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(54) Title: TALBOT-ILLUMINATED IMAGING DEVICES, SYSTEMS, AND METHODS FOR FOCAL PLANE TUNING

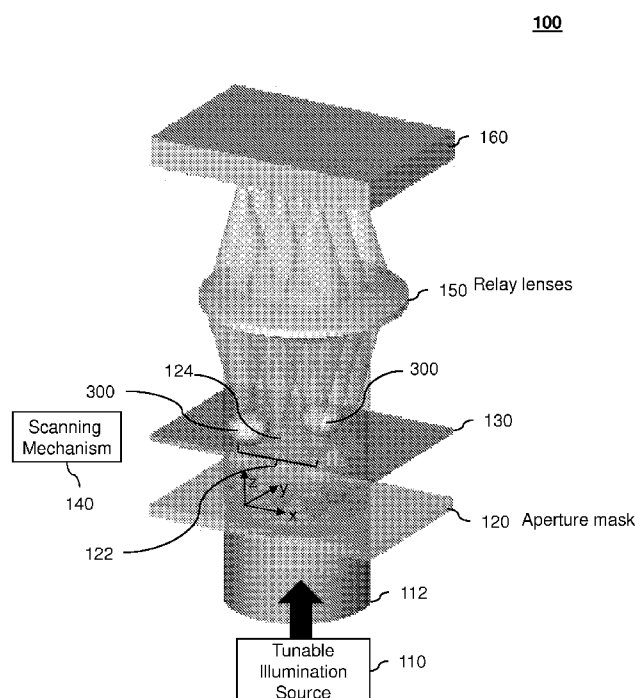


FIG. 3

(57) Abstract: A Talbot-illuminated imaging system for focal plane tuning, the device comprising a Talbot element, a tunable illumination source, a scanning mechanism, a light detector, and a processor. The element generate scan array of focused light spots at a focal plane. The tunable illumination source shifts the focal plane to a plane of interest by adjusting a wavelength of light incident the Talbot element. The scanning mechanism scans an object across an array of focused light spots in a scanning direction. The light detector determines time-varying light data associated with the array of focused light spots as the object scans across the array of light spots. The processor constructs an image of the object based on the time-varying data.



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TALBOT-ILLUMINATED IMAGING DEVICES, SYSTEMS, AND METHODS FOR FOCAL PLANE TUNING

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This is a non-provisional application of, and claims priority to, U.S. Provisional Patent Application No. 61/477,502 entitled “Wide Field-of-View Microscopy Based on Talbot Illumination with Focal Plane Tuning Capability,” filed on April 20, 2011. This provisional application is hereby incorporated by reference in its 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. 12/903,650 entitled “Holographically Illuminated Imaging Devices” filed on October 13, 2010.
- U.S. Patent Application No. 13/415,718 entitled “Talbot Imaging Devices and Systems” filed on March 8, 2012.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0003] The U.S. Government has certain rights in this invention pursuant to Grant No. W81XWH-09-1-0051 awarded by the US Army Medical Research and Material Command.

BACKGROUND OF THE INVENTION

[0004] Embodiments of the present invention generally relate wide field-of-view, high resolution imaging devices. More specifically, certain embodiments relate to Talbot-illuminated imaging (TII) devices, systems, and methods for focal plane tuning, used for wide field-of-view imaging in areas such as, for example, microscopy and photography.

[0005] Wide field-of-view microscopic imaging is highly desired in applications such as digital pathology or high throughput screening. Some examples of digital pathology and high throughput screening applications that may use wide field-of-view imaging are described in Ho, J., Parwani, A.V., Jukic, D.M., Yagi, Y., Anthony, L., and Gilbertson, J.R., "Use of whole slide imaging in surgical pathology quality assurance: design and pilot validation studies," *Human Pathology* 37, pp. 322-331 (2006) and Oheim, M., "High-throughput microscopy must re-invent the microscope rather than speed up its functions," *British Journal of Pharmacology* 152, 1-4 (2007), which are hereby incorporated by reference in their entirety for all purposes.

[0006] Some recent efforts address the need for wide field-of-view imaging, but present significant technical limitations. Some examples of these efforts can be found in Bishara, W., Su, T.W., Coskun, A.F., and Ozcan, A., "Lensfree on-chip microscopy over a wide field-of-view using pixel super-resolution," *Optics Express* 18, pp. 11181-11191 (2010) and Rojo, M.G., Garcia, G.G., Mateos, C.P., Garcia, J.G., and Vicente, M.C., "Critical comparison of 31 commercially available digital slide systems in pathology," *International Journal of Surgical Pathology* 14, pp. 285-305 (2006), which are hereby incorporated by reference in their entirety for all purposes. Another example is the recent development of wide field-of-view microscopes based on holographic focus grid illumination. Some examples of recently developed microscopes based on holographic focus grid illumination can be found in Wu, J., Cui, X., Zheng, J, Wang, Y.M., Lee, L.M., and Yang, C., "Wide field-of-view microscope based on holographic focus grid illumination," *Optics Letters* 35, pp. 2188-2190 (2010) and Wu, J., Lee, L.M., and Yang, C., "Focus grid generation by in-line holography," *Optics Express* 18, pp. 14366-14374 (2010), which are hereby incorporated by reference in their entirety for all purposes.

BRIEF SUMMARY OF THE INVENTION

[0007] Embodiments of the present invention relate to TII devices, systems, and methods for focal plane tuning (e.g., z-axis scanning). Embodiments include a TII device

having a Talbot element, a tunable illumination source illuminating the Talbot element, a scanning mechanism, and a light detector (e.g., imaging sensor). The Talbot element is a patterned structure (e.g., aperture mask) that generates an array of focused light spots (focus grid) at repeated distances away from the Talbot element due to the Talbot effect. The tunable illumination source shifts the focal plane of the array of focused light spots by adjusting the wavelength of a collimated light beam incident the Talbot element. In some cases, the tunable illumination source tunes the focal plane at a plane of interest, such a plane through an object being imaged. The scanning mechanism scans the object across the focused light spots as the light detector measures time varying light data (e.g., linescans) altered and unaltered by the object. A processor can reconstruct an image of the object from the time varying light data.

[0008] One embodiment is directed to a Talbot-illuminated imaging device for focal plane tuning. The device comprises a Talbot element and a tunable illumination source. The Talbot element is configured to generate an array of focused light spots at a focal plane. The tunable illumination source is configured to shift the focal plane to a plane of interest by adjusting a wavelength of light incident the Talbot element. In some cases, the device also includes a scanning mechanism and a light detector. The scanning mechanism is configured to move an object relative to the array of focused light spots in a scanning direction. The light detector is configured to determine time-varying light data associated with the array of focused light spots as the object moves relative to the array of light spots.

[0009] One embodiment is directed to a Talbot-illuminated imaging system for focal plane tuning. The system comprises a Talbot-illuminated imaging device and a processor. The Talbot-illuminated imaging device includes a Talbot element, a tunable illumination source, a scanning mechanism, and a light detector. The Talbot element generates an array of focused light spots at a focal plane. The tunable illumination source is configured to shift the focal plane to a plane of interest by adjusting a wavelength of light incident the Talbot element. The scanning mechanism is configured to move an object relative to the array of focused light spots in a scanning direction. The light detector is configured to determine time-varying light data associated with the array of focused light spots as the object moves relative to the array of light spots. The processor is configured to reconstruct an image of the object based on the time-varying light data.

[0010] One embodiment is directed to a method of focal plane tuning using a Talbot-illuminated imaging system having a Talbot element, a tunable illumination source, a scanning mechanism and a light detector. The method includes providing light of an incident wavelength to the Talbot element to generate an array of focused light spots. The method also includes adjusting, by the tunable illumination source, the incident wavelength to shift the focal plane of the array of focused light spots. The method also includes capturing time-varying light data associated with the array of focused light spots as an object moves relative to the array of focused light spots. In addition, the method includes constructing an image of the object based on the time varying light data.

[0011] These and other embodiments of the invention are described in further detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **FIG. 1(a)** is a series of images of evolving Talbot patterns at different planes around the Talbot plane at $z = Z_{1.5, \lambda_1}$ where $\lambda_1 = 702$ nm, based on numerically simulated results, according to embodiments of the invention.

[0013] **FIG. 1(b)** is a series of images of evolving Talbot patterns at different planes around the Talbot plane at $z = Z_{1.5, \lambda_1}$ for $\lambda_1 = 702$ nm based on experimental characterization results as observed under a conventional microscope with 60X objective, according to embodiments of the invention.

[0014] **FIG. 1(c)** is a series of images of evolving Talbot patterns at different planes around the Talbot plane at $z = Z_{1.5, \lambda_1}$ for $\lambda_1 = 692$ nm, based on experimental characterization results as observed under a conventional microscope with 60X objective, according to embodiments of the invention.

[0015] **FIG. 2** is a block diagram of components of a TII system for focal plane tuning, according to embodiments of the invention.

[0016] **FIG. 3** is a isometric view of some components of a TII device for focal plane tuning, according to embodiments of the invention.

[0017] **FIG. 4** is a cross-sectional side view of a portion of a TII device for focal plane tuning, according to embodiments of the invention.

[0018] **FIG. 5(a)** is a illustration of an array of focused light spots on the Talbot element plane as generated by a Talbot element of a TII device, according to embodiments of the invention.

[0019] **FIG. 5(b)** is a illustration of an array of focused light spots on the Talbot element plane as generated by a Talbot element of a TII device, according to embodiments of the invention.

[0020] **FIG. 6(a)** is an image of a portion of the two-dimensional array of apertures in the Talbot element of the TII device, according to an embodiment of the invention.

[0021] **FIG. 6(b)** is an image of a portion of the focus grid at $z = Z_{1.5}$, according to an embodiment of the invention.

[0022] **FIG. 7(a)** is a wide field-of-view image of the U.S. Air Force (USAF) target acquired by the TII system, according to an embodiment of the invention.

[0023] **FIG. 7(b)(1)** is an expanded view of the region indicated in **FIG. 7(a)**, according to an embodiment of the invention.

[0024] **FIG. 7(b)(2)** is a cross-sectional intensity profile of group 8, element 6 from the expanded view of the region indicated in **FIG. 7(b)(1)**, according to an embodiment of the invention.

[0025] **FIG. 8(a)** is an image of the mixed green algae acquired by the TII system using the incident wavelengths $\lambda_I = 692$ nm, according to an embodiment of the invention.

[0026] **FIG. 8(b)** is an expanded view of the region indicated in **FIG. 8(a)**, according to an embodiment of the invention.

[0027] **FIG. 8(c)** is an image of the mixed green algae acquired by the TII system using the incident wavelengths $\lambda_I = 702$ nm, according to an embodiment of the invention.

[0028] **FIG. 8(d)** is an expanded view of the region indicated in **FIG. 8(c)**, according to an embodiment of the invention.

[0029] **FIG. 9** is a flowchart of a method of focal plane tuning using a TII device, according to an embodiment of the invention.

[0030] **FIG. 10** is a block diagram of subsystems that may be present in the TII system, according to embodiments of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0031] Embodiments of the present invention will be described below with reference to the accompanying drawings. Some embodiments include TII devices, TII systems, and methods for focal plane tuning. Focal plane tuning refers to adjusting the wavelength of light incident a Talbot element to move the focal plane of a repeated Talbot image to a plane of interest. Embodiments include a TII device having a Talbot element, and a tunable illumination source (e.g., tunable laser) providing collimated light to the Talbot element. The Talbot element is a patterned structure (e.g., aperture mask) capable of generating an array of focused light spots at repeated distances away from the Talbot element based on the Talbot effect. The tunable illumination source can adjust the wavelength of incident light to move the focal plane of the array of focused light spots to a plane of interest. The TII device also includes a scanning mechanism and a light detector. The scanning mechanism moves an object being imaged across the array of focused light spots in a scanning direction. The light detector receives light from the array of focused light spots that may be altered and unaltered by the object. The light detector measures time-varying light data associated with each of the focused light spots. The processor reconstructs an image of the object at the plane of interest by compiling the time-varying light data from each focused light spot and properly shifting the data according to the scanning speed. In some cases, the incident wavelength can be tuned to change the focal plane multiple times (z-axis scanning) to obtain images at different focal planes.

