



(51) International Patent Classification:

A61B 5/00 (2006.01)

(21) International Application Number:

PCT/US2019/046574

(22) International Filing Date:

14 August 2019 (14.08.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/718,777

14 August 2018 (14.08.2018)

US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: MULTIFOCAL PHOTOACOUSTIC MICROSCOPY THROUGH AN ERGODIC RELAY

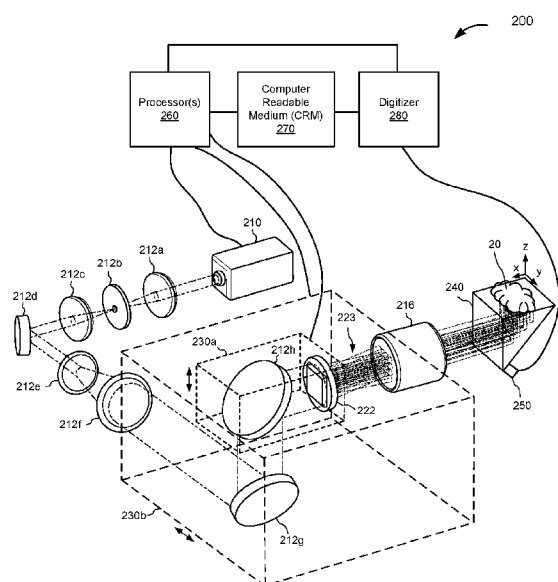


FIG. 2

(57) Abstract: A multifocal photoacoustic imaging system comprises a pattern-generating device (e.g., a microlens array, a spatial light modulator, a digital micromirror device, etc.), an ergodic relay (e.g., prism, plate, silicon wafer, etc.) in optical communication with the pattern-generating device, and an ultrasonic transducer device (e.g., single element transducer) in acoustic communication with the ergodic relay.

Published:

— *with international search report (Art. 21(3))*

MULTIFOCAL PHOTOACOUSTIC MICROSCOPY THROUGH AN ERGODIC RELAY

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims priority to and benefit of U.S. Provisional Patent Application No. 62/718,777, titled “Multifocal Photoacoustic Microscopy through an ergodic relay,” and filed on August 14, 2018, which is hereby incorporated by reference in its entirety and for all purposes.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant No. EY026228 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD

[0003] Certain implementations pertain generally to photoacoustic imaging, and more specifically, to photoacoustic imaging systems and methods that include an ergodic relay for encoding photoacoustic signals.

BACKGROUND

[0004] Photoacoustic imaging is based on the photoacoustic effect where pulsed or modulated radiation is delivered to a sample and some of the delivered energy is absorbed and converted into heat, leading to transient thermoelastic expansion generating ultrasonic emissions. The ultrasonic emissions can be detected by an ultrasonic transducer device and analyzed to produce photoacoustic images. A photoacoustic image depends on the optical absorption properties of the sample being imaged. As a consequence, it offers greater molecular specificity than conventional ultrasound imaging with the ability to detect hemoglobin, lipids, water and other light-absorbing chromophores, but with greater penetration depth than pure optical imaging modalities that rely on ballistic photons. These attributes lend photoacoustic imaging to a wide variety of applications in clinical medicine, preclinical research and basic biology for studying cancer, cardiovascular disease, abnormalities of the microcirculation and other conditions.

SUMMARY

[0005] Certain implementations pertain to a multifocal photoacoustic imaging system comprising a pattern-generating device (e.g., a microlens array, a spatial light modulator, a digital micromirror device, etc.), an ergodic relay (e.g., prism, plate, silicon wafer, etc.) in optical communication with the pattern-generating device, and an ultrasonic transducer device

(e.g., single element transducer) in acoustic communication with the ergodic relay. The pattern-generating device is configured to generate a plurality of illumination beams, wherein the plurality of illumination beams generate a plurality of focal spots in a corresponding plurality of optical foci regions in a field-of-view of a sample being imaged. The ergodic relay is also acoustically coupled to the sample. The ergodic relay is configured to encode photoacoustic signals received from the illuminated optical foci regions. In one implementation, the multifocal photoacoustic imaging system further comprises one or more optical elements configured to direct the plurality of illumination beams from the pattern-generating device to the field-of-view. In another implementation, the ergodic relay is further configured to reflect the plurality of illumination beams to the field-of view. In one aspect, the multifocal photoacoustic imaging system further comprises a scanning mechanism (e.g., scanning mirror or one or more motors) configured to scan the plurality of illumination beams in the field-of-view, wherein the focal spot of each illumination beam is scanned to a plurality of scan locations in the corresponding optical foci region. In some cases, the multifocal photoacoustic imaging system further comprises one or more processors configured to execute instructions configured to reconstruct a photoacoustic image from the encoded photoacoustic signals. For example, the one or more processors may be configured to execute instructions configured to: generate a plurality of snapshot photoacoustic images from the encoded photoacoustic signals, each snapshot photoacoustic image corresponding to one of the plurality of scan locations, for each of the plurality of snapshot photoacoustic images, localize pixel values within each focal spot of each optical foci region to generate a localized snapshot image, and combine the localized snapshot images to generate a multifocal optical resolution photoacoustic image. In one implementation, the pattern-generating device is in optical communication with a light source (e.g., pulsed laser or one or more light emitting diodes) configured to produce a light beam of pulsed and/or modulated radiation, wherein the pattern-generating device is configured to generate the plurality of illumination beams from the light beam.

[0006] In one aspect, a multifocal photoacoustic imaging system is configured to reconstruct a photoacoustic image having an acoustic spatial resolution of less than 220 μm . In another aspect, a multifocal photoacoustic imaging system is configured to reconstruct a photoacoustic image having an acoustic spatial resolution of less than 220 μm .

[0007] In one aspect, a multifocal photoacoustic imaging system is configured to reconstruct a photoacoustic image based on photoacoustic signals encoded by an ergodic relay in less than 10 seconds for a field-of-view of 100 mm^2 . In another aspect, a multifocal photoacoustic imaging

system is configured to reconstruct a photoacoustic image based on photoacoustic signals encoded by an ergodic relay in less than 1000 seconds for a field-of-view of 100 mm².

[0008] In one implementation, the pattern-generating device is in optical communication with a light source (e.g., pulsed laser or one or more light emitting diodes) configured to produce a light beam of pulsed and/or modulated radiation, wherein the pattern-generating device is configured to generate the plurality of illumination beams from the light beam.

[0009] Certain implementations pertain to a multifocal photoacoustic imaging method comprising (a) communicating control signals to a pattern-generating device to cause generation of a plurality of illumination beams with focal spots illuminating a corresponding plurality of optical foci regions in a field-of-view of a sample being imaged, (b) receiving encoded photoacoustic signals detected by an ultrasound transducer device in acoustic communication with an ergodic relay, wherein the ergodic relay is in acoustic communication with the sample being imaged, and (c) reconstructing a snapshot photoacoustic image using calibrated system responses and the encoded photoacoustic signals. In one implementation, the multifocal photoacoustic imaging method further comprises generating a localized photoacoustic image by assigning a calculated value (e.g., a maximum value or a mean value of pixels within the focal spot) to pixels within the focal spot of each optical foci region in the snapshot photoacoustic image and/or zeroing pixels outside of the focal spot of each optical foci region.

[0010] In one implementation, the multifocal photoacoustic imaging method further comprises communicating control signals to a scanning mechanism to cause scanning of the plurality of illumination beams, wherein each illumination beam is scanned to a plurality of scan locations within a corresponding optical foci region. In certain aspects, the multifocal photoacoustic imaging method further comprises repeating (c) for each of the plurality of scan locations to reconstruct a plurality of snapshot photoacoustic images. In one aspect, the multifocal photoacoustic imaging method further comprises for each of the plurality of snapshot photoacoustic images, generating a localized photoacoustic image. In one implementation, the multifocal photoacoustic imaging method further comprises combining the plurality of localized photoacoustic images to generate a multifocal optical resolution photoacoustic image of the field-of-view.

[0011] Certain implementations pertain to a multifocal photoacoustic imaging method comprising: (a) receiving encoded photoacoustic signals detected by an ultrasound transducer device in acoustic communication with an ergodic relay, the photoacoustic signals detected while a plurality of illumination beams is scanned over a field-of-view being imaged, wherein a focal

spot of each illumination beam is scanned to a plurality of locations within an optical foci region of a plurality of optical foci regions in the field-of-view, (b) reconstructing a plurality of snapshot photoacoustic images, each snapshot image reconstructed using calibrated system responses and encoded photoacoustic signals detected by the ultrasound transducer device while the plurality of illumination beams is at one of a plurality of scan locations, (c) for each of the plurality of snapshot photoacoustic images, generating a localized photoacoustic image by assigning pixels within the focal spot of each optical foci region to a calculated value (e.g., a maximum value or a mean value of pixels within the focal spot of each optical foci region), and (d) combining the plurality of localized photoacoustic images to generate a multifocal optical resolution photoacoustic image of the field-of-view. In one implementation, the multifocal photoacoustic imaging method further includes zeroing pixels outside of the focal spot of each optical foci region.

