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Biocavity Lasers

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Laser technology has advanced dramatically and is an integral part of today's healthcare delivery system. Lasers are used in the laboratory analysis of human blood samples and serve as surgical tools that kill, burn or cut tissue. Recent semiconductor microtechnology has reduced the size of a laser to the size of a biological cell or even a virus particle. By integrating these ultra small lasers with biological systems, it is possible to create micro-electrical mechanical systems that may revolutionize health care delivery.

Introduction

Micro-electrical mechanical systems (MEMs) are rapidly finding new applications for chemical analysis, molecular detection, and health care.[1-4] One such device is known as the "biological microcavity laser" or biocavity laser[5-10] for short, and is the result of research conducted at Sandia National Laboratories in Albuquerque, NM. This new laser is part of a set of nanoscopic lasers that is emerging from the materials science/laser engineering communities.[7] These lasers confine intense light into an extremely small interaction volume. They have been shrunk to the astonishing scale of nanometers, even smaller than the wavelength of the light they produce. At such sizes, less that one hundredth the thickness of a human hair, curious aspects of quantum physics begin to take over. By exploiting this quantum behavior, researchers can tailor the basic characteristics of the devices to achieve even greater efficiencies and faster speeds.

Nanolasers have myriad future applications in optical computers, where light would replace electricity for transporting, processing and storing information, and in fiber-optic communications. Devices for the latter application are now in commercial production. Some of these devices have recently been applied to biomedical research. In preliminary experiments to assess cell structure, the laser has shown the ability to probe the human immune system by using optical size effects in the laser spectra to caliper cell and nucleus dimensions of lymphocytes.[5] Further, the laser has been used to characterize genetic disorders (quantify sickled and normal red blood cell shapes and hemoglobin content)[10] and distinguish cancerous from normal cells.[6]

Semiconductor microtechnology is rapidly expanding its role in fiber optic communications, illumination, and medicine. The technology is mostly centered around the material silicon which is well developed for electrical devices for switching, memory

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and light reception. Beyond these uses, silicon serves as an excellent material for microfabrication of microfluidic devices, micro-test tube arrays for pharmaceutical development and implantable biomedical devices.[1] All of these advances have been enabled by concurrent developments in materials science, microlithography, and material processing. Another class of compound semiconductors, derived from columns III and V of the periodic table is becoming important technologically for its ability to generate, transmit, modulate, and detect light. These compounds like GaAs, AlAs, InP, InGaN etc. have undergone extensive research and development in the last decade and are beginning to show remarkable potential for illumination, data transmission, and new biomedical applications such as photodynamic therapy,[12] optical tomography,[13] cell micromanipulation,[14,15] and laser cytometry.[16-18]

Using GaAs surface-emitting lasers,[8] a revolutionary new method for analyzing biological cells has been demonstrated. The method employs a semiconductor laser with a single human cell acting as an internal component of the laser. The cell actually aids the light-generating process, so the emitted laser beam is impressed with information about the cell. The new biological microcavity laser provides the basis for new biomedical analyses of cell structure. This includes both living and fixed cells from humans, animals and plants. The technique does not require the customary chemical staining procedure to render its structure visible. Further, the cells can be connected in tissues, as long as the tissue is a monolayer and able to fit within the laser cavity. Thus, the laser has potential uses for a novel form of microelectronic cytometry and histopathology.

Lasers and conventional application to biomedical diagnostics

A laser operates by exciting electrons in a gain medium to higher energy levels. When the electrons return to the lower energy state, they emit light. By confining the light in a reflective cavity, the light bounces back and forth in the media to trigger the return of other excited electrons to a lower level. A chain reaction can be set off to intensify the light in the cavity. Some of the light leaks from the cavity to form an intense external beam that can be used to irradiate tissue. In this case the laser functions as an intense light source with the tissue at some distance external to the laser cavity.