[0032] Embodiments of the invention provide one or more technical advantages over other wide field-of-view imaging systems. One advantage over a holographically illuminated method is that accurate generation of an array of light spots is more repeatable and robust because the Talbot element can be an aperture mask fabricated using microfabrication techniques. Another advantage is that, unlike previous holographically illuminated imaging methods, there is no zero-order transmission of the incident beam in the Talbot effect. This will enhance the contrast of the light spots on the light detector and thus the signal-to-noise ratio of the image. Another advantage is that the focused light spots are more uniform in their powers (spot-to-spot comparison), which can improve the accuracy of the image. A main advantage of embodiments of the TII system is the capability of focal plane tuning by adjusting the incident wavelength. Some previous holographically illuminated imaging systems require mechanical translation of the holographic element to image at different focal planes, which could be time consuming and mechanically complex. In embodiments of the

invention, the incident wavelength of the tunable illumination source can be tuned to change the focal plane multiple times in order to obtain images at different focal planes. The elimination of mechanical scanning along the focal axis (z-axis scanning) can be a significant advantage for fast scanning microscopy applications.

[0033] I. Introduction to Talbot Effect and Focal Plane Tuning

[0034] The Talbot effect is a phenomenon in which certain patterned structures (e.g., aperture mask with a periodic grid of apertures) repeat the patterned image at regular distance intervals away from the patterned structures (Talbot element). The Talbot effect has been described, for example, in Talbot, H. F., "*LXXVI. Facts relating to optical science. No. IV*," Philosophical Magazine Series 3 9, pp. 401-407 (1836), which is hereby incorporated by reference in its entirety for all purposes. The distance interval between the repeated patterned images is referred to as a Talbot distance (Z_T) or the half Talbot distance ($Z_{T/2}$). For a square periodic grid (two-dimensional array) patterned structure, the Talbot distance can be calculated as:

$$Z_T = 2d^2/\lambda \quad (\text{Eqn. 1})$$

where d is the pattern period and λ is the wavelength of the incident light, as has been shown, for example, in Montgomery, W. D. "*Self-Imaging Objects of Infinite Aperture*," J. Opt. Soc. Am. 57, pp. 772-775 (1967), which is hereby incorporated by reference in its entirety for all purposes. Patterned images will be repeated at Talbot planes at integer multiples of the Talbot distances:

$$z = mZ_T = m2d^2/\lambda \quad (\text{Eqn. 2})$$

where m is an integer.

[0035] Also, phase-reversed patterned images will be repeated at half Talbot distances half way between the Talbot planes of Eqn. 2. A half Talbot distance can be calculated as:

$$Z_{T/2} = d^2/\lambda \quad (\text{Eqn. 3})$$

where d is the pattern period and λ is the wavelength of the incident light. The phase-reversed patterned images will be repeated at Talbot planes:

$$z = (m-1/2)2d^2/\lambda \quad (\text{Eqn. 4})$$

where m is an integer.

[0036] Furthermore, a fractional Talbot effect generates a shrunken patterned image between the Talbot planes described in Eqns. 2 and 4. For a Talbot element of a square periodic grid patterned structure, the fractional Talbot effect generates a focused light spot grid that has a smaller pitch than the original patterned structure. The fractional Talbot effect is described in Besold, B. and Lindlein, N. "Fractional Talbot effect for periodic microlens arrays", Optics Engineering 36, 1099-1105 (1997), which is hereby incorporated by reference in its entirety for all purposes. By utilizing the fractional Talbot effect, the TII system **10** could generate more focused light spots than the aperture number of the Talbot element.

[0037] However, strict Application of the Talbot effect described in Eqns. 1-4 has limitations in some cases. For example, the 'self-imaging' ability of the Talbot effect is only a paraxial approximation as discussed in Paturski, K., "*The self-imaging phenomenon and its applications*," Progress in Optics 27, pp. 3-108 (1989), which is hereby incorporated by reference in its entirety for all purposes. In other words, the Talbot effect will 'self-image' the apertures in a mask well only if the aperture diameter d is much larger than λ , as discussed in Besold, B., and Lindlein, N., Fractional Talbot effect for periodic microlens array," Optics Engineering 36, pp. 1099-1105 (1997) and Di Mambro, E., Haidar, R., Guérineau, N., and Primot, J., "Sharpness limitations in the projection of thin lines by use of the Talbot experiment," J. Opt. Soc. Am. A 21, pp. 2276-2282 (2004), which are hereby incorporated by reference in their entirety for all purposes. The Talbot effect will fail and result in 'self-image' light spots that are substantially larger (less focused) if the aperture diameter is comparable to λ .

[0038] Since image resolution obtainable by the TII system **10** at least partially depends on the size of the focused light spots generated by the Talbot effect, generating tightly focused light spots is highly desired by the TII system **10**. Strict application of the Talbot effect may not work well in some cases and may not provide tightly focused light spots at the Talbot planes described in Eqns. 2 and 4. It has been determined, however, that the Talbot field propagation generates tightly focused light spots in planes that are slightly away (above or below) from the Talbot planes described in Eqns. 2 and 4 if the aperture diameter is comparable to λ .

[0039] **FIG. 1(a)** is a series of images of evolving Talbot patterns at different planes around the Talbot plane at $z = Z_{1.5, \lambda_1}$ where $\lambda_1 = 702$ nm, based on numerically simulated

results, according to embodiments of the invention. **FIG. 1(b)** is a series of images of evolving Talbot patterns at different planes around the Talbot plane at $z = Z_{1.5, \lambda_1}$ for $\lambda_1 = 702$ nm based on experimental characterization results as observed under a conventional microscope with 60X objective, according to embodiments of the invention. **FIG. 1(c)** is a series of images of evolving Talbot patterns at different planes around the Talbot plane at $z = Z_{1.5, \lambda_2}$ for $\lambda_2 = 692$ nm, based on experimental characterization results as observed under a conventional microscope with 60X objective, according to embodiments of the invention. The Talbot element used in the numerical simulation and experimental characterization of **FIGS. 1(a)**, **1(b)** and **1(c)** was an aperture mask with a periodicity d of 30 μm and uniform aperture size a of 800 nm. **FIGS. 1(a)**, **1(b)**, and **1(c)** include the z positions and w , the full width at half maximum (FWHM), of the lights spots except those in the last column.

[0040] The last column in **FIGS. 1(a)**, **1(b)**, and **1(c)** corresponds to the plane at $z = Z_{1.5}$. As shown in the last column, the Talbot effect fails to generate the most tightly focused light spots at the Talbot plane at $z = Z_{1.5}$. That is, the self-imaging approximation does not appear to be valid for this regime where aperture size a of 800 nm is comparable to the wavelength of $\lambda_1 = 702$ nm or $\lambda_2 = 692$ nm.

[0041] Based on Eqns. 1 and 3, the Talbot distance Z_T and Talbot half distance $Z_{T/2}$ of a Talbot element with a square periodic grid pattern are inversely proportional to the incident wavelength, λ . For small changes of incident wavelength, i.e., $\Delta\lambda \ll \lambda$, the Talbot distance change (i.e. change in focal plane of square periodic grid pattern) can be calculated as:

$$\Delta Z_T = -2d^2 \Delta\lambda / \lambda^2 \quad (\text{Eqn. 5})$$

Based on Eqn. 5, the focal plane shift Δz of a focal plane of an array of focused light spots can be calculated as: $\Delta z = 2d^2 \Delta\lambda / \lambda^2$ for a change of incident wavelength of $\Delta\lambda$. For example, a square periodic grid patterned structure with a periodicity of $d = 30$ μm and a nominal wavelength of 700 nm may have a plane shift of 55 μm at $z = Z_{1.5}$ for a small wavelength change of 10 nm.

[0042] In **FIG. 1(c)**, the incident wavelength was adjusted by $\Delta\lambda = 692$ nm - 702 nm = - 10 nm from **FIG. 1(b)**. **FIG. 1(c)** shows the spot pattern closely resembling the spot pattern from **FIG. 1(b)**. However, the focused light spots have shifted by Δz of 55 μm to another focal plane away from the Talbot element. This shift is consistent with the prediction from Eqn. 5 ($Z_{1.5, \lambda_2} - Z_{1.5, \lambda_1} = 55$ μm).

[0043] **FIGS. 1(a), 1(b), and 1(c)** also show that each light spot generally consists of a bright central spot and associated concentric rings. As the Z position increases, the bright center spot get brighter and larger, while the concentric rings get smaller and finally disappear. From observing the evolving of the light spots, it is shown that the best light spots for imaging are those around $z = Z_{1.5} - 90 \mu\text{m}$. For smaller Z positions, the center spot is too weak for a good contrast imaging. While for larger Z positions, the center spot is larger and cannot provide high-resolution images. The observed light spots show that the TII system **10** of embodiments may achieve a resolution of $\sim 1 \mu\text{m}$, which was also verified by acquiring images of a USAF target in the experiment. The size of each pattern figure in **FIGS. 1(a), 1(b)** and **1(c)** is $30 \times 30 \mu\text{m}$.

[0044] In embodiments, the TII system **10** tunes (shifts) the focal plane of the light spots to move the plane to or close to a plane of interest such as a plane through an object being examined. By tuning the focal plane, the TII system **10** can adjust the Talbot distance so that the illumination pattern at the plane of interest has more focused (tighter) light spots. The TII system **10** uses a tunable illumination source to adjust the incident wavelength to tune the focal plane. This tuning ability can be used to accomplish non-mechanical focal plane tuning in the TII system **10**.

[0045] II. TII System

[0046] **FIG. 2** is a block diagram of components of a TII system **10** for focal plane tuning, according to embodiments of the invention. The TII system **10** includes a TII device **100** and a computing device **200** in electronic communication with the TII device **100**. Although the illustrated embodiment includes a single TII device **100** and a single computing device **200**, other embodiments may include two or more TII devices **100** and/or computing devices **200**.

[0047] The TII device **100** includes a tunable illumination source **110**, a Talbot element **120**, a platform **130**, a scanning mechanism **140**, relay lenses **150**, and a light detector **160**. The tunable illumination source **110** is in communication with the Talbot element **120** to provide collimated light (beam) to the Talbot element **120**. The Talbot element **120** generates a light field with an array of focused light spots (Talbot image) at a focal plane at a distance away from the Talbot element **130**. The tunable illumination source **110** can move the focal plane of the array of focused light spots to a plane of interest by adjusting the wavelength of the collimated light. The platform **130** can receive one or more

objects being imaged by the TII system **10**. Although not shown, the scanning mechanism **140** may be in communication with the platform **130** or with the object(s) to move the object(s) relative to the array of focused light spots and/or may be in communication with the Talbot element **120** to move the array of focused light spots relative to the object(s). The relay lenses **150** is in communication with the platform **130** to collect and relay light as may or may not be altered by the object(s) on the platform **130**. The light detector **160** is in communication with the relay lenses **150** to receive the light from the relay lenses **150** and capture time-varying light data as the object(s) moves relative to the array of focused light spots.

[0048] The computing device **200** includes a processor **210**, a computer readable medium (CRM) **220**, and display **230**. The display **230** and CRM **220** are in electronic communication with the processor **210**. The display **230** is in electronic communication with the processor **210** to receive image data and other data for display. The processor **210** is in electronic communication with the CRM **220** to retrieve/store code with instructions for completing functions of the TII system **10**. The processor **210** is in electronic communication with the light detector **160** to receive one or more signals with the time-varying light data. Although not shown, the processor **210** may also be in communication with the tunable illumination source **110** to send signal(s) to control the wavelength of the collimated light to the Talbot element **120** to tune the focal plane of the array of focused light spots. The processor **210** reconstructs one or more images of the object(s) from the time-varying light data.