[0012] These and other features are described in more detail below with reference to the associated drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 is a schematic illustration of an MFOR-PAMER system, according to various implementations.

[0014] FIG. 2 is a schematic illustration of an MFOR-PAMER system, according to various implementations.

[0015] FIG. 3 is a schematic illustration of a side view of an ergodic relay in the form of a right-angle prism, according to certain aspects.

[0016] FIG. 4 is a schematic illustration of a field-of-view being imaged by an MFOR-PAMER system, according to certain aspects.

[0017] FIG. 5 is a schematic drawing of a plan view and a cross-sectional A-A view of a microlens array, according to one aspect.

[0018] FIG. 6 is a flowchart depicting operations of an MFOR-PAMER method, according to certain aspects.

[0019] FIG. 7 is a flowchart depicting operations of an MFOR-PAMER method, according to certain aspects.

[0020] FIG. 8A is an illustration of the actual positions of simulated microlens focal spots of an MFOR-PAMER system, according to an aspect.

[0021] FIG. 8B is a reconstructed image of the simulated microlens array pattern of an MFOR-PAMER system, according to an aspect.

[0022] FIG. 8C is a reconstructed image, according to an aspect.

[0023] FIG. 8D is an illustration of separation distance for two spots in an MFOR-PAMER system, according to certain implementations.

5 [0024] FIG. 9A is an illustration of the quantification of the acoustical lateral resolution of an imaging system without localization.

[0025] FIG. 9B is a graph of the contrast-to-noise ratio versus the distance between the two light spots.

[0026] FIG. 9C is an illustration of the quantification of the optical lateral resolution of an MFOR-PAMER system, according to one implementation.

10 [0027] FIG. 9D is an illustration of the edge-spread function used to calculate the line-spread function of an MFOR-PAMER system, according to one implementation.

[0028] FIG. 10A is a single-frame reconstructed image from a single scanning step of the microlens array excitation pattern of an of an MFOR-PAMER system, according to one implementation.

15 [0029] FIG. 10B is an image created by an imaging system without localization.

[0030] FIG. 10C is an MFOR-PAMER image that was constructed by localizing the optical foci from the scanning steps, according to an aspect.

[0031] FIG. 11A is an AR-PAMER image of the ear vasculature with a zoomed in portion.

20 [0032] FIG. 11B is an MFOR-PAMER image of the ear vasculature with a zoomed in portion, according to an aspect.

[0033] FIG. 12 is a graph of line profiles across the two white dashed lines in the boxed regions in the zoomed in views from FIG. 11A-B.

DETAILED DESCRIPTION

[0034] Different aspects are described below with reference to the accompanying drawings.

25 The features illustrated in the drawings may not be to scale. Certain implementations pertain to multifocal optical-resolution photoacoustic microscopy through an ergodic relay (MFOR-

PAMER) systems and methods. In one implementation, an MFOR-PAMER system includes a pattern-generating device such as a microlens array that can generate multiple illumination beams from a beam such as a pulsed laser beam. The illumination beams are scanned over a

30 field-of-view of a sample being imaged. The focal spot of each illumination beam is scanned to a plurality of scan locations over a corresponding optical foci region of a plurality of optical foci regions in the field-of-view. An ergodic relay is acoustically coupled to the sample to receive photoacoustic signals generated at the illuminated optical foci regions based on the photoacoustic

effect. The ergodic relay reflects the photoacoustic signals internally, which scrambles the photoacoustic signals based on their input locations. An ultrasonic transducer device acoustically-coupled to the ergodic relay can detect the coded photoacoustic signals. Using the calibrated system responses, the system can determine a plurality of snapshot photoacoustic images for the corresponding plurality of scan locations. In certain aspects, the system can localize the pixel values in the optical foci regions of each snapshot photoacoustic image, e.g., by assigning the pixel values within each focal spot to a maximum or mean value of the pixels in that focal spot and zeroing out the pixel values around the focal spot in that optical foci region. The system combines the plurality of localized snapshot photoacoustic images to generate an MFOR-PAMER image.

[0035] In the following description, numerous specific details are set forth in order to provide a thorough understanding of the presented embodiments. The disclosed embodiments may be practiced without one or more of these specific details. In other instances, well-known operations have not been described in detail to avoid unnecessarily obscuring the disclosed embodiments.

While the disclosed embodiments will be described in conjunction with the specific embodiments, it will be understood that it is not intended to limit the disclosed embodiments.

I. INTRODUCTION

[0036] Optical-resolution photoacoustic microscopy (OR-PAM) has demonstrated relatively high spatial resolution imaging of optical absorption in biological tissue. OR-PAM systems have found broad application in biomedical imaging technologies based on their ability to image rich optical absorption contrast in biological tissues. Examples of OR-PAM systems are described Wang, L. V & Yao, J., “A practical guide to photoacoustic tomography in the life sciences,” *Nat. Methods* **13**, 627–638 (2016) and Wang, L. V., “Multiscale photoacoustic microscopy and computed tomography,” *Nat. Photon.* **3**, 503–509 (2009), which are hereby incorporated by reference in their entireties. To date, OR-PAM systems typically rely on either mechanical scanning of a single beam with its confocally-aligned ultrasonic transducer for sensitive acoustic detection or mechanically scanning of the object to form an image, limiting the wide-field imaging speed of these systems. Some examples of such systems are described in Yao, J., Wang, L., Yang, J., Maslov, K.I., Wong, T.T.W., Li, L., Huang, C., Zou, J., and Wang, L.V., “High-speed label-free functional photoacoustic microscopy of mouse brain in action,” *Nat. Methods* **12**, 407–410 (2015) and Wong, T.T.W., Zhang, R., Zhang, C., Hsu, H.-C., Maslov, K., Wang, L., Shi, J., Chen, R., Shung, K.K., Zhou, Q., and Wang, L.V., “Label-free automated three-

dimensional imaging of whole organ by microtomy-assisted photoacoustic microscopy,” *Nat. Comm.* **8**, (2017), which are hereby incorporated by reference in their entireties.

[0037] Although several multifocal optical-resolution photoacoustic (MFOR-PA) systems have attempted to address the limitation on wide field imaging speed, these systems have been hindered by their complex design in a constrained physical space. For example, multifocal optical-resolution photoacoustic computed tomography (MFOR-PACT) systems use a microlens array with multiple optical foci and an ultrasonic transducer array to detect photoacoustic signals. Examples of MFOR-PACT systems can be found in Li, G., Maslov, K.I., and Wang, L.V., “Reflection-mode multifocal optical-resolution photoacoustic microscopy,” *J. Biomed. Opt.* **18**, 030501 (2013) and Xia, J., Li, G., Wang, L., Nasiriavanaki, M., Maslov, K., Engelbach, J.A., Garbow, J.R., Wang, L.V., “Wide-field two-dimensional multifocal optical-resolution photoacoustic-computed microscopy,” *Opt. Lett.* **38**, 5236–6239 (2013), which are hereby incorporated by reference in their entireties. These MFOR-PACT systems are complex and costly due to their implementation of an ultrasonic transducer array and the associated multi-channel data acquisition system. For example, in an MFOR-PACT system, an ultrasonic transducer array with 512 sensing elements is used. To achieve real-time imaging, the sensing elements require their own amplifiers and data acquisition channels (i.e., 512 amplifiers and channels), making it not easy to implement. Also, as mentioned, the applications of the MFOR-PA systems are limited due to the space needed.

[0038] Various implementations pertain to systems and methods of multifocal optical-resolution photoacoustic microscopy through an ergodic relay (MFOR-PAMER). In some cases, the MFOR-PAMER systems include a microlens array or other pattern-generating device that can generate optical excitation at multiple focal spots in a field-of-view of a sample being imaged and an acoustic ergodic relay that can simultaneously detect the photoacoustic responses to the multifocal optical illuminations with an ultrasonic transducer device such as a single-element ultrasonic transducer.