Laser and optical spectroscopy is rapidly expanding its use in biomedical diagnostic evaluation of cells and tissues.[19] Fluorescent imaging and spectroscopy is by far the most popular method of evaluating diseased tissues. Usually, dyes are introduced into the tissue and gross spectroscopic changes are sought for distinguishing normal and diseased tissue. These optical changes in tissue usually arise from microscopic optical changes in individual cells. The connection between tissue and cells can be better understood by studying individual cells. Optical properties of individual cells are usually studied by flow cytometry or microscopic imaging of individual cells or cells in culture. Detailed images of cells are frequently studied using fluorescent molecular probes to selectively label certain cell components. Of course, the labelling process for both cells and tissues consumes time and may alter the cell biochemistry or physiology.

In contrast to the extrinsic fluorescent methods described above, intrinsic fluorescence, reflection, absorption, Raman, and ultrafast spectroscopy are also under investigation.[20] In these techniques, subtle changes in the intrinsic optical properties of the tissues and cells are sought. Usually, the changes between normal and diseased tissue are very small and methods for high sensitivity must be developed.

Anatomy of the biological microcavity laser

The biocavity laser is based upon vertical-cavity surface emitting laser (VCSEL) technology. VCSELs are small lasers and have emerged as the ideal light source for fiber optics communications. The biocavity laser uses compound semiconductor materials that efficiently emit light (Fig. 1). Two surfaces enclose the cavity and a gain medium. One surface is the top of a AlGaAs/GaAs surface-emitting semiconductor wafer. This component acts to amplify light signals. The other surface is a dielectric mirror atop a glass substrate. This component serves to reflect light and leak a small portion out of the cavity. Between these mirrors is gap where a sample is placed. The sample may be whole blood, cells from culture, tissue from a biopsy or a scraping of body tissue (such as that obtained during a cervical cancer test). In contrast to conventional laser methods described above, the cells are internal to the laser cavity and become part of the lasing process. Inside the cavity, the cells is sampled hundreds of times as the light bounces between the mirrors. As a result, the laser ouput light is sensitively dependent upon the cell optical properties.

Most samples, when placed into the biocavity laser display nearly ideal optical elements as light traverses the specimen near 850 nm. At this wavelength light scatter and absorption are minimal, allowing the cell to be transparent. When the chamber of the biocavity laser is excited by an external light source or internally by current, light resonates between the mirrors and the sample acts as a lens focusing light. This concentration of light assists the light-generating process amplifying to such a degree that it escapes through the top mirror. The liberated light can then be analyzed. Thus the sample becomes an intrinsic component of the laser and renders information about itself by acting as an optical waveguide, (i.e. guiding the light waves to a coherent state). The cell acts like a trigger for the production of coherent light.

Sample waveguiding is distinctive and assigns a signature to each specimen, with different cells and tissues having unique signatures. This is due to differences in the dielectric constants between various cell components and the surrounding fluids. Each specimen has various protein and/or nucleic acid concentrations in cellular compartments such as the nucleus, cytoplasm or cell membranes. The variations create original information when placed in the biocavity laser that can be captured and analyzed.

Resonated light is released from the sample chamber and is detected using a high-resolution spectrometer that captures the emitted lasing frequencies. These frequencies are then analyzed as a light spectrum displaying well defined peaks (Fig. 1b). The spectral peaks have both an intensity and spacing distribution that provides individual

spectral signatures for each specimen tested. Computer analysis of the spectral signature can then identify the sample.

Clinical advantages and applications

The goal with any technology for health care delivery is to provide accurate information in a timely fashion using minimally invasive procedures while controlling cost. Semiconductor technology using devices such as the biocavity laser sit on the cutting edge to provide these needed advances in medicine.

Semiconductors can operate efficiently with high throughput and can process large numbers of samples in a short period of time. A considerable amount of information can be gained using a fraction (1/10 to 1/1000) of the routine amount of sample needed for traditional testing. Examples of how this is accomplished include rastering (repeated scanning) of cells or flowing cells through etched channels in the microcavity (Fig. 2). Researchers have been able to fashion grooves precisely in the microcavity materials to dimensions as small as the cells. Technical advances in flow technology can route cells in single file through the channels. In short, this chip now acts as a flow cytometer.

