APPARATUS AND METHOD FOR DETECTING ACTIVITY OF LIVING CELLS

This patent application claims priority to U.S. patent application serial number: 60/722,680, filed September 30, 2005, and is incorporated by reference herein as if set forth in its entirety.

FIELD OF THE INVENTION

[0001] The present invention relates generally to cellular detection, and more particularly to fluorescent detection of neuronal activity with an imaging sensor, and to optical detection of cellular activity of motile cell types.

BACKGROUND

[0002] Cytology is a branch of biology dealing with the study of the formation, structure, and function of cells. As applied in a laboratory setting, scientists and other medical professionals examine cells to, for example, determine the functioning of the cells or make medical diagnoses of a patient's condition.

[0003] Observation of minute biological samples, such as cultured cells, is typically performed by a microscope. When a microscopic image is recorded, a camera or a video camera mounted on the microscope is used. In addition, other equipment may be required, mounted either proximate to or on the microscope station, especially if the temperature and humidity of the sample must be kept constant. Such an arrangement can be costly and complex.

[0004] Various neuroelectronic devices have been developed for monitoring the electrical activity of a small number of cells simultaneously. Generally, these have made use of extra-cellular recording of the action potentials with extra-cellular microcircuit electrode arrays; however, many have involved the use of inter-cellular microelectrodes. The neurons are impaled by micron-size electrodes that penetrate the cell wall. Cell death results from trauma usually within a few hours of electrode insertion. These methods are further limited in that they cannot be extended to very large arrays.

[0005] The neuroelectronic device disclosed in U.S. Patent Publication No. 2004/0219184; the entire disclosure of which is hereby incorporated herein by reference, relies on an electrostatic coupling between the cells and the detector for monitoring neural network activity.

[0006] Additionally, various detection and handling methods and/or diagnostic tools are generally described in U.S. Patent Nos. 4,401,755; 4,985,353; 5,462,856; 5,919,646; 6,631,331; 6,770,449; 6,778,724; and 6,913,877; the entire disclosures of which are hereby incorporated herein by reference.

[0007] It is, therefore, an object of the present invention to provide apparatus, systems, and methods for effectively detecting cellular activity, without complicated and expensive optical components.

SUMMARY OF THE INVENTION

[0008] Generally, the invention relates to apparatus, systems, and methods to sense the neuronal activity of living cells located in close proximity to an imaging sensor. The cells are treated so that they will fluoresce or emit light in response to a stimulus. In one embodiment, the cells used are neurons; however, other cellular materials are contemplated and within the scope of the invention. One advantage of the present invention over the prior art is that there are no optical lenses between the observed medium and the detector. There are no mirrors used to modulate the light path, either. There is a one-to-one correspondence between a detector array and an area sensed by the array.

[0009] In one aspect, the invention relates to a system for detecting cellular activity. The system includes means for receiving a cellular material disposed on or proximate an imaging detector. The means for receiving the cellular material may include a protective layer and/or a diamond-like carbon layer for isolating the cellular material from the imaging detector. In various embodiments, the imaging detector is a sensor or an array of sensor areas, and can include a charge-coupled device (CCD), a complementary metal-oxide semiconductor device (CMOS), a

charge injection device (CD), video camera, diode array, or similar devices. The system could also include a processor for collecting and processing data generated by the system, storage for storing the data, and means for displaying the data.

[0010] The system may also include a light source, such as, for example, a laser, a lamp (for example, incandescent, halogen, or fluorescent), or light emitting diodes. The light source can provide light at various wavelengths or multiple wavelengths. The light source may also include a power source; filters, such as, for example, notch or dichroic filters; and lenses to, for example, focus or collimate the beam(s) of light supplied by the light source.

[0011] In various embodiments, the system includes means for handling and/or treating the cellular material. For example, the system can include means for introducing a gas to the cellular material's environment or means for sampling the cellular material's environment to test, for example, for toxins.

[0012] In another aspect, the invention relates to a method of detecting cellular activity. The method includes the steps of providing light of a wavelength suitable for stimulating fluorescent emission from, for example, doubly-ionized calcium ion (Ca^{++}) tracing dyes in a neuron cell body; collecting at least a portion of the emitted fluorescent light; locating the activated neurons with respect to the sensor array; and characterizing the temporal and/or amplitude of a detected signal.

[0013] In one embodiment, the collection of the emitted light is accomplished with an array of light-sensing sites (pixels) that are located directly below the cells, in the near-field, without any optical transfer assembly, such as lenses or mirrors.

