

SERIAL-LINE-SCAN-ENCODED MULTI-COLOR FLUORESCENCE MICROSCOPY AND IMAGING FLOW CYTOMETRY

[0001] This application claims priority to U.S. Provisional Patent Application No.

5 61/162,072, filed March 20, 2009, and U.S. Provisional Patent Application No. 61/232,113, filed August 7, 2009, the disclosures of which are incorporated herein by reference for all purposes.

BACKGROUND OF THE INVENTION

10 [0002] Cytometry is a technical specialty concerned with the counting and characterization of biological cells. **Figure 1** shows a simplified diagram of one technique known as flow cytometry. In a basic form of flow cytometry, cells **101** are suspended in a fluid and entrained single-file in a narrow transparent tube **102**. The entrainment can be accomplished by any of several methods, including hydrodynamic focusing. A light source **103** illuminates
15 each cell **101** as it passes a measurement location **104**. Light source **103** may be, for example, a laser. Light from light source **103** is scattered by the cell **101** being measured. Some light **105** is scattered generally in the same direction as it traveled to reach the cell **101**. Light **105** is sometimes called “forward scatter”, and may be collected by a forward sensor **106**. Some light may be scattered in other directions as well. This light may be called “side
20 scatter”, and some of the side scattered light **107** may be collected by one or more other sensors **108**. Output signals from sensors **106** and **108** are sent to a computer **109**, which may store and analyze the signals. By analyzing the amount and distribution of the scattered light, it is possible to discern information about each cell, for example its size and some information about its internal structure.

25 [0003] Flow cytometry may measure the scattered light directly, or may make use of fluorescence. In fluorescence cytometry, the cells may be marked with one or more fluorophores, which are excited by light from source **103** to produce light by fluorescence. The nature of the emitted light may reveal additional information about the cells.

[0004] The technique shown in Figure 1 relies entirely on measurements of scattered light
30 to infer information about the cell structure, but does not produce an image of any particular

cell. In another technique, called "image cytometry", an image of an individual cell may be recorded by a camera or microscope.

BRIEF SUMMARY OF THE INVENTION

5 [0005] An improved image cytometry system performs high-speed, high-resolution cytometry using a linear light sensor. In some embodiments, light from a light source is concentrated onto an oblong scanning region, illuminating a cell that is being transported through the scanning region. An optical system focuses an image of a portion of the scanning region onto a linear light sensor. The system repeatedly takes readings of light falling on the
10 linear sensor. The system may include a slit aperture proximate the linear light sensor, such that the system performs semi-confocal imaging.

[0006] In some embodiments, light from a light source illuminates a cell that is being transported through the scanning region. An optical system focuses an image of a portion of the scanning region onto at least two parallel linear light sensors. The system repeatedly
15 takes readings of light falling on the linear light sensors. The system may include a slit aperture proximate the linear light sensor, such that the system performs semi-confocal imaging. In some embodiments, images gathered by the individual linear light sensors are combined to form an image with improved signal-to-noise characteristics as compared with an image gathered by a single linear light sensor. The combination may be performed by
20 digitally combining pixel values from the respective images corresponding to substantially the same respective locations on the cell. The combination may be performed by time delay integration. In some embodiments, light from the light source is concentrated onto an oblong field at the scanning region.

[0007] In another embodiment, a system for performing cytometry comprises a scanning
25 region that is illuminated by light including at least first and second wavelength bands, and means for transporting a cell through the scanning region such that the cell is illuminated. The system further comprises first and second sets of linear light sensors, each set comprising at least one linear light sensor, and an optical system. The optical system selectively directs light emitted from the cell to the two linear light sensor sets such that emitted light in a third
30 wavelength band is primarily directed to the first linear light sensor set, and emitted light in a fourth wavelength band is primarily directed to the second linear light sensor set. The system repeatedly takes readings of light falling on the linear sensors while the cell is transported through the scanning region. Each light sensor set may comprise at least two linear light sensors. The emitted light may be emitted as a result of fluorescence. The system may

include an objective lens that receives and redirects light emitted from the cell, and a mirror that reflects a first portion of the redirected light to the first linear light sensor set and transmits a second portion of the redirected light. The system may comprise a first tube lens that receives the first portion of light and cooperates with the objective lens to form an image of the cell on the first linear light sensor set. In some embodiments, the system may also comprise a second tube lens that receives the second portion of light and cooperates with the objective lens to form an image of the cell on the second linear light sensor set. The system may include a slit aperture proximate at least one of the sets of linear light sensors, such that the system performs semi-confocal imaging. In some embodiments, each set of linear light sensors comprises at least two linear light sensors, and for each set of linear light sensors, images gathered by the individual linear light sensors in the set are combined to form an image with improved signal-to-noise characteristics as compared with an image gathered by a single linear light sensor in the set. The combination may be performed by digitally combining pixel values from the respective images corresponding to substantially the same respective locations on the cell. The combination may be performed by time delay integration.

[0008] In another embodiment, a system for performing cytometry comprises a scanning region that is illuminated by light including at least first and second wavelength bands, and means for transporting a cell through the scanning region such that the cell is illuminated.

The system further includes a set comprising at least one linear light sensor, and an optical system. The optical system selectively directs light emitted from the cell to two portions of the linear light sensor set such that emitted light in a third wavelength band is primarily directed to a first portion of the linear light sensor set, and emitted light in a fourth wavelength band is primarily directed to a second portion of the linear light sensor set. The system repeatedly takes readings of light falling on the linear light sensor set while the cell is transported through the scanning region. The set may comprise at least two linear light sensors. The emitted light may be emitted as a result of fluorescence. The system may include a slit aperture proximate the linear light sensor set, such that the system performs semi-confocal imaging. In some embodiments, the set comprises at least two linear light sensors, and images gathered by the individual linear light sensors in the set are combined to form an image with improved signal-to-noise characteristics as compared with an image gathered by a single linear light sensor in the set. The combination may be performed by digitally combining pixel values from the respective images corresponding to substantially

the same respective locations on the cell. The combination may be performed by time delay integration.

