous emission is weak, exhibiting a dim, featureless image and a broad spectrum (several nanometers). In contrast, intense stimulated emission from the cell appears as a distinct transverse electromagnetic mode. This light has a narrow spectral peak (sub-Ångström) and is highly polarized. These differences between the spontaneous and stimulated light create a high contrast, coherent light image of the cell.

IV. Prototype Microcavity Laser Cytometer

We constructed a prototype biological microcavity laser cytometer that employs the vertical cavity surface-emitting semiconductor for analysis of biological cells or other microstructures. This cytometer allows the lasing modes confined by cells and micron size objects to be studied in several ways. They can either be resolved into narrow spectral peaks, recorded as high-

contrast coherent light images, or detected as time-dependent pulses. We have used these spectra, images, and time resolved data to characterize cell and dielectric microsphere structure, cell morphology, and cell abnormalities. The cytometer, operating in two basic configurations, either probes individual cells and microstructures by spectral analysis of the confined microcavity modes. or rapidly probes large numbers of cells and micron size structures by means of high speed scanning and pulse height spectroscopy.

A schematic of the prototype cytometer is shown in Fig. 5. The pump laser beam is directed through a pair of beam steering mirrors, through a dichroic beam splitter, and through an objective lens that focuses it to the microcavity laser resonator. Light emitted normal to the microcavity mirrors is directed by a beamsplitter and lens to a detector system. In the configuration for analysis of single cells or microstructures, the beam steering mirrors are manually adjusted to move the focused pump laser spot to various locations in the microcavity resonator. Either a solid state camera or a spectrometer/diode array detector combination is used, allowing both images and spectra of the emitted light to be obtained. In the configuration for rapid analysis of large numbers of cells or micron size objects, the beam steering mirrors are in a high speed x-y scanning mode. The output light in this case is collected with a silicon avalanche photodiode to resolve laser pulses generated when the pump beam scans over single or multiple cells or structures in the cavity. In this configuration, output pulses can be collected by a pulse height analyzer for subsequent study.

V. Spectroscopy of Single Cells and Dielectric Microspheres

We have used the first configuration to study spontaneous emission spectra and laser mode spectra of individual cells and dielectric spheres. These cells include red and white blood cells, and normal and malignant cells from tissues. We find that these cells have unique spectral signatures that can be used to identify them and determine their size and shape. Using the spectral separation of transverse modes, we have developed a method for sizing cells and microspheres. The validity of this method is supported by theoretical models that can predict the frequencies of optical modes of Fabry-Perot resonators loaded by differently shaped objects.

V.1 Normal Red Blood Cells

The three dimensional structure and optical properties of a cell or other micron-sized object will govern the waveguide modes it exhibits. Normal red blood cells again provide a good example. As explained above, they have a characteristic toroidal structure about 7 μm in diameter and 2 μm thick. Thus, they serve as a cylindrical type of waveguide in the cavity. In the simplest analysis of cylindrical optical modes in the biocavity, we find the

spacing of a lateral-mode wavelength from a given longitudinal wavelength to be given by[20]

$$\Delta\lambda_{mn} = -(\lambda^3 x^2_{mn}) / (2\pi^2 n_1^2 d^2), \quad (1)$$

where λ_{mn} is the lasing mode wavelength, x_{mn} is the nth zero of an mth order Bessel function, d is the cell diameter, and n_1 is a refractive index. Using this result, we computed a spectrum (vertical lines) of lateral modes for a 7.5 μm diameter cell of refractive index 1.4, with light of wavelength 850 nm, as shown in Fig. 6a. Each mode is represented by a vertical line positioned on the wavelength axis. Above the line, the mode indices, mn, are given. Also shown are experimentally observed lasing images corresponding to the six lowest order modes in normal red blood cells.

The lasing images of normal human red blood cells shown superposed on the computed spectral lines in Fig. 6a demonstrate that the cells support transverse electromagnetic (TEM) modes similar to those observed in other cylindrical laser resonators described in textbooks.[20] These images are recorded with either a cw or nanosecond pulsed pump laser. With these pump sources, the spectral gain is minimal and only one transverse mode is typically observed. Each image corresponds to a single transverse eigenmode of the laser resonator. The frequency of occurrence of a given mode depends on the distribution of cell size and shape. An arbitrarily shaped cell can be decomposed into a unique mixture of mutually exclusive modes. Hence, each cell has a unique spectral signature that cannot be confused with other cells. This situation is analogous to identification of musical instruments based on their unique sounds comprising mixtures of acoustical overtones.

The most frequently occurring mode for normal red blood cells is the TEM_{10} mode. The subscripts, mn, denote the number of angular and radial nodes (dark areas), respectively. The TEM_{10} mode, with its familiar double-lobed intensity distribution, is characteristic of about 50 % of normal red blood cells in plasma in the physiologic state. Another common mode (not shown in Fig. 6a), occurring in about 10 % of the cells, is observed as a ring. This is the transverse electromagnetic mode designated TEM_{10}^*. The star superscript denotes a linear combination of horizontal and vertical versions of the TEM_{10} mode. Both of these modes exhibit nodes (dark areas) near the cell center and are consistent with the toroidal shape of the cells observed under brightfield microscopy (see the light rays in Fig. 4). The fundamental TEM_{00} mode occurs in about 15 % of the cells. These are associated with cells that have lost some of their biconcavity. The higher order modes, TEM_{20} (cloverleaf), TEM_{30} (hexagonal), TEM_{01} (dot and ring) and TEM_{02} (split dot and ring), are observed much less frequently (each accounting for a few percent of

the distribution). These modes occur in the largest cells where higher order modes are stable.

