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(54) Title: REFLECTIVE FOCUSING AND TRANSMISSIVE PROJECTION DEVICE

FIG. 2(a)

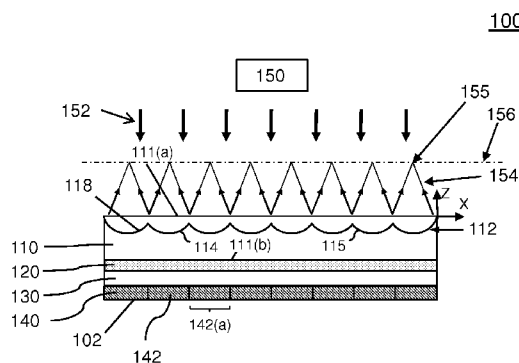
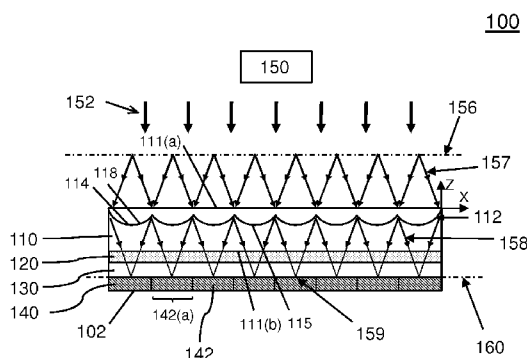


FIG. 2(b)



(57) Abstract: Embodiments of the present invention relate to a reflective focusing and transmissive projection device having a body, a set of reflective-focusing components and a light detector. The body has a surface layer with first and second surfaces, and a detecting layer outside the second surface. The set of reflective-focusing components is in the surface layer. Each reflective-focusing component has a contouring element and a curved reflective element conformed to the contouring element. The curved reflective element is configured to reflect light of a first type, transmit light of a second type and focus the light of the first type outside the first surface of the surface layer. The light detector is in the detecting layer, and is configured to receive light and generate light data associated with the received light. Also, the contouring element can be configured to focus the light of the second type on the light detector.



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REFLECTIVE FOCUSING AND TRANSMISSIVE PROJECTION DEVICE**CROSS-REFERENCES TO RELATED APPLICATIONS**

[0001] This is a non-provisional application of and claims priority to U.S. Provisional Patent Application No. 61/244,363 entitled “Reflective Focusing and Transmissive Projection Device” filed on September 21, 2009 and to U.S. Provisional Patent Application No. 61/251,237 entitled “Approaches for Building Compact Fluorescence Microscopes” filed on October 13, 2009. These provisional applications are hereby incorporated by reference in their entirety for all purposes.

[0002] This non-provisional application is related to the following co-pending and commonly-assigned patent applications, which are hereby incorporated by reference in their entirety for all purposes:

- U.S. Patent Application No. 12/398,050 entitled “Optofluidic Microscope Device with Photosensor Array” filed on March 4, 2009.
- U.S. Patent Application No. 12/399,823 entitled “Scanning Illumination Microscope” filed on March 6, 2009.

BACKGROUND OF THE INVENTION

[0003] Embodiments of the present invention generally relate to reflective focusing and transmissive projection devices. More specifically, certain embodiments relate to reflective focusing and transmissive projection devices used in imaging and sensing applications such as microscopy or photography.

[0004] Microscopes and other optical microscopy devices are used extensively in modern biomedicine and bioscience. Typically, conventional microscopes include an objective lens, a platform for supporting a specimen, and an eyepiece containing lenses for focusing images. These conventional microscope designs have bulky optics, and have proven to be expensive and difficult to miniaturize. Further, since high resolution objective lenses have a limited field of view, the specimen stage must be moved to increase the scope of the view, which can be time-consuming.

[0005] A conventional confocal laser scanning microscope uses a laser beam to provide light which is focused by an objective lens into a small focal volume at the specimen. Scattered and reflected laser light as well as any fluorescence light from the illuminated spot on the specimen is collected by the objective lens. The collected light is transmitted to a beam splitter which separates out the light of interest and passes it to a detection apparatus. As the laser scans over a plane of interest, an image can be obtained of the specimen. This technique can be time-consuming since the laser must scan over the specimen to collect sufficient light data for an image. In addition, the separate component of the beam splitter can make the device bulky.

BRIEF SUMMARY OF THE INVENTION

[0006] Embodiments of the present invention relate to a reflective focusing and transmissive projection device used in imaging and sensing applications such as microscopy or photography.

[0007] One embodiment is directed to a reflective focusing and transmissive projection device comprising a body, a set of reflective-focusing components and a light detector. The body has a surface layer with first and second surfaces, and a detecting layer outside the second surface of the surface layer. The set of reflective-focusing components is in the surface layer. Each reflective-focusing component has a contouring element and a curved reflective element conformed to the contouring element. The curved reflective

element is configured to reflect light of a first type and transmit light of a second type. The curved reflective element is further configured to focus the light of the first type to outside the first surface of the surface layer. The light detector is in the detecting layer, and is configured to receive light and generate light data associated with the received light. In some cases, the contouring element is configured to focus the light of the second type on the light detector.

[0008] Another embodiment is directed to a reflective focusing and transmissive projection system comprising a reflective focusing and transmissive projection device and a processor in electronic communication with the light detector. The reflective focusing and transmissive projection device includes a body, a set of reflective-focusing components and a light detector. The body has a surface layer with first and second surfaces, and a detecting layer outside the second surface of the surface layer. The set of reflective-focusing components is in the surface layer. Each reflective-focusing component has a contouring element and a curved reflective element conformed to the contouring element. The curved reflective element is configured to reflect light of a first type and transmit light of a second type. The curved reflective element is also configured to focus the light of the first type to outside the first surface of the surface layer. The light detector is in the detecting layer, and is configured to receive light and generate light data associated with the received light. The processor is processor configured to receive the light data from the light detector and analyze the light data.

[0009] Another embodiment is directed to a reflective focusing and transmissive projection device comprising a body, a one-dimensional array of reflective-focusing components and a light detector. The body defines a fluid channel and comprises a surface layer having a first surface proximal to the fluid channel and a second surface. The body also comprises a detecting layer outside the second surface of the surface layer. The one-dimensional array of reflective-focusing components is located in the surface layer. Each reflective-focusing component has a contouring element and a curved reflective element conformed to the contouring element. The curved reflective element is configured to reflect light of a first type and transmit light of a second type from the fluid channel. The curved reflective element is also configured to focus the light of the first type to the fluid channel, wherein the one-dimensional array of reflective-focusing components extends substantially across from a first lateral side to a second lateral side of the fluid channel. The light detector

is in the detecting layer, and is configured to receive light and generate time varying data associated with the received light as a specimen passes through the fluid channel.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] **FIG. 1** is a schematic drawing of components of a reflective focusing and transmissive projection system, according to embodiments of the invention.

[0011] **FIG. 2(a)** is a schematic drawing of a cross-sectional view of components of the reflective focusing and transmissive projection device comprising a multi-layered body, and an illumination source providing a uniform light field of light of a first type, according to embodiments of the invention.

[0012] **FIG. 2(b)** is a schematic drawing of a cross-sectional view of components of the reflective focusing and transmissive projection device comprising a multi-layered body, and an illumination source providing a uniform light field of light of a first type, according to embodiments of the invention.

[0013] **FIG. 3** is a cross-sectional view taken along a diagonal line showing components of a reflective focusing and transmissive projection device having optofluidic elements, according to embodiments of the invention.

[0014] **FIG. 4** shows a block diagram of subsystems that may be present in computer devices that are used in a reflective focusing and transmissive projection system, according to embodiments of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0015] Embodiments of the present invention will be described below with reference to the accompanying drawings. Embodiments include a reflective focusing and transmissive projection device having a set of reflective-focusing components for reflecting and focusing light of a first type (e.g., excitation light) into a pattern of tightly focused light spots (e.g., point illuminations) on a specimen outside one side of a surface layer. The set of reflective-focusing components also transmits and/or focuses light of a second type (e.g., specimen emissions) onto a light detector outside the other side of the surface layer. The light detector can generate light data based on the received light, which can be used to image or otherwise analyze the specimen.

[0016] One embodiment of the reflective focusing and transmissive projection device includes a multi-layer body having a surface layer with first and second surfaces (sides), and a detecting layer outside the second surface. A specimen is located outside the first surface. The surface layer has a set of reflective-focusing components. Each reflective-focusing component having a curved reflective element (e.g., reflective coating/film) on a curved surface of the contouring element (e.g., surface of a lenslet array or curved surface array). The curved reflective elements reflect light of a first type (e.g., excitation light) from an illumination source and focus the reflected light into a first light field pattern of tightly focused light spots (e.g., point illuminations) to a focal plane near the specimen. The curved reflective elements receive and transmit light a second type (e.g., emission light) from the specimen, and the contouring elements focus the light into a second light field pattern at the light detector.

[0017] In an exemplary photoluminescence operation, an illumination source provides an excitation light to a reflective focusing and transmissive projection device. The curved reflective elements in the surface layer reflect the excitation light (e.g., single wavelength or polarization) and focus it into a pattern of tightly focused light spots (e.g., point illuminations) on a specimen outside the surface layer. The light spots are scanned (e.g., raster scanned or linearly scanned) over an area larger than the coverage area of the corresponding curved reflective element. In response to the light spots, the specimen emits light of another type (e.g., different wavelength or polarization). The curved reflective elements transmit the emissions and the contouring elements focus the emissions into another light field pattern on the light detector. The light detector generates light data based on the

emissions and uses the light data to image or otherwise analyze the specimen. The focusing and transmissive projection device may optionally include a filter located between the surface layer and the light detector. The filter can block any excitation light that may have passed through the surface layer to help ensure that the light received by the light detector is primarily due to emissions from the specimen.

[0018] Embodiments of the invention provide one or more technical advantages. One advantage is that the device has few components in a simple multi-layered arrangement. Since the body is a multilayered structure, the device can be fabricated inexpensively using standard semiconductor and micro/nanofabrication procedures. Also, a multilayered structure can be easily miniaturized. Another advantage is the use of curved reflective elements (e.g., lenslet array with reflective coating) to make efficient use of the examining (excitation) light field. Some embodiments include curved reflective elements capable of reflecting over 95% of the examining light field into the light spots. The use of curved reflective elements to perform reflective focusing is particularly useful because it has been demonstrated that reflective focusing can actually provide a higher numerical aperture than if a comparable surface is used as a refractive surface for transmissive focusing as shown in F. Merenda, J. Rohner, J. M. Fournier, and R. P. Salathe, "Miniaturized high-NA focusing-mirror multiple optical tweezers," *Optics Express* 15, 6075-6086 (2007), which is hereby incorporated by reference in its entirety for all purposes. By using the curved surfaces, the examining light can be efficiently collected and focused into point illuminations on the specimen. This advantage is particularly useful in fluorescent applications to activate fluorophores. Another advantage is an adjustable plane of focus. The device can be designed to place the focal plane of the light spots at a specific height outside the surface layer. In this way, the light spots can be positioned through a specimen being examined. Designing the focus to be at a plane running through the specimen will provide high quality detection and high resolution images. Another advantage is that the reflective focusing and transmissive projection device can simultaneously capture light data over a wide field of view while generating high resolution images. The device can raster scan or linearly scan the light spots over the specimen to project light over a large field of view. In addition, the emissions can be collected and focused into higher intensity light projections which are easier to detect. With higher intensity light data being received by the light detector, the reflective focusing and transmissive projection device can generate high resolution images and/or other high quality detection analyses. Since the reflective focusing and transmissive projection device can

simultaneously provide high resolution images and capture light data over a wide field of view, the reflective focusing and transmissive projection device can provide rapid processing of high quality images and other analyses.