[0049] A tunable illumination source **110** can refer to any suitable device or combination of devices that can provide collimated light **112** (e.g., collimated beam) to the Talbot element **120** and can adjust the wavelength of the collimated light **112** in a wavelength range $\Delta\lambda$ between an initial wavelength λ_0 and a final wavelength λ_I . In some cases, the tunable illumination source **110** can incrementally adjust the wavelength to wavelengths between the initial wavelength λ_0 and a final wavelength λ_I . Any suitable increment (e.g., 10 nm, 20 nm, 5 nm, etc.) can be used. Any suitable number (e.g., 2, 4, 10, etc.) of increments can be used. An example of a suitable device is a tunable laser (e.g., tunable Ti:Sapphire laser). Suitable devices are commercially available. The tunable illumination source **110** can be placed in any suitable location appropriate for providing collimated light **112** to the Talbot element **120**. The tunable illumination source **110** may be a component of the TII device **100** or may be separate from the TII device **100**. The light properties (wavelength (λ), phase,

intensity, etc.) of the collimated light **112** may have any values suitable for operation of the TII system **10**.

[0050] A Talbot element **120** can refer to any suitable patterned structure(s) capable of creating a light field that repeats an intensity pattern (Talbot image) at distances away from the Talbot element **120** based on the Talbot effect (including fractional Talbot effect). Some examples of suitable patterned structures include a layer with an array of light transmissive regions **122**, a micro-lens array, micro-concave mirror array, phase/amplitude gratings, other patterned diffraction structure, or any suitable combination thereof. The layer may be made of any suitable material(s) (e.g., metallic material) of any suitable thickness(es). The listed arrays may be one-dimensional arrays, two-dimensional arrays, or combination of one-dimensional and two-dimensional arrays with dimensions of suitable values (e.g., 1300 x 1100, 100 x 100, 30 x 50, 100 x 1, etc.).

[0051] The patterned structure of the Talbot element **120** may be a periodic structure(s) or non-periodic structure(s). A periodic structure refers to a structure with a pattern that repeats structural elements (e.g., apertures) on a periodic basis according to pattern period (periodicity), d . The periodic structure may be periodic in a single lateral direction or both lateral directions of the Talbot element **120**. The pattern period, d , may be any suitable value (e.g., 5 microns, 15 microns, 20 microns, 30 microns, 100 microns, etc.). Some examples of suitable periodic structures are one-dimensional and two-dimensional arrays of uniformly spaced structures. Any suitable structure (e.g., apertures, microlenses, etc.) can be used.

[0052] In embodiments, the Talbot element **120** may be an aperture mask (i.e. material layer with apertures) having an array of light transmissive regions **122**. Each light transmissive region **122** may be a vacuum or gas-filled space, or filled with transparent material such as such water or a viscous polymer (e.g., SU-8 resin). The array of light transmissive regions **122** may be a one-dimensional array, a two-dimensional array, or a combination of one and two-dimensional arrays. The array of light transmissive regions **122** has dimensions of any suitable values (e.g., 1300 x 1100, 100 x 100, 30 x 50, 100 x 1, etc.). The light transmissive regions **122(a)** in the array **122** may have any suitable shape (e.g., circular, rectangular, etc.) and any suitable size (e.g., 0.4 microns, 0.6 microns, 0.8 microns, 1 micron, etc.). For example, the light transmissive regions **122(a)** in some examples has a

uniform width, a , which is comparable to the wavelength λ of the incident collimated light **112**.

[0053] In some cases, the aperture mask (i.e. material layer with light transmissive regions) may include a two-dimensional array of light transmissive regions **122**. In one case, the two-dimensional array of light transmissive regions **122** has a uniform pattern period d and uniform size a . A way to generate a two-dimensional array of focused light spots **124** (focus grid) is to illuminate the aperture mask with collimated light **112** (e.g., collimated beam). The focus grid is then generated around Talbot planes at: $z = mZ_T$ or $z = (m-1/2)Z_T$, where Z_T is the Talbot distance, as described by Eqns. 2 and 4. The pitch p of the focus grid will be the same as the pattern period d and the width b of the focused light spots **124(a)** will be similar to the size a for the basic Talbot effect.

[0054] In embodiments, the Talbot element **120** may be a layer with a microlens array. In these embodiments, a main array of focused light spots **124** (main focus grid) may be generated at the main focal plane of the microlens array. The main focus grid may generate more focus grids around Talbot planes at: $z = mZ_T$ or $z = (m-1/2)Z_T$, where Z_T is the Talbot distance as described in Eqns. 2 and 4. The width b of the focused light spots **124(a)** may be limited by the numerical aperture of the microlens in some cases.

[0055] In embodiments, the Talbot element **130** may be designed so that the distance between the light spots **124(a)** is larger than the size (e.g., pixel size) of the light detecting elements **160(a)** in the light detector **160**. With this design, each light detecting element **160(a)** corresponds to a single light spot **124(a)** and the intensity (e.g., emission intensity) from each light spot **124(a)** can be differentiated.

[0056] The intensity pattern can refer to a pattern of repeated elements (e.g., light spots **124(a)**). The intensity pattern may be, for example, an array of focused light spots **124**. The pitch p of the intensity pattern refers to the distance between repeated elements (e.g., light spots **124(a)**) of the intensity pattern. For example, the pitch p of an array of focused light spots **124** is the distance between two adjacent light spots **124(a)** in the array of focused light spots **124**. A width b can refer to the width of the repeated element (e.g., diameter of a circular light spot **124(a)**).

[0057] The geometry of intensity pattern (e.g., an array of focused light spots **124**) may correspond to the periodic pattern in the Talbot element **120** or may correspond to fractional values. For example, the pitch p between the light spots **124(a)** may be the same as

pattern period p of the Talbot element **130**. In a fractional Talbot effect example, the pitch p between the light spots **124(a)** may be a fraction (e.g., $1/2$, $1/4$, etc.) of the pattern period p of the Talbot element **130**. In the first example, the shape and size of the light spots may directly correspond to the shape and size of elements (e.g., apertures) in the periodic structure of the Talbot element **130**.

[0058] The TII system **10** of embodiments uses the array of focused light spots **124** generated by the Talbot effect or fractional Talbot effect for illumination in wide field-of-view imaging. In some cases, the TII system **10** uses a second phase reversed image ($m=2$) of the Talbot effect having a Talbot plane at $Z_{1.5} = 1.5 Z_T$.

[0059] A focal plane of an intensity pattern (e.g. array of focused light spots **124**) can refer to a plane at which the repeated elements (e.g., light spots **124(a)**) are most focused. In some cases, a focal plane **125** may be around a Talbot plane at: $z = mZ_T$ or $z=(m-1/2)Z_T$, where Z_T is the Talbot distance as described in Eqns. 2 and 4. In a case where the incident wavelength λ of the incident collimated light **112** is comparable to the width a of the light transmissive region, the focal plane **125** may be located away from the Talbot plane.

[0060] A plane of interest **126** can refer to a plane at which the TII system **10** or user of the TII system **10** may be imaging or otherwise investigating. A plane of interest **126** may be, for example, a plane (section) through one or more objects being imaged. The plane of interest **126** may be in any suitable direction. In some embodiments, the plane of interest **126** is parallel to the platform surface with the object(s) being imaged and is located at a distance z_I from the Talbot element **120**. Other suitable directions may be used in other embodiments.

[0061] In embodiments, the TII system **10** tunes the focal plane **125** by adjusting the light spots **124(a)** to the plane of interest **126**. That is, the focal plane **125** of the array of focused light spots **126** may be moved to or close to the plane of interest **126**. In the illustrated embodiment in **FIG. 4**, for example, the focal plane **125** of the array of focused light spots **124** is moved from an initial location at a distance z_0 from the Talbot element **120** to the plane of interest **126** at a distance z_I from the Talbot element **120**. In this example, the tunable illumination source **110** adjusted the wavelength of the incident light **112** by $\Delta\lambda$ to tune the focal plane **125** a distance of $z = z_I - z_0$. In some cases, the tunable illumination source **110** may adjust the wavelength λ in small increments ($\Delta\lambda/n$) to finely tune the focal plane **125** between the initial location and the final location.

[0062] A platform **130** can refer to any suitable transparent structure(s) capable of receiving an object(s) **300** for imaging by the TII system **10**. The platform **130** may also include structures or other devices for holding the objects **300**. For example, the platform **130** may be a transparent slide/dish and cover.

[0063] The scanning mechanism **140** can refer to a suitable device(s) capable of moving the object(s) **300** being imaged across the array of focused light spots **124** or moving the array of focused light spots **124** across the object(s) **300**. For example, the scanning mechanism **140** may move the platform with the object(s) **300** across the array of focused light spots **124**, may move the object(s) **300** across the array of focused light spots **124**, may move the Talbot element **120** so that the array of focused light spots **124** move across the object(s) **300**, etc. The scanning mechanism **140** can be based on any suitable method including, for example, microfluidic flow methods, optical tweezing methods, and scanning methods (raster scanning, linear scanning, etc.). In one example, the scanning mechanism **140** may include a raster scanning device for raster scanning the object(s) **300** or platform **130** with the object(s) **300** through the light spots **124(a)** or raster scanning the light spots **124(a)** over the object(s) **300**. An example of a scanning mechanism **140** employing a microfluidic flow method includes a fluid channel having a fluid flow with the object **300** being imaged. The scanning mechanism **130** can be in any suitable location.

[0064] The scanning mechanism **140** moves the object(s) **400** or array of focused light spots **124** in a scanning direction. In embodiments, the scanning direction is at a small angle θ from a x-axis or y-axis of the array of focused light spots **124**. The scanning direction may be, for example, in the direction along an x'-axis at a small angle θ from a x-axis. As another example, the scanning direction may be in the direction along an y'-axis at a small angle θ from a y-axis.

[0065] The relay lenses **150** refer to one or more suitable lenses capable of relaying light from the array of light spots **124** to the light detector **160**. The light from the one or more light spots **124(a)** includes light altered and unaltered by object(s) **300** being imaged by the TII system **10**. In one example, each relay lens **150** may be capable of relaying light from a single light spot **124(a)** to a single light detecting element **160(a)**. The relay lenses **150** may have any suitable numerical aperture value and may have any suitable spacing.

[0066] A light detector **160** can refer to a suitable device or combination of devices capable of receiving light, measuring/recording time-varying light data associated with the

light received, and generating one or more signals with time-varying light data. The one or more signals with time-varying light data may be in the form of an electrical current from the photoelectric effect. In some cases, the light detector **160** may be in the form of a one-dimensional linear array of a two-dimensional array of discrete light detecting elements **160(a)** (as shown in **FIG. 4**) of any suitable size (e.g., 1-10 microns) and any suitable shape (e.g., circular, rectangular, square, etc.). Some examples of suitable devices include a complementary metal oxide semiconductor (CMOS) imaging sensor array, a charge coupled device (CCD), electron multiplying charge coupled device (EMCCD), an avalanche photo-diode (APD) array, a photomultiplier tubes (PMT) array, and a photo-diode (PD) array. These light detectors **160** and others are commercially available. The light detector **160** can be a monochromatic detector or a multi-color detector (e.g., RGB detector).

[0067] Time-varying light data can refer to any suitable information related to the light received and measured by the light detector **160**. If the light detector **160** is the form of multiple discrete light detecting elements **160(a)** (as shown in **FIG. 4**), the time-varying light data may include suitable information related to the light received and measured by the multiple discrete light detecting elements **160(a)** on a time-varying basis. In some cases, each light detecting element **160(a)** can generate a signal with time-varying light data based on light received and measured by the light detecting element **160(a)**. Time-varying light data may include, for example, properties of the light received such as the intensity(ies) of the light, the wavelength(s) of the light, the frequency or frequencies of the light, the polarization(s) of the light, the phase(s) of the light, the spin angular momentum(s) of the light, and/or other light properties associated with the light received by the light detector **160** and/or each light detecting element **160(a)**. Time-varying light data may also include the scanning location, of the location of the light detecting element(s) **160(a)** receiving the light, the time that the light was received, or other information related to the light received. In some cases, each light detecting element **160(a)** can generate time varying information associated with a single focused light spot **124**. The time-varying light data can be compiled to construct an image or images of the object(s) **300**.