[0014] In various embodiments, any cellular process that can sit on top of the detector, or on lop of a clear support plate in close proximity to the imaging detector, can be detected. Additionally, the cellular material can be doped or otherwise treated. For example, some neurons can be treated with a type of fluorescing substance that traces calcium or potassium ions, or the material can be

treated with a voltage-sensitive dye to be used with ion tracing, photon-emitting compounds to track cellular ion channels or movement of ions in living cells.

[0015] These and other objects, along with advantages and features of the present invention herein disclosed, will become apparent through reference to the following description and the accompanying drawings. Furthermore, it is to be understood that the features of the various embodiments described herein are not mutually exclusive and can exist in various combinations and permutations.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] In the drawings, like reference characters generally refer to the same parts throughout the different views. In addition, the drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the invention. In the following description, various embodiments of the present invention are described with reference to the following drawings, in which:

[0017] Figure 1 is a schematic elevation view of a device for detecting cellular activity in accordance with one embodiment of the invention;

[0018] Figure 2 is a schematic representation of an overall system for detecting cellular activity in accordance with one embodiment of the invention;

[0019] Figure 3 is a schematic elevation view of a portion of a device for detecting cellular activity in accordance with one embodiment of the invention; and

[0020] Figure 4 is a flow chart illustrating a method of detecting cellular activity in accordance with one embodiment of the invention.

DETAILED DESCRIPTION

[0021] Embodiments of the present invention are described below. It is, however, expressly noted that the present invention is not limited to these embodiments, but rather the intention is that variations, modifications, and

equivalents that are apparent to the person skilled in the art are also included. For example, the apparatus, systems, and methods disclosed herein are typically described with reference to measuring neuronal activity of cellular material using certain dyes and fluorescent light; however, it is expressly noted that the present invention can be practiced with other types of cellular materials, dyes, and light sources.

[0022] As shown in Figure 1, the neurons (100) are grown or placed on top of an imaging detector, or sensor, (101) without any intervening lenses or mirrors, optical fibers, or any other type of optical relay system. The imaging sensor is fabricated so that it does not damage the neurons through its operation. For example, inert protective layers (102) and a diamond-like carbon layer (111) can be placed between the neurons and the imaging sensor. Examples of inert protective layers and a diamond-like carbon layer are described in U.S. Patent Publication No. 2004/0219184.

[0023] The neurons are grown in a cell growth medium (104) in a liquid cellular incubation nutrient bath (105), within a cellular incubation atmosphere (106). The nutrient bath and atmosphere are encapsulated (103) and supplied by an external source of gas through a port (107) and an external source of liquid through a port (108). The external sources of gas and liquid can be delivered to the appropriate ports via any necessary tubing or similar means of conveyance. The system also includes a cellular liquid drain (109), a cellular atmosphere tap (110), and means for introducing a signal (120) to the cellular material.

[0024] As the action potentials propagate through the cell bodies, fluorescent light is generated. Some of the light will be detected by the imaging sensor, indicating the presence of neuronal activity. It should be noted that the technique described herein uses optical detection of fluorescently emitted light and in no way relies upon electrostatic coupling.

[0025] The imaging detector consists of an array of sensing sites, or pixels, which can sense photons. Such a device could be a CCD, a CMOS active pixel sensor, or any other device with an array of sensing sites appropriate for visible or near infrared imaging. Since photons are emitted in close proximity to the imaging sensor, they propagate in the near-field.

[0026] Figure 2 depicts the overall system layout of one preferred embodiment, including the imaging device and auxiliary components. It is possible to mix in other gases (210) and liquids (220) with the incubation atmosphere and nutrient fluids. In this way, the device can be used to test for toxins or the effects of different chemicals, both liquid and gas, on the cells under culture. Changes in the behavior of the cells that are detectable by the sensor array would indicate a reaction to the chemicals that are introduced. The system includes the necessary valves and plumbing to facilitate the introduction, removal, and monitoring of the various gases and liquids used in the system.

[0027] The following description is a general description of the operation of one embodiment of a system in accordance with the invention. In operation, the imaging device is used in a normal manner (i.e., the imager is cleared of a residual image, if necessary, and the imager is set in an exposure mode, in which it collects photons); the cells are stimulated in some fashion so as to emit light, or they self-stimulate; and the collected photons are converted into a digital form.

[0028] In a particular embodiment, the imager is a CCD, which is first cleared of any accumulated charge. Its clock waveforms would be set to an integrating mode, in which photons are converted to photo-electrons in the pixel wells. A cellular stimulation would be applied, fluorescence-stimulating radiation would illuminate the device, and the exposure would occur. After a period of time, the exposure would end, and the CCD would be read-out in a normal fashion. The resultant image would show bright regions where the fluorescence had occurred.