[0019] Some embodiments of the invention provide specialized advantages when used in fluorescence and phosphorescence applications. In these applications, emission from a specimen can be weak and difficult to detect. An advantage of using the reflective focusing and transmissive projection device is that the reflective-focusing components can efficiently capture emissions from the specimens and focus the emissions to the light detector. Since these reflective-focusing components are located in the surface layer, they can be placed in close proximity to a specimen to efficiently collect the emissions from the specimen. In addition, the emissions can be focused by the reflective-focusing components into focused light projections. Since the focused light projections can be of higher intensity than the emissions from specimen, the emissions will be easier to detect by the light detector, which can improve the quality of the analyses performed and resolution of the images generated.

[0020] **I. Reflective Focusing and Transmissive Projection System**

[0021] **FIG. 1** is a schematic drawing of components of a reflective focusing and transmissive projection system **10**, according to embodiments of the invention. System **10** includes a reflective focusing and transmissive projection device **100** in electronic communication with a computer **200**. The computer **200** can process data communicated from the reflective focusing and transmissive projection device **100**. The computer **200** includes a processor **202** coupled to a computer readable medium (CRM) **204**. In other embodiments, the processor **202** and/or computer readable medium **204** may be a component of the reflective focusing and transmissive projection device **100** or may be integrated into a component of the reflective focusing and transmissive projection device **100**. Although not shown, system **10** can also include other suitable devices such as a display (e.g., a monitor) coupled to the processor **202**, other processors, other filters, an illumination source, a specimen stage, a scanning device, etc. Although the system **10** is shown to have a single focusing and transmissive projection device **100**, other embodiments may have two or more focusing and transmissive projection devices **100**.

[0022] **FIG. 1** includes a cross-sectional view of components of the reflective focusing and transmissive projection device **100** having a body **102** with a first surface **111(a)** and second surface **111(b)**, according to embodiments of the invention. The reflective

focusing and transmissive projection device **100** also includes an x-axis and a z-axis that lie in the cross-sectional plane. The z-axis is orthogonal to the second surface **111(b)**. The x-axis is orthogonal to the z-axis.

[0023] A body **102** can be a multi-layer structure or a single, monolithic structure. In the illustrated example, the body **102** is a multi-layer structure including a surface layer **110**, a filter layer **120**, an intermediate layer **130**, and a detecting layer **140** having a light detector **142**. Each layer of the body **102** may be made of any suitable material(s) having any suitable thickness, and may include any suitable device(s) (e.g., a light detector). For example, the intermediate layer **130** can be made of an opaque or semi-opaque material (e.g., epoxy). Other embodiments may integrate, omit, add, or change the location of one or more layers in the body **102**. For example, some embodiments do not include the filter layer **120** and/or the intermediate layer **130**. As another example, an embodiment may locate the filter layer **120** at the first surface of the surface layer **110**.

[0024] In addition to the first surface **111(a)** and the second surface **111(b)**, the surface layer **110** includes a set of reflective-focusing components **112** having one or more reflective-focusing components **114**. A reflective-focusing component **114** refers to any suitable device(s) capable of reflecting and focusing light of a first type outside the first surface **111(a)** of the surface layer **110**, and transmitting and/or focusing light of a second type outside the second surface **111(b)** of the surface layer **110**. A set of reflective-focusing components **112** refers to one or more reflective-focusing components **114** that are capable of collectively reflecting and focusing light of a first type into a first light field pattern outside the first surface **111(a)** of the surface layer **110**, and transmitting and/or focusing light of a second type into a second light field pattern that is passed through the filter layer **120** to the light detector **142** in the light detecting layer **140**.

[0025] In other embodiments, the surface layer **110** may include additional, alternative or fewer components. For example, the surface layer **110** may have a transparent protective material placed on the set of reflective-focusing components **112**.

[0026] In many embodiments, a reflective focusing and transmissive projection device **100** with a multi-layered body **102** can be fabricated using standard semiconductor and micro/nanofabrication procedures. In one example, the detecting layer **140** comprising a light detector **142** (e.g., CMOS sensor) can be coated with the filter layer **120** capable of blocking the light of a first type with good efficiency. The set of reflective-focusing

components **112** (e.g., lenslet array with reflective coating (e.g., dichroic filter)) can be placed directly on top of the filter layer **120** to form the surface layer **110**.

[0027] Light of a first type refers to light having a first light property or set of light properties. Light of a second type refers to light having a second light property or light properties different from the first light property or properties. Any suitable light property or combination of light properties can be used. Some examples of suitable light properties include polarization, wavelength, frequency, intensity, phase, orbital angular momentum, etc. For example, light of the first type may have a wavelength 1 and light of the second type may have a wavelength that is different from wavelength 1. In one embodiment, light of a first type is light having a first wavelength (e.g., 488 nm) and light of a second type is any light of a different wavelength or light of a select second wavelength (e.g., 510 nm) different from the first wavelength. In another embodiment, light of a first type is light having a wavelength of a plurality of wavelengths (e.g., spectral bandwidth between 488 nm to 500nm) and light of a second type is any light having a different wavelength not belonging to the plurality of wavelengths or light having a particular second wavelength (e.g., 510 nm) that does not belong to the plurality of wavelengths. In yet another embodiment, light of a first type is light having a first polarization and light of a second type is light having different polarization. In exemplary fluorescence or phosphorescence embodiments, light of a first type is an excitation light and light of a second type is an emission light (emissions) from the specimen in response to receiving excitation light. For example, the light of the first type can be fluorescence, 2-photon or higher order fluorescence, Raman, second harmonic or higher order, or other emission mechanism that results in emissions at a different wavelength or other different property than the excitation light.

[0028] In operation, the set of reflective-focusing components **112** receives light of the first type (e.g., excitation light). The set of reflective-focusing components **112** reflects and focuses the received light of the first type into a first light field pattern at a focal plane outside the surface **111(a)** of the surface layer **110**. The set of reflective-focusing components **112** also transmits light of a second type. In some cases, the set of reflective-focusing components **112** also focuses the transmitted light into a second light field pattern at a focal plane outside the second surface **111(b)** of the surface layer **110**. The filter layer **120** between the detecting layer **140** and the surface layer blocks any light of the first type. The light detector **142** in the light detecting layer **140** detects the second light field pattern and

generates light data associated with the detected light. The processor **202** receives the light data from the light detector **142** and analyzes the light data.

[0029] A light field pattern refers to an arrangement of light projections. Some examples of suitable arrangements include a one-dimensional array, a two-dimensional array, and a multiplicity of one-dimensional and/or two-dimensional arrays. The arrays can have any suitable orientation or combination of orientations. A first light field pattern refers to a suitable arrangement of light projections of light of the first type reflected and focused by the set of reflective-focusing components **112** to a first focal plane **156** (shown in FIG. 2(a)). A second light field pattern refers to a suitable arrangement of light projections of light of the second type transmitted and focused by the set of reflective-focusing components **112** to a second focal plane **160** (shown in FIG. 2(b)). In some cases, the first light field pattern includes light projections (e.g., light spots) of excitation light that can be used to activate the fluorophores in a specimen located outside the first surface **111(a)** of surface layer **110**.

[0030] The light projections in the first and second light field patterns can have any suitable dimension and light properties (e.g., phase, amplitude, etc.). Some examples of suitable diameters of light projections are 1 micron, 5 microns, 10 microns, 20 microns, etc. In one embodiment, the light projections are light spots. In some cases, the light spots can be tightly focused light spots (e.g., point illuminations) with small diameters (e.g., 1 micron, 2 microns, etc.). In another embodiment, the light projections may be larger than the size of a light detecting element **142(a)** of the light detector **142**. In some embodiments, the light properties are uniform over the light projections. In other embodiments, the phase and/or amplitude of the light may vary spatially over the light projection.

[0031] In **FIG. 1**, each reflective-focusing component **114** includes a contouring element **118** (e.g., lens or curved surface) and a curved reflective element **115** (e.g., reflective coating) coupled to a curved surface of the contouring element **118**. The curved reflective element **115** may be coupled to the using any suitable method.

[0032] A curved reflective element **115** refers to any suitable component capable of reflecting light of the first type, transmitting light of a second type, and focusing the reflected light into a focused light projection (e.g., light spot) outside the first surface **111(a)** of the surface layer **110**. In many cases, the curved reflective element **115** is a reflective coating/film deposited on a curved surface of the contouring element **118**. The reflective coating/film can be made of any suitable material of any suitable thickness that reflects light

of the first type and transmits light of the second type. The reflective coating/film can have any suitable reflectivity and transmissivity properties. The curved reflective element **115** can be designed with any suitable curvature to provide a suitable focal length to focus the light of the first type to a desired focal plane. In FIG 2(a), a curved reflective element **115** is designed to focus the light to the first focal plane **156** outside the first surface **111(a)** of the surface layer **110**. The use of curved reflective surfaces to perform reflective focusing is particularly useful because it has been demonstrated that reflective focusing can actually provide a higher numerical aperture than if a comparable surface is used as a refractive surface for transmissive focusing as shown in F. Merenda, J. Rohner, J. M. Fournier, and R. P. Salathe, "Miniaturized high-NA focusing-mirror multiple optical tweezers," *Optics Express* 15, 6075-6086 (2007), which is hereby incorporated by reference in its entirety for all purposes. As an example, a lenslet of curvature $0.024 \text{ micron}^{-1}$, refractive index of 1.5 and diameter of 30 micron can focus light to a spot of size 1.4 micron (FWHM). If the same lenslet is reflective coated and is used as a reflective surface, it will focus light to a spot of size 0.4 micron (FWHM).

[0033] A contouring element **118** refers to any suitable structure for providing a curved surface for supporting the curved reflective element **115**. For example, the contouring element **118** may be a lens (e.g., lenslet) or a curved surface molded into a suitable material (e.g., SU-8 film). The contouring element **118** can have any suitable refractive index ratio (e.g., 2, 3, etc.).

[0034] In some embodiments, the contouring element **118** also focuses light passing through the contouring element **118**. In these embodiments, the contouring element **118** may have a high refractive index ratio (e.g., 3, 4, 5, etc.). In one embodiment, the contouring element **118** helps focus the light of the first type into the light projection (e.g., light spot) outside the first surface **111(a)** of the surface layer **110**. In another embodiment, the contouring element **118** focuses the light of the second type outside the second surface **111(b)** of surface layer **110**. For example, the contouring element **118** can be a convex lens (e.g., lenslet) that can focus the light of the second type transmitted through the curved reflective element **115** to outside the second surface **111(b)** of the surface layer **110** to the light detector **142**.