[0068] An object can refer to any suitable entity, such as a biological or inorganic entity, or portion of an entity. Examples of suitable biological entities include cells, cell components (e.g., proteins, nuclei, etc.), microorganisms such as bacteria or viruses, etc. Although one or two objects **300** are shown in illustrated embodiments, any suitable number (e.g., 1, 2, 3, 4, 5, 10, 100, etc.) of objects **300** can be imaged by the TII system **10**.

[0069] A computing device **200** can refer to any suitable combination of devices capable of performing computing and controlling functions of the TII system **10** such as reading out the collected signal(s) from the light detector **160**, triggering the functioning of components of the TII system **10**, reconstructing an image of one or more objects, etc. Some examples of suitable computing devices include a personal computer (desktop, laptop, etc.), a mobile communications device (e.g., smartphone or tablet), or other suitable device. In **FIG. 2**, the TII system **10** includes the computing device **200**. Alternatively, the computing device **200** can be a separate device from the TII system **10**.

[0070] A processor **210** can refer to any suitable processing device (e.g., microprocessor). The processor **210** can receive signals with time-varying light data from the light detector **160** associated with the light received by the light detecting elements **160(a)**. The processor **210** executes code stored on the CRM **220** to perform functions of TII system **10** such as interpreting time-varying light data from the light detector **160** and constructing one or more images from the time-varying light data.

[0071] The CRM **220** (e.g., memory) stores code for performing some functions of system **10**. The code is executable by the processor **210**. For example, the CRM **220** may include code for: c) code for interpreting time-varying light data received in one or more signals from the light detector **160**, d) code for constructing one or more images of object from the time-varying light data, f) code for displaying images on the display **230**, g) and/or any other suitable code for performing functions of the TII system **10**. The CRM **220** may also include code for performing any of the signal processing or other software-related functions that may be created by those of ordinary skill in the art. The code may be in any suitable programming language including C, C++, Pascal, etc. The processor **210** may be in any suitable location. In **FIG. 2**, the processor **210** is located in the computing device **200**. In another embodiment, the processor **210** may be located in the light detector **160**.

[0072] The display **230** can refer to an suitable device for displaying reconstructed images, light data, and other suitable data. Some examples of suitable displays include a computer monitor, cell phone panel, projection, etc. Suitable displays are commercially available. The display **230** may be a monochromatic or multi-color display. Also, the display may be a two-dimensional or three-dimensional display, etc. In one case, the image display **230** may be capable of displaying multiple views.

[0073] **FIG. 3** is a isometric view of some components of a TII device **100** for focal plane tuning, according to embodiments of the invention. The TII device **100** includes a tunable illumination source **110** providing collimated light **112**, a Talbot element **120** generating an array of focused light spots **122**, and a transparent platform **130** with two objects **300** being imaged by the TII system **10**. The Talbot element **120** (e.g., aperture mask) includes a 3 x 3 two-dimensional array of light transmissive regions (e.g., apertures). The array of focused light spots **124** is a 3 x 3 two-dimensional array comprising nine (9) focused light spots **124(a)**. Although the array of focused light spots **122** and array of light transmissive regions are 3 x 3 two-dimensional arrays, other embodiments may have arrays of other suitable dimensions. The TII device **100** also includes a scanning mechanism **140** for moving the objects **300** relative to the array of focused light spots **124**. The TII device **100** also includes relay lenses **150** for relaying light from the array of focused light spots **124**, which may be altered or unaltered by the objects **300**. The TII device **100** also includes a light detector **160** for receiving light from the relay lenses **150** and for generating time-varying light data based on the received light as the objects **300** move relative to the array of focused light spots **124**. The time-varying light data can be compiled by the processor **210** (shown in **FIG. 2**) to construct an image of the objects **300**.

[0074] **FIG. 4** is a cross-sectional side view of a portion of a TII device **100** for focal plane tuning, according to embodiments of the invention. The TII device **100** includes a tunable illumination source **110** providing collimated light **112**, a Talbot element **120** generating an array of focused light spots **124**, a platform **130** with an object **300** being imaged, a scanning mechanism **140**, relay lenses **150**, and a light detector **160** having discrete light detecting elements **160(a)**. The Talbot element **120** includes a 3 x 3 two-dimensional array of light transmissive regions **122** including nine (9) light transmissive regions **122(a)** and an outer surface **121**. Talbot element **120** also includes a x-axis, y-axis (not shown), and a z-axis. The x-axis and y-axis lie in a plane at the outer surface **121** of the Talbot element **120**. The array of light transmissive regions **122** has a period, d , between the light transmissive regions **122(a)**. Each light transmissive region **122(a)** in the array **122** has a width, a , along the x-direction. The Talbot element **120** generates 3 x 3 two-dimensional array of focused light spots **124** comprising nine (9) focused light spots **124(a)**. The array of focused light spots **124** lie at a focal plane **125** at a plane of interest **126** through the object **300**. The plane of interest **126** is at a distance, z_l , from the Talbot element **120**. In the illustrated example, the tunable illumination source **100** has adjusted the wavelength of the

collimated light **112** incident the Talbot element **120** to move the focal plane **125** from an initial distance, z_0 , from the Talbot element **120** to the distance, z_I , at the plane of interest **126**. The change in distance is $\Delta z = z_I - z_0$.

[0075] In **FIG. 4**, the scanning mechanism **140** moves the object **300** relative to the array of focused light spots **124**. Although not shown, the scanning mechanism **140** may be in communication with the object **300** or the platform **130** with the object **300** to move the object **300** relative to the array of focused light spots **124**, or may be in communication with the Talbot element **120** to be able to move the Talbot element **120** and array of focused light spots **124** relative to the object **300**. The relay lenses **150** can relay light from the array of focused light spots **124**, which may be altered or unaltered by the objects **300**. The TII device **100** also includes a light detector **160** for receiving focused light from the relay lenses **150** and for generating time-varying light data based on the received light as the object **300** moves relative to the array of focused light spots **124**. The light detector **160** includes discrete light detecting elements **160(a)**. In this example, each light detecting element **160(a)** may receives light from a single corresponding focused light spot **124(a)**. In other embodiments, two or more light detecting elements **160(a)** may receive light from a single focused light spots **124(a)** or one light detecting element **160(a)** may receive light from two or more focused light spots **124(a)**. The time-varying light data can be compiled by the processor **210** (shown in **FIG. 2**) to construct an image of the object **300**.

[0076] **FIG. 5(a)** is an illustration of an array of focused light spots **124** on the Talbot element plane as generated by a Talbot element **120** of a TII device **100**, according to embodiments of the invention. In the illustrated example, the array of focused light spots **124** is a two-dimensional array of focused light spots **124**. The dimensions of the two-dimensional array may be of any suitable size (1x1, 1x10, 10x10, 30x30, 33x16, 100x100, etc.). The array of focused light spots **124** includes an x-axis and a y-axis along the two dimensions of the two-dimensional array of focused light spots **124**. The array of focused light spots **124** also includes a x'-axis and a y'-axis. The x'-axis and y'-axis are tilted at a small angle θ with respect to the x-axis and y-axis. Any suitable small angle θ (e.g., 1 degree, 2 degrees, 5 degrees, etc.) may be used. In some cases, the x'-axis or the y'-axis can define the scanning direction used by the scanning mechanism **140**. The scanning mechanism **140** may scan the object(s) **300** or the Talbot element **120** in a scanning direction along either the x'-axis or the y'-axis.

[0077] **FIG. 5(b)** is an illustration of an array of focused light spots **124** on the Talbot element plane as generated by a Talbot element **120** of a TII device **100**, according to embodiments of the invention. In **FIG. 5(b)**, the Talbot element **120** has been rotated by a suitable small angle θ . The array of focused light spots **124** is a two-dimensional array of focused light spots **124** having dimensions of any suitable size. The array of focused light spots **124** includes an x -axis and a y -axis. The array of focused light spots **124** is oriented in the x -direction along the x -axis and the y -direction along the y -axis. The array of focused light spots **124** also includes a x' -axis and a y' -axis. The x -axis and y -axis are tilted at a small angle θ with respect to the x' -axis and y' -axis. The scanning direction used by the scanning mechanism **140** is defined by either the x' -axis or the y' -axis. The scanning mechanism **140** may scan the object(s) **300** or the Talbot element **120** in a scanning direction along either the x' -axis or the y' -axis.

[0078] Since many apertures **122(a)** contribute to a focused light spot **124(a)**, the intensity difference of the original array of light transmissive regions **122** will be partially averaged out. During image processing, the processor **210** can generate image uniformity by normalizing each line scan to compensate for the non-uniform intensity distribution of the light spots **124(a)**.

[0079] In an exemplary operation of a TII system **10** of embodiments, a specimen with one or more objects **300** is located on a platform **130**. The processor **210** sends a trigger signal to the tunable illumination source **110** to start generating collimated light **112** with an initial wavelength λ_0 . In some cases, the platform **130** may be located so that a plane of interest **126** (e.g., plane through the objects **300**) is located at a Talbot plane. Upon receiving the collimated light **112**, the Talbot element **120** generates a light field with an array of focused light spots **124** at a focal plane **125** at an initial distance z_0 from the Talbot element **120**. The initial distance z_0 may be away from the Talbot plane if, for example, the initial wavelength λ_0 is comparable to the aperture size a . Next, the processor **210** sends a signal to the scanning mechanism **140** to start scanning and a signal to the light detector **160** to start an initial image acquisition cycle. The scanning mechanism **130** moves the platform **130** with the object(s) **300** in a scanning direction across the array of focused light spots **124** or the Talbot element **120** in a scanning direction across the object(s) **300**. The relay lenses **150** receives light from the array of focused light spots **124** that has been altered and unaltered by the object(s) **300** as the object(s) **300** move relative to the array of focused light spots **124**. The light detector **160** receives light from the relay lenses **150** and records time-varying light

data (e.g., intensity data) of the altered and/or unaltered light as the object(s) move relative to the focused light spots **125**. After the light detector **160** completes the initial acquisition cycle, it sends a handshake signal to the processor **210**. The processor **210** sends a stop signal to the scanning mechanism **140** to stop scanning. The processor **210** receives a signal or signals with the time-varying light data from the light detector **160**. The processor **210** can reconstruct one or more initial images of the object(s) **300** based on the time-varying light data from each focused light spot **124** and the scanning speed.

[0080] Based on the initial images, a user or the processor **210** determines a wavelength change $\Delta\lambda$ using Eqn. 5 and based on z_1 and z_0 . The processor **210** may then send a signal to the tunable illumination source **110** to tune the focal plane **125** to a plane of interest **126** by adjusting the wavelength by a wavelength change $\Delta\lambda$ so that the new wavelength of the incident collimated light **112** is $\lambda_1 = \lambda_0 + \Delta\lambda$. Upon changing the wavelength, the array of focused light spots **124** is shifted to a distance Z_1 , which may be calculated as $z_1 = z_0 - 2d^2\Delta\lambda/\lambda_0^2$. The processor **210** may send additional signals to the tunable illumination source **100** to adjust the wavelength to tune the focal plane **125** to other planes of interest **126**. In some cases, the processor **210** may send signals to the tunable illumination source **100** to incrementally adjust the wavelength until the focal plane **125** is at or approximately at the plane of interest **126**. Any suitable small incremental change (10 nm, 20 nm, etc.) in wavelength can be used.