[0009] In another embodiment a system for performing cytometry includes a scanning region that is illuminated by a light source, a set comprising at least one linear light sensor, and an optical system that focuses an image of a portion of the scanning region onto the linear light sensor set. The system repeatedly takes readings of light falling on the linear light sensor set while a cell is transported through the scanning region and illuminated by the light source. In this embodiment, the system is configurable such that during a first experiment, a first image is created having a first number of pixels in a dimension corresponding to the length of the linear light sensor set, and during a second experiment, a second image is created having a second number of pixels in the dimension corresponding the length of the linear light sensor set, the second number of pixels being fewer than the first. The set may comprise at least two linear light sensors. The number of pixels in the second image may be reduced by selecting fewer than all of the pixels from the linear light sensor set. The number of pixels the second image may be reduced by binning some or all of the pixels from the linear light sensor set. Each reading of light falling on a particular one of the linear sensors may result in a single numerical representation of the amount of light falling on the particular sensor.

[0010] In another embodiment, a system for producing an oblong illumination field includes a laser that produces a beam, a cylindrical lens that receives the beam and causes the beam to converge in only a first axis, and an objective lens that receives the beam after the cylindrical lens. The objective lens is part of an infinity-corrected optical system, and causes the beam to converge in a second axis orthogonal to the first. The system may include a wavelength-selective mirror between the cylindrical lens and the objective lens. In some embodiments, the objective lens is spaced from the cylindrical lens by a distance less than the focal length of the cylindrical lens. In some embodiments, the objective lens is spaced from the cylindrical lens by a distance greater than the focal length of the cylindrical lens. In some embodiments, the beam is diverging in the first axis as it leaves the objective lens. In some embodiments, the beam is converging in the first axis as it leaves the objective lens.

[0011] In another embodiment, a method of performing cytometry comprises illuminating, using a light source, an oblong field at a scanning region, wherein illuminating the oblong field further includes concentrating, using a light shaping element, light from the light source onto the oblong field. The method further comprises focusing, using an optical system, an image of a portion of the scanning region onto a linear light sensor, and repeatedly taking

readings of light falling on the linear sensor while a cell is transported through the scanning region and illuminated by the light source. In some embodiments, the method further comprises passing the light falling on the linear light sensor through a slit aperture proximate the linear light sensor, such that the system performs semi-confocal imaging.

- 5 [0012] In another embodiment, a method of performing cytometry comprises illuminating a scanning region using a light source, and focusing, using an optical system, an image of a portion of the scanning region onto at least two parallel linear light sensors. The method of this embodiment further comprises repeatedly taking readings of light falling on the two parallel light sensors while a cell is transported through the scanning region and illuminated
10 by the light source. In some embodiments, the method further comprises combining images gathered by the individual linear light sensors to form an image with improved signal-to-noise characteristics as compared with an image gathered by a single linear light sensor. Combining images may further include digitally combining pixel values from the respective images corresponding to substantially the same respective locations on the cell. Combining
15 images may further include combining images using time delay integration. In some embodiments, the light source produces illumination including light in at least first and second wavelength bands, and the method further comprises directing, using the optical system, light emitted from the cell in a third wavelength band primarily to one of the two parallel light sensors, and directing, using the optical system, light emitted from the cell in a
20 fourth wavelength band primarily to the other of the two parallel linear light sensors. In some embodiments, the method further comprises focusing, using the optical system, an image of a portion of the scanning region onto at least two sets of parallel linear light sensors, each set comprising at least two linear light sensors.

25 BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 shows a simplified diagram of a technique known as flow cytometry.

[0014] Figure 2 shows a simplified conceptual diagram of a high-speed, high-resolution line scan image cytometry system in accordance with an embodiment.

[0015] Figures 3A-3C illustrate an image forming process.

- 30 [0016] Figure 4 shows an orthogonal view of a system in accordance with another embodiment of the invention.

[0017] Figure 5 illustrates an orthogonal view of a system in accordance with another embodiment of the invention.

[0018] Figure 6 illustrates an orthogonal view of a system in accordance with another embodiment of the invention.

5 [0019] Figure 7 illustrates an orthogonal view of a system in accordance with still another embodiment of the invention.

[0020] Figure 8 illustrates an orthogonal view of a system in accordance with another embodiment.

10 [0021] Figures 9A-9C illustrate embodiments of a system for producing an oblong illumination field.

DETAILED DESCRIPTION OF THE INVENTION

[0022] **Figure 2** shows a simplified conceptual diagram of a high-speed, high-resolution line scan image cytometry system **200** in accordance with an embodiment. The system of Figure 2 is a flow cytometry system, although one of skill in the art will recognize that
15 embodiments of the invention may be utilized in other kinds of cytometry as well.

[0023] Cells **101** are entrained in fluid to progress through tube **102** in single file. The system may be used to characterize cells of many different kinds, but in a typical application, cells **101** may be, for example, about 10 to 20 micrometers across, and may progress through
20 tube **102** at a speed of, for example, 10 millimeters per second. A light source **201** provides field of light **203** onto tube **102**. Light source **201** may be a laser, a light-emitting diode, an incandescent light source, a fluorescent light source or another kind of light source. Light source **201** may produce substantially monochromatic light, broad spectrum light, or light containing two or more narrow bands of wavelengths. Optional light shaping element **202**
25 may include various lenses, prisms, reflectors, or other optical components to concentrate light from light source **201** into oblong or slit-shaped field **203**, through which cells **101** are transported. Because, as is described below, only a narrow line image will be scanned, only a narrow field need be illuminated, in contrast to traditional epi-illumination in which the entire objective field is illuminated. The concentration provided by light shaping element **202** can
30 increase the effective illumination level by as much as two to six orders of magnitude as compared with normal, symmetric epi-illumination.