[0035] The set of reflective-focusing components **112** may be in any suitable form. Some examples of suitable forms include a lens array having a reflective coating/film on a

surface of each lens, or a curved surface array having a reflective coating/film on the curved surfaces. In a simple embodiment, the set of reflective-focusing components **112** is in the form of a lenslet array. One of the outer surfaces of the lenslets is coated with a reflective coating for reflecting light of a first type (e.g., light of wavelength 1). When light of the first type is shined on the reflective focusing and transmissive projection device **100**, the curved reflective element **115** reflects and focuses the light of the first type to form a number of tightly focused light spots **155**. The tightly focused spots **155** can be used to scan samples (specimens) by either moving the light spots **155** over the samples or by moving the samples over the light spots **155**. The emissions from the samples at the location of the light spots **155** can be transmitted and brought to a focus at the sensor (light detector **142**). In some cases, the emissions from each light spot **155** can be uniquely distinguished as long as the emissions (light projections) do not overlap significantly. The emissions can be fluorescence, 2-photon or higher order fluorescence, Raman, second harmonic or higher order, or other emission mechanism that results in emissions at a different wavelength or other different property than the excitation light.

[0036] In one exemplary embodiment, the set of reflective-focusing components **112** is in the form of a convex lens array (e.g. lenslet array) having the reflective coating/film deposited or otherwise coupled to one of the curved surfaces of the convex lenses (e.g., lenslets). Each reflective-focusing component **114** is a convex lens having a first curved surface proximal to or comprising the first surface **111(a)** of the surface layer **110** and a second curved surface distal the first surface **111(a)** and proximal to the second surface **111(b)**. The reflective coating/film is deposited on the second curved lens surface most distal to the first surface **111(a)**. In this embodiment, the curved reflective element **115** is the reflective coating (e.g., dichroic filter) deposited on the curved second lens surface of each of the lenses. The curved reflective element **115** reflects light of the first type toward the first surface **111(a)** of the surface layer **110** and transmits light of the second type toward the second surface **111(b)** of the surface layer **110**. The curved reflective element **115** and the contouring element **118** focus the light of the first type into focused light projections outside the first surface **111(a)** of the surface layer **110**. If the set of reflective-focusing components **112** is located at a suitable distance from the first surface **111(a)**, the light of the first type will be focused into a first light pattern having light projections (e.g., light spots) to a focal plane **156** outside the first surface **111(a)** of the surface layer **110**.

[0037] In another exemplary embodiment, the set of reflective-focusing components **112** may be in the form of a curved surface array (e.g., surface with an array of curved indentations) formed in a suitable material with a reflective coating/film deposited on or otherwise coating to the curved surfaces to reflect light of a first type and transmit light of a second type. The curved surfaces may be formed in the material using any suitable method. In one case, a lens array (e.g., lenslet array) may be used as a mold for form the shape of the curved indentations in a moldable material and then the reflective coating may be deposited or otherwise coupled to the inside of the molded curved surfaces. In this embodiment, the reflective coating/film on the curved surfaces is the curved reflective element **115**, and the molded material is the contouring element **118**. If the material has a refractive index variation, the contouring element **118** can also focus the light of a second type transmitted through the curved reflective elements **115**.

[0038] In many embodiments, the primary enabling concept is the use of a curved surface that is treated to reflect light (e.g., coated with a reflective coating) of a first type (e.g., wavelength 1 or polarization 1) to project an interesting first light field pattern, and 2) to use the same surface in combination with one or more other curved surfaces or other elements with material refractive index variations to transmit and project a second light field pattern onto the light detector **142**. In one case, the first light field pattern is a grid of tightly focused light spots of light of the first type and the second light field pattern is a grid of tightly focused light spots of light other than light of the first type.

[0039] Any suitable number of reflective-focusing components (e.g., 1, 10, 50, 100, 200, etc.) can be used in any suitable arrangement. Some examples of suitable arrangements include a one-dimensional array, a two-dimensional array, and a multiplicity of one-dimensional and/or two-dimensional arrays. The arrays can have any suitable orientation or combination of orientations. In an optofluidic application, the set of reflective-focusing components **112** may be arranged in one or more one-dimensional arrays extending diagonally at an angle, α with respect to the x-axis. Although the illustrated embodiment shows adjacent reflective-focusing components **114** in the set of reflective-focusing components **112** located next to each other without a separation, other embodiments may have a set of reflective-focusing components **112** with a separation between reflective-focusing components **114**. A separation between adjacent reflective-focusing components **114** can allow the system **10** to more easily ascribe the detected light projections to the appropriate light spot. In some cases, the number of reflective-focusing components **114** in

the set of reflective-focusing components **112** that is required in the device **100** can be determined from the dimensions of the wide area view covered by the device **100** at any one time.

[0040] The set of reflective-focusing components **112** can have any suitable location/orientation in the surface layer **110**. The set of reflective-focusing components **112** may be located parallel to and at a suitable distance from the first surface **111(a)** of the surface layer **110** in order to generate a first light field pattern at a first focal plane **156** outside the first surface **111(a)** of the surface layer **110**. In some embodiments, the location of the reflective-focusing components **114** may be changed to vary the location of the first focal plane **156**. The reflective-focusing components **112** may be located at a suitable distance from the second surface **111(b)** of the surface layer **110** in order to generate the second light field pattern at a second focal plane **160** outside the second surface **111(b)** of the surface layer **110**. The second focal plane **160** may be a surface of the light detector **142**. In an optofluidic example, the set of reflective-focusing components **112** may be located in a diagonal orientation across the width of the surface layer **110** to generate light spots in a diagonal pattern.

[0041] The curved reflective element **115** can be made of any suitable form of material or combination of materials of any suitable dimensions and having any suitable reflectivity and transmissive properties. For example, the curved reflective element **115** may be a reflective coating or film (e.g. dichroic filter, dielectric filter, etc.) on a curved surface. In one case, the curved reflective element **115** has a reflectivity of greater than 95% for select wavelengths or polarizations.

[0042] The body **102** of the reflective focusing and transmissive projection device **100** may optionally include a filter layer **120** located between the surface layer **110** and the intermediate layer **130**. The filter layer **130** may include one or more filters. A filter refers to any suitable device (e.g., an optical filter) capable of selectively transmitting light having certain properties (e.g., wavelength, spectral bandwidth, polarization, range of polarizations) while blocking the remainder of the light. Any suitable type of filter may be used. Some examples of suitable types of filters include a polarization filter, dichroic filter, a monochromatic filter, monochromatic filters, etc. In many embodiments, the filter layer **130** includes one or more filters (e.g., an interference filter and an absorption filter) designed to block light of the first type. In one exemplary embodiment, the curved reflective element **115**

and the filter(s) in the filter layer **130** are designed to block light of the first type from reaching the light detector **142**.

[0043] The body **102** also includes a detecting layer **140** located between the surface layer **110** and the intermediate layer **130**. The detecting layer **140** includes a light detector **142**. A light detector **142** (e.g., photosensor) refers to any suitable device capable of detecting light and generating signals with data about the intensity, wavelength, and/or other information about the light being detected. The signals from the light detector **142** can be in the form of electrical current that results from the photoelectric effect. Some examples of suitable light detectors **140** include a charge coupled device (CCD) or a one-dimensional or two-dimensional array of photodiodes (e.g., avalanche photodiodes (APDs)). The light detector **142** can also be a complementary metal-oxide-semiconductor (CMOS) or photomultiplier tubes (PMTs). Other suitable light detectors **140** are commercially available.

[0044] The light detector **142** includes one or more discrete light detecting elements **142(a)** of any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square). For example, light detecting elements in CMOSs and CCDs may be 1-10 microns and in APDs and PMTs may be as large as 1-4 mm. The light detecting elements **142(a)** can be arranged in any suitable form such as a one-dimensional array, a two-dimensional array, or a multiplicity of one-dimensional and/or two-dimensional arrays. The arrays can be in any suitable orientation or combination of orientations. In some cases, the light detecting elements **142(a)** can be arranged to correspond to the reflective-focusing components **114**. For example, the light detecting elements **142(a)** can have the same form as the set of reflective-focusing component **112** so that one or more light detecting elements **142(a)** corresponds to a reflective-focusing component **114**. In **FIG. 1**, each light detecting element **142(a)** corresponds to a reflective-focusing component **114**.

[0045] System **10** also includes a computer **200** communicatively coupled to the light detector **142**. The computer **200** comprises a processor **202** and a computer readable medium **204** (CRM). Alternatively, the computer **200** can be a separate device.

[0046] The processor **202** receives signals with light data from the light detector **142** associated with the light received by the light detector **142**. The light data may include the intensity of the light, the wavelength(s) of the light, the polarization(s) of the light and/or other information about the light received by the light detector **142**. The processor **202** executes code stored on the CRM **204** to perform some of the functions of system **10** such as

interpreting the light data from the light detector **142**, performing analyses from the light data and/or generating images from the light data.

[0047] The CRM **204** (e.g., memory) stores code for performing some functions of system **10**. The code is executable by the processor **202**. In one embodiment, the CRM **204** comprises: a) code for interpreting the light data received from the light detector **142**, b) code for performing analyses based on the light data, c) code for generating one or more images from the light data, d) code for displaying one or more images, and e) any other code for performing the functions of system **10**.

[0048] Although not shown, system **10** may include other suitable components such as a display, a scanning device, other processors, etc. For example, some embodiments may include a display communicatively coupled to the processor **204**. Any suitable display may be used. In some cases, the display may be a part of the reflective focusing and transmissive projection device **100**. The display may provide information such as images to a user of the system **10**.

[0049] In many embodiments, the system **10** also includes a suitable raster or linear scanning device to perform wide field of view imaging or sensing. In one case, system **10** may include a scanning device capable of raster scanning or linearly scanning the light spots of a first type (e.g., wavelength **1**) over an area of the specimen equal to or larger than the area covered by each reflective-focusing component **114**. Alternatively, the scanning device may raster scan or linearly scan the specimen over the light spots. Any type of scanning can be used, and in any orientation. For example, the scanning device may linearly scan the specimen in a direction at an angle to the x-axis.

[0050] In one embodiment, the reflective focusing and transmissive projection device **100** may use electromagnetic radiation or acoustic waves instead of light. In this embodiment, the light field **152** may be a suitable electromagnetic field or an acoustic wave in any medium. Some examples of suitable electromagnetic fields include an X-ray, terahertz field, radio wave, etc. In this embodiment, the reflective-focusing component **114** is a suitable device(s) for reflecting and focusing the electromagnetic radiation or acoustic waves of a first type outside the surface **111(a)** of the surface layer **110**, and transmitting and/or focusing the electromagnetic radiation or acoustic waves of a second type outside the second surface **111(b)** of the surface layer **110**. The set of reflective-focusing components **112** collectively reflects and focuses the electromagnetic radiation or acoustic waves of a first

type into a first pattern outside the first surface **111(a)** of the surface layer **110**, and transmitting and/or focusing the electromagnetic radiation or acoustic waves of a second type into a second pattern that is passed through the filter layer **120** to the detector **142** of electromagnetic radiation or acoustic waves. In this embodiment, the reflective coating/film reflects the electromagnetic radiation or acoustic waves of the first type and transmits the electromagnetic radiation or acoustic waves of the second type. The filter layer **120** in this embodiment will include a filter for blocking the electromagnetic radiation or acoustic waves of the first type.