The laser can also be operated with femtosecond pumping. In this case the gain is spectrally very broad and many transverse modes can simultaneously support lasing. The observed distribution of spectral intensities of the modes will depend on the relative gain and loss for each mode. The relative loss will be determined by the geometry and optical diffraction of the cell. Also, the spectral separation of the modes will decrease with cell diameter as predicted by Eq. 1. Different cells have a unique spectral distribution of transverse modes that can be used to caliper the cell dimensions on an ultrashort time scale.

Images (insets) and spectra of three normal red blood cells with femtosecond pumping are shown in Fig. 6b. The three cells correspond to different diameters ranging from 5 to 7.5 μm. In each case, the image and spectrum comprise many different transverse modes operating simultaneously. The spectra are displayed on a log scale (spanning 3 orders of magnitude from background to highest peak) to enhance weaker modes. The 5.0 μm diameter cell's spectrum in the lower photo exhibits a dominant TEM_{00} mode and a less intense TEM_{10} mode. Consistent with this distribution, the inset image appears as a Gaussian disk. This smaller cell has little biconcavity and favors the fundamental mode. The modes exhibit a wide 5.5 nm spectral separation due to the small cell size. The 5.7 μm diameter cell's spectrum in the center photo exhibits a dominant TEM_{10} mode, a less intense TEM_{00} mode, and a very weak TEM_{20} mode. The dominant mode is evident in the double-lobed image. This cell has more biconcavity to favor the first overtone. The peaks in this spectrum have a smaller 4.6 nm separation due to the larger cell size.

The uppermost photo shows an image and spectrum of a larger 7.5 μm diameter cell. The spectrum reveals 7 operating modes. The dominant mode is TEM_{10} and is identified by the lasing image near threshold (not shown). The higher order modes decrease almost monotonically to shorter wavelength. The separation of these modes is small (typically 1 to 2 nm) due the larger cell size. The spacings of the lowest order modes are in general agreement with computed line spacings in Fig. 6a. Note in particular the close spacing of the TEM_{20} and TEM_{01} modes (2 large peaks nearest the dominant peak). The spacings of the three highest order modes are much less than those predicted by the simple theoretical model of Eq. 1. This is expected since the optical field intensity for these modes leaks outside the cell boundaries as discussed above.

V.2 Sickled Red Blood Cells

We also tested the biocavity laser for its sensitivity to abnormal blood cells. We selected sickled red blood cells since they correspond to one of the best understood molecular diseases.[21] In this disease, the hemoglobin molecule (HbS) has one amino acid substitution in the β globulin chain which causes the HbS protein to partially crystallize within the cell. With solid and liquid phases present, the cell acquires the characteristic shape of a sickle.

A theoretical spectrum was computed and is displayed in Fig. 7a. In this calculation, the sickled cell is assumed to take a long, thin shape in contrast to the earlier circular cell analysis. Computed transverse modes are shown in the spectrum of Fig. 7a for a 9 μm by 4 μm cell. Superposed on the spectral lines are schematic images of the six lowest order transverse modes. The modes are labelled by indices representing the number of antinodes (one more than the number of nodes) along the short and long axes, respectively.

The experimental images and spectra for three sickled cells of different lengths are shown in Fig. 7b. All of the lasing images reveal the linear geometry of the sickled cells which have long and short axes. Each image exhibits nodes along the length of the sickled cells. These images resemble the lower set of images drawn in the theoretical spectrum of Fig. 7a. The number of nodes increases from 2 for the 8.2 μm long cell to 3 for the 9.0 μm long cell to 7 for the 11.6 μm long cell. The images suggest that the lowest order modes (TEM_{11} or TEM_{21} with 1 or 0 nodes) are not present. The spectra in Fig. 7b confirm this suggestion. All of the images have 2 or more nodes.

In the lower spectrum of Fig. 7b, for the 8.2 μm long cell, the longest wavelength peaks on the right side are broad and weak. These peaks correspond to the TEM_{11} and TEM_{21} modes and do not support lasing. The dominant peak TEM_{31}, third from the right, corresponds to the observed image with 2 nodes. Two other lasing peaks occur at shorter wavelengths. The peaks are widely and equally separated by about 2 nm.

The central spectrum of Fig. 7b for the large 9.0 μm long cell comprises 3 broad, weak peaks at long wavelengths and 3 strong peaks at shorter wavelengths. These peaks correspond to the TEM_{11}, TEM_{21}, and TEM_{31} modes which are not supporting lasing or marginally supporting lasing. The dominant peak is the TEM_{41} mode, fourth from the right, and corresponds to the image with 3 nodes. The peaks are more closely but equally spaced by about 1.7 nm.