[0081] Once the focal plane **125** is at or approximately at the plane of interest **124**, the processor **210** sends a signal to the scanning mechanism **140** to start scanning and a signal to the light detector **160** to start image acquisition. The scanning mechanism **130** moves the platform **130** with the object(s) **300** in a scanning direction across the array of focused light spots **124**. Alternatively, the scanning mechanism **140** could move the Talbot element **120** in a scanning direction to move the array of focused light spots **124** across the object(s) **300**. The relay lenses **150** receives light from the array of focused light spots **124** that has been altered and unaltered by the object(s) **300** as the object(s) **300** move relative to the array of focused light spots **124**. The light detector **160** receives light from the relay lenses **150** and records time-varying light data (e.g., intensity data) of the altered and/or unaltered light as the object(s) move relative to the focused light spots **125**. After the light detector **160** completes the acquisition cycle, it sends a handshake signal to the processor **210**. The processor **210** sends a stop signal to the scanning mechanism **140** to stop scanning. The processor **210** receives a signal or signals with the time-varying light data from the light detector **160**. The

processor **210** can reconstruct one or more images of the object(s) **300** based on the time-varying light data from each focused light spot **124** and the scanning speed. The processor **210** can display the one or more images at the plane of interest on the display **230**.

[0082] In one case, the TII system **10** can perform z-axis scanning. In this case, the tunable illumination source **110** can be tuned to change the focal plane multiple times. For example, the tunable illumination source **110** may incrementally adjust the incident wavelength by a small value (e.g., 10 nm, 20 nm, etc.). After each adjustment, the TII system **10** can acquire an image. In these embodiment, the focal plane is tuned to multiple planes of interest and images are acquired at the multiple planes.

[0083] In one embodiment, the TII device **100** includes a tunable illumination source **110** providing collimated light **112**, a Talbot element **120** generating an array of focused light spots **122**, a platform **130** with object(s) **300**, a scanning mechanism **140**, relay lenses **150**, and a light detector **160**. In this embodiment, the tunable illumination source **110** and Talbot element **120** are located outside a first side of the transparent platform **130**. The tunable illumination source **110** provides collimated light **112** to the Talbot element **120** to generate an array of focused light spots **124** to an object(s) **300** located outside the first side of the transparent platform **130**. The light detector **160** and/or relay lenses **150** may be located on a second opposing side of the transparent platform **130**.

[0084] In one embodiment, a TII device **100** may include a Talbot element **120** that has an array of light transmissive regions that are designed to diffract more light at large angles to improve in tighter light spots generation.

[0085] Modifications, additions, or omissions may be made to the TII device **100** and/or TII system **10** of embodiments without departing from the scope of the disclosure. For example, the tunable illumination source **110** may be separate from the TII device **100** and/or TII system **10** in an embodiments. As another example, the TII device **100** and/or TII system **10** may omit the lens **150** as a component. As another example, a TII device **100** and/or TII system **10** may add a filter between the object(s) and the light detector **160**, for example, as a coating on side of the light detector **160** proximal the objects(s).

[0086] In addition, components of the TII device **100** and/or TII system **10** of embodiments may be integrated or separated according to particular needs. For example, the processor **210** may be integrated into the light detector **160** so that the light detector **160** performs one or more of the functions of the processor **210** in another TII system **10**. As

another example, the processor **210**, CRM **220**, and display **230** may be components of a computer separate from a TII system **10** and in communication with the TII system **10**. As another example, the processor **210**, CRM **220**, and/or display **230** may be integrated into components of the TII device **100**.

[0087] III. Experimental Results

[0088] In experiments, a TII system **10** with a TII device **100** having components arranged as illustrated in **FIG. 3** was used. The Talbot element **120** was an aperture mask having an array of light transmissive regions **122** that was a two-dimensional array of apertures. The Talbot element **120** was illuminated by collimated light **112** introduced by a tunable illumination source **110** (e.g., tunable Ti: Sapphire laser). The aperture mask had a pattern period of $d = 30 \mu\text{m}$ and aperture size $a = 800 \text{ nm}$. The incident wavelength λ was adjusted from 692 nm (λ_0) to 702 nm (λ_I). That is, the wavelength change was $\Delta\lambda = 692 \text{ nm} - 702 \text{ nm} = -10 \text{ nm}$. The plane of interest **126** was at a sample. The distance between the Talbot element **120** and the plane of interest **126** at the sample was set at $z_I = Z_{I,5}$ where $m = 2$, during an initial alignment. The tunable illumination source was first set to an initial incident wavelength λ_0 . At λ_0 , the focal plane **125** was located at z_0 . Subsequently, the tunable illumination source **100** finely adjusted the incident wavelength to a final incident wavelength λ_I . At this wavelength, the focal plane **125** is located at the plane of interest **126** through the object **300**.

[0089] According to Eqn. 5, the tuning range Δz of the focal plane position was $55 \mu\text{m}$ in the experiment. The sample with objects **300** was then illuminated by the array of focused light spots **124** (focus grid) and the transmission of the light spots **124** was projected by the relay lenses **150** (e.g., the Edmund Optics.® NT45-760 relay lenses) onto an light detector **160** (e.g., Lumenera ® imaging sensor). The sample was scanned across the focus grid in the scanning direction (y' -direction). The scanning direction (y' -direction) is slightly tilted at a small angle θ with respect to the focus grid, which is oriented at x - and y -direction. The image can be reconstructed by compiling line scans from each focused lights spots **124** and properly shifting each the time varying light data according to the scanning speed.

[0090] **FIG. 6(a)** is an image of a portion of the two-dimensional array of apertures in the Talbot element **110** of the TII device **100**, according to an embodiment of the invention. **FIG. 6(b)** is an image of a portion of the focus grid at $z = Z_{I,5}$, according to an embodiment of the invention. The intensity uniformity of the focus grid was more uniform than the original

array of apertures. The averaged relative adjacent spot intensity variation is 4% for the focus grid and 20% for the original array of apertures (aperture grid). Since many apertures contribute to a focused light spot **124(a)**, the intensity difference of the original array of apertures will be partially averaged out, which creates this increased intensity uniformity. During image processing, the processor **210** can generate image uniformity by normalizing each line scan to compensate for the non-uniform intensity distribution of the focused light spots **124(a)**.

[0091] Next, the TII system **10** was used in an imaging demonstration. The array of focused light spots **124** was a two-dimensional array having dimensions of 213×60 (in x -direction and y -direction, respectively), and the scanning was performed in the y' -direction. Thus the reconstructed image is oriented in the x' and y' -direction. The image will have a saw-tooth shape at the starting and ending part because of the linear scanning characteristics. The effective field-of-view in the x' -direction is $213 * d \approx 6.4$ mm. In the experiments, the sample was moved at a speed of $v = 0.165$ mm/s for $L = 6$ mm. At the same time, the light detector **160** acquired 12,000 frames at $F = 330$ frames/s. The exposure time of the light detector **160** was 0.5 ms. Thus, the sampling distance in the y' -direction is $v/F = 0.5$ μ m. The sampling distance in the x' -direction is determined by the angle θ between the scanning direction (y' -direction) and the grid orientation (y -direction), which was set to be 0.0167. And thus, the sampling distance in x' -direction is $d * \sin(\theta) = 0.5$ μ m. The effective FOV in the y' -direction can be calculated by $L - H = 4.2$ mm, where $H = 60 * d = 1.8$ mm is the extent of focus grid in y -direction.

[0092] Next, the TII system **10** was used in a resolution test to image a U.S. Air Force (USAF) target. **FIG. 7(a)** is a wide field-of-view image of the U.S. Air Force (USAF) target acquired by the TII system **10**, according to an embodiment of the invention. The effective field-of-view of the TII system **10** was 6.4×4.2 mm. The effective field-of-view is indicated in the large dashed rectangle in **FIG. 7(a)**. **FIG. 7(b)(1)** is an expanded view of the region indicated in **FIG. 7(a)**, according to an embodiment of the invention. **FIG. 7(b)(2)** is a cross-sectional intensity profile of group 8, element 6 from the expanded view of the region indicated in **FIG. 7(b)(1)**, according to an embodiment of the invention. The features size with a line width of 1.1 μ m (group 8, element 6) can be resolved, which agrees with the measured spot size of ~ 1 μ m shown in **FIG. 1**. The focal spot size was measured to be ~ 1 μ m under a conventional microscope.

[0093] Next, the TII system **10** was used to acquire images of a mixed green algae microscope slide. Images were acquired at different focal planes corresponding to different incident wavelengths $\lambda_I = 692$ nm and $\lambda_2 = 702$ nm. **FIG. 8(a)** is an image of the mixed green algae acquired by the TII system **10** using the incident wavelengths $\lambda_I = 692$ nm, according to an embodiment of the invention. **FIG. 8(b)** is an expanded view of the region indicated in **FIG. 8(a)**, according to an embodiment of the invention. **FIG. 8(c)** is an image of the mixed green algae acquired by the TII system **10** using the incident wavelengths $\lambda_I = 702$ nm, according to an embodiment of the invention. **FIG. 8(d)** is an expanded view of the region indicated in **FIG. 8(c)**, according to an embodiment of the invention. As shown in **FIGS. 8(b)** and **8(d)**, the TII system **10** focused at different focal planes and rendered high resolution images of the sample at the different focal planes.

[0094] IV. Flowchart

[0095] **FIG. 9** is a flow chart of an exemplary method of focal plane tuning using a TII system **10**, according to embodiments of the invention. In **step 310**, the object **300** is introduced into the TII device **100** and received by the platform **130**. The plane of interest is at a distance z_I from the Talbot element **120**, as shown, for example, in **FIG. 4**. In some cases, the platform **130** may be located so that a plane of interest **126** is located at a distance z_I calculated as an integer multiple of a full or half Talbot distance from the Talbot element **120** such as shown in **FIG. 4**. If the Talbot element **120** is a square grid pattern the distance z_I can be calculated using Eqn. 1 or Eqn. 3 where d is the pattern period of the square grid pattern and $\lambda = \lambda_0$, which is the initial incident wavelength.

[0096] In **step 320**, the tunable illumination source **110** is set to provide collimated light **112** to the Talbot element **120** at the initial incident wavelength of λ_0 . In one case, the processor **210** may send a trigger signal to the tunable illumination source **110** to start generating collimated light **112** with an initial wavelength λ_0 . The Talbot element **120** generates a light field with an array of focused light spots **124** located at a focal plane **125** at an initial distance z_0 from the Talbot element **120**, as shown, for example, in **FIG. 4**. The initial distance z_0 may be away from a Talbot plane **120** if, for example, the initial wavelength λ_0 is comparable to the aperture size a of a Talbot element **120** comprising an array of light transmissive regions. In **FIG. 4**, the initial wavelength λ_0 is comparable to the aperture size a of the Talbot element **120** and the initial distance z_0 and calculated distance z_I at the Talbot plane are separated by a distance $\Delta z = z_I - z_0$.

[0097] In **step 330**, the scanning mechanism **140** scans the object **300** (or the platform **130** with the object) in a scanning direction across the array of focused light spots **124** or scans the Talbot element **120** in a scanning direction across the object **300**. The scanning direction can be any suitable direction. For example, the scanning direction may be in the x' -direction along the x' -axis or in the y' -direction along the y' -axis, as shown in **FIG. 5**. In one case, the processor **210** may send a start signal to the scanning mechanism **140** to start scanning and a signal to the light detector **160** to start an initial image acquisition cycle.

[0098] The object **300** alters light from the array of focused light spots **124**. In one case, the relay lenses **150** receives light from the array of focused light spots **124** that has been altered and unaltered by the object **300** as the object **300** move relative to the array of focused light spots **124**. The relay lenses **150** relay light to the light detector **160**.

[0099] In **step 340**, the light detector **160** receives light altered/unaltered by the object **300** and captures time-varying light data (e.g., intensity data) of the altered and/or unaltered light as the object **300** moves relative to the array of focused light spots **124**.

[0100] In **step 350**, the processor **210** can reconstruct one or more images at the focal plane **125** through the object **300** based on the time-varying light data from each focused light spot **124** and the scanning speed. The processor **210** can display the one or more images at the focal plane **125** on the display **230**. The processor **210** may be separate from the light detector **210** or may be a part of the light detector **210**. After image acquisition, the processor **210** may sent a stop signal to the scanning mechanism **140** to stop scanning.