[0029] A series of images could be acquired and differences between various images would illustrate changes in the cellular activity. This is similar to examining successive photographs in order to discern changes, a technique long-employed in many different scientific fields. For instance, this technique was used early in the 20th century by Henrietta Leavitt to discover variable stars in nearby galaxies. By measuring the brightness of stars that changed in brightness, and making a table of their locations and brightness variations with time, she was able to catalog more than 1000 variable stars.

[0030] Various embodiments of the invention include using ion indicating fluorescent dyes from Invitrogen (Carlsbad, CA), in the form of the fluo-3 dye. This is a calcium ion tracing dye, which consists of antibodies that attach to doubly-ionized calcium ions (Ca^{++}). One of ordinary skill in the art will understand that other ion, pH or voltage indicating dyes which function in the same general manner can also be used in the context of the present invention. An action potential stimulating signal (112) can be applied to a neuron. As a neuron transmits an action potential along its cell body to a synapse, the Ca^{++} ions pool in the region of the cell body where the action potential will cross the adjacent synapse. By illuminating the cell medium with light of the appropriate wavelength, nominally about 490 nm, light of a wavelength from about *505* to about 535 nm, peaking at about 515 nm, will be emitted by the Ca++ ions. That is, the 515 nm light will indicate the location of neurons that have fired. This 515 nm light can be distinguished from the 490 nm light by the placement of an optical filter that blocks light below 500 nm but transmits light above 500 nm. This filter, in this embodiment, would be placed between the imaging detector (101) (in this case, a CCD) and the protective layers (102). The total distance from the bottom of the neural cells to the top of the imaging sensor would be less than about 1 mm (see Figure 3). Figure 4 illustrates the above described process in a flowchart.

[0031] In an alternative embodiment similar to that just described, no optical filter would be used. Instead, the slightly elevated light signal from the stimulated

cells detected in the pixels immediately below the cells would indicate where in the array neural signals have passed.

[0032] In still another embodiment of the invention, voltage-activated dyes can be used instead of fluorescent dyes. Voltage-activated dyes respond to changes in voltage and emit light. The voltage-sensitive dyes could be injected into the cells or introduced via the bath of nutrient fluid. As the action potential propagates across the individual neuron under examination, light will be emitted by the dye. The light will similarly be emitted by successive neurons that are activated.

[0033] In yet another embodiment of the invention, cell types other than neurons can be used. These cells will be treated so that they will fluoresce or emit other signals in response to a stimulus or a lack of stimulus. The device will allow a user of the system (for example, a scientist) to quickly establish and quantify the metabolic effect of a compound on the cells being studied. Cells that could be used include, for example, cardiac muscle cells, endothelial cells, epithelial cells, and reproductive cells.

[0034] A further embodiment of the invention will utilize motile cell types, such as muscle cells. Dynamic motion of the cell or cells will be used as an indicator of metabolic activity. Detection of cell motion can be through bright field, fluorescent, or other means of illumination of the cells.

[0035] In the foregoing embodiment, a CCD can be used to sense the contraction rate and amplitude of cardiac myocytes lying directly on top of the pixels (chip sensors). Toxic agents could be sensed by a change in the contraction rate and amplitude (increase or decrease). The stimulus for inducing cardiac cell contraction could be, but is not limited to, electric field stimulation, chemical stimulation, or increasing extra-cellular potassium, either alone or in combination with other stimuli. Sensing of the contraction could be achieved by shining a light (bright field illumination) over the cardiac cell laced CCD. The CCD would act as a camera to record the stimulus-induced contraction of the cells. In addition to

a CCD sensor, other sensors that can be placed on a chip are contemplated, such as nanowires or other voltage sensitive devices which can measure the action potentials created by the myocytes or other cells.

[0036] The light source described above could be any bright field direct current powered illuminator. One advantage of this technology over other technologies is that neither fluorescence nor electrostatic detection methods need to be employed in order to resolve effects of toxins on ion channels. Because the present invention does not require fluorescence, there is no need for a fluorescent light source and an optical filter to see only fluorescent light emitted by a dye. Furthermore, elimination of the need for electrostatic detection eliminates the need for a chip that is sensitive to nerve or muscle cell discharge (action potential). The invention could use either single myocytes or sheets of myocytes. Contraction data, such as rate and amplitude at a particular frequency of stimulation, could be analyzed in both the absence and presence of toxins.