In the uppermost spectrum of Fig. 7b, for the 11.6 μm long cell, many closely spaced peaks are evident. This spectrum is more complicated than the previous two. The peaks are not uniformly spaced and the distribution of lasing modes is less well defined. One peak at the extreme left dominates the spectrum. The postion in the spectrum identifies it as the TEM_{71} mode. This mode has 6 nodes and is consistent with the image superposed on this spectrum. Two weaker lasing modes are evident in the central part of the spectrum. The other broad peaks are modes that do not support lasing.

The images and spectra of sickled cells are quite different from those for normal red blood cells. In normal cells, the fundamental and first overtones support lasing. In the sickled cells, only higher overtones support lasing. No lasing fundamental mode was observed in any sickled cell. This is consistent with the linear structure of the sickled cell. The bright antinodes in images are almost always bright circular or oval spots. The ratio of the horizontal and vertical dimensions of these spots does not vary by more than a factor of ~2. The fundamental oblong mode is quite unstable and linear spots are not observed because of the large anisotropy of the optical diffraction arising from long and short cell axes. Thus, long objects tend to break up into segmented antinodes.

We also found that the lasing threshold pump power for sickled cells was higher by 50% than that for normal cells. This is probably a consequence of the larger dimension of the sickled cell. A circular pump spot is more effective in pumping a circular cell area. Another explanation is that the sickled cells are expected to be optically turbid entities that absorb or scatter light to lower the quality of the lasing resonance. Light scattering is expected from phase boundaries of the crystalline/liquid hemoglobin in the sickled cell. Increased absorption may be due to the crystalline phase of hemoglobin.

V.3 Nucleated Cells

Nucleated cells such as yeast and white blood cells are, in contrast to red blood cells, larger and include a nucleus comprising concentrated DNA/protein complexes. The nuclear complex has a higher refractive index than the surrounding cytoplasm. In turn, the cytoplasm contained by the cell has a higher refractive index than the blood plasma. As a consequence of these three different indices, white blood cells, for example, have two distinct types of optical modes that are illustrated in Fig. 8a. One type, referred to as the nuclear mode, has light confined within the nucleus. These nuclear modes are similar to those in the red blood cell, including the fundamental TEM_{00}, first overtone TEM_{10} and higher order overtones.

Another distinct set of modes arises from light confined inside the cell but outside the nucleus. These modes have the light skimming around the periphery of the cell inside the cytoplasm. They are referred to as whispering

gallery modes, an analogy to sound waves in Saint Paul's Cathedral first described by Lord Rayleigh. These modes exhibit zero intensity in the nucleus and a spoke-wheel-like distribution of antinodes along the cell boundary. They correspond to high order Bessel functions such as TEM_{06} and TEM_{07}, as illustrated in Fig. 8b.

Fig. 9 shows images from a nucleated white blood cell (polymorphonuclear leukocyte). Note the large nucleus and complex internal structure indicated by image (a), formed by light microscopy. In image (d), which is at low power below threshold for lasing, the periphery of the cell is rendered bright compared to the cytoplasm and external plasma. At higher power, amplified spontaneous light is confined and the nucleus begins to support lasing. In image (b), the focused pump laser beam is placed concentrically while, in image (c), the laser focal point is closer to the periphery of the cell. The spectra corresponding to these images are also shown. At low power, the spontaneous emission has a very broad peak. At high power, the spectrum narrows to form very sharp peaks, characteristic of the lasing process, and is many orders of magnitude more intense than spontaneous emission as seen in image (d).

V.4 Dielectric Microspheres

We used the microcavity laser to study the mode spectra of polystyrene microspheres. These dielectric spheres have index of refraction n=1.59, are readily available in well-controlled sizes, and are highly transparent (transmittance > 94%) in the 850 nm lasing region. We studied spheres with nominal diameters of 4 μm, 6 μm, 10 μm, and 22 μm (determined by the manufacturer, Polysciences) immersed in de-ionized water. The natural resonances of microspheres[22] as well as lasing from dye-doped microspheres[22-24] have been of interest for some time. As far as we know, this is the first time the effects of microspheres within a planar Fabry-Perot resonator have been investigated.

The basic behavior of the laser for 3 sphere sizes under cw pumping is shown by the light-light curves in Fig. 10a. All three sphere sizes show a sharp threshold at low power densities. This is followed by a fairly high efficiency region (~5%) in which a single transverse mode oscillates. The curves then roll-off as multiple transverse modes begin oscillating. Thus, there are three distinct regions of interest: threshold, single mode operation, and multimode operation. The curves also show that in multimode operation, the total output power increases approximately as the square of the diameter, i.e. in proportion to the semiconductor area under the sphere.

Fig. 10b shows a plot of threshold cw pump power vs. sphere size. For all sphere sizes, the cw pump spot diameter was ~ 20 μm. The curve exhibits

a minimum for spheres somewhere near 6 μm in diameter. For sphere sizes smaller than this, the rapid threshold power increase is due to diffraction losses. For larger sphere sizes, the threshold power increase with diameter is slow, in contrast to the total power output in Fig. 10a.