[0051] II. Operating Concept

[0052] FIGS. 2(a) and 2(b) illustrate an operating concept of a reflective focusing and transmissive projection device **100**, according to embodiments of the invention.

[0053] FIG. 2(a) and FIG. 2(b) include a cross-sectional view of components of the reflective focusing and transmissive projection device **100** comprising a multi-layered body **102** and an illumination source **150** providing a uniform light field of light of a first type **152** (e.g., a single wavelength, a single polarization, a spectral bandwidth, etc.). The reflective focusing and transmissive projection device **100** also includes an x-axis and a z-axis that lie in the cross-sectional plane. The z-axis is orthogonal to the second surface **111(b)**. The x-axis is orthogonal to the z-axis.

[0054] In the illustrated example, the body **102** is a multi-layered structure including a surface layer **110** having a first surface **111(a)** and an opposing second surface **111(b)**, an optional filter layer **120**, an optional intermediate layer **130** and a detecting layer **140** having a light detector **142** (e.g., a photosensor). Each layer of the body **102** may be made of any suitable material or materials having any suitable thickness, and may include any suitable device(s) (e.g., light detector **142**). In one case, the intermediate layer **130** can be made of an opaque or semi-opaque material (e.g., epoxy). Other embodiments may integrate, omit, add, or change the location of one or more layers of the body **102**.

[0055] In addition to the first surface **111(a)** and the second surface **111(b)**, the surface layer **110** also includes a set of reflective-focusing components **112** including eight (8) reflective-focusing components **114**. Although eight (8) reflective-focusing components **114** are included in the illustrated embodiment, any suitable number of reflective-focusing components **114** can be included (e.g., 1, 10, 100, etc.) in the set **112**. Also, other embodiments may have a body **102** which omits, integrates, modifies, or adds components.

[0056] The set of reflective-focusing components **112** reflects and focuses the light of the first type into a first light field pattern having eight light spots **155** on a first focal plane **156** outside the first surface **111(a)** of the surface layer **110**, and transmits and focuses light of the second type into a second light field pattern having eight light spots **159** at a second focal plane **160** outside the second surface **111(b)** of the surface layer **110**. In the illustrated embodiment, the set of reflective-focusing components **112** is arranged in a one-dimensional array. In other embodiments, other suitable arrangements can be used such as a two-dimensional array or a multiplicity of one-dimensional and/or two-dimensional arrays. The arrays can have any suitable orientation or combination of orientations.

[0057] **FIG. 2(a)** shows the trajectory of the light field of the first type in an exemplary operation of the focusing and transmissive projection device **100**, according to embodiments of the invention. In the illustration, the illumination source **150** provides the uniform light field of light of the first type **152** (e.g., excitation light) to the reflective focusing and transmissive projection device **100**. The set of reflective-focusing components **112** receives the light of the first type, and reflects and focuses the received light of into a first pattern of light spots **155** (e.g., point illuminations) at a first focal plane **156** outside the first surface **111(a)** of the surface layer **110**. The first trajectory **154** illustrates the path of the light of the first type as reflected and focused by the set of reflective-focusing components **112** into the light spots **155**. Although the illustrated example shows light of the second type originating from the sites of the light spots **155**, light of the second type may also originate from other areas or sites in other examples.

[0058] **FIG. 2(b)** shows the trajectory of the light field of the second type in the exemplary operation shown in **FIG. 2(a)**, according to embodiments of the invention. In this example, a second trajectory **157** illustrates the path of the light of the second type (e.g., emission light) from the focal spots **155** at the first focal plane **156** and spreading out to the surface **111(a)** of the surface layer **110**. The set of reflective-focusing components **112** receives the light of the second type, and transmits and focuses the received light of the second type into a second pattern of light spots **159** at a second focal plane **160** outside the second surface **111(b)** of the surface layer **110**. The third trajectory **158** illustrates the path of the light of the second type as transmitted and focused by the set of reflective-focusing components **112** into the light spots **159** at the second focal plane **160**. In many cases, the focal plane **160** is coincident with the surface of the light detector **142**. Although light spots

are shown in the illustrated embodiments, other less focused or unfocused light projections can be generated in other embodiments.

[0059] In **FIGS. 2(a)** and **2(b)**, the set of reflective-focusing components **112** reflects light of a first type provided by the illumination source **150** and focuses the reflected light into a first light field pattern at the first focal plane **156** outside the first surface **111(a)** of the surface layer **110**. The set of reflective-focusing components **112** transmits and focuses light of the second type into a second light field pattern at a second focal plane **160** outside the second surface **111(b)** of the surface layer **110**. The second focal plane **160** is coincident with a surface of the light detector **142**. In other embodiments, the first light field pattern and second light field pattern may be focused to other focal planes.

[0060] In some embodiments, reflective focusing and transmissive projection devices **100** are designed for focal plane ranging. These devices **100** are designed to locate the first focal plane **156** at a predefined distance away from the first surface **111(a)**. This can help locate the first focal plane **156** in close proximity to the specimen or on or in the specimen, which can help improve the quality of detection or resolution of the images generated of the specimen. In these embodiments, the set of reflective-focusing components **112** is positioned at a distance from the first surface **111(a)** equal to the focal length of the reflective-focusing components **114** less the predefined distance. By this design, the light spots **155** may be located at the focal plane **156** above the first surface **111(a)**.

[0061] Light of the first type is light having a suitable light property or combination of light properties (e.g., a single wavelength/polarization, range of wavelengths/polarizations, single phase, etc.) selected for reflection. Light of the second type can be light having light properties different from the select light property or properties of the light of the first type. In an exemplary bioluminescence (e.g., fluorescence or phosphorescence) embodiment, light of a first type is an excitation light for activating the fluorophores in a specimen **180** located outside the first surface **111(a)** of the surface layer **110**. The light of the first type may have a select wavelength/polarization. The light of a second type is the emitted light from the fluorophores. The light of the second type may have a different wavelength/polarization from the wavelength/polarization of the light of the first type. Some examples of suitable excitations lights include a fluorescence, two-photon or higher order fluorescence, Raman, second harmonic or higher order, or other emission mechanism that results in emissions at a different wavelength or other light property than the excitation light.

[0062] In the illustrated embodiment, the first light field pattern includes a one-dimensional array of eight light spots **155** focused at the first focal plane **156** and the second light field pattern of a one-dimensional array of eight light spots **159** focused at the second focal plane **160**. The light spots **155**, **159** may have any suitable dimensions. In one case, the light spots **155**, **159** may be tightly confined light spots (e.g., point illuminations). The light properties may be uniform or vary spatially over each light spot. Although the illustrated embodiment shows a light pattern of eight focused light spots in a one-dimensional array, any suitable light pattern (e.g., one-dimensional array, two dimensional array, multiplicity of one and/or two-dimensional arrays having any suitable orientation or orientations) can be used. Also, any suitable number of light spots (e.g., 1, 10, 100, 200, 500, etc.) can be used.

[0063] In **FIGS. 2(a)** and **2(b)**, each reflective-focusing component **114** includes a contouring element **118** (e.g., lens or material formed with curved surface) and a curved reflective element **115** (e.g., reflective coating) coupled to a curved surface of the contouring element **118**. The curved reflective element **115** may be coupled using any suitable method. The curved reflective element **115** can be any suitable component. For example, the curved reflective element **115** may be a reflective coating/film with suitable reflectivity and transmissivity properties. In **FIGS 2(a)** and **2(b)**, each curved reflective element **115** can reflect and focus the light of the first type to a light spot **155** at the first focal plane **156** outside the first surface **111(a)** of the surface layer **110**. In **FIGS 2(a)** and **2(b)**, the contouring element **118** can provide the curved surface for application of the curved reflective element **115** and can focus the light of the second type into a light spot **159** at the second focal plane **160** at the surface of the light detector **142** in the detecting layer **140**. In some cases, the contouring element **118** may also help focus the light of the first type into the light spot **155**. Some examples of suitable contouring elements **118** include a lens, a material molded to have a curved surface, etc.

[0064] The set of reflective-focusing components **112** may be in any suitable form. Some examples of suitable forms include a lens array (e.g., lenslet array) having a reflective coating/film on a surface of one or more lenses, or a curved surface array having a reflective coating/film on its curved surfaces.

[0065] The body **102** of the reflective focusing and transmissive projection device **100** may optionally include a filter layer **120** located between the set of reflective-focusing components **112** and the light detector **142**. The filter layer **120** has one or more filters for

blocking light of the first type. Any suitable filter may be used. Some examples of suitable types include a polarization filter, dichroic filter, a monochromatic filter, monochromatic filters, etc. The curved reflective elements **115** and the filter(s) in the filter layer **120** help prevent the light of the first type (e.g., wavelength 1) from reaching the light detector **142** (e.g., photosensor) in the detecting layer **140**. In some cases, the filter(s) in the filter layer **120** blocks any light of the first type that may have been transmitted through the set of reflective-focusing components **112** to help ensure that the light collected by the light detector **142** is primarily due to emissions from the specimen.

[0066] The body **102** also includes a detecting layer **140** having a light detector **142** (e.g., CCD, APDs, CMOS or PMTs) for detecting light and generating signals with data about the intensity, wavelength, and/or other information about the light being detected. The signals from the light detector **142** can be in the form of electrical current that results from the photoelectric effect.

[0067] The light detector **142** includes discrete light detecting elements **142(a)**. The light detecting elements **142(a)** can have any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square). For example, light detecting elements in CMOSs and CCDs may be 1-10 microns and in APDs and PMTs may be as large as 1-4 mm. In the illustrated example, each light detecting element **142(a)** corresponds to a reflective-focusing component **114**. In the illustrated example, the light detecting elements **142(a)** are arranged in a one-dimensional array to correspond to the one dimensional array of reflective-focusing components **114**. That is, each light detecting element **142(a)** corresponds to a reflective-focusing component **114**. The one-dimensional array may have any suitable orientation. In other embodiments, the light detecting elements **142(a)** can be arranged in other forms such as a two-dimensional array or a multiplicity of one-dimensional and two-dimensional arrays. The arrays can be in any suitable orientation or combination of orientations.

[0068] An illumination source **150** refers to any suitable light source or sources (e.g., a 100 mW 488 nm Cyan CW laser) providing a light field of light of the first type. In the illustrated example, the illumination source **150** provides a uniform light field of light of the first type **152** to the reflective focusing and transmissive projection device **100**. Some suitable sources of light include a LED, laser, white illumination source, etc. In some cases, the illumination source **150** comprises a suitable source of light and a filter for selectively transmitting light having the property or properties of light of the first type **152**. For

example, the illumination source **150** may include a filter that transmits only the excitation light for activating fluorophores in a specimen **180** being examined by the reflective focusing and transmissive projection device **100**. The illumination source **150** may be a component of the reflective focusing and transmissive projection device **100** or may be a separate component. In an exemplary embodiment, the illumination source **150** provides light of a type (e.g., wavelength or polarization) that will cause activation of fluorophores in a specimen being examined or imaged by the reflective focusing and transmissive projection device **100**. Although a light (illumination) field is used in many embodiments, other fields can be substituted for the light field in other embodiments. For example, a suitable electromagnetic field can be used. Some examples of suitable electromagnetic fields include an X-ray, terahertz field, radio wave, etc. As another example, the light field can be substituted with an acoustic wave in any medium.