[0101] In **step 360**, the processor **210** determines whether to shift the focal plane **125**. The processor **210** may make the determination based on input from a user of the TII system **10** or makes the determination based on an analysis. For example, the user of the TII system **10** may determine that the current cross-sectional image of the object **300** is not at the desired plane of interest. In **FIG. 4**, for example, the first image taken at the focal plane at z_0 may not include the area of concern (e.g., nucleus) of the object **300** (e.g., cell) and the user may determine that another image at a distance slightly above the current focal plane is desired. As another example, the processor **210** may be able to determine that the cross sectional image is not in focus using an auto-focusing scheme at the area of concern.

[0102] If the processor **210** determines that the image is not at the plane of interest and to shift the focal plane, the method continues to step **370**. The processor **210** may determine the plane shift Δz based on input from a user of the TII system **10** or makes the

determination based on an analysis. For example, the user of the TII system **10** may determine that the focal plane must be shifted along the z-axis by an increment Δz . As another example, the processor **210** may calculate the Δz based on z_1 and z_0 . In one embodiment, the processor **210** may automatically determine the Δz in an autofocus scheme. The processor **210** calculates the $\Delta \lambda$ based on the determined Δz and current incident wavelength using Eqn. 5.

[0103] At step **380**, the processor **210** may then send a signal to the tunable illumination source **110** to tune the focal plane **125** to a new focal plane **125** by adjusting the wavelength by the wavelength change $\Delta \lambda$. The tunable illumination source **110** will then adjust the incident wavelength by the wavelength change $\Delta \lambda$ to shift the focal plane. If the processor **210** determines that the image is at the plane of interest and not to shift the focal plane at step **360**, the method ends at step **370**.

[0104] V. Subsystems

[0105] **FIG. 10** is a block diagram of subsystems that may be present in the TII system **10**, according to embodiments of the invention. For example, the TII system **10** includes a processor **210**. The processor **210** may be a component of the light detector **160** in some cases.

[0106] The various components previously described in the Figures may operate using one or more of the subsystems to facilitate the functions described herein. Any of the components in the Figures may use any suitable number of subsystems to facilitate the functions described herein. Examples of such subsystems and/or components are shown in a **FIG. 10**. The subsystems shown in **FIG. 10** are interconnected via a system bus **425**. Additional subsystems such as a printer **430**, keyboard **432**, fixed disk **434** (or other memory comprising computer readable media), display **230**, which is coupled to display adapter **438**, and others are shown. The display **230** may include the illuminating display **116** and/or the image display **230**. Peripherals and input/output (I/O) devices, which couple to I/O controller **440**, can be connected to the computer system by any number of means known in the art, such as serial port **442**. For example, serial port **442** or external interface **444** 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 processor **210** to communicate with each subsystem and to control the execution of instructions from system memory **446** or the fixed disk **434**, as well as the exchange of information between

subsystems. The system memory **446** and/or the fixed disk **434** may embody a CRM **220**. Any of these elements may be present in the previously described features.

[0107] In some embodiments, an output device such as the printer **430** or display **230** of the TII system **10** can output various forms of data. For example, the TII system **10** can output 2D/3D color/monochromatic images, data associated with these images, or other data associated with analyses performed by the TII system **10**.

[0108] 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.

[0109] 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 CRM, 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 CRM may reside on or within a single computational apparatus, and may be present on or within different computational apparatuses within a system or network.

[0110] A recitation of “a”, “an” or “the” is intended to mean “one or more” unless specifically indicated to the contrary.

[0111] 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.

[0112] 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.

[0113] 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 Talbot-illuminated imaging device for focal plane tuning, the device comprising:
 - a Talbot element configured to generate an array of focused light spots at a focal plane; and
 - a tunable illumination source configured to shift the focal plane to a plane of interest by adjusting a wavelength of light incident the Talbot element.
2. The Talbot-illuminated imaging device for focal plane tuning of claim 1, further comprising:
 - a scanning mechanism configured to move an object relative to the array of focused light spots in a scanning direction; and
 - a light detector configured to determine time-varying light data associated with the array of focused light spots as the object moves relative to the array of light spots, wherein the object is located between the Talbot element and the light detector.
3. The Talbot-illuminated imaging device for focal plane tuning of claim 2, further comprising a processor configured to reconstruct an image of the object based on the time-varying light data.
4. The Talbot-illuminated imaging device for focal plane tuning of claim 2, wherein the light detector is further configured to reconstruct an image of the object based on the time-varying light data.
5. The Talbot-illuminated imaging device for focal plane tuning of claim 2, further comprising a platform between the object and the Talbot element, the platform for receiving the object.
6. The Talbot-illuminated imaging device for focal plane tuning of claim 2, further comprising relay lenses between the object and the light detector.

8. The Talbot-illuminated imaging device for focal plane tuning of claim 1, wherein the focal plane is located away from a Talbot plane.

9. The Talbot-illuminated imaging device for focal plane tuning of claim 1, wherein the Talbot element is an array of light transmissive regions.

10. The Talbot-illuminated imaging device for focal plane tuning of claim 1, wherein the Talbot element is a microlens array.

11. A Talbot-illuminated imaging system for focal plane tuning, the system comprising:

a Talbot-illuminated imaging device comprising

a Talbot element configured to generate an array of focused light spots at a focal plane,

a tunable illumination source configured to shift the focal plane to a plane of interest by adjusting a wavelength of light incident the Talbot element,

a scanning mechanism configured to move an object relative to the array of focused light spots in a scanning direction, and

a light detector configured to determine time-varying light data associated with the array of focused light spots as the object moves relative to the array of light spots; and

a processor configured to reconstruct an image of the object based on the time-varying light data.

12. The Talbot-illuminated imaging system for focal plane tuning of claim 11, wherein the object is located between the Talbot element and the light detector.

13. The Talbot-illuminated imaging system for focal plane tuning of claim 11, wherein the light detector includes the processor.

14. The Talbot-illuminated imaging device for focal plane tuning of claim 1, wherein the focal plane is located away from a Talbot plane.

15. A method of focal plane tuning using a Talbot-illuminated imaging system having a Talbot element, a tunable illumination source, a scanning mechanism and a light detector, the method comprising:

providing light of an incident wavelength to the Talbot element to generate an array of focused light spots;

adjusting, by the tunable illumination source, the incident wavelength to shift the focal plane of the array of focused light spots;

capturing time-varying light data associated with the array of focused light spots as an object moves relative to the array of focused light spots; and

constructing an image of the object based on the time varying light data.

16. The method of focal plane tuning of claim 15, wherein the tunable illumination source adjusts the incident wavelength to tune the focal plane to a plane of interest.