In the low gain regime, only one lateral mode lases. The observed modes under low gain conditions are summarized in Table I. For all sphere sizes, the TEM_{00} is the dominant mode. As size increases, the number of higher order modes increases. The 6 μm diameter spheres have nearly equal numbers of TEM_{00} and TEM_{10} modes and no higher order modes.

With a femtosecond pump laser, the gain is spectrally wide enough to support several lasing modes. As the pump power is increased from zero, the behavior parallels that of the cw pumping. The femtosecond pump at low power (gain) causes the same dominant mode to oscillate as the cw pumping. Above this power additional modes begin to appear. As these modes appear, the previously sharp spatial image rapidly becomes a bright, featureless spot due to the superposition of all the modes. Spectrally, a new peak rises as each new mode oscillates.

Fig. 11 shows the mode spectra for 6, 10, and 22 μm diameter spheres under high gain. All spectra are referenced to 845nm (zero wavelength shift), since the absolute mode wavelength varies slightly due to small variations in wafer layer thickness. In each spectrum, the rightmost peak corresponds to the fundamental TEM_{00} mode followed by the first overtone TEM_{10} mode at shorter wavelengths. At even shorter wavelengths, other higher spectral overtones are evident in the case of the 22 μm diameter sphere. Note the rapid decrease in mode separation with increasing sphere diameter. As the separation decreases, more lateral modes can fit within the gain spectrum.

V.5 Sizing of Cells and Dielectric Microspheres by Laser Mode Spectroscopy

We studied the relationship between the microcavity laser mode spectra and the size and shape of cells and microspheres. The cells included red and white blood cells as well as normal and malignant cells from tissues. We found that the unique spectral signature of a cell or microsphere can indeed be used not only to identify it but also to determine its size and shape. Using the spectral separation of transverse modes, we developed a method for sizing both cells and microspheres.

Eq. 1 above expresses the spacing of a lateral-mode wavelength from a given longitudinal wavelength according to the analysis of cylindrical optical modes in a microcavity[20]. Such cylindrical waveguiding is characteristic of a structure that is basically 2-dimensional; that is, whose length and width are comparable and considerably larger than the height. For 3-dimensional

structures, the situation is different. In the case of spherical objects placed within a Fabry-Perot cavity, for example, the optical modes will contain symmetries of both the longitudinal cavity and the sphere. Taking this into account, we have shown. in a derivation analogous to that for cylindrical modes,[21] that the separation between the TEM_{00} and TEM_{10} modes for a sphere in a microcavity is

$$\Delta\lambda = 4\xi n (x_{10} - x_{00}) (L/p)^2 / \pi d, \qquad (2)$$

where ξ is a geometrical factor averaged over the spherical volume and has a value ≤ 1, n is the refractive index of the sphere, x_{ln} is the nth zero of the lth Hankel function, L is the effective cavity length, p is the longitudinal mode index, and d is the sphere diameter. This approximation is valid when the index difference between the sphere and surrounding fluid is sufficiently large ($\Delta n/n \approx 0.2$ for polystyrene spheres in water). Eq. (2) shows an inverse dependence on the sphere diameter since $L/p \approx \lambda/2n$ is approximately constant and fixed by the bandgap wavelength λ. In contrast, the cylindrical mode separation of Eq. (1) shows an inverse square dependence on the diameter of the cylindrical waveguide structure.

We recorded intracavity lasing spectra of red and white blood cells, platelets, yeast cells and dielectric spheres. The laser was operated below the lasing threshold, allowing us to examine the spontaneous spectra. In this case, all of the transverse modes of a cell or sphere loaded cavity can be observed simultaneously. Since the spontaneous emission is relatively weak, a dominant longitudinal mode of the unloaded cavity is also present in the spectra. This provides a convenient reference line for measuring the wavelength shifts of the transverse cell and microsphere modes. These shifts allow a precise determination of the refractive indices of the cells and spheres relative to the fluid surrounding them. The indices of the fluids are easily measured by refractometry. Thus, the absolute values of the cell and microsphere indices can be accurately determined by this method. Further, the spectral separations of the transverse modes can be used to quantify cell and microsphere sizes.

A spectrum for the cavity loaded with a 6 micron diameter polystyrene sphere in water is shown in Fig. 12a. Spectra for red and white blood cells in whole blood plasma diluted with saline are shown in Fig. 12b. Also shown in these figures are reference spectra recorded under bare cavity conditions, where fluid is present, but no cell or sphere. The reference spectra differ due to changes in the fluid, cavity length, and wafers used. The spectral changes from the bare cavity to the cell-loaded cavity are evident as additional modal peaks near 850 nm. The number of modes, their spacings, intensity distribution, and red shifts from the bare cavity mode are distinctive for the sphere and each cell type. The average spacings of transverse modes (open

points) are plotted against cell diameters on a log-log scale in Fig. 13. These data are well described by the simple 2-dimensional mode theory according to Eq. 1. The 2-diminsional mode theory is indicated by the solid line labeled B/d^2 where B is a constant and d is the cell diameter. This dependence is in contrast with the mode spacings observed for polystyrene spheres and also plotted in Fig. 13. In those data (solid points), the modes are well described by the 3-dimensional mode spacing according to Eq. 2 and indicated by the solid line labeled A/d. These data indicate that the spectra are sensitive to 3-dimensional cell/sphere shape as well as size.