Flow cytometry is currently used both in research and medical testing. One example of how this technique works is counting the number of CD4+ cells in patients with HIV. CD4 count informs the physician about the severity of disease because the count decreases as AIDS worsens. Samples used for testing are currently obtained by venipuncture, then processed and stained with special reagents to visualize the cells. This entire process is labor intensive and time consuming. It is anticipated that devices such as the biocavity laser will eliminate the need for timely and costly processing. Furthermore, the biocavity laser uses smaller sample volumes and this translates into less invasive sampling from a patient. A finger puncture can provide similar information to venipuncture.

Given the speed and efficiency of the device, another meaningful application involves cellular analysis to find rare events. These events may include detecting a malignant cell, dying cell or contaminating cell from a large volume of sample. Applications may include examining cells from cervical tissue to find precancerous cells or sampling a blood specimen to test for a blood cancer. The biocavity laser might revolutionize stem cell transplantation, a therapy used in treating blood cancers and other serious disease. This technique relies upon the isolation of stem cells (cells that have the potential to give rise to a large number of mature blood cells). Stem cells comprise a very small portion of circulating blood cells, hence large volumes of blood are processed to collect small numbers of these important cells. A device such as the biocavity laser can assist in finding these rare cells in a more rapid and efficient manner.

Measurement of intracellular hemoglobin in red blood cells

Estimates suggest that 20% of the world's population suffers from various anemias arising from dietary or genetic deficiencies. Although the disease has many causes, the

net effect is a decrease in the concentration of hemoglobin in the red blood cells. Standard methods for measuring anemia involve lysing relatively large volumes (10 ml) of whole blood, oxidizing with potassium ferricyanide and potassium cyanide, and performing spectroscopic absorption at 540 nm (position of the oxidized hemoglobin absorption peak). This method determines the average hemoglobin concentration in whole blood, but cannot distinguish cellular or plasma hemoglobin, or determine the distribution among the cells. Using the semiconductor microcavity as a optical probe of protein, a new analysis of single cell hemoglobin has been developed (Fig. 2). Such microlaser techniques have the potential to be rapid, inexpensive alternatives to conventional hematologic methods that require large instruments.

The biocavity method determines the concentration of hemoglobin and its variation among red blood cells using only 10 picoliters of whole blood diluted in saline. The stimulated emission spectra were recorded with and without the cell present, respectively. In typical spectra, there was a dominant peak at 827 nm corresponding to the longitudinal mode of the wet cavity containing only the blood plasma. With the cell present, a series of spectral peaks in the lasing spectra. By knowing the effective cavity length and using literature values of the refractive index of hemoglobin solutions, the hemoglobin concentration was determined from the spectrum. Histograms of such hemoglobin measurements in red cells from a normal male and an anemic female were recorded. Blood samples from both individuals were measured by standard hematology methods for reference. The normal red cell histogram shows a mean hemoglobin concentration of about 34 grams per deciliter and a standard deviation of 7%. By contrast, the anemic red cell histogram shows a much lower mean concentration of 13 g/dL and a standard deviation of 25%.

Detecting cancer cell growth rates quickly and accurately

In the U. S. A. alone, over 600,000 people die each year from cancer. Fortunately modern surgical techniques and therapies are able to extent the lives of many cancer patients. However, little progress has been made in reducing the cancer death rate, which remains the second leading cause of death in the U. S A. We are investigating the biocavity laser for high throughput screening of tumor cells in a sensitive nanodevice that has the potential to quickly identify a cell population that has begun the rapid protein synthesis and division characteristics of cancer cell proliferation. This technology has the potential to quickly and accurately diagnose cancer in its early stages to enable a decisive intervention for treating this deadly disease.