[0024] Some light from source **201** is transmitted through or scattered by one of cells **101**, at least a portion of which is within field **203**. Some of the light is redirected by one or more lenses **204** onto a linear sensor **205**. Linear sensor **205** may be, for example a charge-coupled device (CCD) sensor, a complementary metal oxide semiconductor (CMOS) sensor, or
5 another kind of sensor having a plurality of light-sensitive sites arranged in a row. Lens **204** and sensor **205** may be, for example parts of a line scan camera such as a Basler Sprint line scan CMOS camera available from Basler AG of Ahrensburg, Germany. The individual sensor sites are sometimes called “pixels”. The corresponding sites at the scan line sensed by the sensor pixels are also sometimes called pixels. Sensor **205** may comprise, for example,
10 one or more rows of pixels, each row containing 512, 1024, 2048, or another appropriate number of pixels. The intensity of light falling on the row of pixels may be read by clearing the pixel array, allowing charge to accumulate in the pixel sites for a predetermined exposure time, and then converting the accumulated charge amounts to numerical values representing the light intensities. This process is performed repeatedly as the cells pass the scan area. In
15 one example embodiment, the system may take a reading (“scan a line”) every 20 microseconds, or at a scan rate of 50 kHz. Using a cell transport speed of 10 millimeters per second and a scan rate of 50 kHz results in an imaging pixel size of 200 nm. Other transport speeds and scan rates are possible, and may result in other imaging pixel sizes. The resulting array of measurements can be reassembled into an approximate image of a cell.

[0025] Figures 3A-3C illustrate the image forming process. In Figure 3A, a scan line **301** includes pixels a, b, c, d, and e. A cell **101** is transported past scan line **301**, as shown in Figure 3B, which shows scan line **301** superimposed on cell **101** at consecutive sample times T_1 - T_7 . (While Figure 3B shows the cell traversing exactly one pixel per sample time, this is not a requirement, and in fact will only occur for certain combinations of cell travel speed,
25 sample rate, and pixel size. In practice, consecutive scanned lines may overlap on the cell being imaged, or there may be a gap between areas of the cell read by consecutive scan lines.) The light levels read by pixels a, b, c, d, and e are affected by the structure of cell **101**. For example, when no cell crosses scan line **301**, relatively high light levels are registered. When a relatively transparent part of cell **101** crosses a pixel, the light level registered by that
30 pixel is somewhat reduced. When the nucleus of cell **101** is within a pixel, the light level registered at that pixel is may be significantly reduced. Figure 3C shows traces of the light levels (on an arbitrary scale ranging from 0 to 1) registered at pixels a, b, and c as a function of time. Figure 3D shows a reconstructed image, formed by stacking together data scanned during several consecutive line scans, and representing each numerical light reading by a

printed gray level. While Figure 3D is constructed using only a few pixels sampled at a few times and therefore shows a relatively crude depiction of cell **101**, in practice a system according to an embodiment of the invention may scan more or fewer lines during the passage of each cell, and each line may contain more or fewer pixels than shown. In one

5 embodiment the system may scan approximately 50 lines during the passage of each cell, and each line may contain approximately 50 pixels. The exact number of lines scanned and pixels affected for each cell will depend on the size of the cells, the line scan frequency, the speed at which cells flow past the scan line, and the particular sensor and optical components used.

10 **[0026]** The theoretical resolution of the system depends mainly on the quality of the objective lens. The practical scanning resolution of the system also depends on the scan rate, the speed of transport of the cells past the scan line, and the particular sensor and optical system used. The pixel resolution in the Y direction is determined by the imaging system, including the particular lens and sensor used. Pixel resolution in the X direction is equal to

15 $v \cdot dt$, where v is the sample delivery speed and dt is the camera's exposure time. Preferably, v is a known parameter, either pre-determined before a particular flow experiment or measured during the course of a cell's passage through the system. Ideally, a cell being scanned should be rotation-free and jittering-free during its passage of the scan line.

[0027] The operation of the system of Figure 2 is described above in the context of direct

20 light imaging, where scattered light from source **201** is measured by sensor **205**. A system operating on the same principles could be used to perform fluorescence imaging as well, and in fact, the system may be especially helpful in fluorescence imaging.. In that case, light from source **201** would excite fluorescence in the cell **101** being measured, and resulting emitted light would be collected and measured by sensor **205**. The emitted light will

25 generally be at a longer wavelength than the excitation light from source **201**. In fluorescence imaging, it may be desirable to shield sensor **205** from receiving light from source **201**, using various filters or geometric arrangements of components, so that the source light does not overwhelm or interfere with the measurement of the light emitted by fluorescence. Typically, the light emitted by fluorescence will be less intense than the source

30 light, and longer exposure times, stronger illumination, or more sensitive sensors may be required for fluorescence imaging than for direct imaging. Also, the shape of the temporal signal changes shown in Figure 3C may be different in fluorescent imaging than in direct imaging. In direct imaging, additional structure in a cell tends to result in less light being received by the corresponding pixel of sensor **205**. In fluorescence imaging, additional

structure may carry additional fluorophores, and may result in more light reaching the corresponding sensor pixel, as compared with a pixel corresponding to a cell portion with little structure.