V.6 Cluster Plot Technique for Cell Identification

We used these results to develop a novel spectral method for identifying cells. Fig. 14 shows a cluster plot derived from three spectral parameters; namely, mode shift, mode spacing, and relative intensity of the spectral peaks. The plot shows data for four different cell types: red and white blood cells, platelets and spindle nuclei from a placental tumor. The cluster plot shows that the cells can be distinguished on the basis of spectral features alone, without need of images. This result has significant implications. Cell identification can be quantified by computer algorithms that process simple features of 1-dimensional spectra rather than complex, data-intensive 2-dimensional images. Consequently, higher cell identification rates can be achieved.

VI. Multicell Pulse Height Spectroscopy

Using the prototype microcavity laser cytometer in the rapid scanning mode, we acquired pulse height spectra of single and multiple cells and dielectric spheres under low and high field of view conditions, respectively. In this case, the biocavity was operated well above the lasing threshold with high output intensity. To interpret these data, we developed a simple model that relates scanning excitation of a single cell mode to the shape of a single cell pulse height spectrum. The experimental data are in qualitative agreement with this model, which also provides a basis to interpret pulse height distribution of populations of cells.

When the scanning pump laser spot passes over a cell in the resonator, as illustrated in Fig. 15, the cell acts as a dielectric waveguide, confining the pump light and lowering the laser threshold so that an intense pulse of laser light is emitted from the cavity. Since the cavity lifetime is the order of 10 picoseconds, the light pulse can be very short. Thus, many cells can be probed in a short period of time with high signal-to-noise light intensity. Using this principle, we have accumulated pulse height spectra of cell and dielectric sphere populations within a few seconds to minutes. Images and pulse height spectra for populations of polystyrene spheres and blood cells are

shown in Figs. 16 and 17, respectively. These spectra exhibit distinctive peaks that reflect the cell/sphere size and uniformity in the populations.

VI.1 Raster Scan and Cell Sampling

The raster scan, illustrated in Fig. 15, has horizontal and vertical frequencies, f_x and f_y, respectively. The horizontal speed of the laser spot is $v_x = f_x A_x$ where A_x is the horizontal scan amplitude in the object plane. Likewise the vertical speed is $v_y = f_y A_y$ where A_y is the vertical scan amplitude in the object plane. The vertical separation of adjacent scan lines in the same is $s = f_y A_y / f_x$. The separation s can be adjusted relative to the cell size D. If $s >> D$, the cell is unlikely to be sampled. If $s << D$, the cell is sampled many times and the cell structure can be measured. Few cells can, however, be studied since data collection for a large number of cells consumes more time. For simply counting the cells, a good choice is the Nyquist sampling condition $s \approx D$, where each cell is sampled at least twice.

VI.2 Laser Pulse Frequency

A pulsed laser excitation source is useful for pumping the laser cavity. The laser pulse repetition frequency f_o and pulse width t_p influence the horizontal sampling of the cell. The horizontal scan length during the laser pulse is $l = v_x t_p$ and the spacing between pulses $L = v_x/f_o$. To ensure satisfaction of the Nyquist condition in horizontal sampling, $L = D/2$. Combining x and y sampling conditions, we obtain a condition on the laser pulse repetition frequency $f_o = (f_x^2/f_y)(A_x/A_y)$. If the amplitude ratio is set to 5:4 for a standard TV monitor, and a 4kHz resonant scanner is used in 1000:1 frequency ratio, we obtain $f_o = 5$ MHz. Thus, high pulse repetition frequencies are required. In most of the experiments described here $f_0 = 4$MHz.

VI.3 Scan amplitude and Laser Spot Size

There is a compromise between the scanning field of view and the spot size. For small angles, the amplitude in the object plane is $A = F\theta$ where F is the objective focal length and θ is the angle of the scan subtended by the objective. The laser spot size is $\delta = F\lambda/d$ where λ is the wavelength of the laser light and d is the beam diameter. Thus, the field of view and the spot size scale together. Typical values for the sampling parameters discussed above are summarized in table I.

TABLE I. RASTER SCAN PARAMETERS

field/spot conditions	scanning parameters for D=10μm cell size, f_o=4 MHZ, t_p=12 ns						
F (mm)/δ(μm)	f_x (Hz)	A_x (mm)	v_x (m/s)	s (μm)	L(μm)	l(μm)	
	f_y (Hz)	A_y (mm)	v_y (mm/s)				
Small field 10/2	4000	0.25	1	0.2	0.25	0.012	
	4	0.20	0.8				
Large field 80/16	4000	5	20	4	5	0.24	
	4	4	16				

VI.4 Excitation of Optical Modes in the Biocavity

In a simple analysis, we have shown that the optimum excitation of the fundamental mode occurs when the spot size is about 70% of the cell diameter. This is in close agreement with experiments where the spot size was adjusted with a prefocus lens to achieve optimal output intensity. The spot size for optimal intensity is close to the line scan separation required for the Nyquist condition.