[0037] Having described certain embodiments of the invention, it will be apparent to those of ordinary skill in the art that other embodiments incorporating the concepts disclosed herein may be used without departing from the spirit and scope of the invention. The described embodiments are to be considered in all respects as only illustrative and not restrictive.

Claims

1. An apparatus for detecting activity of living cells, the apparatus comprising a solid support having:

a) a cell growth medium layer;

b) an inert protective layer;

c) a light source; and

d) a detector,

e) a processor for collecting and processing data generated by the system,

f) storage means for storing the data, and

g) display means for displaying the data,

wherein said activity is detected by the apparatus without any optical transfer assembly such as lenses or mirrors.

2. The apparatus of claim 1, wherein said support is enclosed and is capable of containing liquid growth media.

3. The apparatus of claim 2, wherein the support has at least one inlet/outlet port.

4. The apparatus of claim 1, further comprising a layer d) of diamond like carbon interposed between the cell growth medium layer a) and the inert protective layer b).

5. The apparatus of claim 1, wherein said detector is capable of detecting light emission or transmission, changes in voltage, and cellular motion.

6. The apparatus of claim 5, wherein the detector is selected from the group consisting of: a charge-coupled device (CCD); a complementary metal-oxide semiconductor device (CMOS); a charge injection device (CD); and a diode array, or similar solid state photonic detection device.

7. An apparatus for detecting the activity of living cells comprising an imaging sensor (101), an inert protective layer (102) an encapsulating support (103), a cell growth medium (104), a liquid cellular incubation nutrient bath (105), a cellular incubation atmosphere (106) a first port (107) and a second port (108), a cellular liquid drain (109), a cellular atmosphere tap (110), a light source (130), and means for introducing a signal (120) to the cellular material.

8. The apparatus of claim 7, further comprising a diamond like carbon layer (111) interposed between the cell growth medium layer (104) and the inert protective layer (102).

9. The apparatus of claim 7, further comprising an optical filter (300) interposed between the imaging sensor (101) and the cells to be detected (100).

10. The apparatus of claim 7, further comprising a first gas port (205) coupled to a gas mixing valve (210) which is also connected to a secondary gas port (200), the gas mixing valve outlet being connected to a gas inlet valve (215) which is then connected to gas inlet port (107), the apparatus also having a gas outlet port (110) connected to an outlet valve (220) which communicates to the outside of the apparatus (103), additionally said apparatus including a first liquid port (230) coupled to a liquid mixing valve (235) which is also connected to a secondary liquid port (225), the liquid mixing valve outlet being connected to a liquid inlet valve (240) which is then connected to liquid inlet port (108), the apparatus also having a liquid outlet port (109) connected to a liquid outlet valve (245) which communicates to the outside of the apparatus (103).

11. The apparatus of claim 7, further comprising a first gas port which communicates to the outside of the apparatus (103) connected through a filter to a fluid inlet port (109), the apparatus also having a gas outlet port

(107) which communicates to the outside of the apparatus (103), the apparatus also having a liquid outlet port (108).

12. A method for detection of activity in living cells, comprising the steps of:

a) placing cells of interest into the apparatus of claim 1;

b) allowing the cells of interest to take up a fluorescent indicator dye for a sufficient time;

c) exposing the cells of interest to a light source at a wavelength which corresponds to the excitation wavelength of said indicator dye;

d) detecting the emission of fluoresced light at a wavelength which corresponds to the emission wavelength of the indicator dye; and

e) analyzing the amount of light emitted.

13. The method of claim 12, wherein said indicator dye is fluo-3 or fluo-4 and the excitation wavelength is between 490 nm and 510 nm and the emission wavelength is between about 505 nm and 535 nm, preferably about 515 nm.

14. The method of claim 12, wherein the indicator dye is a calcium indicator dye.

15. The method of claim 14, wherein the calcium indicator dye is selected from the group consisting of fluo derivative dyes, fura derivative dyes, indo derivative dyes, rhod-2, calcium green and quin-2.

16. The method of claim 12, wherein the indicator dye is an ion indicator dye.

17. The method of claim 12, wherein the indicator dye is a voltage or pH indicator dye.

18. The method of claim 12, wherein the cells of interest are neuronal cells.

19. The method of claim 12, wherein the cells of interest are non-neuronal cells.

20. A method for detection of activity in living cells, comprising the steps of:

a) placing cells of interest into the apparatus of claim 1;

c) exposing the cells of interest to a light source;

d) taking an image of the cells of interest at specific time intervals and storing the image for later analysis; and

e) analyzing the differences in the images.