[0028] Figure 4 shows an orthogonal view of a system 400 in accordance with another embodiment of the invention. The embodiment of Figure 4 may be especially suited to single-color fluorescence imaging cytometry. In the embodiment of Figure 4, a light source 401 emits light. Light source 401 may be a laser, a light-emitting diode, an incandescent light source, a fluorescent light source or another kind of light source. Light source 401 may produce substantially monochromatic light, broad spectrum light, or light containing two or more narrow bands of wavelengths. In one example embodiment, light source 401 is a laser that emits light at a nominal wavelength of 488 nm. An excitation filter 402 may be utilized to further narrow the band of wavelengths of light utilized by the system, especially if light source 401 is a broad spectrum light, or otherwise produces wavelengths that are undesirable for a particular cytometry experiment. An optional light shaping element or condenser lens 403 may concentrate the emitted light at a scanning region 404, through which a cell 101 is being transported. Preferably, cell 101 has been marked with one or more fluorophores that fluoresce when excited by the light from light source 401. Many different fluorophores are known, including the ALEXA FLUOR™ series of fluorophores available from Life Technologies Corporation of Carlsbad, California, USA. The concentration provided by light shaping element or condenser lens 403 improves the effective illumination of cell 101, and results in a stronger fluorescent signal. The stronger signal results in less restriction on the exposure time of the sensor used in the system. The oblong or slit-shaped illumination field is well suited to light sources that have naturally asymmetric illumination patterns, for example semiconductor lasers or light emitting diodes.

[0029] Light scattered from cell 101 is gathered and redirected by objective lens 405, reflects from dichroic mirror 406, passes through tube lens 408, and reaches line scan camera 409, where sequential line images of scan region 404 are gathered for analysis by processing unit 410. An emission filter 407 may be placed in the system to narrow the band of light wavelengths delivered to camera 409. Dichroic mirror 406 may also provide filtering. This filtering may reduce the effect of direct light from source 401 that may be scattered by cell 101. Objective lens 405 and tube lens 408 preferably form an infinity-corrected optical system, such that an “infinity space” is created between them. In such a system (known in the art), the performance of the system is relatively insensitive to the distance between the

objective lens and the tube lens, allowing space for the insertion of other components such as dichroic mirror **406** and emission filter **407**.

[0030] **Figure 5** illustrates an orthogonal view of a system **500** in accordance with another embodiment of the invention. The system of **Figure 5** is configured for simultaneous two-color fluorescence imaging cytometry. In the system of **Figure 5**, excitation light comprising two bands of wavelengths is provided to the cell **101** being imaged. This is represented in **Figure 5** by two light sources **501** producing light of different wavelengths indicated by the solid and dashed lines. The light may be further conditioned by one or more filters **502**. Other arrangements are possible. For example, a single broad-spectrum light source may be utilized, and particular bands of wavelengths preferentially selected by filters **502**. Or a single light source could be used to excite two different fluorescent wavelengths. In a preferred embodiment, light sources **501** comprise two lasers, one producing light in a first narrow band at a nominal wavelength of 532 nm and the other producing light in a second narrow band at a nominal wavelength of 633 nm. The light may be concentrated at the scan region **504** by a light shaping element or condenser lens **503**. Element **503** may comprise various lenses, prisms, reflectors, or other optical components, singly or in combination, and preferably concentrates the light produced by sources **501** onto an oblong area at the scan region **504**.

[0031] Preferably, cell **101** is marked with one or more fluorophores, such that when excitation light from sources **501** reaches cell **101**, light of at least two different color characteristics is produced by fluorescence. For example, one fluorophore may react strongly to the 532 nm excitation light, producing emitted light with an emission peak at about 550 nm, and a second fluorophore may react strongly to the 633 nm excitation, producing emitted light with an emission peak at about 650 nm. These different emissions are approximately represented in **Figure 5** using dashed and solid lines in a way similar to the way the two colors of excitation light are represented, although it is to be understood that light represented by a particular line type after emission does not generally have the same spectral characteristics as excitation light represented by the same line type.

[0032] Light from scan region **504** is then gathered by objective lens **505**, and directed to dichroic mirror **506**. Mirror **506** may provide some filtering, such that light principally from a band of wavelengths is reflected from mirror **506**, and the remaining light passed through. The light reflected from mirror **506** may pass through another emission filter **507** to further restrict the spectral characteristics of the light, and then pass through tube lens **508** and reach camera **509**. Thus, camera **509** preferentially receives light emitted by a first fluorophore

marker in cell **101**, with little contamination by light from either of sources **510** or from light emitted by a second fluorophore marker. That is, the light reaching camera **509** preferably falls within a third band of wavelengths selected from the fluorescent emissions of the first fluorophore.

5 **[0033]** The light passed through dichroic mirror **506** is then reflected from another dichroic mirror **510**, may pass through another dichroic emission filter **511**, passes through a second tube lens **512** and to camera **513**. Thus, camera **509** preferentially receives light emitted by the second fluorophore marker in cell **101**, with little contamination by light from either of sources **510** or from light emitted by the first fluorophore marker. That is, the light reaching
10 camera **513** preferably falls within a fourth band of wavelengths selected from the fluorescent emissions of the second fluorophore.

[0034] Cameras **509** and **513** then can scan simultaneous images of cell **101** in different emission spectra. The outputs of cameras **509** and **513** are passed to processing unit **514** for storage, analysis, display, or other purposes. Processing unit **514** may be, for example, a
15 computer system or other processor-based system capable of processing the image data. Processing unit **514** may be an external stand-alone device, or integrated into a testing instrument.

[0035] Many variations are possible for the system. For example, dichroic mirror **510** may be eliminated and filter **511**, tube lens **512**, and camera **513** positioned to directly receive the
20 light that has passed through dichroic mirror **506**. Some of the filters in the system may be optional, depending on the particular light sources and fluorescent materials used. Additional sets of light sources, filters, mirrors, lenses, or cameras may be added so that simultaneous imaging may be performed in three, four, or even more different spectral bands.