VI.5 Output Pulse Detection

The pulses emitted by the biocavity are detected by a silicon photodiode, either a fast pin structure or an avalanche type having response times of several ns. In the experiments presented here, the excitation laser pulse width was 12 ns. The biocavity response time is ~10 ps so it can easily follow the excitation pulse. Thus, the biocavity output pulse is 12 ns. The output pulses are passed through a pulse-shaping amplifier with time constant of 1-3 μs and into a 200 MHz Wilkinson-type multichannel analyzer with 512 channels. The conversion time for the analyzer is 2.5 μs per channel, slightly longer than the amplifier time constant. Thus, the upper limit on the cell detection rate is about 200 kHz. Note that the intrinsic sampling rate of the biocavity is about 2 orders of magnitude higher than the detector and about 5 orders of magnitude higher than the electronic converter. Even with faster electronics, the upper limit is the laser pulse frequency, in this case 4 Mhz.

Since the laser frequency is 4 MHz, the 12 ns output pulses are separated in time by 250 ns. The transit time of the laser spot across the cell varies from 0.5 to 5 μs, depending on the horizontal velocity and cell size. Thus, anywhere from N = 2 to 20 pulses per transit are obtained. The resulting pulse train is integrated according to the amplifier RC time constant, leaving only a single ~ 5 μs pulse per cell. The intensity of the integrated pulse S will be proportional to the number $N = Df_0/A_xf_x$ of excitation pulses during the transit. As a result, the pulse amplitude is proportional to the cell size.

In the pulse height spectra, the position of the peaks will be proportional to intensity, and the width of the peaks will be proportional to fluctuations in intensity. The fluctuations will depend on pump laser noise (usually less than 5%), mechanical vibrations in the optical hardware, and intracavity effects like cell motion and laser heating. Care must be taken to minimize all of these.

VI.6 Single Cell Pulse Height Spectroscopy

The pulses were accumulated in the multichannel analyzer to assess the distribution in pulse heights. The pulse height spectrum will be influenced by the variety of cell sizes and shapes. To understand how this is effected, we first consider the pulse height spectrum from a single cell sampled by a raster scan with horizontal line spacing s much less than the cell diameter. During successive line scans, the intersection of scan line and cell can range from near miss to direct hit. At different times, the line will be offset by R(t) from the cell center, and the pulse amplitude will, to first order, be $I \sim \exp(-\beta R^2(t))$. Thus, successive scans will give a pulse train with a Gaussian envelope. Within this train, the probability of a given pulse height h is $P(h) = K\int dt\delta(I(t)-h) = K/\left|dI/dt\right|_{I=h}$ where K is a constant. For Gaussian pulses, P(h) has cusps at h = 0 and $h = I_{max}$. Experimentally, these cusps will be rounded off by the finite resolution of the digital/analog converter. Consequently, the single cell spectrum will appear as a saddle-like function with peaks near h = 0 and $h = I_{max}$.

Experimental data for a sphere and a red blood cell are shown in Figs. 16a and 17a, respectively. The spectrum for the sphere corresponds closely to a saddle-shaped curve with cusp-like features near the minimum and maximum values of the pulse heights. This is consistent with the Gaussian shape of the pulse train envelope shown by the upper right inset oscilloscope trace in Fig. 16a. In contrast, the spectrum for a red blood cell in Fig. 17a reveals a double-peaked structure at high pulse intensity and a monotonically decreasing tail to lower intensities. The photo inset of Fig. 17a clarifies these differences: the pulse train envelope is far from Gaussian. In contrast to the

Gaussian envelope of Fig. 16a, the envelope of Fig. 17a is not symmetric, has more structure, and the tails are truncated. This pulse envelope is typical of the red blood cells. The cells are less symmetric than spheres and exhibit more internal structure. All of these differences are reflected in the pulse height spectra shown in Fig. 17a.

VI.7 Multicell/Multisphere Pulse Height Spectroscopy

If many randomly distributed, identical cells are sampled with a raster where $s \sim D$, the pulse height spectrum will be identical (aside from a multiplying factor) to the single cell spectrum. This is so because there is no correlation between the scan pattern and the relative position of the cells. All values of the offset R between the scan line and cell center are equally probable. If the cell positions are correlated with the scan pattern, the spectrum will be enhanced or suppressed at certain pulse heights. However, this situation is not likely to occur.

The most common case occurs when the cells are randomly distributed and are not identical. When a population of such cells is examined, the pulse output will vary from cell to cell. These variations are of interest for assessing the characteristics of the population. The overall shape, positions and peak modulation are indicative of the variations in the cell population. Here, we expect the pulse height spectrum to be a composite of distinct single cell spectra. The cells will vary in size, shape, and internal structure giving rise to distinctive saddle-like spectra for each different cell type. Larger cells and cells with low internal scattering, will have a cusp/peak at higher pulse heights. Small cells and cells with high lasing thresholds will produce a cusp/peak shifted to lower pulse heights. All of the cells will produce a cusp/peak near $h = 0$. Consequently, the composite spectrum will be very strongly peaked near $h = 0$ and will have other distinct peaks at higher pulse heights. The number of these peaks will depend on the number of distinct cell types that dominate the distribution. If there exists a continuous variation in the cell characteristics, we expect the distinct peaks to be smeared over a continuous range of pulse heights.