[0039] In various implementations, the MFOR-PAMER system includes an ergodic relay (also sometimes referred to herein as an “acoustic ergodic relay” or an “acoustic ergodic cavity”). The ergodic relay is a low-loss acoustic propagation medium that scrambles input acoustic waves inside the medium by internally reflecting the waves at boundaries which results in distinct time delay characteristics at an output location for each input location. The waves are reflected at the boundaries due to the discontinuity in acoustic transmissivity between the medium of the ergodic relay and the air or other medium outside the boundaries. Such time delay characteristics are

discussed in Draeger, C., Fink, M., “One-channel time reversal of elastic waves in a chaotic 2D-silicon cavity,” *Phys. Rev. Lett.* 79, 407–410 (1997), Ing, R. K., Quieffin, N., Catheline, S., Fink, M., “In solid localization of finger impacts using acoustic time-reversal process,” *Appl. Phys. Lett.* 87, 204104 (2005), and Montaldo, G., Palacio, D., Tanter, M., Fink, M., “Building three-dimensional images using time-reversal chaotic cavity,” *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* **52**, 1489-1497 (2005), which are hereby incorporated by reference in their entirety. If the ergodic relay is considered lossless and the boundaries perfect reflectors, an acoustic wave at a particular input location propagates to an output location along a unique path relative to the paths of other acoustic waves at other input locations. Since an ergodic relay is generally linear and temporally shift-invariant, its time delay characteristics for these paths can be calibrated and used to analyze the impulse response in the output photoacoustic signals to determine their input locations.

[0040] In certain implementations, MFOR-PAMER techniques can use calibrated time delay characteristics of the ergodic relay and/or the sample and to detect acoustic waves generated at multifocal optically illuminated regions (also sometimes referred to herein as “optical foci regions”) in the field-of-view in parallel using an ultrasound transducer device acoustically coupled to the ergodic relay. In some cases, the ergodic relay can also project one or more illumination beams to the optical foci regions to generate optical excitation. For example, an MFOR-PAMER system may include an ergodic relay that projects one or more input illumination beams generated by a pattern-generating device to corresponding optical foci regions in a field-of-view at a sample plane of a sample being imaged and receive at different input locations the acoustic waves generated by the photoacoustic effect at the illuminated optical foci regions.

[0041] An example of an ergodic relay is a light-transparent prism (e.g., prism made of fused silica) such as a right-angle prism. A schematically-illustrated example of an ergodic relay in the form of right-angle prism is the ergodic relay **340** shown in **FIG. 3**. Some examples of suitable commercially-available ergodic relays are ultraviolet fused silica right-angle prisms (e.g., PS611, PS612, PS608, PS613, etc.) sold by Thorlabs, Inc. of Newton, New Jersey. The PS611 right-angle prism made by Thorlabs, Inc. of Newton, New Jersey has a 99.99% normal-incidence acoustic reflectivity by amplitude at the boundary between the prism and air and an acoustic attenuation coefficient is 1.23 nepers/m at 10 MHz, which indicates negligible attenuation in the prism, which has a right-angle edge length of 2.5 cm. With such high boundary reflectivity and low acoustic attenuation, the photoacoustic signals reflected within the PS611 right angle prism

can be assumed to be lossless and sufficiently scrambled. Although examples herein generally describe MFOR-PAMER systems with ergodic relays in the form of right-angle prisms or other prisms, it would be understood that these systems can implement other types of ergodic relays according to other implementations. Some examples of other types of suitable ergodic relays include glass/quartz plates (e.g., slides, coverslips), silicon wafers, etc.

[0042] For photoacoustic imaging, an ergodic relay can be used as an encoder to transform photoacoustic signals from acoustic waves at different input positions into unique temporal signals. Examples of systems that use ergodic relays are described in Cox, B., Beard, P., “Photoacoustic tomography with a single detector in a reverberant cavity” *J. Acoust. Soc. Am.*

10 **125**, 1426 (2009) and Li, Y., Li, L., Zhu, L., Maslov, K., Shi, J., Yao, J., Liang, J., Wang, L., and Wang L.V., “Snapshot photoacoustic topography through an ergodic relay for high-throughput imaging of optical absorption,” which are hereby incorporated by reference in their entireties. By recording the system impulse response of each input position in advance during a calibration or training process, the time delay characteristics of the ergodic delay can be established and the photoacoustic signals from a plurality of illuminated optical foci regions in the field-of-view can be detected by the ultrasound transducer device in parallel based on a single input beam such as a single pulsed laser shot. For example, a pattern generating device can separate a single input beam into multiple illumination beams directed to illuminate a plurality of optical foci regions at a sample plane in the field-of-view. According to certain aspects, the encoded photoacoustic signals can then be decoded mathematically to reconstruct a two-dimensional photoacoustic image of the object.

[0043] In various implementations, an MFOR-PAMER system includes an ergodic relay that is acoustically coupled at one or more output locations to an ultrasound transducer device to detect encoded photoacoustic signals based on the time delay characteristics of the ergodic relay and/or the sample. In other words, the ergodic relay receives a plurality of encoded photoacoustic signals from a plurality of illuminated optical foci regions in the field-of-view of the sample being imaged and delivers a photoacoustic signal for each illumination beam after multiple characteristic time delays relative to the delivery of the illumination beam at the input location to the ergodic relay which corresponds to the location at the sample plane illuminated by the illumination beam. In this way, the ergodic relay encodes the spatial location of the source of the photoacoustic signal as a characteristic delay between the time of delivery of the illumination beam and the time at which the photoacoustic signal is received from the ergodic relay by the ultrasound transducer device. Some examples of suitable ultrasound transducer devices include a

single-element transducer (e.g., a pin-shaped ultrasound transducer), one or more linear transducer arrays, one or more two-dimensional transducer arrays, or any combination thereof. An example of a suitable single-element transducer is a pin-shaped ultrasound transducer such as, e.g., the commercially-available VP-0.5 transducer made by CTS Electronics, Inc., which has a 10 MHz central frequency and 0.5 mm element size or the commercially-available VP-0.5 -20 MHz transducer made by CTS Electronics, Inc., which has a 20 MHz central frequency, 56% one-way bandwidth, and 0.5 mm element size. Generally, the ultrasound transducer device is asymmetrically acoustically coupled (i.e. at a location or locations that provide asymmetry in the geometric relationship between the ergodic relay and the ultrasound transducer device, e.g., at location/s not on the line of symmetry) to the ergodic relay to increase the distinctions among the received photoacoustic signals from different input locations. For example, a single-element ultrasound transducer may be coupled to an ergodic relay at a corner to maximize the distinctions among the received signals from the different input locations. The ergodic relay may be acoustically coupled directly to the ergodic relay or via acoustic coupling material (e.g., polyester resin) to the ergodic relay.

[0044] In various implementations, an MFOR-PAMER system also includes a pattern-generating device capable of generating a plurality of N illumination beams with N focal spots in an optical foci pattern (also sometimes referred to herein simply as a “pattern”) where the illumination beams are capable of optical excitation at the focal spots that will generate acoustic waves by the photoacoustic effect in the sample being imaged. Some examples of suitable patterns include a rectangular array, a linear array, a circular array, etc. In certain implementations, the illumination beams are propagated from the pattern-generating device to the ergodic relay and the ergodic relay projects the illumination beams to form the optical foci pattern in a field-of-view of the sample being imaged. In other cases, the illumination beams are propagated directly from the pattern-generating device to the field-of-view. For example, the pattern-generating device may be located above a sample located on a surface of the ergodic relay or above the surface with an acoustic gel between the surface and the sample. In yet other cases, the illumination beams are propagated via one or more optical elements from the pattern-generating device to the field-of-view. An example of a rectangular grid pattern of four-hundred (400) focal spots **412a** from illumination beams propagated, directly or via other optical elements, from a pattern-generating device in the form of a 20 x 20 microlens array to a sample plane to illuminate a corresponding four-hundred (400) regions **401a** in a field-of-view **400** is shown in **FIG. 4**. During operation, the ultrasonic transducer device coupled to the ergodic delay

detects the encoded photoacoustic signals and the data from these signals can be mapped using the system responses from the calibration data to the known locations of the focal spots in the pattern. The encoded photoacoustic signals are decoded using the system responses (or impulse responses) from the calibration data. The reconstructed photoacoustic amplitude image can be calibrated by the RMS amplitude of the system responses from the calibration data.

[0045] In various implementations, the pattern-generating device is an arrangement of optical elements capable of generating a plurality of illumination beams from a single beam that is propagated directly or via one or more optical elements from a light source. Some examples of suitable pattern-generating devices include a microlens array, a digital mirror, optical fibers, a spatial light modulator such as a liquid-crystal spatial light modulator, and any combination thereof.

[0046] In implementations that include a pattern-generating device in the form of a microlens array, the microlens array is arranged to separate a single beam into multiple illumination beams and/or focus the illumination beams to corresponding focal spots. In some cases, the illumination beams are projected directly from the microlens array to the sample plane, and in other cases via one or more optical elements (e.g., a relay lens). The number of illumination beams produced by the microlens array corresponds to the number of microlenses in the array. In one aspect, a microlens array has at least 100 microlenses. In another aspect, a microlens array has at least 200 microlenses. Some examples of suitable pitch between adjacent microlenses in the array include 75 μm , 100 μm , 200 μm , 300 μm , 400 μm , and 500 μm . In one aspect, the pitch of the microlens array is greater than 220 μm . In another aspect, the pitch of the microlens array is greater than 75 μm .