[0069] In an exemplary operation, the illumination source **150** provides a uniform light field of light of the first type (e.g., light of wavelength **1**) **152** to the surface **111(a)** of the surface layer **110**. The set of reflective-focusing components **112** receives the light and reflects and focuses the light of the first type into a first light field pattern of light spots **155** at the focal plane **156** through a specimen **180** (shown in FIG. 3). The light spots **155** are scanned (e.g., raster scanned or linearly scanned) over areas of the first focal plane **156** corresponding to the reflective-focusing components **114** or over a larger area. By scanning, the device **100** can perform wide field of view imaging or sensing. Either the reflective focusing and transmissive projection device **100** or the specimen stage can be fixed during scanning. In response to receiving the light spots **155**, the specimen transmits (e.g., emits) light of a second type. The light of the second type is emitted from the specimen **180** at a greater intensity where the specimen receives the light spots **155**. Each reflective-focusing component **114** receives light of the second type associated with corresponding light spots. The set of reflective-focusing components **112** transmits and focuses the received light of the second type into a second light field pattern with light spots **159** on corresponding light detecting elements **142(a)** of the light detector **142**. The filter layer **120** blocks any light of the first type received by the filter layer **120** and transmits light of the second type to help ensure that only light of the second type reaches the light detecting elements **142(a)**. The light detector **142** generates signals with light data associated with the received light. The signals are communicated to a processor **202** in the light detector **142** or in a separate

component. The processor **202** uses the light data to compile an image of the specimen **180** or otherwise analyze the specimen **180**.

[0070] In one embodiment, polarization is used as a selection criterion for the light of the first type and second type instead of wavelength or other light property. In this embodiment, the reflective focusing and transmissive projection device **100** has a set of reflective-focusing components **112** that is designed to reflect and focus light having a polarization 1. Each reflective-focusing component **114** includes a curved reflective element **115** designed to selectively reflect light having a polarization 1 and transmit light of other polarization. Each reflective-focusing component **114** also includes a contouring element **118** configured to focus the reflected light having a polarization 1 into light spots on a specimen located outside the first surface **111(a)** of the surface layer **110**. The specimen **180** emits or otherwise transmits light of other polarization. Light of other polarization is transmitted through the curved reflective element **115** to generate a pattern of light projections on the light detector **142**. The light detector **142** receives the light including the light projections and generates signals with light data associated with the light received by the light detector **142**. The signals are communicated to a processor **202** in the light detector **142** or in a separate component. The processor **202** uses the light data to compile an image of the specimen **180** or otherwise analyze the specimen **180**. In some cases, a scanning device can be used to raster scan or linearly scan the first light field pattern of light projections (e.g., light spots) over the area covered by the corresponding reflective-focusing component **114**. In one example, the reflective focusing and transmissive projection device **100** can be fixed and the specimen stage is moved. In another example, the reflective focusing and transmissive projection device **100** can be moved and the specimen stage is fixed. In these cases, the light data generated by the light detector **142** can be used to generate birefringence or scattering images of the specimen.

[0071] **IV. Optofluidic Application**

[0072] In many embodiments, a reflective focusing and transmissive projection device **100** efficiently uses an excitation light field by reflecting and focusing the excitation light into tightly focused, high intensity light spots (e.g., point illuminations). In addition, the reflective focusing and transmissive projection device **100** can be designed to focus the light spots to a focal plane above the floor of the fluid channel **170** where specimens may be located. By focusing the high intensity light spots at a focal plane through the specimens, the

device **100** can achieve high resolution detection/imaging of the specimens. Further, the reflective focusing and transmissive projection device **100** can efficiently collect emissions by focusing and collecting the emissions at the surface layer **110** in close proximity to specimens.

[0073] Incorporating elements of an optofluidic system into the reflective focusing and transmissive projection device **100** provides a highly compact device with highly automatable, high-throughput, and high-resolution (e.g., 0.6 micron or better) bioluminescence emission detection and imaging. Due to its compact size, low-cost and automatable nature, the reflective focusing and transmissive projection device **100** with optofluidic element can be multiplexed into large parallel arrays for cost-effective and high-throughput analyses. Using optofluidic elements also provides advantages such as being able to directly process samples without slide preparation, rapidly image morphological information for specialized analyses (e.g., phenotype characterization), manage large volume analyses in a rapid and cost-effective manner, etc.

[0074] **FIG. 3** is a cross-sectional view taken along a diagonal line showing components of a reflective focusing and transmissive projection device **100** having optofluidic elements, according to embodiments of the invention. In the illustrated embodiment, the reflective focusing and transmissive projection device **100** comprises a multi-layer body **102** and an illumination source **150** providing a uniform light field of light of a first type **152** (e.g., a single wavelength, a single polarization, a range of wavelengths, etc.). The reflective focusing and transmissive projection device **100** also includes an x-axis and a y-axis. The y-axis lies along a longitudinal axis of the fluid channel **170**. The z-axis is orthogonal to the y-axis.

[0075] The body **102** includes a surface layer **110** with a first surface **111(a)** and second surface **111(b)**, a filter layer **120** having an interference filter **120(a)** and an absorption filter **120(b)**, and a detecting layer **140** having a light detector **142**. In addition, the body **102** defines or includes a fluid channel **170** which has a first channel surface and an opposing second channel surface which coincides with the first surface **111(a)** of the surface layer **110**. The body **102** also includes a channel surface layer **172**. The layers of the body **102** may include any suitable material or combination of materials having any suitable thickness, and may include any suitable devices (e.g., light detector). For example, the channel surface layer **172** may be made of Polydimethylsiloxane (PDMS). Other

embodiments may integrate, omit, or add one or more layers or change the location of one or more layers in the body **102**.

[0076] In the illustrated example, two specimens **180** are shown at a specific time ($t = t_1$) as the specimens **180** move through the fluid channel **170** generally in the y-direction. In this example, the reflective focusing and transmissive projection device **100** is using fluorescence or phosphorescence to image or detect the specimens **180**. Although the specimens **180** are shown as cells in the illustrated embodiment, other embodiments may include another suitable object(s). Some examples of suitable objects include biological or inorganic entities. Some examples of biological entities include whole cells, cell components, microorganisms such as bacteria or viruses, cell components such as proteins, etc. Inorganic entities may also be detected or imaged by embodiment of the invention. Although two specimens **180** are shown, any suitable number of specimens may be analyzed by the reflective focusing and transmissive projection device **100**.

[0077] The fluid channel **170** may have any suitable dimensions. For example, the width and/or height of the fluid channel **170** may each be less than about 10 microns, 5 microns, or 1 micron. In some cases, the fluid channel **170** may be sized based on the size of the specimens **180** being detected by the reflective focusing and transmissive projection device **100**. For example, the height of the fluid channel **170** may be at least 10 microns where the specimens **180** being examined are 8 microns to keep the specimens **180** close to the focal plane **156**, which may help improve the quality of the detection or resolution of the imaging.

[0078] In addition to the first surface **111(a)** and the second surface **111(b)**, the surface layer **110** includes a set of reflective-focusing components **112** located at an angle, θ (90 degrees minus α) with respect to the y-axis. The angle, θ can be any suitable angle. In other embodiments, the surface layer **110** may include additional, alternative or fewer components.

[0079] In **FIG. 3**, the set of reflective-focusing components **112** reflects light of the first type provided by the illumination source **150** and focuses the reflected light into a first light field pattern of light spots **155** at the first focal plane **156** outside the first surface **111(a)** of the surface layer **110**. After the first focal plane **156**, the light of the first type spreads. The specimens **180** pass along the first focal plane **156** and the sites of two light spots **155**. In response to the illumination, the specimens **180** emit light of a second type from the sites

of the two light spots **155**. The set of reflective-focusing components **112** transmits and focuses the light of the second type to light projections at the light detector **142**. In the illustrated embodiment, the focused light projections are larger than the light detecting elements **142(a)**. In other embodiments, the first light field pattern and second light field pattern may be focused to other focal planes and may have other light projections.

[0080] In the illustrated example, the light of the first type is an excitation light and the light of the second type is an emission light (e.g., fluorescence, phosphorescence, etc.). The excitation light can have any suitable light property (e.g., a single wavelength, single polarization, etc.) for activating the fluorophores in the specimens. Some examples of suitable excitations lights include a fluorescence, two-photon or higher order fluorescence, Raman, second harmonic or higher order, or other emission mechanism that results in emissions at a different wavelength or other light property than the excitation light. The light of the second type may have a different wavelength/polarization from the select single wavelength/polarization of the light of the first type.

[0081] In the illustrated embodiment, the first light field pattern includes a one-dimensional array of light spots **155** at an angle, θ with respect to the y-axis. The angle, θ can be any suitable angle. The light spots **155** are focused at the focal plane **156**. The second light field pattern includes a two wider light projections at the light detector **142**. The light properties may be uniform or vary spatially over the light spots and wider light projections. Although the illustrated embodiment shows light patterns of one-dimensional arrays, other suitable light patterns (e.g., two dimensional array, multiplicity of one and/or two-dimensional arrays having any suitable orientation or orientations) can be used. Also, any suitable number of light spots or other light projections (e.g., 1, 10, 100, 200, 500, etc.) can be used. Further, the light spots and wider light projections can have any suitable dimension. In addition, the light properties may be uniform or vary spatially over each light spot and/or wider light projections.

[0082] In **FIG. 3**, each reflective-focusing component **114** includes a contouring element **118** (e.g., lens, material formed with curved surface, etc.) and a curved reflective element **115** (e.g., reflective coating) coupled to a curved surface of the contouring element **118**. The curved reflective element **115** may be coupled using any suitable method. The curved reflective element **115** can be any suitable material or combination of materials. For example, the curved reflective element **115** may be a reflective coating/film with suitable

reflectivity and transmissivity properties. In **FIG. 3**, each curved surface element **115** can reflect and focus the light of the first type to a light spot **155** at the first focal plane **156** outside the first surface **111(a)** of the surface layer **110**. In **FIG. 3**, the contouring element **118** can provide the curved surface for application of the curved reflective element **115** and can focus the light of the second type into a light spot **159** at the second focal plane **160** at the surface of the light detector **142** in the detecting layer **140**. In some cases, the contouring element **118** may also help focus the light of the first type into the light spot **155**. Some examples of suitable contouring elements **118** include a lens, a material molded to have a curved surface, etc.

[0083] The set of reflective-focusing components **112** may be in any suitable form. Some examples of suitable forms include a lens array (e.g., lenslet array) having a reflective coating/film on a surface of each lens, or a curved surface array having a reflective coating/film on the curved surfaces.