Currently, pathologists rely on microscopic examination of cell morphology using -old staining methods that are labor-intensive, time-consuming and frequently in error. New micro-analytical methods for automated, real time screening without chemical modification are critically needed to advance pathology and improve diagnoses. We have teamed scientists with physicians to create a microlaser biochip which evaluates tumor cells by quantifying their growth kinetics. The key new discovery was demonstrating that the lasing spectra are sensitive to the biomolecular mass in the cell,

which changes the speed of light in the laser microcavity. Initial results with normal and cancerous human brain cells show that only a few hundred cells - the equivalent of a billionth of a liter - are required to detect abnormal growth. The ability to detect cancer in such a minute tissue sample is crucial for resecting a tumor margin or grading highly localized tumor malignancy.

Biomolecular concentration measurements for studying the cell cycle

The biocavity laser spectrum provides a quantitative measure of total biomolecular concentration in the cell. This measurement can be explained as follows. Typical mammalian cells are composed of water (70%), proteins (18%), lipids (5%), metabolites (3%), sugars (2%), and RNA/DNA (1-2%).[21] Simpler molecules such as H_2O and sugars comprise chemical bonds that have weaker dipole oscillator strengths in the UV absorption spectrum and contribute less to the refractive index in the spectral region 850 nm, where the laser operates. However, more complex molecules like protein and RNA/DNA, comprise many carbon-carbon and carbon-nitrogen double bonds and have strong oscillator strengths in the UV absorption spectrum. These optical absorptions give rise to strong enhancements of the refractive index at longer wavelengths.[22] As proteins are the most abundant complex molecules in the cell, and as the complex molecules contribute most strongly to the refractive index, the lasing spectrum is most sensitive to the protein content of the cell.

Biological cell cycle

It is feasible to use the biomolecular concentration to assess the stages of the cell cycle (fig. 3a), and to identify possible failures in regulation of the cycle. During interphase, the cell must reproduce genetic material and the other protein components before it undergoes mitosis and cytokinesis.[21] It is during this period that the cell is most actively transcribing and translating genetic information. G1 is a gap phase in the cell cycle during which there is gene expression and biochemical activity but no replication of DNA. During the S phase, the nucleus replicates its chromatin and cellular proteins. Thus the amount of DNA and protein must double. G2 is a second gap phase in the cell cycle that follows the S phase. During G2, the cell is resting before it proceeds to divide by mitosis. Most cells spend very little time in G2, so few cells would be found in this phase. Moreover, irregular cell cycles induced by oncogenes or other perturbations would alter the relative population of cells found in G1 and G2 phases. By measuring biomolecular concentration (or biomolecular mass in a fixed cell volume), it is possible to quantify the number of cells in G1 and G2 and assess the cell growth rate.

Micocavity laser measurements of cell protein

To measure the biomolecular mass cells are flowed through the microlaser (Fig. 2). When a liquid is placed inside a laser microcavity, optical resonance occurs when the roundtrip light path is a whole number of light wavelengths. These resonance conditions appear as sharp peaks in the emission spectrum of the laser. When a cell with higher DNA/protein concentration flows through the laser, the resonance peaks shift to longer wavelengths

and can be precisely measured with a spectrometer. By knowing the specific refractive increment (the index change for a given concentration of molecules), the average biomolecular mass can be determined from the shift.

Results for Normal and Cancerous Brain Cells

Figure 4 (left) shows a histogram of spectral shifts for a population of normal human astrocytes. The main peak near 4.5 nm is assigned to cells in G1, comprising 98% of the population. The smaller peak near 9 nm is assigned to the remaining 2% of cells in G2. This distribution of cells indicates that the cells are growing slowly with two well-defined population groups. Figure 4 (right) also shows results for glioblastoma cancer cells and reveal two subpopulations at 5 and 10 nm shifts corresponding to G1 and G2 phases. Here, there are considerably more cells (~5%) in the G2 phase. Further, the population is much more broadly distributed between the two phases, indicating many intermediate cells in the synthesis phase. These data indicate that the glioblastoma cells are proliferating at a much higher rate than the normal astrocytes, as expected for tumor cells. This observation is consistent with the measured rate of cell growth in culture and conventional flow cytometry data using protein markers in tumor cells.