[0047] In various aspects, the pitch of a microlens array or other pattern-generating device is determined for a particular implementation based on a tradeoff between a desired acoustic resolution and a desired imaging speed. Generally, the pitch values have to be larger than the acoustic resolution. For example, if an MFOR-PAMER system has an acoustic resolution of 220 μm , the pitch must be greater than 220 μm . The imaging speed of an MFOR-PAMER system can be increased by reducing the pitch so that the area of the optical foci region of the field-of-view scanned by a single illumination beam is smaller.

[0048] According to certain aspects, the microlens array is a rectangular array, a circular or curved array, a linear array, or combination thereof. In one aspect, the microlens array is a 20 x 20 rectangular array with four hundred (400) microlenses. In another aspect, the microlens array is a 10 x 10 rectangular array with one hundred (100) microlenses. In another aspect, the

microlens array is a rectangular array with each dimension being at least 10 microlenses. In another aspect, the microlens array is a rectangular array with each dimension being at least 20 microlenses. In another aspect, a microlens array has at least 100 microlenses. In another aspect, a microlens array has at least 200 microlenses. An example of a commercially-available microlens array is the 64-479 microlens array made by Edmund Optic of Barrington, New Jersey, which is a rectangular microlens array having dimensions 20 x 20, a 500 μm pitch between microlenses, and 1.2 degree divergence angle. An example of a microlens array is illustrated in **FIG 5**.

[0049] In implementations that include a pattern-generating device in the form of a digital micromirror device (also sometimes referred to herein as a “DVD”), the digital mirror device is capable of generating multiple illumination beams from a single beam and/or focusing the generated illumination beams to a corresponding plurality of focal spots. Each micromirror of a digital mirror device can be individually rotated to an angle, α . In this way, each micromirror can be transitioned to either an aperture setting at angle, α , or to a field setting at no rotation, or vice versa. An example of a suitable commercially-available digital micromirror device is the DLP9000/DLP9000X DLP® Digital Mirror Device by Texas Instruments®. Although micromirrors are usually arranged in a rectangular array, other arrangements can be used. The pitch value of a digital mirror device can be controlled digitally, for example, more or fewer mirror units can be turned on to adjust the pitch value. The size of each micromirror is related to the highest acoustic resolution that can be achieved by the digital micromirror device. In certain aspects, a relay lens can be used to adjust (improve or degrade) the acoustic resolution.

[0050] In implementations that include a pattern-generating device in the form of a spatial light modulator, the spatial light modulator is capable of separating a single beam into multiple illumination beams. In some cases, a spatial light modulator uses an electrical and/or optical signal from a spatial light modulator light source to modulate phase, ϕ , and/or amplitude of light. In some cases, the spatial light modulator light source is a collimated light source such as a laser (e.g., Excelsior® 532 SM). In other cases, the spatial light modulator light source may be spatially filtered light from a light emitting diode. The pitch value of a spatial light modulator can be adjusted by turning on or off more or fewer elements. An example of a commercially-available spatial light modulator is the reflective HOLOEYE® spatial light modulator sold by Pluto, which is a phase only LCoS with 8 μm pixel size and a 1080 x 1920 pixels display.

[0051] In certain implementations, an MFOR-PAMER system combines the powerful capability of an ergodic relay with a single-element ultrasonic transducer to detect multiple

photoacoustic signals in parallel with a simple system setup. In these cases, the MFOR-PAMER system is a low-cost alternative to systems that implement a transducer array. Additionally or alternatively, an MFOR-PAMER system may implement a microlens array or other pattern-generating device to shape a wide-field laser beam into multiple optical focal spots to reduce the scanning time. Since the excitation pattern through the microlens array or other pattern-generating device is known, the response from each optical focal spot can be computationally localized to improve the acoustically defined image resolution. By combining the pattern-generating device with the ergodic relay, the image resolution is improved to the optically defined image resolution and the imaging speed is reduced by a factor equal to the number of microlens elements. For example, one implementation of an MFOR-PAMER system includes a pattern-generating device in the form of a two-dimensional microlens array having 20×20 microlens elements having a $500 \mu\text{m}$ pitch between the elements. During operation, the microlens array receives a single pulsed laser beam and can generate four hundred (400) illumination beams that are projected through the ergodic relay to generate four hundred (400) optical foci in the field-of-view at a sample plane of the object. In this implementation, the MFOR-PAMER system also includes a single element transducer element coupled to a corner of the ergodic relay that can detect photoacoustic signals from the illuminated four-hundred (400) optical foci regions in the field-of-view in a single laser shot. This MFOR-PAMER system improves the imaging resolution from $220 \mu\text{m}$ to $13 \mu\text{m}$, which is a factor of ~ 17 , as compared to OR-PAM systems. Moreover, this MFOR-PAMER system achieves a 400 times improvement in imaging speed over a $10 \text{ mm} \times 10 \text{ mm}$ field-of-view as compared to OR-PAM systems at the same resolution and laser repetition rate.

[0052] Some examples of microlens arrays can be found in Prevedal, R., Yoon, Y., Hoffmann, M., Pak, N., Wetzstein, G., Kato, S., Schrodel, T., Raskar, R., Zimmer, M., Boyden, E.S., and Vaziri, A., "Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy," *Nat. Methods* **11**, 727-730 (2014) and Fujitaa, K., Nakamura, O., Kanekoa, T., Oyamadab, M., Takamatsub, T., and Kawataa, S. "Confocal multipoint multiphoton excitation microscope with microlens and pinhole arrays," *Opt. Comm.* **174**, 7-12 (2000), which are hereby incorporated by reference in their entireties.

[0053] Certain implementations of the MFOR-PAMER systems and methods have one or more technical advantages. For example, certain implementations of an MFOR-PAMER system include a pattern-generating device that enable a simple and compact design that allows the system to be more flexible and portable which is compatible with various applications.

As another example, by implementing a pattern-generating device, one implementation of an MFOR-PAMER system improves the imaging resolution from 220 μm to 13 μm as compared to OR-PAM systems that do not have a pattern-generating device. As another example, one implementation of an MFOR-PAMER system may improve the imaging speed over a 10 mm x 10 mm field-of-view by 400 times as compared to an OR-PAM system at the same resolution and laser repetition rate. Generally speaking, the MFOR-PAMER systems and methods have promising potential use in many biomedical applications, such as utilizing ultra-violet illumination for high-speed, label-free histological study of biological tissues. According to certain aspects, using MFOR-PAMER systems and methods can reduce the imaging time from several hours to less than a minute (as compared to ultra-violet optical resolution photoacoustic microscopy (OR-PAM) systems), which, if implemented, may significantly improve the efficiency of clinical histology and diagnostics.

[0054] According to certain aspects, an MFOR-PAMER system includes one or more light sources that can provide pulsed or modulated radiation with properties that can generate temporal data and cause optical excitation resulting in the photoacoustic effect in the sample being imaged. Some examples of suitable pulsed light sources include: pulsed (1) laser, (2) flash lamp, (3) laser diode, (4) light emitting diode, etc. Some examples of suitable modulated light sources include modulated (i) continuous-wave laser, (ii) flash lamp, (iii) laser diode, (4) light-emitting diode, etc.

[0055] In certain aspects, a light source in the form of a pulsed laser or other pulsed light source that can deliver a series of light pulses suitable for photoacoustic imaging. The light pulse wavelength, duration, and pulse repetition rate (pulses/sec) are selected based on one or more factors such as, e.g., selective absorbance of pulse wavelength by structures or objects of interest in the sample being imaged, scattering of the pulse wavelength through the sample being imaged, sufficient pulse duration to produce detectable photo acoustic signals, etc. In some aspects, the light pulses are in a range of wavelengths between 200 nm and 6500 nm. In one aspect, the wavelength of the light pulses is about 266 nm. In another aspect, the wavelength of the light pulses is about 532 nm. In another aspect, the wavelength of the light pulses is about 559 nm. In another aspect, the wavelength of the light pulses is about 650 nm. In another aspect, the wavelength of the light pulses is about 680 nm. In another aspect, the wavelength of the light pulses is about 930 nm. In another aspect, the wavelength of the light pulses is about 1064 nm. In another aspect, the wavelength of the light pulses is about 1210 nm. In another aspect, the wavelength of the light pulses is about 1710 nm. Some examples of suitable ranges of

wavelengths that can be used in implementations include: ranges between 200 nm and 6500 nm, in particular (some examples) 266 nm, 532 nm, 559 nm, 650 nm, 680 nm, 930 nm, 1064 nm, 1210 nm, 1710 nm. In some aspects, the light pulses have a pulse width less than 10 ns. In one aspect, the light pulses have a pulse width of 3 ns. In one aspect, the light pulses have a pulse width of 100 ps. In one aspect, the light pulses have a pulse width of 20 ps. In one aspect, the light pulses have a pulse width of 200 fs. In certain aspects, a pulsed light source provides light pulses at a repetition rate in the range between about 10 Hz and about 10 kHz. In one aspect, the pulsed light source provides light pulses at a repetition rate at or above 2 kHz. In another aspect, the pulsed light source provides light pulses at a repetition rate at or above 10 kHz. In another aspect, the pulsed light source provides light pulses at a repetition rate at or above 10 Hz. In another aspect, the pulsed light source provides light pulses at a repetition rate at or above 100 Hz. In another aspect, the pulsed light source provides light pulses at a repetition rate at or above 1 kHz. For example, a suitable light source is a pulsed laser configured to deliver a series of laser pulses at a pulse wavelength of 532 nm. An example of a suitable commercially-available pulsed laser is the INNOSAB IS811-E sold by Edgewave® GmbH with a 2 KHz pulse repetition rate and 5-ns pulse width.