[0036] One of skill in the art will recognize that the dichroic mirrors and filters thus far
25 described do not have perfect wavelength discrimination or perfect efficiency. Some light in the wavelength bands intended to be passed by a particular filter may be absorbed or reflected. Some light in wavelength bands intended to be blocked by a particular filter may be passed or reflected. However, the filters and mirrors perform sufficiently well to preferentially pass or block designated wavelengths that the system can discriminate different
30 emitted light colors effectively. In other variations, components other than dichroics may be used for color separation, including prisms, gratings, or other optical components.

[0037] **Figure 6** illustrates an orthogonal view of view of a system **600** in accordance with another embodiment of the invention. System **600** is similar to system **500**, with the addition

of slit apertures **601** and **602** placed in front of cameras **509** and **513** respectively. Slit apertures **601** and **602** have the effect of tending to block or exclude some light gathered from locations other than in the focal plane of the system from reaching the respective camera. This effect is illustrated in Figure 6 by finely-dashed pencil of rays **603**, emanating from an out-of-focus location above cell **101**. The resulting pencil of rays **604** emerging from lens **508** will focus more closely to lens **508** than does the light from the focal plane of the system. By the time the light in pencil **604** reaches slit aperture **601**, pencil **604** has already started to diverge, so that only a small portion of the center of pencil **604** can pass through slit **601** and reach camera **509**. Thus, the system preferentially receives light from the focal plane of the system at cell **101**, and excludes at least some light received from other depth locations.

[0038] When a small circular aperture is used in this way to limit the light received by a single sensor, this technique is called confocal imaging. In the system of Figure 6, apertures **601** and **602** are slits, and therefore exclude light in only one axis. For the purposes of this disclosure, this is referred to as “semi-confocal” imaging. This technique improves the contrast of images recorded by the system as compared with images recorded by a system not utilizing semi-confocal imaging.

[0039] Another advantage of a cytometry system embodying the invention is that it may be modified or made configurable into a point-detector style system, where either only a few pixels in the middle of the linear detector are in operation or some or all the pixels in the row are binned into one pixel or a few pixels. This results in an image of reduced resolution in a dimension corresponding to the length of the linear light sensor (the Y direction in Figure 6). Each exposure of the light sensor may even result in a single numerical representation of the amount of light falling on the sensor, for example if all of the sensor pixels are binned. Optionally, the illumination field could be shaped to a much smaller circle or ellipse, to enhance the speed of the system when operating in that mode. An advantage of this kind of system is that a very high speed single cross-section image of a cell can be generated. This kind of system may be especially useful when electronic communication bandwidth is limited, but ample illumination is available. A system configurable in this way may be applicable to both line-scan imaging cytometry, and to non-imaging flow cytometry.

[0040] Figure 7 illustrates an orthogonal view of view of a system **700** in accordance with still another embodiment of the invention. In the system of Figure 7, simultaneous two-color fluorescence imaging cytometry is enabled using only one linear light sensor or line-scan camera. The illumination system in system **700** may be, for example, any of the illumination systems described above with respect to system **500** shown in Figure 5. That is, one or more

light sources excites two different fluorescence spectra, for example from two different fluorophores in cell **101**. Some of the light emitted by fluorescence from cell **101** is captured and redirected by objective lens **505** toward dichroic mirror **701**. The solid and dashed lines in Figure 7 indicate that light containing two different fluorescence spectra reach dichroic mirror **701**. Mirror **702** selectively filters the light, so that one band of wavelengths preferentially reflects from mirror **701**, and other wavelengths preferentially pass through mirror **701** and continue toward mirror **702**. Additional mirrors and filters may be placed in the optical system directing and conditioning the light as desired. For example, mirror **703** redirects the light from mirror **702** toward tube lens **705**, and mirror **703** may also provide additional filtering. Similarly, mirror **704** redirects the light from mirror **701** toward tube lens **705**, and mirror **704** may also provide filtering. One or more additional filters such as emission filters **707** and **708** may be placed in the optical path. Tube lens **705** refocuses the light onto linear light sensor **706**, which may be part of a line scan camera, and can be read by processing unit **514**.

[0041] The arrangement of mirrors provides a geometric offset between the two bands of light reaching sensor **706**, so that part of sensor **706** receives light in one wavelength band, selected from the light emitted in one of the fluorescence spectra, and another part of sensor **706** receives light in the other wavelength band, selected from light emitted in the other fluorescence spectrum. For example, if sensor **706** comprises 512 pixels arranged in a row, then approximately the first 256 pixels may receive light in one band of wavelengths, while approximately the remaining 256 pixels may receive light in the other wavelength band. As above, processing unit **514** receives repeated line scans from sensor **706**, and can reconstruct two images of cell **101**, one image for each wavelength band. Such a system requires only one linear light sensor or line scan camera, and may be constructed at reduced cost as compared with a system having two linear light sensors or line scan cameras. Other kinds of optical systems may also be used to direct light in two wavelength bands to separate portions of a linear light sensor. For example, such an optical system may comprise an optical grating. A slit aperture may be included in a system such as system **700**, so that the system performs semi-confocal imaging.

[0042] Figure 8 illustrates an orthogonal view of view of a system **800** in accordance with still another embodiment. System **800** is illustrated as a variant of system **400**, shown in Figure 4, but one of skill in the art will recognize that the additional features of system **800** may be employed in other systems, including ones that perform multi-color imaging, fluorescence imaging, or other techniques.

[0043] System 800 employs an exemplary camera 801 having three closely spaced parallel rows of sensors 802, 803, 804. (The sensor rows are shown end-on in Figure 8.) By virtue of the operation of the optics of the system, each of the rows images a different “stripe” on cell 101. Camera 801 thus has three different opportunities to image any particular part of cell 101 as cell 101 passes by the scanning region 404. That is, a particular part of cell 101 will be imaged onto row 802 at a first time. That same part of cell 101 will be imaged onto row 803 at a later time, and onto row 804 at a still later time. In Figure 8, only the central rays of pencils connecting cell 101 with sensor rows 802, 803, 804 are shown, so as not to obscure the operation of the system in unnecessary detail.