Experimental spectra for multicell and multisphere scans are recorded in Figs. 16b and 17b, respectively. These spectra represent pulse heights for about a hundred spheres or cells. The spectrum for the spheres exhibits about seven distinct peaks that have widths comparable to the single sphere spectrum of Fig. 16a. A dominant peak exists near the pulse height value of 3. Generally, the spectrum falls with increasing pulse height. Contrasting this spectrum, the red blood cell spectrum is nearly an exponentially decreasing function of pulse height with very slight peaks at selected pulse heights. These data indicate a much larger variation of cell size.

The spectra presented in Figs. 16 and 17 demonstrate that distinctive pulse height features for cells and spheres can be obtained simply by scanning a laser spot across the cell or sphere in the biocavity and integrating the output intensity over wavelength and cell transit time. The multicell and multisphere spectra are markedly different from their corresponding single cell or sphere spectra both in overall shape and detailed features. The observed differences in the single and multicell/sphere spectra are consistent with predictions by the simple analysis presented here.

VII. Technical Challenges

Ways of introducing cells or microparticles in a fluid environment into the Fabry-Perot resonator region are fundamental to microcavity laser cytometry. Many configurations, both with and without fluid flow, are possible. In all cases, methods for reliably transporting fluids on and off the semiconductor wafer must be developed. This includes buffer solutions and stimulants as well as the fluid media carrying the cells. All of this must occur without clogging the microplumbing leading to the analysis chamber.

Another hurdle is the development of sealing methods for dissimilar materials to prevent cross contamination from different microflow channels.

VII.1 Biocompatibility Issues

Biocompatibility of the glass and semiconductors is another issue that must be addressed. Biocompatibility refers to the influence of the biocavity materials on the cells and vice versa. For example, the materials must be robust to withstand enzymes that may attack the surfaces. We observed semiconductor surface degradation in the form of erosion or scratches after the cavity had been assembled many times and exposed to biological fluids. On the otherhand, some semiconductor materials may alter or cause damage to cells. In any case, semiconductor surfaces must be protected and/or passivated with inert materials that are benign to the cells and that do not adversely affect the operation of the microcavity laser.

In typical devices for optical analysis of cells, biological fluids are in contact with glass windows which may have anti-reflection dielectric coatings that consist of only a few coating layers. Such coatings, when deposited by electron beam evaporation methods, are actually fairly porous and hydroscopic, but this is not a problem when the coatings involve just a few layers. In microcavity laser cytometers, however, the dielectric mirrors are multilayer because they must have specific dichroic transmission and reflection properties, and these multilayer coatings are in direct contact with biological fluids. The porous and hydroscopic nature of such coatings cannot be overlooked. We observed that such dielectric mirror coatings, after repeated exposure to blood samples (plasma fluid and cells), become stained,

indicating they have absorbed biological fluids. This could have an adverse effect on the performance of these dichroic mirrors. Dielectric coatings deposited by ion beam evaporation with ion beam assistance are much denser and less likely to absorb biological fluids, and may be required for practical versions of microcavity laser cytometers.

We investigated a class of biocompatibility issues; namely, the effects of semiconductor surfaces on cell growth. Several semiconductor substrates (Si, GaAs, GaSb, GaP, InAs, InSb, and InP) were placed in a solution containing rat lung epithelial cells. After incubation for approximately 24 hours at a temperature of 37 C, the samples were dried and the resulting cell growth observed. Both confocal microscopy as well as scanning electron microscopy techniques were used to image the cells on the semiconductor. Most of the samples demonstrated healthy cell proliferation, as shown in Fig. 18a. However, the compound semiconductors containing Ga produced severely inhibited cell growth. Few cells survived and those that did, like the one shown in Fig. 18b, were greatly deformed. We were able to passivate the Ga based semiconductor surfaces by plasma coating them with silicon nitride. Cell growth on the silicon nitride coated Ga based semiconductor surfaces was normal. On the otherhand, the silicon nitride coating caused the lasing threshold to increase by 10 - 20 %, indicating it introduced some optical scatter.

VII.2 Toward Detection of a Small Number of Molecules

As explained in Section III, the cell's waveguide properties, and corresponding laser mode spectrum, are functions of the differences in index of refraction between the cell and surrounding fluid as well as by the cell's size and shape. The cell's index of refraction is in turn determined by its molecular constituents. Take, as an example, a red blood cell. It contains about 10^9 hemoglobin molecules. (Here, we assume a cell of 7 μm diameter and 2 μm thickness, and near close packing of hemoglobin molecules of about 4 nm diameter). This large number of hemoglobin molecules gives the cell its index of refraction of 1.4 as compared to the 1.35 index of surrounding blood plasma. The corresponding shift of the laser mode spectrum under low gain conditions is 20 nm. If hemoglobin molecules were removed from the red blood cell and replaced with plasma, eventually the cell would acquire the index of pure plasma, and there would be no mode shift.

Barer[25] has shown the validity of a linear relationship between the index of refraction of a liquid and the concentration of molecules of a given type dissolved in the liquid; namely, $n = n_o + \alpha C$, where n_o is the index of the liquid without dissolved molecules, C is the concentration (in gr per 100 ml) of dissolved molecules, and α is a constant characteristic of the solute molecule. This relationship provides a basis for estimating the minimum

number of molecules of a given type in a cell that can be detected by means of mode shift spectroscopy.