[0056] In various examples, a pattern-generating device generates a plurality of illumination beams from a single light beam propagated directly, or via one or more optical elements, from a light source (e.g., a pulsed laser, a light-emitting diode, etc.). In the examples where the light beam is propagated via one or more optical elements to the pattern-generating device, the one or more optical elements may, in some cases, modify the light beam delivered to the pattern-generating device, e.g., by adjusting the amount or properties of the light energy delivered. Some examples of suitable optical elements include optical fibers, lenses, mirrors, beam splitters, optical filters, etc. For example, one of the optical elements may be an aperture (e.g., pinhole) that can be contracted or dilated to spatially filter the light beam. As another example, two of the optical elements may be a pair of lenses that act together to expand the light beam. In yet another example, one or more of the optical elements may be a mirror or a beam splitter that reflects the beam or a portion of the light beam in another direction. In yet another example, one or more of the optical elements may be a lens, e.g., a focusing lens to focus the light beam to narrow the lateral dimensions of the beam or two lenses to widen the lateral dimensions of the beam. An illustrated example of one or more optical elements **212a, 212b, 212c, 212d, 212e, 212f, 212g, and 212h** between the pattern-generating device **214** in the form of a microlens array and the light source **210** in the form of a pulse laser are shown in **FIG. 2**.

In this example, a pinhole mechanism **212b** is used to spatially filter a pulsed laser beam and the pair of lenses **212e**, **212f** to expand the lateral dimensions of the pulsed laser beam. In one implementation having a light source in the form of a pulsed laser, the MFOR-PAMER system includes a beam sampler to monitor the energy of the light pulses from the pulsed laser. The energy of each light pulse is measured and the measurements are used to apply corrections to the measured photoacoustic signals.

[0057] In certain implementations, an MFOR-PAMER system includes a scanning mechanism capable of scanning one or more elements of the MFOR-PAMER system to cause the plurality of illumination beams generated by the pattern-generating device to be scanned. Each of the illumination beams is scanned so that the its focal spot is moved to a plurality of locations in an optical foci region of the field-of-view being imaged. In some cases, the scanning mechanism scans the focal spots of the illumination beams in one direction in the sample plane such as in a direction along a local x-axis in the corresponding optical foci regions. In other cases, the scanning mechanism scans the focal spots in two directions in the sample plane, e.g., a first direction along the local x-axis and a second direction along a local y-axis in the corresponding optical foci regions. In these implementations, each of the focal spots is scanned to a plurality of locations in the sample plane over the corresponding optical region of the field-of-view. In some cases, at each scan location, the ultrasonic transducer device coupled to the ergodic relay simultaneously detects encoded photoacoustic signals. In one aspect, the data from the encoded photoacoustic signals can be mapped to pixel locations in the field-of-view using the system responses from the calibration data. Some examples of suitable scanning mechanisms are an x-y stage, a galvanometric mirror (non-resonant/resonant), an acousto-optic device, one or more motorized scanning stages, a spinning polygon mirror, etc. In one embodiment, the scanning mechanism includes two motorized translation stages. An example of a commercially-available motorized translation stage is a PLS-85 stage made by PI Inc.® with US headquarters located in Auburn, Massachusetts.

[0058] In various implementations, an MFOR-PAMER system includes a digitizer such as, e.g., a two-channel digitizer, that can record data from the photoacoustic signals received from an ultrasonic transducer device. In some cases, the digitizer may also record measurements taken by other system components. A commercially-available two-channel digitizer is the ATS9350 two-channel digitizer made by Alazar Tech Inc.® with a 50 MS/s sampling rate and 16384 samples/A-line sample length. In cases where the ultrasound transducer device is an

analog transducer, the digitizer may also function to convert the electrical signals into digital data.

[0059] In various implementations, an MFOR-PAMER system includes one or more processor(s) and a computer readable medium CRM. The processor(s) execute instructions stored on the CRM to perform one or more operations of the MFOR-PAMER system. For example, the processor(s) of the MFOR-PAMER system and/or one or more external processors may execute instructions that construct a photoacoustic image of the field-of-view of the sample from the data in the photoacoustic signals received from the ultrasonic transducer device. In addition or alternatively, the processor(s) of the MFOR-PAMER system and/or one or more external processors may execute instructions that cause the communication of control signals to control operations of one or more system components (e.g., control light pulses delivered by the light source, control the scanning movement from a scanning mechanism, etc.). Some examples of suitable types of processors that can be used include, e.g., a general purpose processor (CPU), an application-specific integrated circuit, a programmable logic device (PLD) such as a field-programmable gate array (FPGA), and a System-on-Chip (SoC). In some cases, the CRM includes a non-volatile memory array for storing processor-executable code (or “instructions”) that is retrieved by the processor(s) to perform various functions or operations described herein for carrying out various logic or other operations on the photoacoustic signals or image data. The CRM can also store raw data and/or processed image data. In some cases, the CRM or a separate memory can additionally or alternatively include a volatile memory for temporarily storing code to be executed as well as image data to be processed, stored, or displayed.

[0060] In some implementations, the MFOR-PAMER system includes one or more communication interfaces (e.g., a universal serial bus (USB) interface). Communication interfaces can be used, for example, to connect various peripherals and input/output (I/O) devices such as a wired keyboard or mouse or to connect a dongle for use in wirelessly connecting various wireless-enabled peripherals. Such additional interfaces also can include serial interfaces such as, for example, an interface to connect to a ribbon cable. It should also be appreciated that the various system components can be electrically coupled to communicate with various components over one or more of a variety of suitable interfaces and cables such as, for example, USB interfaces and cables, ribbon cables, Ethernet cables, among other suitable interfaces and cables.

II. Examples of MFOR-PAMER systems

[0061] FIG. 1 is a schematic illustration of an MFOR-PAMER system 100, according to various implementations. The MFOR-PAMER system 100 includes a light source 110 (e.g., a pulsed laser or a light emitting diode) that can produce a light beam 111 of pulsed or modulated radiation. The MFOR-PAMER system 100 also includes an optical system 120 with a pattern-generating device 122 (e.g., a microlens array, a digital mirror, or a spatial light modulator) in optical communication with the light source 110 to receive the light beam 111. In one aspect, the optical system 120 further includes one or more optical elements, e.g., one or more mirrors, that direct the light beam 111 from the light source 110 to the pattern-generating device 122 and/or modify the light beam 111, e.g., an aperture that can spatially filter the light beam 111 and/or an two or more lenses that can expand the light beam 111. In another aspect, the pattern-generating device 122 receives the beam directly from the light source 110.

[0062] In this illustrated example, the pattern-generating device 122 is configured or configurable to generate a plurality of illumination beams 123 from the light beam 111 (unmodified or unmodified by intervening optical elements) from the light source 110. For simplicity, the pattern-generating device 122 is illustrated as generating twelve (12) illumination beams. It would be understood that the pattern-generating device 122 can generate greater numbers of illumination beams and in any suitable pattern. For example, the pattern-generating device 122 may in certain implementations generate greater than one hundred (100) beams, greater than two hundred (200) beams, greater than three hundred (300) beams etc. In one example, the pattern-generating device 122 is a 20 x 20 microlens array that generates two hundred (200) beams in a rectangular grid pattern.