[0044] In one technique, three different images may be gathered of cell 101, one made by each of sensor rows 802, 803, 804. The different images are shifted in time with respect to each other, or may also be thought of as shifted in space, in the X direction. These multiple images may be used to create a composite image with improved signal-to-noise characteristics. For example, if the three images are digitally shifted back into alignment and pixel values from the three images corresponding to substantially the same locations on cell 101 added, the resulting composite image will have a signal-to-noise ratio improved by a factor of approximately $\sqrt{3}$ as compared with any one of the individual images. While camera 801 has been illustrated as having three scan lines, it may have 2, 4, or any usable number n . A composite image produced by this digital addition or averaging technique from a camera having n lines will have a signal-to-noise ratio improved by a factor of approximately $\sqrt{n}$ as compared with a single image. The combination of the images may be done “on the fly” as the scanned image lines are available, so that no complete image of a particular cell made by a single linear sensor is constructed.

[0045] Camera 801, having multiple rows of pixels, may additionally or alternatively be configured to perform time delay integration (TDI). In TDI, the electrical charges in the various pixels resulting from an exposure to cell 101 are accumulated within the pixel rows before conversion to digital values. The exposures of the sensors to cell 101 are substantially synchronized such that a particular location on cell 101 is exposed to sensor row 802 during one exposure, to sensor row 803 during the next exposure, and to sensor row 804 during the next exposure. Charges accumulated in row 802 during the first exposure are shifted into row 803 and added to by the second exposure, and the resulting charges are shifted into row 804 and added to by the third exposure. The accumulated charges are then converted to digital

values. TDI also results in an approximately $\sqrt{n}$ improvement in signal-to-noise ratio as compared with a single image.

[0046] One advantage of scanning simultaneous parallel image lines, whether for use with digital image combination or TDI, is that the technique takes better advantage of the available illumination. A light shaping element such as element 403 will not generally focus light onto a single-pixel-wide strip at the scan line. The illumination field will have some appreciable width, and some of the illumination may be wasted in a single-line camera system.

[0047] Another advantage of such a system is that the resolution is not compromised, as it may be in systems that simply bin pixels in order to improve signal-to-noise characteristics.

[0048] One of skill in the art will recognize that a system such as system 500 shown in Figure 5 could also be adapted such that each camera 509, 513 includes a set of two or more linear light sensors. Imaging would be performed by each camera 509, 513 as described above with respect to camera 801, so that multi-color imaging may be accomplished by digital image combination or TDI.

[0049] Similarly, a system such as system 700 shown in Figure 7 could be adapted so that sensor 706 is replaced by a set of at least two linear light sensors. The system would then direct wavelength-selected light separately to two portions of the set of linear light sensors.

[0050] The systems of Figures 2, 4, 5, 6, and 7 may be thought of as including a “set” having a single linear light sensor.

[0051] Additionally, combining images from at least two parallel linear light sensors, whether by digital combination or by time delay integration, can be combined with binning or other resolution-reducing techniques. Binning may produce an image with further improved signal-to-noise characteristics, albeit at a reduced resolution.

[0052] Figures 9A-9C illustrate additional techniques for providing an oblong illumination field convenient for performing line-scan cytometry.

[0053] The line-scan cytometry technique may not require the use of an oblong illumination field in all embodiments. Conventional circular epi-illumination may be utilized, providing the illumination power is sufficiently high. For imaging using scattered, non-fluorescent light, sufficient power of the illumination source may not be difficult to achieve. However, for practical sensing of light emitted by fluorescence, concentrating the excitation light into an oblong field can be much more energy-efficient, for example reducing

the required excitation laser power from a level measured in tens or hundreds of watts to a level measured in tens or hundreds of milliwatts.

[0054] Figure 9A illustrates one view of a system **900** that includes an embodiment of a technique for providing an oblong illumination field. System **900** uses some components and arrangements similar to those of system **400** shown in Figure 4, but one of skill in the art will recognize that the illumination technique illustrated in Figure 9A may be used with other sensing arrangements as well. In system **900**, illumination is provided by a laser **901** from the same direction as from which sensing is performed, so that the space above the sample is left unobstructed. This arrangement may therefore accommodate much larger samples than the arrangements previously described, which may be limited to samples no thicker than the distance between the sample stage and the condenser lens. Another advantage of the system of Figure 9A is that objective lens **405** participates in the formation of the illumination field. Objective lens **405** may typically be a very high-quality lens, so that the illumination field it produces may be very sharply defined.

[0055] In the example system of Figure 9A, laser **901** produces a beam **902**, directed at cell **101**. Beam **902** passes through a cylindrical lens **903**. For the purposes of this disclosure, a cylindrical lens is any lens that has curvature in only one dimension. A cylindrical lens may but need not have curved surfaces defined by circular cylinders. In the view of Figure 9A, cylindrical lens **903** is positioned with its cylindrical axis parallel to the X direction, and lens **903** appears to have no effect on beam **902**. Beam **902** continues through dichroic mirror **406** to objective lens **405**, which focuses the beam onto cell **101**. Light emanated from cell **101** passes through objective lens **405**, preferentially reflects from mirror **406**, may encounter one or more filters **407**, passes through lens **408**, and reaches camera **409**.