[0084] In operation, the illumination source **150** provides a uniform light field of light of the first type (e.g., excitation light) to the fluid channel **170**. The set of reflective-focusing components **112** receives light of the first type, and reflects and focuses the received light into a first light field pattern of light spots **155** at the first focal plane **156** outside the surface **111(a)** of the surface layer **110**. The light of the first type spreads to a larger light distribution after passing through the focal plane **156**. The trajectory **154** shows the path of the light field of the light of the first type as reflected and focused by each reflective-focusing component **114** to the first focal plane **156** and then spreading to a larger distribution. As fluid flows through the fluid channel **170**, the two specimens **180** in the fluid pass through the first light field pattern of light spots **155**. The specimens **180** emit light of a second type at the sites of the light spots **155**. The two light emissions **190** show the emitted light of the second type spreading from the sites of the light spots **155** to two reflective-focusing components **114**. The two reflective-focusing components **114** transmit and slightly focus it. The two light emissions **192** show that the light passing through the reflective-focusing components **115** is slightly more focused. The filter layer **120** blocks any remaining light of the first type and passes the light to the detecting layer **140**. Two light detecting elements **142(a)** of the light detector **142** receive and detect the second light field pattern having two light projections **192**. In this example, the two light projections **192** are larger than the light detecting elements **142(a)**. The light detector **142** generates time varying light data associated with the light detected as the specimens **180** pass through the light spots **155**. The

processor **202** receives the time varying light data from the light detector **142** and analyzes the time varying light data and/or generates images of the specimens **180** from the time varying data.

[0085] The body **102** of the reflective focusing and transmissive projection device **100** also includes a filter layer having an interference filter **120(a)** and an absorption filter **120(b)**. An interference filter **120(a)** refers to a filter that removes light of the first type by interference and transmits other light. An absorption filter **120(b)** refers to a filter that removes light of the first type by absorption and transmits other light. Other embodiments may include other filters. The interference filter **120(a)** and an absorption filter **120(b)** are designed to block light of the first type from reaching the light detector **142**.

[0086] The body **102** also includes a detecting layer **140** having a light detector **142** (e.g., CCD, APDs, CMOS or PMTs) for detecting light and generating signals with data about the intensity, wavelength, and/or other information about the light being detected. The signals from the light detector **142** can be in the form of electrical current that results from the photoelectric effect.

[0087] The light detector **142** includes discrete light detecting elements **142(a)**. The light detecting elements **142(a)** can have any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular or square). For example, light detecting elements in CMOSs and CCDs may be 1-10 microns and in APDs and PMTs may be as large as 1-4 mm. In the illustrated example, each light detecting element **142(a)** corresponds to a reflective-focusing component **114**. In the illustrated example, the light detecting elements **142(a)** are arranged in a one-dimensional array to correspond to the one dimensional array of reflective-focusing components **114**. That is, each light detecting element **142(a)** corresponds to a reflective-focusing component **114**. The one-dimensional array may have any suitable orientation. In other embodiments, the light detecting elements **142(a)** can be arranged in other forms such as a two-dimensional array or a multiplicity of one-dimensional and two-dimensional arrays. The arrays can be in any suitable orientation or combination of orientations.

[0088] An illumination source **150** refers to any suitable light source or sources (e.g., a 100 mW 488 nm Cyan CW laser) providing a light field of light of the first type. In the illustrated example, the illumination source **150** provides a uniform light field of light of the first type **152** to the reflective focusing and transmissive projection device **100**. Some suitable sources of light include a LED, laser, white illumination source, etc. In some cases,

the illumination source **150** comprises a suitable source of light and a filter for selectively transmitting light having the property or properties of light of the first type **152**. For example, the illumination source **150** may include a filter that transmits only the excitation light for activating fluorophores in specimens **180** being examined by the reflective focusing and transmissive projection device **100**. The illumination source **150** may be a component of the reflective focusing and transmissive projection device **100** or may be a separate component. In an exemplary embodiment, the illumination source **150** provides light of a type (e.g., wavelength or polarization) that will cause activation of fluorophores in specimens **180** being examined or imaged by the reflective focusing and transmissive projection device **100**. Although a light (illumination) field is used in many embodiments, other fields can be substituted for the light field in other embodiments. For example, a suitable electromagnetic field can be used. Some examples of suitable electromagnetic fields include an X-ray, terahertz field, radio wave, etc. As another example, the light field can be substituted with an acoustic wave in any medium.

[0089] In an exemplary bioluminescence operation, a reagent may be mixed with a sample comprising the specimens **180**. The reagent may be a suitable chemical that can tag portions (e.g., molecules of a cell nucleus) of the specimens **180** with fluorophores. The resulting fluid is introduced into the device **100**.

[0090] The fluid within which the specimens **180** are suspended, flows through the fluid channel **170**. Any suitable mode or modes of controlling the flow or fluid and/or movement of the specimens **180** can be employed. Any suitable devices such as micropumps, DC electrokinetic devices, dielectrophoresis electrodes, and/or hydrodynamic focusing channels can be used to control the flow of fluid and/or the movement of the specimens **180** through the fluid channel **170**.

[0091] The illumination source **150** provides a uniform light field **152** of light of a first type (e.g., excitation light) to the fluid channel **170**. The set of reflective-focusing components **112** generates a first light field pattern of light spots **155** (e.g., point illumination sources) at the first focal plane **156**. As the fluid flows with the specimens through the fluid channel **170**, the specimen **170** passes through the light spots **155** at $t = t_1$. The light spots excite the fluorophores in portions of the specimen **180**. In response, the fluorophores emit light of the second type from the vicinity of the sites of the light spots **155**. The set of

reflective-focusing components **112** transmits and focuses the emitted light onto the light detector **142**.

[0092] As the specimens **180** passes through the fluid channel **170**, the light detecting elements **142(a)** take data (e.g., intensity, wavelength, phase, etc.) of light over time. The time varying data can be used to image or otherwise analyze the specimens **180**. In the illustrated embodiment, each reflective-focusing component **114** and light spot **155** uniquely maps to a single light detecting element **142(a)**. Each discrete light detecting element **142(a)** in the light detector **142** generates time varying data that can be used to generate a line scan associated with locations along the x-axis. The time varying data is communicated in the form of a signal. The time varying data from the light detecting element **142(a)** is dependent on the fluorophores in the specimens **180**. For example, the time varying data that corresponds to a low intensity of light of the second type at a position for a determined period of time may provide data regarding the length of the specimen at the position along the x-axis in the fluid channel **170**. The time varying data can be processed using a processor **202** to construct image(s) of the specimens **180** using the line scans and, optionally, other data. In some cases, certain data can be used to determine the rotation and velocity of the specimens **180** as they flow through the channel **170**. The time varying data for each light detecting element **142(a)** can then be processed to form images or otherwise analyze the specimens **180** while accounting for the rotation and/or the velocity of the specimens **180**.

[0093] In some embodiments, the specimens **180** are illuminated with both the first uniform light field **152** and with the light spots **155** which generates a general signal at the light detector **142**. The uniform light field generates a time-stable background signal that can be subtracted out of the general signal by the processor **202**. In many cases, the light spots **155** will be at least three orders of magnitude greater in intensity when compared to the uniform light field **152**.

[0094] In one embodiment, a system **10** may include multiple reflective focusing and transmissive projection device **100** having optofluidic elements, located in a series to examine specimens **180** at a different planes through the specimens **180**. For example, the system **10** may include a series of devices **100** along a fluid channel **170**. Each device **100** may be designed to examine (e.g. image or analyze) the specimens **180** at a different focal planes **156**. In each case, the device **100** may have the set of reflective-focusing components **112** located at a different distances from the first surface **111(a)** of the surface layer **110** so

that each device **100** is focused at a different focal plane **156**. The time varying data from the light detectors **142** in each device **100** can be compiled by processor(s) **202** to generate analyses or images at multiple focal planes (e.g., three-dimensional analyses).

[0095] In one embodiment, the set of reflective-focusing components **112** is in the form of a one-dimensional lenslet array having a reflective coating/film (e.g., dichoric filter). The lenslet array is oriented at an angle with respect to the y-axis of the fluid channel **170**. The light of the first type (e.g., excitation light) is reflected by the curved surfaces of the lenslet array and comes into sharp focus within the fluid channel **170**. The array of light spots can then be used to effectively excite fluorophores in the specimens **180** passing through the fluid channel **170**. The light of the second type (e.g., fluorescent or phosphorescent emissions) can then be collected by the lenslet array and channeled onto the light detector **142**. The reflective coating/film and the filter(s) in the filter layer **120** may screen the light of the first type from reaching the light detector **142**. By monitoring the time-varying signal associated with each light spot while specimens **180** passes across the array of light spots, the processor **202** can generate OFM images of the specimens **180**.

[0096] In one embodiment, a system **10** includes multiple reflective focusing and transmissive projection devices **100** having optofluidic elements, located in a series or in parallel to examine the emissions from specimens based on different excitation light (e.g., light having different wavelengths or polarizations). The system **10** can perform multi-spectral fluorescence or phosphorescence imaging or other analyses. Conventional multi-spectral fluorescence imaging systems typically required swapping of chromatic filters between each acquisition step. As the number of slots in a filter carousel is limited, the acquisition of more than 5 wavelength bands typically requires a carousel swap. In the system **10** with multi-spectral fluorescence or phosphorescence imaging, the acquisition of multi-spectral images can be implemented automatically by connecting several multiple reflective focusing and transmissive projection devices **100** having optofluidic elements in series. The multiple reflective focusing and transmissive projection devices **100** will have different filters (e.g., bandpass filters) in the filter layer **120** for transmitting light of different spectral bandwidths so that the light detecting elements **142(a)** will only detect light of a specific spectral bandwidth (e.g., 420-490nm, 550-610nm, and 620-680nm). The sample with specimens **180** can be exposed to multiple fluorescent or phosphorescent dyes associated with the different spectral bandwidths. In this way, a single passage of specimens **180**

through the fluid channel **170** will allow the collection of a complete set of fluorescence images.

[0097] In many embodiments, the specimens **180** are illuminated by both the uniform light field **152** and the light spots **155** which generates a general signal at the light detector **142**. The uniform light field typically generates a time-stable background signal at the light detector **142**. Since the specimens **180** are moving through the light spots **155** in a fluid channel **170**, the specimens **180** are being illuminated by different light spots at different times and the emissions from the specimens **180** can vary with time. The light spots typically can generate a time-varying signal at the light detector **142**. The processor **202** can determine the time-variable signal associated with light spots **155** by subtracting the time stable background signal from the general signal. In many cases, the light spots **155** may be at least three orders of magnitude greater in intensity when compared to the uniform light field **152**. In these cases, the processor **202** may be able to determine the time-variable signal associated with light spots **155** by subtracting the lower intensity background signal from the general signal.

[0098] V. Fabrication Techniques

[0099] In many embodiments, a reflective focusing and transmissive projection device **100** having a multi-layered body **102** can be fabricated using standard semiconductor and micro/nanofabrication procedures.

[0100] In one embodiment, a reflective focusing and transmissive projection device **100** has a set of reflective-focusing components **112** in the form of a lens (e.g., lenslet) array with a reflective coating, which can be fabricated using standard semiconductor and micro/nanofabrication procedures. For example, the detecting layer **140** comprising a light detector **142** (e.g., CMOS sensor) can be coated with the one or more filters of the filter layer **120** capable of blocking the light of the first type (excitation light) with good efficiency. The lens array can be coated with the reflective coating/film. The lens (e.g., lenslet) array with reflective coating can be placed directly on top of the filter layer **120** to form the surface layer **110**. In one case, a lenslet array having a lens curvature $0.024 \text{ micron}^{-1}$ and a lens diameter of 30 micron can be used. In an OFM application shown in FIG. 3, the channel surface layer **172** may be used to form the fluid channel **170** or a formed fluid channel **170** may be placed on top of the surface layer **110**.