[0063] The MFOR-PAMER system 100 also includes an ergodic relay 140 in acoustic communication with the sample 10 to receive acoustic waves from regions in a field-of-view of the sample 10 illuminated by the illumination beams 123. The ergodic relay 140 is either touching or coupled via a coupling material (e.g., acoustic gel) to a surface of the sample 10 being imaged during an imaging process. The MFOR-PAMER system 100 also includes an ultrasonic transducer device 150 coupled to or otherwise in acoustic communication with the ergodic relay 140 at output location(s) to detect photoacoustic signals from the illuminated regions of the sample 10. In this illustrated example, the ergodic relay 140 is also in optical communication with the pattern-generating device 122 to receive the plurality of illumination beams 123 from the pattern-generating device 122 and project the illumination beams 123 such that a pattern of focal spots 124 of the illumination beams 111 is projected to a sample plane 11

of a sample **10** being imaged. In this case, the pattern-generating device **122** is located so that its focusing plane is imaged onto the imaging face of the ergodic relay **140** to illuminate the sample plane **11** of the sample **10**. In other implementations, one or more other optical elements are implemented to propagate the plurality of illumination beams **123** to the sample **10**. In these cases, the ergodic relay **140** need not be in optical communication with the pattern-generating device **122**. In yet other implementations, one or more optical elements are in the optical path between the pattern-generating device **122** and the ergodic relay **140**. For example, a relay lens may be in optical communication with the pattern-generating device **122** to receive the plurality of illumination beams **123** and extend their focal length to the sample plane **11**. The illustrated ergodic relay **140** is in the form of a right-angle prism such as, e.g., the commercially-available right-angle prism PS611 sold by Thorlabs, Inc. of Newton, New Jersey with 2,203 kg/m³ density and 73.6 GPa Young's modulus. In other implementations, other types of suitable ergodic relays can be used such as glass/quartz plates (e.g., slides, coverslips), silicon wafers, etc. The illustrated example also includes an x-axis and a y-axis (not shown) in the sample plane **11**, and a z-axis. The y-axis is orthogonal to the x-z plane.

[0064] In the illustrated example, the ergodic relay **140** is in acoustic communication with the sample **10** to receive acoustic waves from the illuminated regions of the sample **10** illuminated by the pattern of focal spots **124** of the illumination beams **123**. The ergodic relay **140** is a low-loss acoustic propagation medium that scrambles input acoustic waves from the sample **10** and encodes the photoacoustic signals detected by the ultrasonic transducer device **150** with distinct time delay characteristics for each input location of acoustic waves from an illuminated region of the sample **10**. The ultrasonic transducer device **150**, e.g., a single element transducer, is in acoustic communication with the ergodic relay **150** to detect the encoded photoacoustic signals with the distinct time delay characteristics for each input location.

[0065] In various implementations, the ultrasonic transducer device is in acoustic communication with, e.g., coupled directly or acoustically connected via an acoustic material (e.g., an acoustic gel) to, the ergodic relay at a location or multiple locations that creates an asymmetric relationship between the ultrasonic transducer device and ergodic relay. The asymmetric relationship increases the distinctions among the photoacoustic signals received from different input locations of the ergodic relay. In **FIG. 1**, the illustrated ergodic relay **150** is schematically depicted as coupled to a face of the ergodic relay **140** at the corner to provide such an asymmetric relationship between the ergodic relay **150** and the ultrasonic transducer device **150** to increase the distinctions among the photoacoustic signals from acoustic waves received at

different input locations along the surface of the ergodic relay **150** coupled to the sample **10**.

Although the ultrasonic transducer device **150** is shown coupled directly to the ergodic relay **140**, it would be understood that there may be an acoustic material (e.g., an acoustic gel, a needle, etc.) located between the ultrasonic transducer device **150** and the ergodic relay **140**.

5 **[0066]** The MFOR-PAMER system **100** also includes a scanning mechanism **130** coupled to one or more elements of the optical system **120**. Optionally (denoted by a dotted line) the scanning mechanism **130** is coupled to the light source **110**, in addition to or in the alternative to, the one or more elements of the optical system **120**. The scanning mechanism **130** is coupled to one or more components of the MFOR-PAMER system **100** to be able to move the focal spots
10 **124** of the illumination beams **123** to different locations at the sample plane **11**. Each of the illumination beams **123** is scanned to locate its focal spot **124** at a plurality of locations in a corresponding optical foci region in the field-of-view being imaged. An illustration depicting focal spots of 400 illumination beams being moved such that each illumination beam is moved to a plurality of locations in a corresponding optical foci region is shown in **FIG. 4**. In one
15 example, the scanning mechanism **130** moves the focal spot **124** in a first direction along the x-axis and/or in a second direction along the y-axis at the sample plane. In one implementation, the scanning mechanism **130** includes one or more mechanical motors to move the one or more system components. In one implementation, the scanning mechanism **130** includes a first motor or other mechanism to move one or more components in a first direction and a second motor or
20 other mechanism to move one or more components in a second direction. Some examples of suitable scanning mechanisms include an x-y stage, a galvanometric mirror (non-resonant/resonant), an acousto-optic device, one or more motorized scanning stages, a spinning polygon mirror, etc.

[0067] In **FIG. 1**, the MFOR-PAMER system **100** also includes a digitizer **180** (e.g., a two-
25 channel digitizer) in electrical communication with the ultrasonic transducer device **150** to receive and record photoacoustic signals. The MFOR-PAMER system **100** also includes one or more processors **160** and a computer readable medium (CRM) **170** in electronic communication with the processor(s) **160**. The processor **160** is also in electronic communication with the scanning mechanism **130**, the light source **110**, and the digitizer **180** to be able to send control
30 signals. The digitizer **180** is in electronic communication with the CRM **170** to store the recorded photoacoustic signal data. The processor(s) **160** executes instructions stored on the CRM **170** to perform one or more operations of the MFOR-PAMER system **100**. In some cases, the processor(s) **160** and/or one or more external processors execute instructions that construct a

photoacoustic image of the field-of-view of the sample **10** from the data in the photoacoustic signals received from the ultrasonic transducer device **150** and/or execute instructions that communicate control signals to system components. For example, the processor(s) **160** and/or one or more external processors may execute instructions that communicate control signals to the scanning mechanism **130** to scan focal spots **124** of the illumination beams **123** to different locations in the regions of the field-of-view and communicate control signals to the digitizer **180** to simultaneously record photoacoustic signals relayed through the ergodic relay **140** from the illuminated regions of the sample **10** for each of the locations in the regions. In addition or alternatively, the processor(s) **160** and/or one or more external processors may execute instructions that communicate control signals to the light source **110** to control the light pulses or other modulated light delivered by the light source **110**. The described electrical communications between components of the MFOR-PAMER system **100** are able to provide power and/or communicate signals with data.

[0068] Returning to **FIG. 1**, the MFOR-PAMER system **100** also includes an optional (denoted by dotted lines) first communication interface **191**, one or more optional input devices **190** in communication with the processor(s) **160** through the first communication interface **191**, a second communication interface **192**, and a display **192** in communication with the processor(s) **160** through the second communication interface **193**. The optional input device(s) **190** are in communication with the processor(s) **160** through the first communication interface **191** to be able to send a signal with imaging operational parameters or display parameters based on input received at the input device(s) **190**. The processor(s) **160** is configured or configurable to communicate data over the second communication interface **193** for display on the display **192** including, e.g., raw data from the photoacoustic signals and a constructed photoacoustic image. The second communication interface **192** is in communication with the input device(s) **190** to receive input, e.g., from an operator.

[0069] It would be understood that electrical communication between components of the various MFOR-PAMER systems described herein can be in wired or wireless form. For simplicity, the sample **10** is illustrated as a block, it would be understood that the sample **10** can be in a variety of shapes and may have one or more objects of interest.

[0070] During a data acquisition phase of an imaging process of the MFOR-PAMER system **100** according to one implementation, the processor(s) **160** executes instructions that send control signals to the light source **110** to deliver a beam of pulsed or modulated radiation which are converted into a plurality of illumination beams **123**, control signals to the scanning

mechanism **130** to scan the illumination beams **123**, control signals to the digitizer **180** to record photoacoustic signals received from the ultrasonic transducer device **150**. The digitizer **180** records photoacoustic signals for each of the locations of the focal spots of the illumination beams **123** in regions of the field-of-view of the sample **10** being imaged. During an image construction phase, the processor(s) **160** executes instructions to perform operations to construct a photoacoustic image from the data in the photoacoustic signals. First, the calibrated system responses are used to map the amplitudes of the recorded photoacoustic signals to: i) pixels within the areas of the focal spots in the optical foci regions of the field-of-view, or ii) the pixels within the field-of-view. This first operation is done for each illumination pattern (i.e., for each scanning location of the illumination beams). Second, a localization operation is performed for the pixels in each optical foci region that: i) determines a maximum or mean amplitude value of the pixel values within the area of each focal spot, and ii) assigns the pixels within the area of the focal spot to the determined maximum or mean amplitude value and zeroes out the pixel values around the focal spot. The localization operation is performed for each illumination pattern to generate a plurality of localized images. Third, the pixel values from the plurality of localized images are summed to generate a photoacoustic image of the field-of-view of the sample **10**.