These initial data reveal cells with single and doubled biomolecular concentration, and resemble conventional flow cytometry histograms of DNA content in cells. The cells with the single concentration correspond to cells in the G1 phase of the cell cycle, and the cells with double concentration correspond to the G2 phase after DNA and protein synthesis. The identity of the G1 and G2 peaks in the biocavity laser data was confirmed by flowing G1 and G2 cells that had been sorted by conventional flow cytometry. This suggests that the microcavity technique provides a complementary method to conventional flow cytometry for cell analysis. Whereas conventional cytometry requires a fluorescent molecular tag and measures DNA content, the biocavity laser uses cells in the native state and is a measure of total biomolecular content (primarily protein).

It remains an open question if the total biomolecular content measurement used here is able to determine cancer in an individual cell. However, the flow data show that only a few hundred cells are required to detect abnormal growth in a small cell population. Such a quantity of cells corresponds to a tissue volume of only one nanoliter. The ability to detect rapidly proliferating cells in such a minute tissue sample could be of significant value in resecting a tumor margin or grading the malignancy at a localized point in a tumor.

Summary

'Point of care' is a concept in medicine that is becoming lost. A visit to a physician's office may result in the patient making unexpected trips to other facilities in order to provide a blood or urine sample. Samples that are obtained in the doctors office are sent to a large reference laboratory for analysis. Turn around time for the delivery of results from the blood tests can take days. Therefore it becomes useful to have an instrument

that can provide near instantaneous results at the point of care once the specimen has been obtained. MEMS devices can be manufactured relatively inexpensively in compact sizes. This allows testing to be accomplished within a physicians office and provide important and often anxiety relieving information to the patient. One can imagine the relief of learning that the results of a breast biopsy shows no indication of cancer minutes after the sample is obtained rather than days later as is currently required using traditional methods.

The biocavity laser is a MEMS device using VCSEL technology that offers many advantages over existing technologies. These advantages include miniaturization, cost containment, decreased time to test results, improved point of care delivery and increased diagnostic yield. Compact diagnostic devices could be used in physician's office, third world countries or deployed in areas devastated by war or other disasters. The healthcare/biotechnology fields will discover the usefulness of these instruments as researchers continue to refine and validate these devices. A revolution has occurred in biotechnology that will steer medicine into the next millennium.

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Fig. 1. Left side: schematic of the biocavity laser. Right side: images and spectra of stationary normal and sickled red blood cells obtained with the biocavity laser.

Fig. 2. Upper left: schematic of flow chamber and and top view of flowing red blood cells recorded by high speed video microscopy. Upper right: laser scanning confocal micrograph showing red and white blood cells. Lower left: portable spectrometer for reading the microlaser cytometer. Lower right: measurements of hemoglobin in normal and anemic blood cells.