17. The method of focal plane tuning of claim 15,, wherein the Talbot element is an array of light transmissive regions.

18. The method of focal plane tuning of claim 15, wherein the Talbot element is a microlens array.

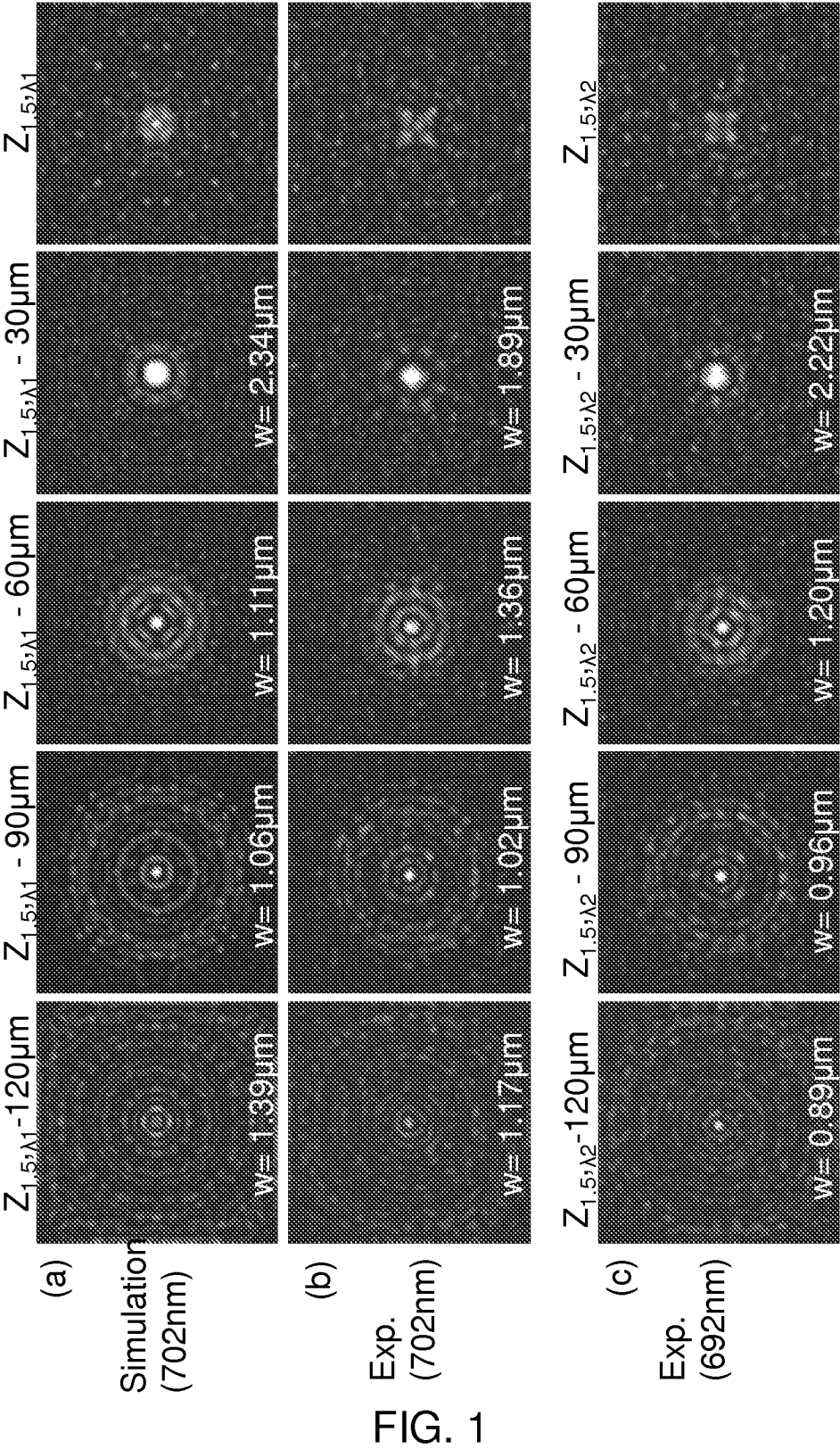


FIG. 1

2/10

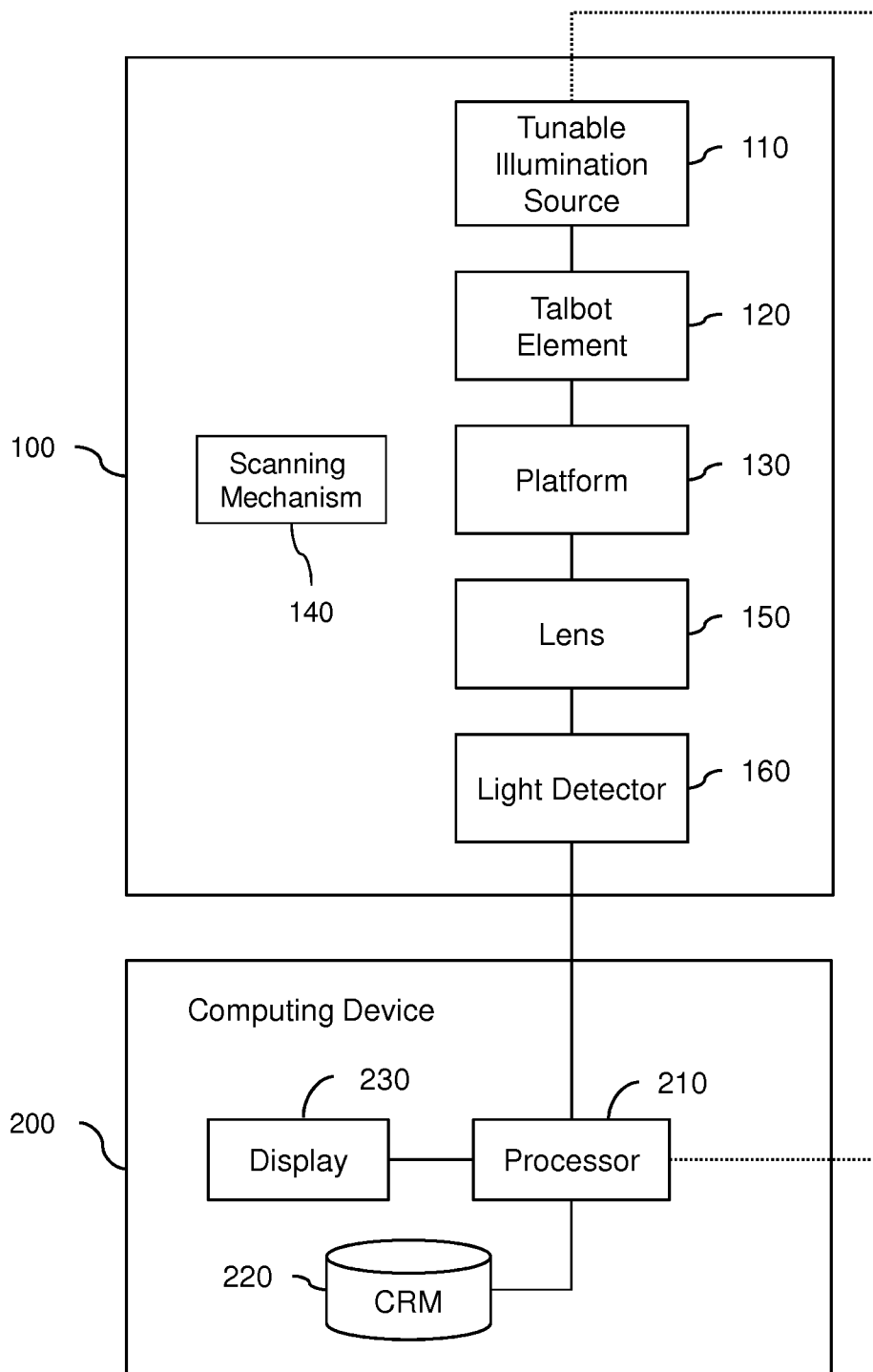
10

FIG. 2

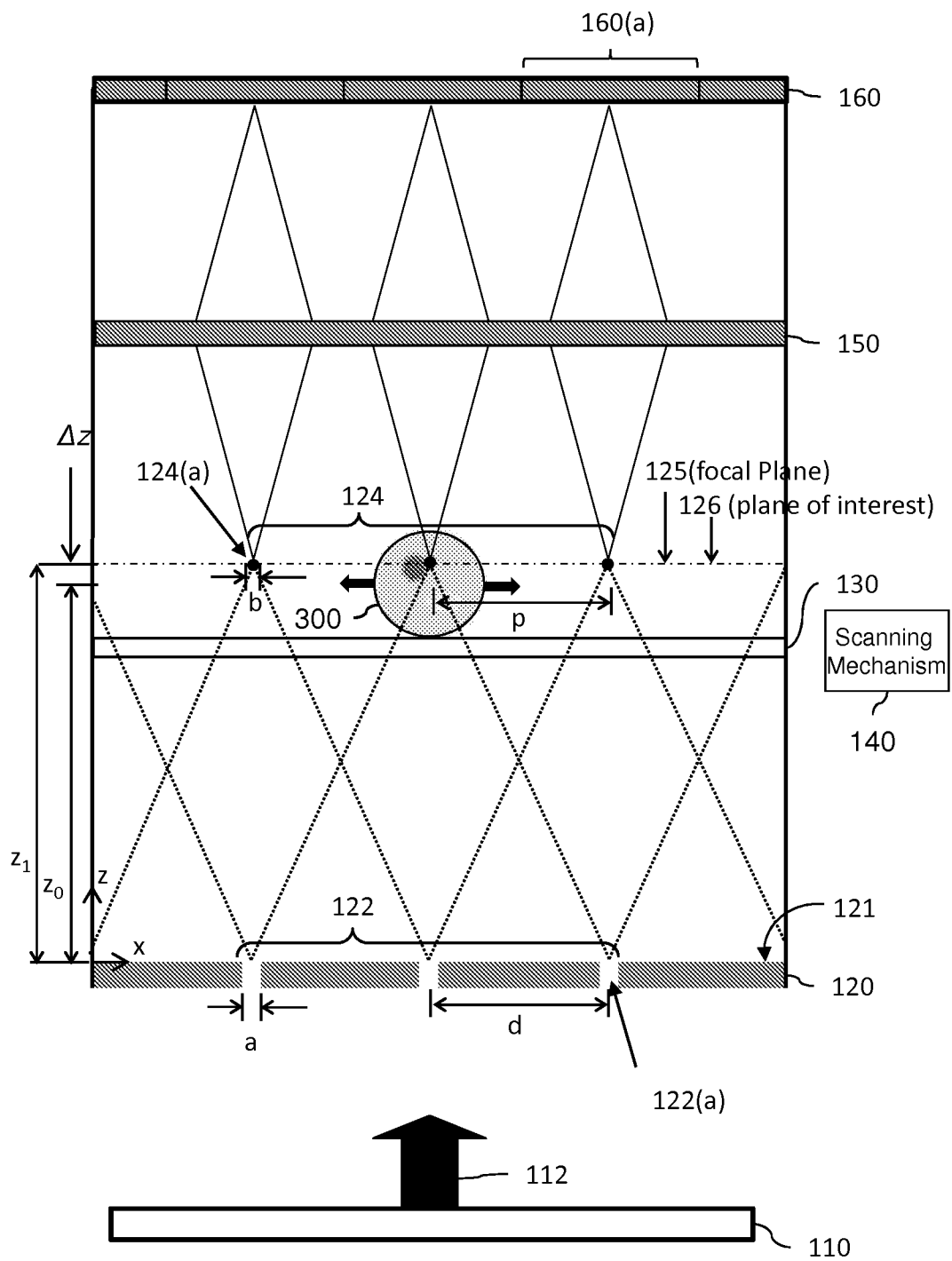


FIG. 4

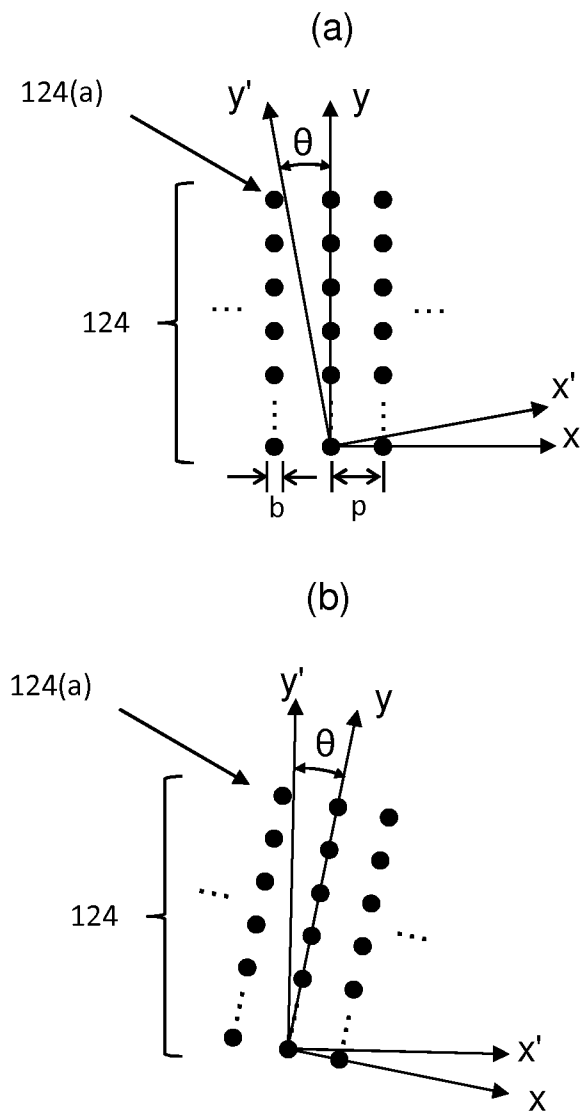
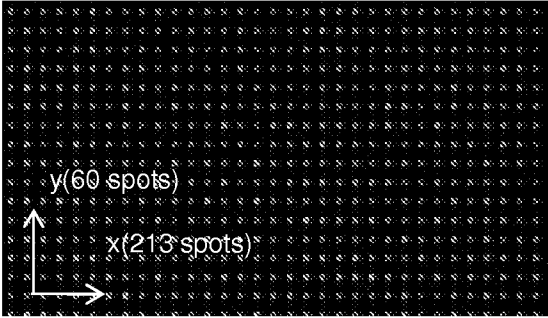


FIG. 5

(a)



(b)