[0071] FIG. 2 is a schematic illustration of an MFOR-PAMER system **200**, according to various implementations. The MFOR-PAMER system **200** includes a light source **210** in the form of a pulsed laser that can produce laser pulses for optical excitation in a sample **20** being imaged. An example of a suitable commercially-available pulsed laser is the INNOSAB IS811-E sold by Edgewave ® GmbH with a 2 KHz pulse repetition rate and 5-ns pulse width. The illustrated example also includes an x-axis, a y-axis, and a z-axis. The x-axis and y-axis are in the sample plane.

[0072] The MFOR-PAMER system **200** also includes an optical system with a first lens **212a** configured to focus the beam from the light source **210** and an aperture (e.g., pinhole) **212b** configured to spatially filter the beam from the first lens **212a**. An example of a suitable commercially-available first lens **212a** that can focus the beam to a 5 µm laser spot is the LA1509 lens made by Thorlabs, Inc. ® with a 25.4 mm diameter and 100 mm focal length. The optical system also includes a second lens **212c** configured to collimate the beam spatially-filtered by the aperture **212b**, a first mirror **212d** configured to reflect the beam collimated by the second lens **212c**, a third lens **212e** configured to propagate the beam reflected from the first mirror **212d**, a fourth lens **212f** configured to collimate the beam, a second mirror **212g** configured to reflect the beam from the fourth lens **212f**, and a third mirror **212h** configured to

reflect the beam from the second mirror **212g**. The third lens **212e** and the fourth lens **212f** act together to laterally expand the beam. The optical system also includes a pattern-generating device **222** (e.g., a microlens array, a digital mirror, or a spatial light modulator) located to receive the pulsed beam reflected by the third mirror **212h** and generate a plurality of

5 illumination beams **223**, and a relay lens **216** configured to extend the focal lengths of the plurality of illumination beams **223**. Generally, the lenses and mirrors of an MFOR-PAMER system are selected based on the illumination wavelength. For the MFOR-PAMER system **200**, the focal lengths of a lens pair controls how much the light beam will be expanded. To expand the beam, for example, if lens **212f** has a focal length of 100 mm, and lens **212e** has a focal

10 length of 20 mm, the beam after passing through this lens pair **212e**, **212f** will be expanded by $100 \text{ mm} / 20 \text{ mm} = 5$ times. For spatial filtering, lenses **212a** and **212c** can have the same focal length.

[0073] The pattern-generating device **222** is configured or configurable to generate a plurality of illumination beams **223** from the adjusted pulsed laser beam reflected by the third mirror

15 **212h**. For simplicity, the pattern-generating device **222** is illustrated as generating a small number of illumination beams. It would be understood that the pattern-generating device **222** can generate greater numbers of illumination beams and in any suitable pattern. For example, the pattern-generating device **222** may in certain implementations generate greater than one hundred (100) beams, greater than two hundred (200) beams, greater than three hundred (300) beams etc.

20 In one example, the pattern-generating device **222** is a 20 x 20 microlens array that generates two hundred (200) beams in a rectangular grid pattern.

[0074] The MFOR-PAMER system **200** also includes an ergodic relay **240**. The ergodic relay **240** is depicted in the illustration in the form of a right-angle prism. An example of a suitable

25 commercially-available ergodic relay that can be implemented is the PS611 right-angle prism sold by Thorlabs, Inc. of Newton, New Jersey with $2,203 \text{ kg/m}^3$ density and 73.6 GPa Young's modulus. Other examples of a right-angle prisms are described in Li, Y., Li, L., Zhu, L., Maslov, K., Shi, J., Yao, J., Liang, J., Wang, L., and Wang L.V., "Snapshot photoacoustic topography

through an ergodic relay for high-throughput imaging of optical absorption," which is hereby incorporated by reference in its entirety. Although the MFOR-PAMER system **200** is shown in

30 the illustrated example with a right-angle prism, other types of ergodic relay scan be used in other implementations such as glass/quartz plates (e.g., slides, coverslips), silicon wafers, etc.

[0075] In the illustrated example, the ergodic relay **240** is also in optical communication with the relay lens **216** to receive the plurality of illumination beams **223** and propagate the plurality

of illumination beams **123** to the sample **10**. In other implementations, one or more other optical elements are implemented to propagate the plurality of illumination beams **223** to the sample **20**.

[0076] In the illustrated example, the relay lens **216** is in optical communication with the pattern-generating device **222** to receive the plurality of illumination beams **223** from the

pattern-generating device **222** and extend their focal length to the sample plane **21**. An example of a suitable commercially-available relay lens is the 272EN II camera lens sold by Tamron

Inc.® with a 0.29 m minimum focus distance and 1:1 maximum magnification ratio. The pattern-generating device **222** is located so that its focusing plane is imaged onto the imaging

face of the ergodic relay **240** to illuminate the sample plane of the sample **20** with a pattern of focal spots of the illumination beams **223**. In another implementation, the relay lens **216** is not

needed and can be removed where the focal length of the illumination beams **223** from the pattern-generating device **222** is sufficient to allow the focusing plane of the pattern-generating

device **222** to be imaged onto the imaging face of the ergodic relay **240** without the relay lens.

[0077] The sample **20** being imaged is touching the surface of the ergodic relay **240** and/or

acoustically coupled via a coupling material (e.g., acoustic gel) to the surface of the ergodic relay **240**. The MFOR-PAMER system **200** also includes an ultrasonic transducer device **250** coupled

to or otherwise in acoustic communication with the ergodic relay **240** at a corner to break

symmetry. An example of a suitable ultrasonic transducer device **250** is a miniature single element miniature ultrasonic transducer such as the XMS-10 ultrasonic transducer by Olympus,

Inc.® with a 10 MHz central frequency and 0.3 mm element size. The ultrasonic transducer device **250** is configured to detect photoacoustic signals received at input locations from the

illuminated regions of the sample **20**. The ergodic relay **240** is a low-loss acoustic propagation medium that scrambles input acoustic waves from the sample **20** and encodes the photoacoustic

signals detected by the ultrasonic transducer device **250** with distinct time delay characteristics

for each input location of acoustic waves from an illuminated region of the sample **20**. The

ultrasonic transducer device **250**, e.g., a single element transducer, is in acoustic communication with the ergodic relay **250** to detect the encoded photoacoustic signals with the distinct time

delay characteristics for each input location. Although the ultrasonic transducer device **250** is shown coupled directly to the ergodic relay **240**, it would be understood that there may be an

acoustic material may be located between the ultrasonic transducer device **250** and the ergodic relay **240**.

[0078] The MFOR-PAMER system **200** also includes an x-scanning motor **230a** coupled to the third mirror **212h** and the pattern-generating device **222** and a y-scanning motor **230b**

coupled to the second mirror **212g**. The x-scanning motor **230a** is configured to move the third mirror **212h** and the pattern-generating device **222** to cause the focal spots of the illumination beams **223** to move in the x-direction in the sample plane of the sample **20**. The y-scanning motor **230a** is configured to move the second mirror **212g** to cause the focal spots of the illumination beams **223** to move in the y-direction in the sample plane of the sample **20**. The x-scanning motor **230a** and y-scanning motor **230b** are configured to scan the illumination beams **223** in the sample plane such that the focal spot of each illumination beam **223** is moved to a plurality of locations in the corresponding optical foci region of the field-of-view being imaged. An illustration depicting focal spots of four hundred (400) illumination beams being scanned in a corresponding plurality of optical foci regions is shown in **FIG. 4**.

[0079] The MFOR-PAMER system **200** also includes a digitizer **280** (e.g., a two-channel digitizer) in electrical communication with the ultrasonic transducer device **250** to record the encoded photoacoustic signals. An example of a suitable commercially-available digitizer is the ATS9350 two-channel digitizer made by AlazarTech, Inc. that can be adjusted to have up to a 100 MS/s sampling rate.

[0080] The MFOR-PAMER system **200** also includes one or more processors **260** and a computer readable medium (CRM) **270** in electronic communication with the processor(s) **260**. The processor(s) **260** is also in electronic communication with the x-scanning motor **230a**, the y-scanning motor **230b**, the digitizer **280**, and the light source **210** to send control signals. The processor(s) **260** is in electronic communication with the digitizer **280** to send control signals to activate the digitizer **280** to record the encoded photoacoustic signals. The digitizer **280** is in electronic communication with the CRM **270** to store the recorded photoacoustic signal data.