[0056] Figure 9B illustrates an embodiment of the illumination portion of Figure 9A, from a view along the X axis. That is, Figure 9B shows a view rotated 90 degrees from the view of Figure 9A. In this view, tube **102** projects as a circle, and cylindrical lens **903** shows as having a curved profile. In the example embodiment of Figure 9B, the materials and dimensions of cylindrical lens **903** are selected such that lens **903** has a relatively long focal length – greater than the distance between the cylindrical lens and the objective lens. After passing through cylindrical lens **903**, beam **902** is seen to relatively gradually converge, as seen in this view. Objective lens **405** then converges and reexpands the beam in the Y direction, such that the illumination field is widened. As is shown in Figure 9A, objective lens **405** simultaneously focuses the beam in the X direction. The resulting illumination field may have a sharply-defined oblong shape as it encounters cell **101**.

[0057] Figure 9C illustrates another embodiment of the illumination portion of Figure 9A, from a view along the X axis. In this embodiment, the materials and dimensions of cylindrical lens 903 are selected such that lens 903 has a relatively short focal length – shorter than the distance between the cylindrical lens and the objective lens. After passing through
5 cylindrical lens 903, beam 902 is seen to converge and then rediverge before reaching objective lens 405. Objective lens 405 redirects beam 902 such that it again converges, but slowly enough that when beam 902 reaches cell 101, beam 902 is still sufficiently wide to span at least a portion of the line being scanned by camera 409. Again, objective lens 405 simultaneously focuses the beam in the X direction. The resulting illumination field may
10 have a sharply-defined oblong shape as it encounters cell 101.

[0058] While embodiments of the invention have been illustrated as scanning cells confined in a linear tube, one of skill in the art will recognize that embodiments of the invention may be utilized in systems using any of a wide range of cell delivery techniques, including electrophoresis, pressure driven flow, optical tweezers, motorized translation stage,
15 and others. Cells may be conveyed as a payload in an oil emulsion, in an electrowetting-actuated droplet, or via magnetic transport assisted by magnetic bead tagging. It is intended that the claims not be limited by the cell delivery method utilized.

[0059] In the claims appended hereto, the term “a” or “an” is intended to mean “one or more.” The term “comprise” and variations thereof such as “comprises” and “comprising,”
20 when preceding the recitation of a step or an element, are intended to mean that the addition of further steps or elements is optional and not excluded. The invention has now been described in detail for the purposes of clarity and understanding. However, those skilled in the art will appreciate that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

- 1 1. A system for performing cytometry, the system comprising:
2 a light source;
3 a light shaping element that concentrates light from the light source onto an
4 oblong field at a scanning region;
5 a linear light sensor; and
6 an optical system that focuses an image of a portion of the scanning region
7 onto a linear light sensor;
8 wherein the system repeatedly takes readings of light falling on the linear
9 sensor while a cell is transported through the scanning region and illuminated by the light
10 source.
- 1 2. The system of claim 1, further comprising a slit aperture proximate the
2 linear sensor, such that the system performs semi-confocal imaging.
- 1 3. A system for performing cytometry, the system comprising:
2 a light source that illuminates a scanning region;
3 at least two parallel linear light sensors; and
4 an optical system that focuses an image of a portion of the scanning region
5 onto the at least two parallel linear light sensors;
6 wherein the system repeatedly takes readings of light falling on the linear
7 sensors while a cell is transported through the scanning region and illuminated by the light
8 source.
- 1 4. The system of claim 3, further comprising a slit aperture proximate the
2 linear sensors, such that the system performs semi-confocal imaging.
- 1 5. The system of claim 3, wherein images gathered by the individual
2 linear light sensors are combined to form an image with improved signal-to-noise
3 characteristics as compared with an image gathered by a single linear light sensor.

1 6. The system of claim 5, wherein the images are combined by digitally
2 combining pixel values from the respective images corresponding to substantially the same
3 respective locations on the cell.

1 7. The system of claim 5, wherein the images are combined by time delay
2 integration.

1 8. The system of claim 3, further comprising a light shaping element that
2 concentrates light from the light source onto an oblong field at the scanning region.

1 9. A system for performing cytometry, the system comprising:
2 a scanning region that is illuminated by light including at least first and second
3 wavelength bands;

4 means for transporting a cell through the scanning region such that the cell is
5 illuminated;

6 first and second sets of linear light sensors, each set comprising at least one
7 linear light sensor; and

8 an optical system that selectively directs light emitted from the cell to the two
9 linear light sensor sets such that emitted light in a third wavelength band is primarily directed
10 to the first linear light sensor set, and emitted light in a fourth wavelength band is primarily
11 directed to the second linear light sensor set;

12 wherein the system repeatedly takes readings of light falling on the linear
13 sensors while the cell is transported through the scanning region.

1 10. The system of claim 9, wherein each linear light sensor set comprises
2 at least two linear light sensors.

1 11. The system of claim 9, wherein the emitted light is emitted as a result
2 of fluorescence.

1 12. The system of claim 9, wherein the optical system further comprises:
2 an objective lens that receives and redirects light emitted from the cell;
3 a mirror that reflects a first portion of the redirected light to the first linear
4 light sensor set and transmits a second portion of the redirected light.

1 13. The system of claim 12, further comprising:

2 a first tube lens that receives the first portion of light and cooperates with the
3 objective lens to form an image of the cell on the first linear light sensor set.

1 14. The system of claim 13, further comprising a second tube lens that
2 receives the second portion of light and cooperates with the objective lens to form an image
3 of the cell on the second set of linear light sensors.

1 15. The system of claim 9, further comprising a slit aperture proximate at
2 least one of the sets of linear light sensors, such that the system performs semi-confocal
3 imaging.

1 16. The system of claim 9, wherein each set of linear light sensors
2 comprises at least two linear light sensors, and wherein for each set of linear light sensors,
3 images gathered by the individual linear light sensors in the set are combined to form an
4 image with improved signal-to-noise characteristics as compared with an image gathered by a
5 single linear light sensor in the set.

1 17. The system of claim 16, wherein the images are combined by digitally
2 combining pixel values from the respective images corresponding to substantially the same
3 respective locations on the cell.