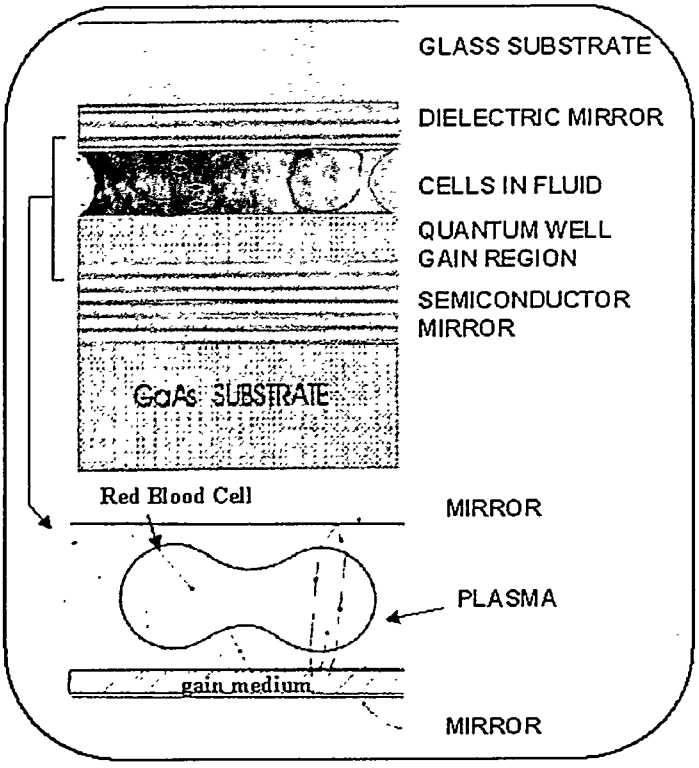
Fig. 3. Left diagram shows the cell growth and replication cycle, including mitosis M when the cell divides, a dormant gap phase G_0, growth and regulation gap phase G_1, synthesis phase S, and final gap phase G_2. The top right diagram shows how the protein mass of the cell doubles with time during the normal cell cycle. Cells in a population are distributed in phase according to the duration of the phase (red curve showing most cells in G_1 phase). The bottom right diagram shows the growth curve for cancer cells that exhibit a shortened G_1 phase and a larger percentage of cells in the G_2 phase.

Fig. 4. Histograms for normal human astrocytes (left) and glioblastoma cells (right) showing the distribution of cells in wavelength shift of the laser line relative to the microcavity with fluid but without the cell. The wavelength shift is approximately proportional to the total protein content of the cell, since proteins are the major biomolecular component of the cell mass.

BIOCAVITY LASER - A New Tool to Diagnose Disease

Biological cells form part of a semiconductor laser and impress cell information on the laser's optical output

Unique spectral signatures detect and identify diseased cells



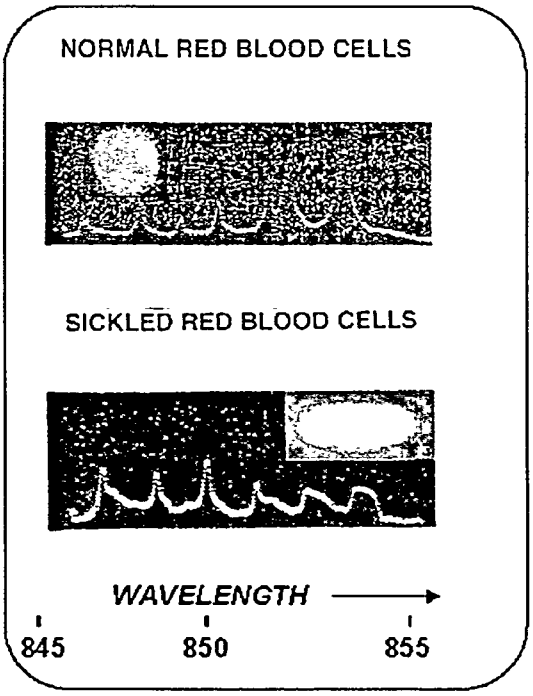
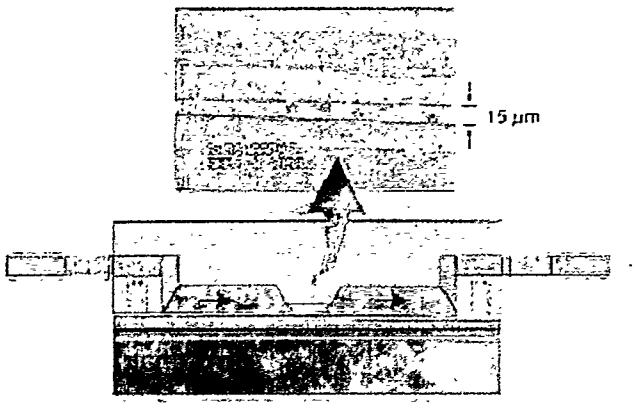