[0101] In another embodiment, a reflective focusing and transmissive projection device **100** with a set of reflective-focusing components **112** in the form of curved surface array with a reflective coating, which can be fabricated using standard semiconductor and micro/nanofabrication procedures. For example, the detecting layer **140** comprising a light detector **142** (e.g., CMOS sensor) can be coated with the one or more filters of the filter layer **120** capable of blocking the light of the first type (excitation light) with good efficiency. A layer of a moldable material (e.g., epoxy) can be coated on the filter layer **120**. Next, a lens array or other curved surface form is pressed into the moldable material to create an indentation casting of concave indentations. After the moldable material hardens the reflective coating/film can be coated on the concave indentations. In an OFM application shown in FIG. 3, the channel surface layer **172** may be used to form the fluid channel **170** or a formed fluid channel **170** may be placed on top of the surface layer **110**.

[0102] **VI. Computer devices**

[0103] **FIG. 4** shows a block diagram of subsystems that may be present in computer devices that are used in reflective focusing and transmissive projection system **10**, according to embodiments of the invention. For example, the computer **200** in communication with the reflective focusing and transmissive projection device **100** may have any suitable combination of components in **FIG. 4**.

[0104] The various components previously described in the Figures may operate using one or more computer devices to facilitate the functions described herein. Any of the elements in the Figures may use any suitable number of subsystems to facilitate the functions described herein. Examples of such subsystems or components are shown in a **FIG. 4**. The subsystems shown in **FIG. 4** are interconnected via a system bus **375**. Additional subsystems such as a printer **374**, keyboard **378**, fixed disk **379** (or other memory comprising computer readable media), monitor **376**, which is coupled to display adapter **382**, and others are shown. Peripherals and input/output (I/O) devices, which couple to I/O controller **371**, can be connected to the computer system by any number of means known in the art, such as serial port **357**. For example, serial port **377** or external interface **381** can be used to connect the computer apparatus to a wide area network such as the Internet, a mouse input device, or a scanner. The interconnection via system bus allows the central processor **202** to communicate with each subsystem and to control the execution of instructions from system memory **204** or the fixed disk **379**, as well as the exchange of information between

subsystems. The system memory **204** and/or the fixed disk **379** may embody a computer readable medium. Any of these elements may be present in the previously described features. A computer readable medium according to an embodiment of the invention may comprise code for performing any of the functions described above.

[0105] In some embodiments, an output device (e.g., the printer **374**) of the reflective focusing and transmissive projection system **10** can output various forms of data. For example, the reflective focusing and transmissive projection system **10** can output a fluorescence image of a specimen or other results of analysis.

[0106] It should be understood that the present invention as described above can be implemented in the form of control logic using computer software in a modular or integrated manner. Based on the disclosure and teachings provided herein, a person of ordinary skill in the art will know and appreciate other ways and/or methods to implement the present invention using hardware and a combination of hardware and software.

[0107] Any of the software components or functions described in this application, may be implemented as software code to be executed by a processor using any suitable computer language such as, for example, Java, C++ or Perl using, for example, conventional or object-oriented techniques. The software code may be stored as a series of instructions, or commands on a computer readable medium, such as a random access memory (RAM), a read only memory (ROM), a magnetic medium such as a hard-drive or a floppy disk, or an optical medium such as a CD-ROM. Any such computer readable medium may reside on or within a single computational apparatus, and may be present on or within different computational apparatuses within a system or network.

[0108] A recitation of “a”, “an” or “the” is intended to mean “one or more” unless specifically indicated to the contrary.

[0109] The above description is illustrative and is not restrictive. Many variations of the disclosure will become apparent to those skilled in the art upon review of the disclosure. The scope of the disclosure should, therefore, be determined not with reference to the above description, but instead should be determined with reference to the pending claims along with their full scope or equivalents.

[0110] One or more features from any embodiment may be combined with one or more features of any other embodiment without departing from the scope of the disclosure.

Further, modifications, additions, or omissions may be made to any embodiment without departing from the scope of the disclosure. The components of any embodiment may be integrated or separated according to particular needs without departing from the scope of the disclosure.

[0111] All patents, patent applications, publications, and descriptions mentioned above are hereby incorporated by reference in their entirety for all purposes. None is admitted to be prior art.

WHAT IS CLAIMED IS:

1. A reflective focusing and transmissive projection device comprising:
a body having a surface layer with first and second surfaces, and a detecting layer outside the second surface of the surface layer;
a set of reflective-focusing components in the surface layer, each reflective-focusing component having a contouring element and a curved reflective element conformed to the contouring element, the curved reflective element configured to reflect light of a first type and transmit light of a second type, the curved reflective element further configured to focus the light of the first type to outside the first surface of the surface layer; and
a light detector in the detecting layer, and configured to receive light and generate light data associated with the received light.
2. The reflective focusing and transmissive projection device of claim 1, wherein the contouring element is configured to focus the light of the second type on the light detector.
3. The reflective focusing and transmissive projection device of claim 2, wherein the light of the first type is focused to a first focal plane and the light of the second type is focused to a second focal plane parallel to the first focal plane.
4. The reflective focusing and transmissive projection device of claim 1, wherein the reflective focusing component comprises a reflective coating on a curved surface of the contouring element.
5. The reflective focusing and transmissive projection device of claim 1, wherein the contouring element is configured to focus the light of the first type.
6. The reflective focusing and transmissive projection device of claim 1, wherein the reflected light of the first type is focused into a first pattern of light projections.
7. The reflective focusing and transmissive projection device of claim 6, wherein the light projections are light spots.
8. The reflective focusing and transmissive projection device of claim 1, wherein the light of the second type is focused into a second pattern of light projections outside the second surface of the surface layer.

9. The reflective focusing and transmissive projection device of claim 1, wherein light of the second type is light emitted from a specimen in response to receiving the focused light of the first type.

10. The reflective focusing and transmissive projection device of claim 1, wherein the set of reflective-focusing components is a lenslet array having a reflective coating.

11. The reflective focusing and transmissive projection device of claim 1, wherein the set of reflective-focusing components is a curved surface array having a reflective coating.

12. The reflective focusing and transmissive projection device of claim 1, further comprising a filter layer located between the surface layer and the light detector, the filter layer configured to block light of the first type.

13. The reflective focusing and transmissive projection device of claim 1, wherein the light of the first type is light having a first wavelength, and wherein the light of the second type is light having a second wavelength different from the first wavelength.

14. The reflective focusing and transmissive projection device of claim 1, wherein the light of the first type is light having a first polarization, and wherein the light of the second type is light having a second polarization different from the first polarization.

15. A reflective focusing and transmissive projection system comprising:
a reflective focusing and transmissive projection device including
a body having a surface layer with first and second surfaces, and a detecting layer outside the second surface of the surface layer,
a set of reflective-focusing components in the surface layer, each reflective-focusing component having a contouring element and a curved reflective element conformed to the contouring element, the curved reflective element configured to reflect light of a first type and transmit light of a second type, the curved reflective element also configured to focus the light of the first type to outside the first surface of the surface layer, and

a light detector in the detecting layer, and configured to receive light and generate light data associated with the received light; and

a processor in electronic communication with the light detector, the processor configured to receive the light data from the light detector and analyze the light data.

16. The reflective focusing and transmissive projection system of claim 15, wherein the contouring element is configured to focus the light of the second type on the light detector.

17. The reflective focusing and transmissive projection system of claim 15, wherein the reflected light of the first type is focused into a first pattern of light spots.

18. The reflective focusing and transmissive projection system of claim 15, wherein the light of the second type is focused into a second pattern of light projections outside the second surface of the surface layer.

19. A reflective focusing and transmissive projection device comprising:
a body defining a fluid channel, and comprising a surface layer having a first surface proximal to the fluid channel and a second surface, the body further comprising a detecting layer outside the second surface of the surface layer;

a one-dimensional array of reflective-focusing components located in the surface layer, each reflective-focusing component having a contouring element and a curved reflective element conformed to the contouring element, the curved reflective element configured to reflect light of a first type and transmit light of a second type from the fluid channel, the curved reflective element also configured to focus the light of the first type to the fluid channel, wherein the one-dimensional array of reflective-focusing components extends substantially across from a first lateral side to a second lateral side of the fluid channel; and

a light detector in the detecting layer, and configured to receive light and generate time varying data associated with the received light as a specimen passes through the fluid channel.

20. The reflective focusing and transmissive projection device of claim 19, wherein the contouring element is configured to focus the light of the second type on the light detector.

1/4

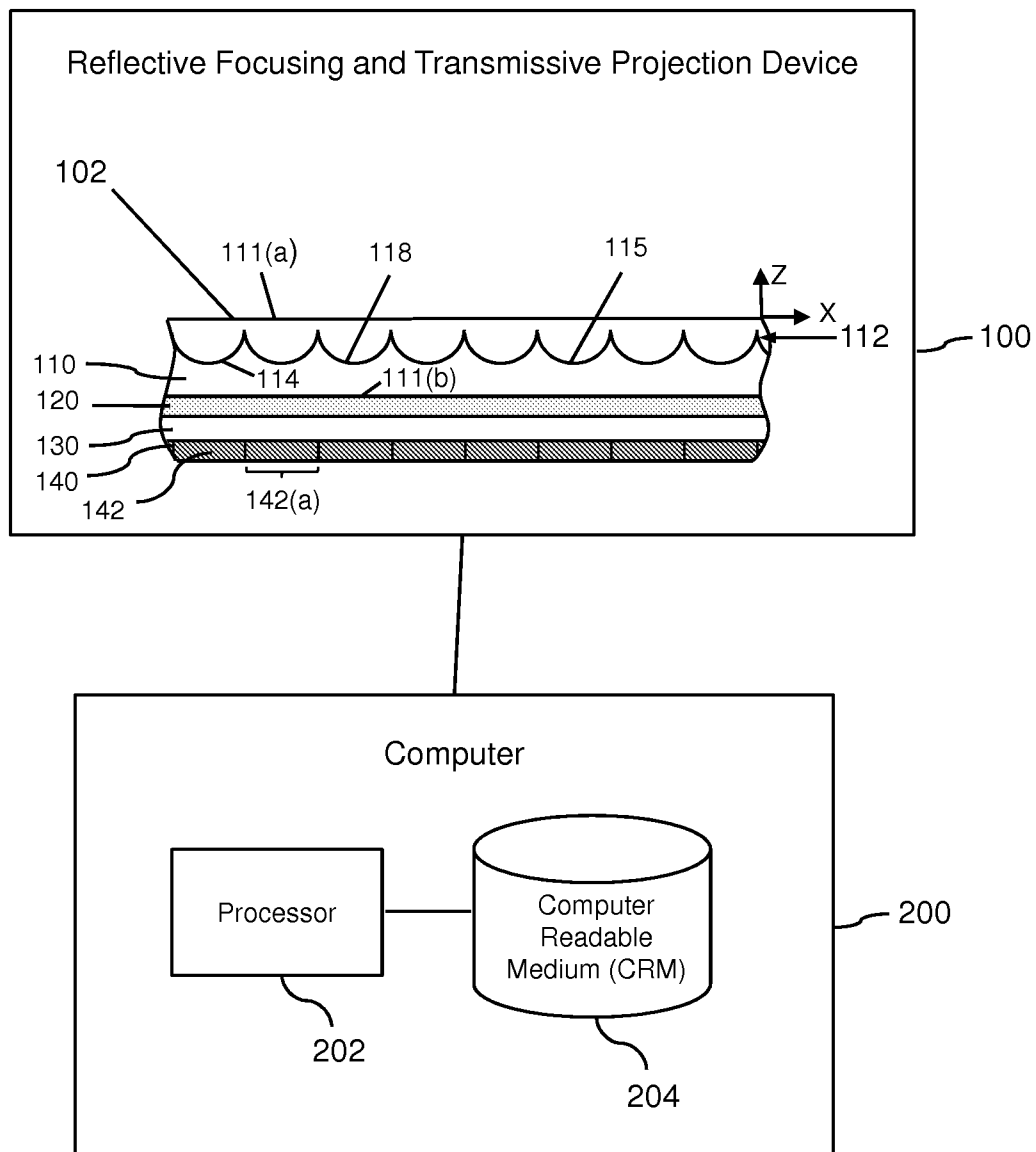
10

FIG. 1

2/4

FIG. 2(a)