The minimum relative change in index, $\Delta n/n_o$, that can be measured in terms of a spectral mode shift is $\Delta\lambda/\lambda_o$, where $\Delta\lambda$ is the resolution of the spectrometer and λ is the operating wavelength. Thus, the resolution of the spectrometer governs the minimum index change that is measurable. The number of molecules of a given type that would need to be added to a cell in order to cause its refractive index to change by this minimum amount is the minimum number detectable by spectral shifts. For example, in the case of 0.1 nm resolution at 850 nm, $\Delta\lambda/\lambda \sim \Delta n/n_o \sim 1.2 \times 10^{-4}$. For red blood cells containing only plasma, $n_o = 1.35$. As hemoglobin is added to a red blood cell, the minimum detectable index change will occur when $\Delta n = \alpha C = 1.6 \times 10^{-4}$. For hemoglobin, $\alpha \sim 0.0019$[25]. and the molecular weight is ~ 8000 atomic units. Thus, the minimum detectable number of hemoglobin molecules in a red blood cell would be about 5×10^6.

VIII. Advantages and Future Applications

With the technical hurdles overcome, a practical cytometer could operate in two basic configurations: either as a flow device or as static fluid device scanned by a probe laser. Individual cells could be examined in detail or large populations of cells could be rapidly studied by flow combined with laser-scanning. This later configuration may be useful for understanding of cellular and molecular events that occur during blood cell production. Quantitative measurements of blood cells and precursor stem cells would help assess the effectiveness of gene therapy or bone marrow transplants in the treatment of leukemia. Currently, there is no assay for human stem cells which account for 0.01% of bone marrow cells. The biocavity laser has the potential to probe 10^5-10^6 cells per second, making such an assay possible.

Conventional flow cytometers tend to large, expensive instruments requiring highly trained operators. The instruments rely on laser-induced fluorescence and light scattering to assess cell types and structural characteristics like cell size and granularity. The scattered light is distributed over an angular range that is inversely related to cell size. However, it is difficult to unambiguously assign specific internal cell structure to a given scattering pattern. A more-developed microelectronic cytometer may be relatively inexpensive to manufacture, easy to operate, and permit accurate assessment of cell structure.

Such technology may also aid the histopathologic examination of tumors. Conventional methods of sectioning, staining, and microscopic examination of abnormal cells rely on qualitative human vision and

interpretation of color images. These methods are operator dependent and subject to frequent misidentification. Improvements are brought by digital imaging techniques that rely on quantitative machine vision and sophisticated computation to characterize cells. However, 2-dimensional color images comprise large data sets that are time-consuming to process. The microelectronic laser technique is both quantitative and fast.

Considerable advantages over existing technologies are possible with this microcavity laser sensor. It can be operated at very high frequencies since the light is generated by a stimulated emission process. Photons generated by the semiconductor sample the cell hundreds of times as they bounce between opposing mirrors. This multiple sampling serves to amplify subtle refractive, absorptive or diffractive effects in the cell. The durations of the light pulses are limited only by the speed of light traversing the cavity. With newly developed mirror scanning and light detection methods, large populations of cells can be sampled in a short period of time.

Further, the stimulated emission occurs in a narrow beam that can be directed toward a small area, high speed detector. Thus, the light collection process is simple and efficient. And, the light signal is very intense providing high signal-to-noise ratio and enhanced signal sensitivity. The top dichroic mirror is reflective in the near infrared 850 nm, but transparent in the UV and visible. Thus, the cells can be probed with other light wavelengths, say from fluorescent tags,[17-20] while the cell is supporting lasing.

IX. Summary and Impact of the Research Project

We have examined the lasing modes and spectra of blood cells configured as a biological microcavity laser. Operation of this laser provides high contrast, coherent light images which are localized in the cell. The images correspond to lasing modes which are defined by the contrast in the refractive index of the cell relative to its surrounding fluid. Corresponding to these images are lasing spectra with very sharp lines sensitive to slight changes in the cell diameter and shape. We found that operation of the laser under low gain conditions produced simple spectra corresponding to only one lasing mode. Under high gain conditions, many lasing modes operated simultaneously. The spectral positions of these modes were sensitive to cell size and shape and not the pumping intenstiy. The spectrum of modes was very different for normal and sickled cells. The lowest order modes were preferred in normal circular cells while higher order modes were preferred by the linear structure of the sickled cell.

This biomedical laser sensor has the potential to rapidly quantify size and shape of different kinds of living human cells for early detection of disease. The laser device provides high contrast, coherent light images and spectra of cells and intracellular structures and has several critical advantages

over conventional cell analysis methods. We have demonstrated that the biocavity laser can caliper cell and nucleus dimensions of nucleated cells, quantify sickled and normal red blood cell shapes, and determine the size of small (few micron) dielectric particles. Beyond these applications this laser may also have uses for high speed analyses of liquids, gases, and particulates for environmental monitoring or for ultra sensitive detection of single molecules.

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