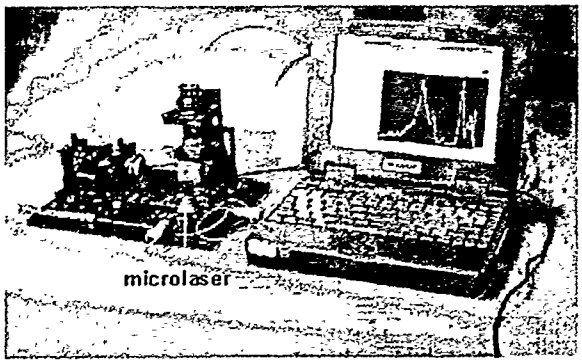
Fig.1

Microlaser Cytometer Diagnoses Blood Disorders

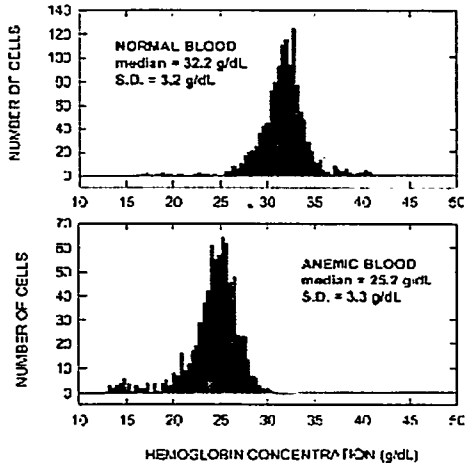


Red blood cells flow through the microcavity laser

Red blood cells surrounding a white blood cell



Compact spectrometer reads microlaser output



Results for normal and anemic blood

Fig.2

The replication kinetics of a single cell can be inferred from the distribution of cells in a population