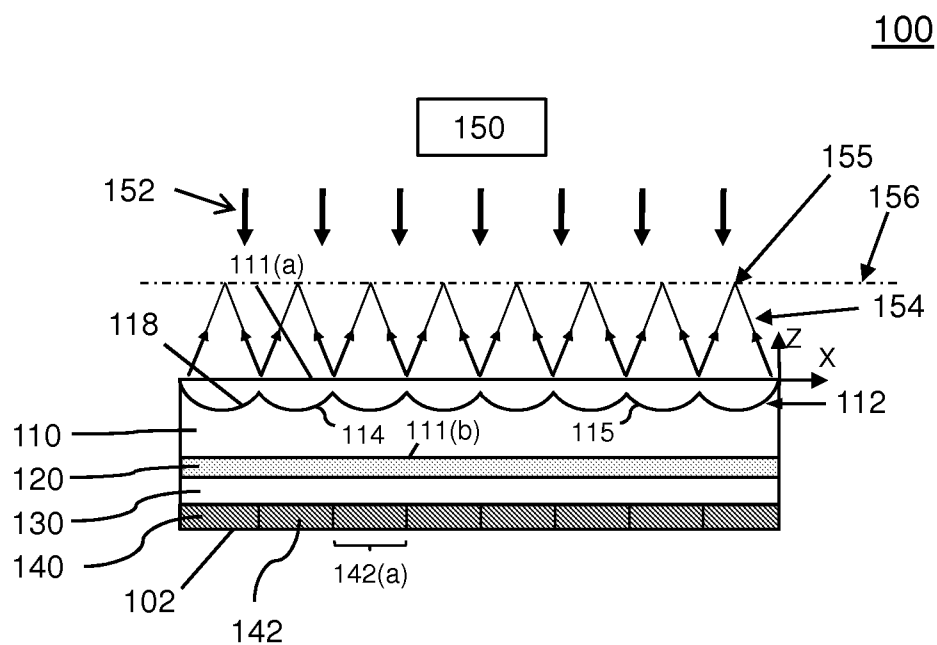
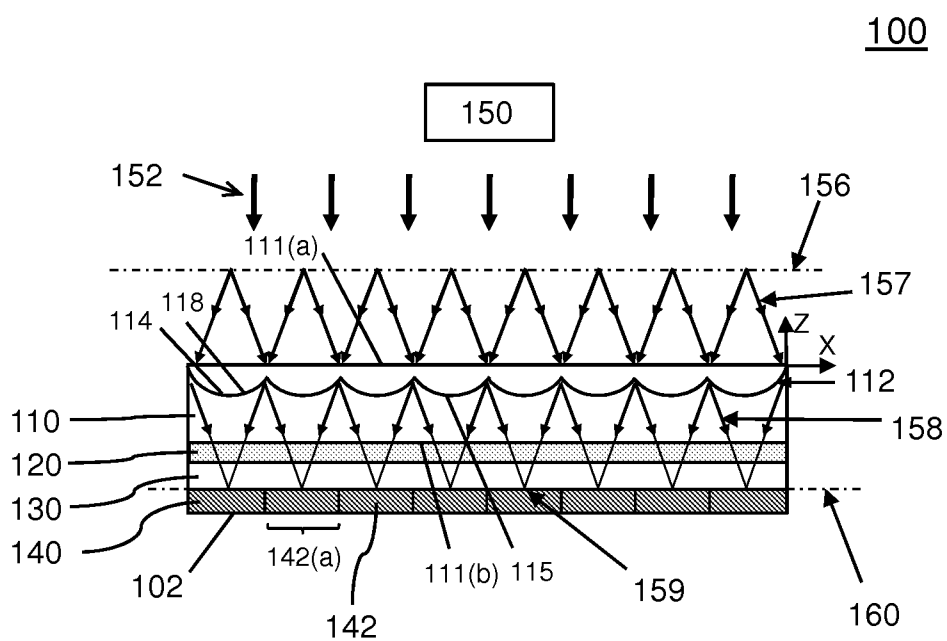


FIG. 2(b)



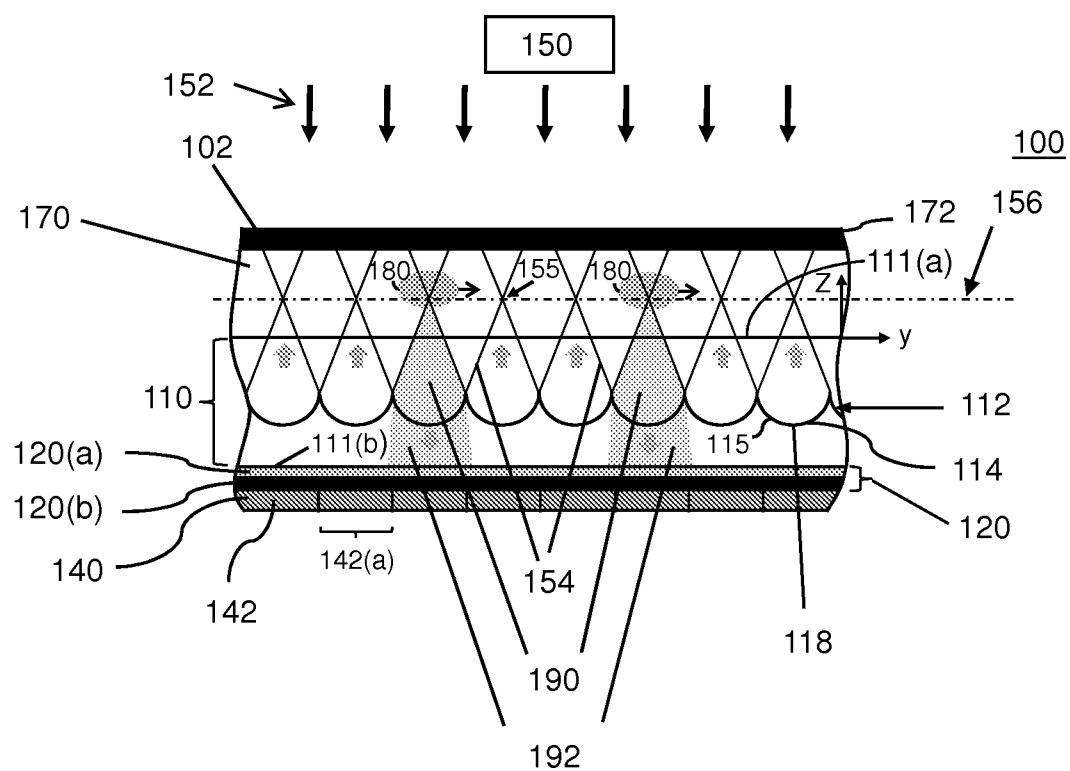


FIG. 3

4/4

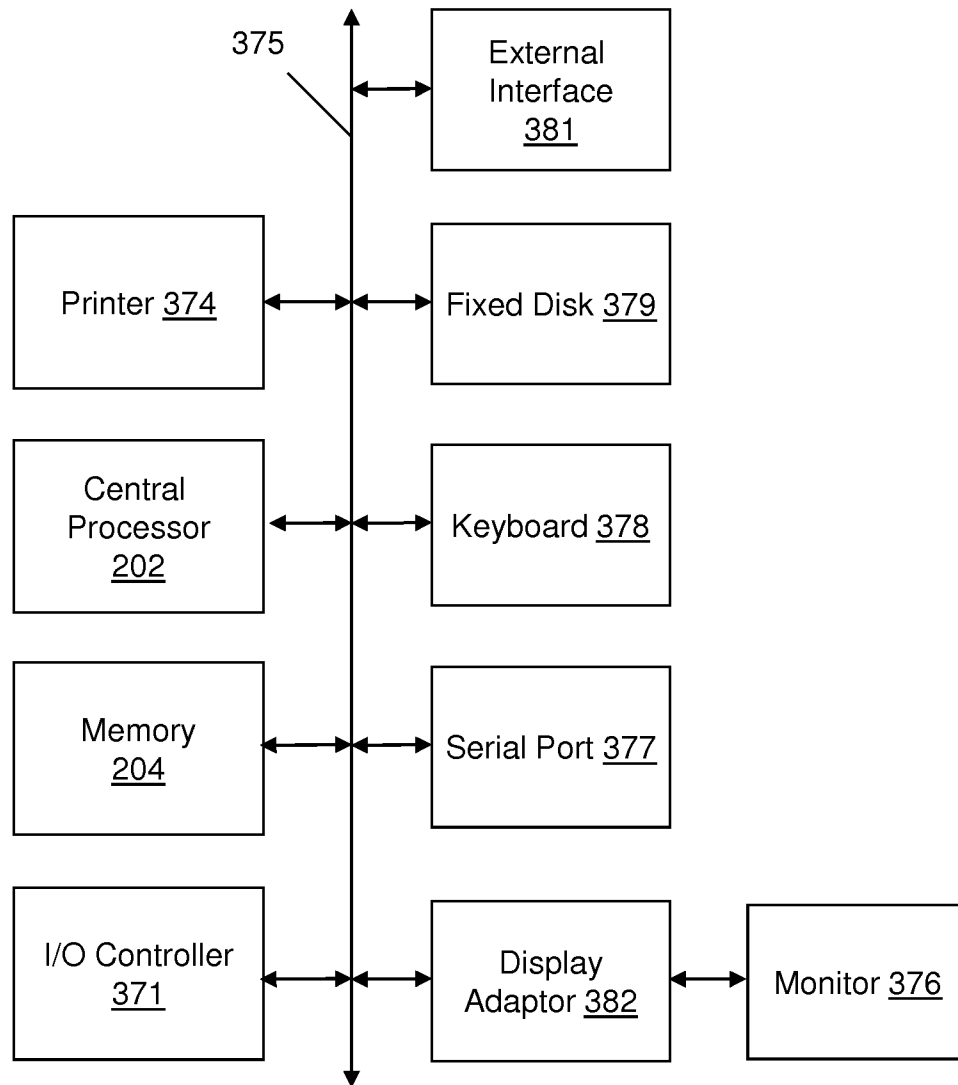


FIG.4