1 18. The system of claim 16, wherein the images are combined by time
2 delay integration.

1 19. A system for performing cytometry, the system comprising:
2 a scanning region that is illuminated by light including at least first and second
3 wavelength bands;

4 means for transporting a cell through the scanning region such that the cell is
5 illuminated;

6 a set comprising at least one linear light sensor; and

7 an optical system that selectively directs light emitted from the cell to two
8 portions of the linear light sensor set such that emitted light in a third wavelength band is
9 primarily directed to a first portion of the linear light sensor set, and emitted light in a fourth
10 wavelength band is primarily directed to a second portion of the linear light sensor set;

11 wherein the system repeatedly takes readings of light falling on the linear light
12 sensor set while the cell is transported through the scanning region.

1 20. The system of claim 19, wherein the set comprises at least two linear
2 light sensors.

1 21. The system of claim 19, wherein the emitted light is emitted as a result
2 of fluorescence.

1 22. The system of claim 19, further comprising a slit aperture proximate
2 the linear light sensor set, such that the system performs semi-confocal imaging.

1 23. The system of claim 19, wherein the set comprises at least two linear
2 light sensors, and wherein images gathered by the individual linear light sensors in the set are
3 combined to form an image with improved signal-to-noise characteristics as compared with
4 an image gathered by a single linear light sensor in the set.

1 24. The system of claim 23, wherein the images are combined by digitally
2 combining pixel values from the respective images corresponding to substantially the same
3 respective locations on the cell.

1 25. The system of claim 23, wherein the images are combined by time
2 delay integration.

1 26. A system for performing cytometry, the system comprising:
2 a scanning region that is illuminated by a light source;
3 a set comprising at least one linear light sensor; and
4 an optical system that focuses an image of a portion of the scanning region
5 onto the linear light sensor set;
6 wherein the system repeatedly takes readings of light falling on the linear light
7 sensor set while a cell is transported through the scanning region and illuminated by the light
8 source;

9 and wherein the system is configurable such that during a first experiment, a
10 first image is created having a first number of pixels in a dimension corresponding to the
11 length of the linear light sensor set, and during a second experiment, a second image is
12 created having a second number of pixels in the dimension corresponding the length of the
13 linear light sensor set, the second number of pixels being fewer than the first.

1 27. The system of claim 26, wherein the set comprises at least two linear
2 light sensors.

1 28. The system of claim 26, wherein the number of pixels in the second
2 image is reduced by selecting fewer than all of the pixels from the linear light sensor set.

1 29. The system of claim 26, wherein the number of pixels the second
2 image is reduced by binning some or all of the pixels from the linear light sensor set.

1 30. The system of claim 26, wherein each reading of light falling on a
2 particular linear sensor results in a single numerical representation of the amount of light
3 falling on the particular sensor.

1 31. A system for producing an oblong illumination field, the system
2 comprising:
3 a laser that produces a beam;
4 a cylindrical lens that receives the beam and causes the beam to converge in
5 only a first axis;
6 an objective lens that is part of an infinity-corrected optical system, the
7 objective lens receiving the beam after the cylindrical lens and converging the beam in a
8 second axis orthogonal to the first.

1 32. The system of claim 31, further comprising a wavelength-selective
2 mirror between the cylindrical lens and the objective lens.

1 33. The system of claim 31, wherein the objective lens is spaced from the
2 cylindrical lens by a distance less than the focal length of the cylindrical lens.

1 34. The system of claim 31, wherein the objective lens is spaced from the
2 cylindrical lens by a distance greater than the focal length of the cylindrical lens.

1 35. The system of claim 31, wherein the beam is diverging in the first axis
2 as it leaves the objective lens.

1 36. The system of claim 31, wherein the beam is converging in the first
2 axis as it leaves the objective lens.

1 37. A method of performing cytometry, the method comprising:
2 illuminating, using a light source, an oblong field at a scanning region,
3 wherein illuminating the oblong field further includes concentrating, using a light shaping
4 element, light from the light source onto the oblong field;

5 focusing, using an optical system, an image of a portion of the scanning region
6 onto a linear light sensor; and
7 repeatedly taking readings of light falling on the linear sensor while a cell is
8 transported through the scanning region and illuminated by the light source.

1 38. The method of claim 37, further comprising passing the light falling on
2 the linear light sensor through a slit aperture proximate the linear light sensor, such that the
3 system performs semi-confocal imaging.

1 39. A method of performing cytometry, the method comprising:
2 illuminating, using a light source, a scanning region;
3 focusing, using an optical system, an image of a portion of the scanning region
4 onto at least two parallel linear light sensors;
5 repeatedly taking readings of light falling on the two parallel light sensors
6 while a cell is transported through the scanning region and illuminated by the light source.

1 40. The method of claim 39, further comprising combining images
2 gathered by the individual linear light sensors to form an image with improved signal-to-
3 noise characteristics as compared with an image gathered by a single linear light sensor.

1 41. The method of claim 40, wherein combining images further comprises
2 digitally combining pixel values from the respective images corresponding to substantially
3 the same respective locations on the cell.

1 42. The method of claim 40, wherein combining images further comprises
2 combining images using time delay integration.

1 43. The method of claim 39, wherein the light source produces
2 illumination including light in at least first and second wavelength bands, and wherein the
3 method further comprises:
4 directing, using the optical system, light emitted from the cell in a third
5 wavelength band primarily to one of the two parallel light sensors, and directing, using the
6 optical system, light emitted from the cell in a fourth wavelength band primarily to the other
7 of the two parallel linear light sensors.

1 44. The method of claim 39, further comprising focusing, using the optical
2 system, an image of a portion of the scanning region onto at least two sets of parallel linear
3 light sensors, each set comprising at least two linear light sensors.