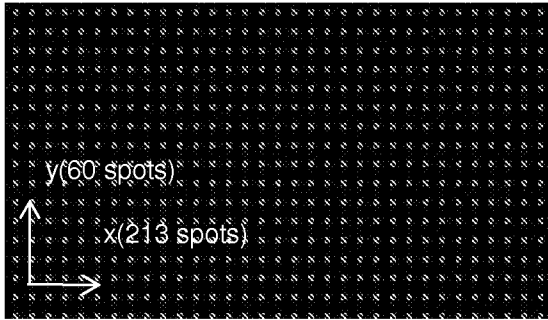


FIG. 6

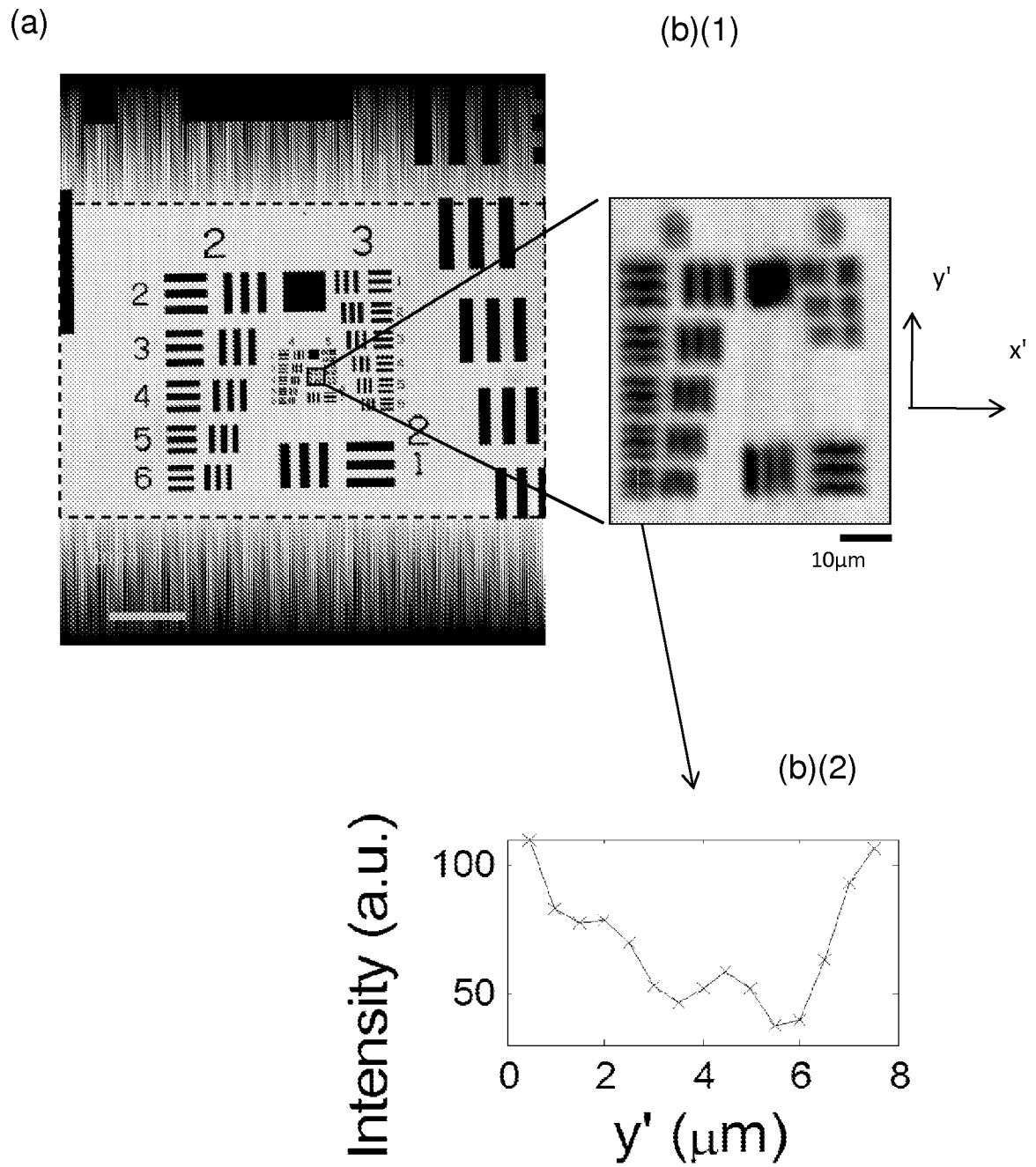


FIG. 7

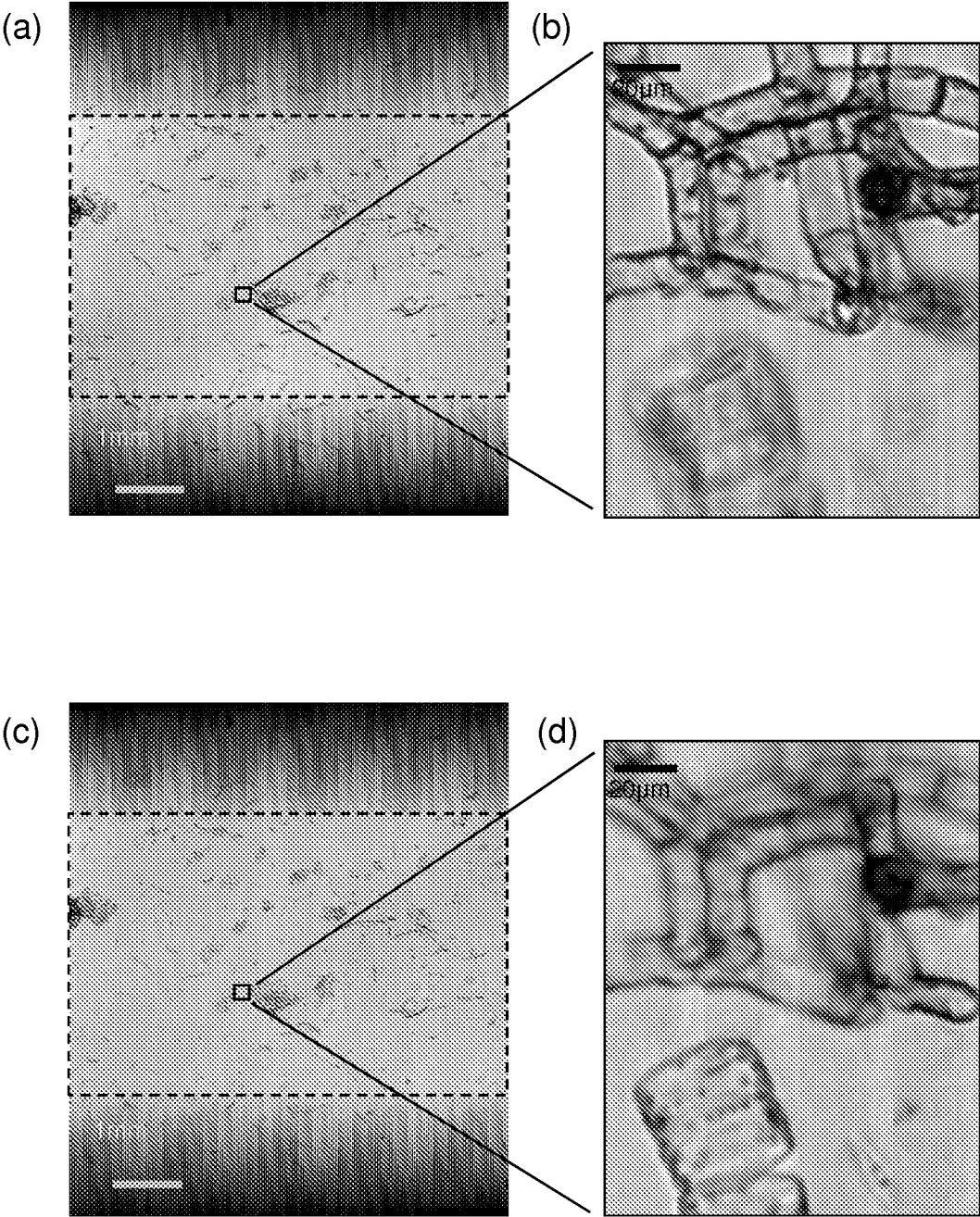


FIG.8

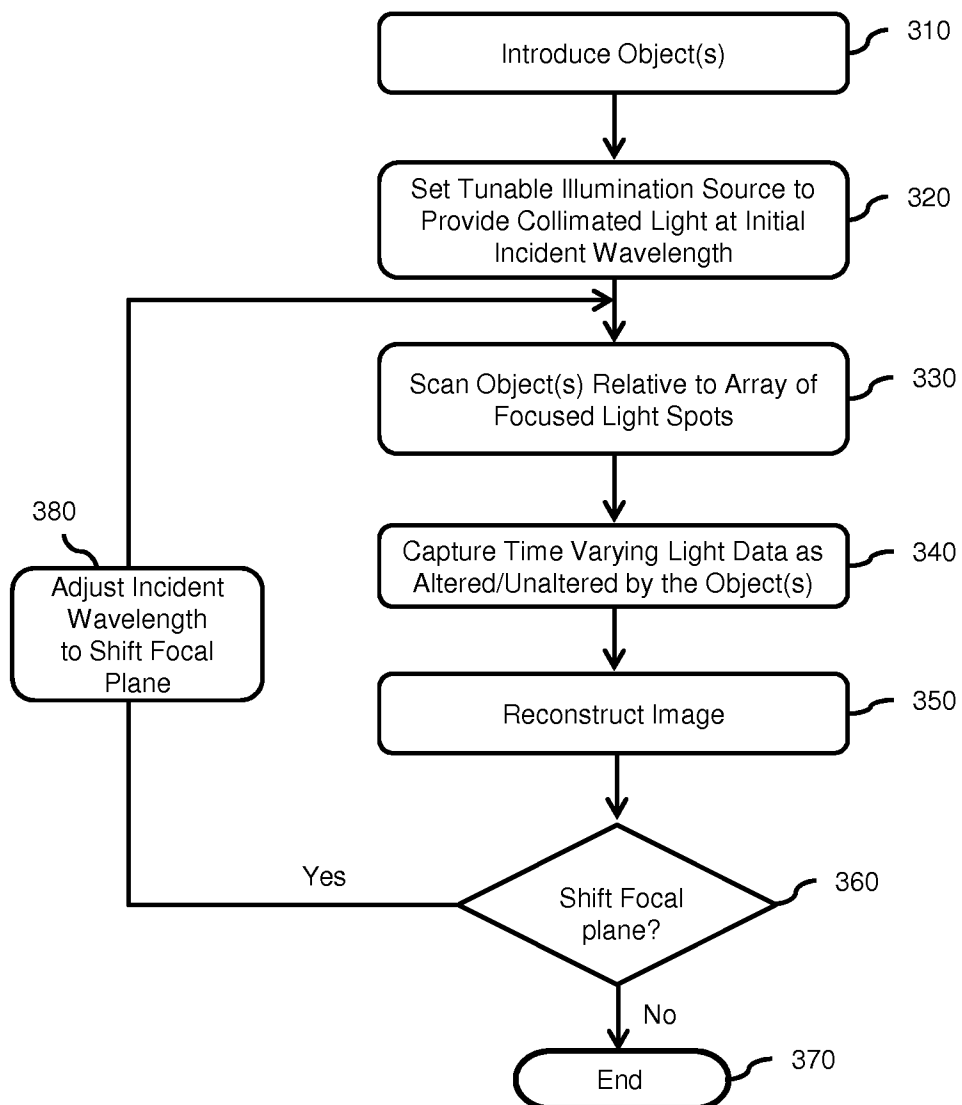


FIG. 9

10/10

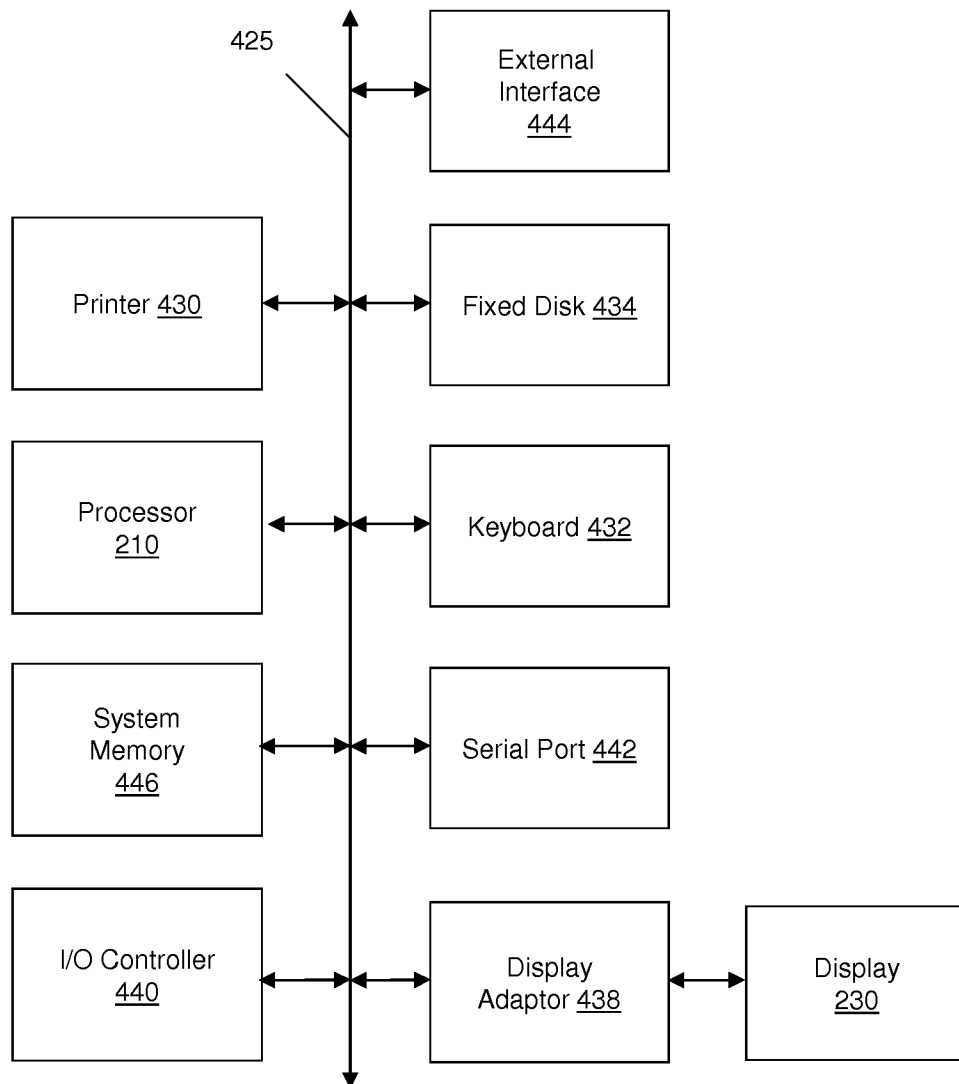


FIG. 10