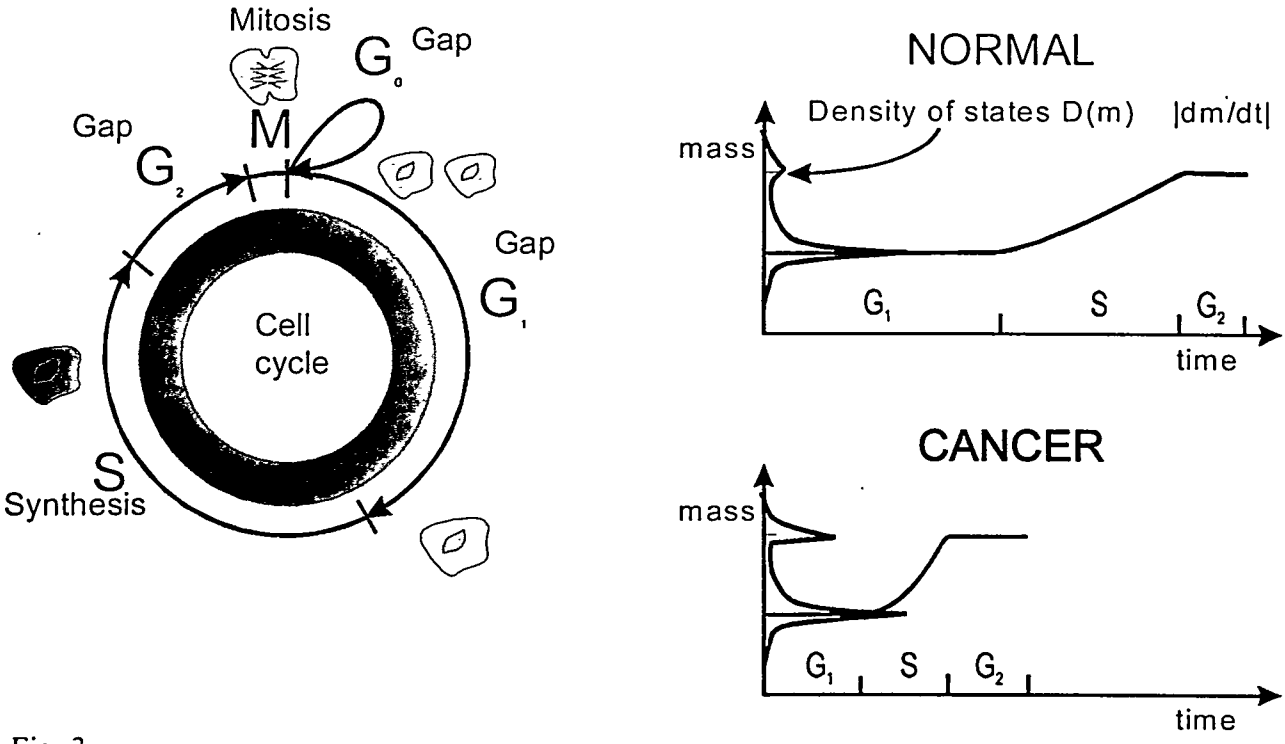
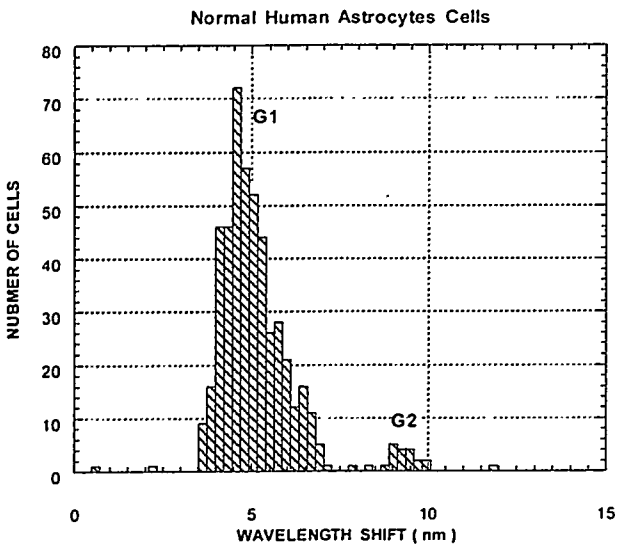


Fig. 3


Normal Human Astrocytes Cells
NUBMER OF CELLS
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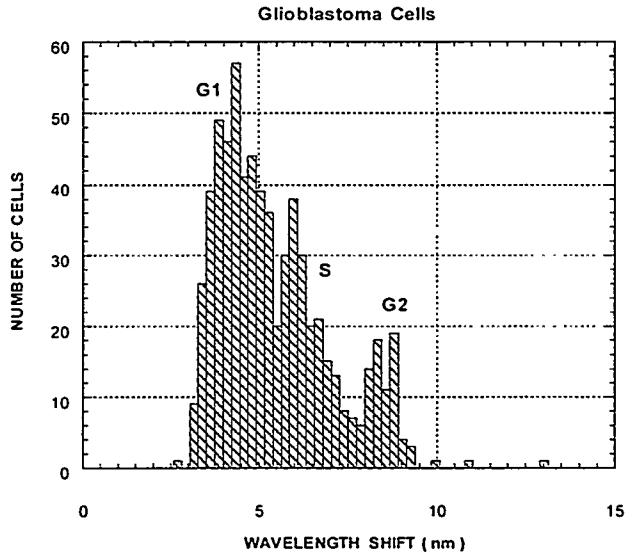
70
60
50
40
30
20
10
0
G1
G2
0
5
10
15
WAVELENGTH SHIFT (nm)


Glioblastoma Cells
NUMBER OF CELLS
60
50
40
30
20
10
0
G1
S
G2
0
5
10
15
WAVELENGTH SHIFT (nm)