[0081] The processor(s) **260** executes instructions stored on the CRM **270** to perform one or more operations of the MFOR-PAMER system **200**. In some cases, the processor(s) **260** and/or one or more external processors execute instructions that construct a photoacoustic image of the field-of-view of the sample **20** from the data in the photoacoustic signals recorded by the from the ultrasonic transducer device **250** and/or execute instructions that communicate control signals to system components. For example, the processor(s) **260** and/or one or more external processors may execute instructions that communicate control signals to the x-scanning motor **230a** and the y-scanning motor **230b** to cause the scanning of the focal spots of the illumination beams **223** to different (x,y) locations in the regions of the field-of-view and to communicate control signals to the digitizer **280** to simultaneously record the encoded photoacoustic signals relayed through the ergodic relay **240** from the illuminated regions of the sample **20** for each of

the locations in the regions. In addition or alternatively, the processor(s) **260** and/or one or more external processors may execute instructions that communicate control signals to the light source **210** to control the light pulses or other modulated light delivered by the light source **210**. The described electrical communications between components of the MFOR-PAMER system **200** are able to provide power and/or communicate signals with data.

[0082] It would be understood that electrical communication between components of the various MFOR-PAMER systems described herein can be in wired or wireless form. It would also be understood that the sample **20** being imaged by the MFOR-PAMER system **100** can be of any variety of shapes and have one or more objects of interest.

[0083] During a data acquisition phase of an imaging process of the MFOR-PAMER system **100** according to one implementation, the processor(s) **260** executes instructions that send control signals to the light source **210** to generate a pulsed beam which is spatially filtered and expanded and propagates to the pattern-generating device **222**. The pattern-generating device **222** generates a plurality of plurality of illumination beams **123** from the beam. The processor(s) **260** also executes instructions processor(s) **260** that send control signals to the x-scanning motor **230a** and y-scanning motor that cause the focal spots of the illumination beams **123** to be scanned over the field-of-view of the sample **20** being imaged. The processor(s) **260** also executes instructions processor(s) **260** that send control signals to the digitizer **180** to simultaneously record photoacoustic signals received from the ultrasonic transducer device **150**. The digitizer **280** records photoacoustic signals for each of the locations of the focal spots of the illumination beams **223** in regions of the field-of-view of the sample **20** being imaged. During an image construction phase, the processor(s) **260** executes instructions to perform operations to construct a photoacoustic image from the data in the photoacoustic signals. First, the calibrated characteristic time delays of the ergodic relay **240** are used to map the RMS amplitudes of system responses to: i) the pixels within the areas of the focal spots in the optical foci regions of the field-of-view, or ii) the pixels within the field-of-view. This first operation is done for each illumination pattern (i.e., for each scanning location of the illumination beams). Second, a localization operation is performed that: i) determines a maximum or mean amplitude value of the pixel values within the area of each focal spot, and ii) assigns the pixels within the areas of each focal spot to the determined maximum or mean amplitude value and zeroes out the pixel values around the focal spot. The localization operation is performed for each illumination pattern to generate a plurality of localized images. Third, the pixel values from the plurality of

localized images are summed to generate a photoacoustic image of the field-of-view of the sample **10**.

– *Example of an ergodic relay in the form of a right-angle prism*

[0084] FIG. 3 is a schematic illustration of a side view of an ergodic relay **340** in the form of a right-angle prism, according to certain aspects. The ergodic relay **340** includes a first face **342**, a second face **344**, and a third face **346**. The illustrated example is shown at an instant in time during a data acquisition phase during which a plurality of illumination beams, including a first illumination beam **323a** with a first focal spot **324a** and a second illumination beam **323b** with a second focal spot **324b**, are simultaneously received at the first face **342** of the ergodic relay **340**.

The first illumination beam **323a** and the second illumination beam **323b** are propagating through the ergodic relay **340** and the second face **344** reflects the illumination beams **202 a,b** toward the third face **346** and enters the sample **30** being imaged. A single element ultrasonic transducer device **350** is coupled to a location at the corner of the ergodic relay **340** along the second face **344**.

[0085] For simplicity, only two illumination beams **323a,b** are shown being applied simultaneously. It would be understood that a greater number of illumination beams would typically be implemented, e.g., one or more illumination beams between the first illumination beam **202a** and the second illumination beam **202b**. During data acquisition, the focal spots of the plurality of illumination beams are scanned along a sample plane **31** over a field-of-view **328** of the sample **30** being imaged. Each focal spot is scanned in a corresponding optical focal region of the field-of-view **328**.

[0086] The illumination beams **323a,b** are directed into the sample **30** through the third face **346** and carry sufficient energy to induce localized heating of sample **30** illuminated by the illumination beams **323a,b**, resulting in the production of photoacoustic signals due to the photoacoustic effect. The photoacoustic signals in the form of ultrasound waves induced by an illumination beam propagate in all directions away from the illuminated region of the sample **30** including in the direction of the third face **346** of the ergodic relay **340**. In this illustrated example, an acoustic coupling gel **38** has been applied to the sample **30** and/or the surface of the third face **346** to enhance the efficiency of transfer of the photoacoustic signals into the third face **346**.

[0087] For purposes of illustration, an example of a photoacoustic signal **326a** is shown entering the ergodic relay **340** through the third face **346** propagating across the material of the ergodic relay **340** and internally reflected due to the discontinuity in the acoustic transmissivity

of the material between the ergodic relay **340** and the surrounding air. It would be understood that a plurality of photoacoustic signals are generated at the areas of the sample being illuminated by the plurality of focal spots of the illumination beams and that the plurality of photoacoustic signals are being propagating through the ergodic relay **340** during each scanning step in the data acquisition phase.

[0088] Each photoacoustic signal reflects internally within ergodic relay **340** between the internal surfaces of the first face **342**, second face **344**, and third face **346**. The internal path of each photoacoustic signal from a particular location of the region illuminated at the sample plane **31** to the single element ultrasonic transducer device **350** will have a distinct time delay characteristics based on the internal reflections. In the illustration, the internal path of the photoacoustic signal **326a** within the ergodic relay **340** directs the photoacoustic signal **326a** to the single element ultrasonic transducer device **350** coupled to the corner of the ergodic relay **340**. The ultrasonic transducer device **350** can detect the incoming photoacoustic signal **326a** encoded with a time delay due to its internal path. Since the internal path is associated with a particular location of the illuminated region of the sample **30**, the photoacoustic signal **326a** can be mapped to the location of the sample based on the time delay in the photoacoustic signal **326a**.

– *Example of optical foci regions in a field-of-view and a microlens array*

[0089] **FIG. 4** is a schematic illustration of a field-of-view **400** being imaged by an MFOR-PAMER system that includes a pattern-generating device in the form of a 20 x 20 microlens array, according to certain aspects. The illustrated example is shown at an instant in time, t_i , during a data acquisition phase when the plurality of four-hundred (400) illumination beams are projected to the sample plane and form an optical foci pattern (also sometimes referred to herein as “pattern”) in a rectangular grid pattern of focal spots at a sample plane. The field-of-view **400** has four-hundred (400) optical foci regions. Over the course of the data acquisition phase, the illumination beams are scanned so that each focal spot in the pattern is scanned to a plurality of locations in a corresponding optical foci region of the four-hundred (400) optical foci regions of the field-of-view **400**. The number of optical foci regions of the field-of-view **400** corresponds to the number of illumination beams being scanned. An example of a 20 x 20 microlens array capable of generating a plurality of four-hundred (400) illumination beams with focal spots in a rectangular grid pattern is the microlens array **500** shown in **FIG. 5**.

[0090] The illustrated example shows an enlarged portion of the field-of-view **400** at the upper right corner with an optical foci region **410a** with a focal spot **412a** (also sometimes referred to

herein as an “optical foci”) of the corresponding illumination beam at an x, y location **420a** at this instant in time, t_i , during the scanning of the illumination beams. Although the reference x,y location **420a** of the focal spot **412a** is defined as the center of the focal spot **412a** in this example, it would be understood that the x, y location **420a** can be defined as other locations within the area of the focal spot **412a** in other implementations.

[0091] The illustrated example also includes a local x-axis and a y-axis of the field-of-view **400**. As depicted by the arrows in the x-direction and the y-direction, the illumination beams are scanned in the x-direction and the y-direction so that their focal spots positioned at a plurality of N locations within the corresponding optical foci region **410a** over the course of the data acquisition phase. While the plurality of illumination beams are scanned to the x,y locations, an ultrasonic transducer device detects photoacoustic signals encoded with time delay characteristics, which can be used to map each photoacoustic signal to the x-y location illuminated in the field-of-view **400** of the sample that generated the acoustic waves.

[0092] It would be understood that the optical foci region **410a** is representative of the four-hundred (400) optical foci regions shown in the field-of-view **400** and that in other implementations there may be fewer or greater numbers of optical foci regions depending on the number of illumination beams being scanned in the field-of-view **400**. In addition or alternatively, the optical foci regions may have different areas. For example, reducing the area of the optical foci regions reduces the duration of the data acquisition phase and the imaging time.

[0093] **FIG. 5** includes a schematic drawing of a plan view and a cross-sectional A-A view of a microlens array **500**, according to one aspect. The illustrated example also includes an x-axis and a y-axis. The microlens array **500** has plan dimensions of 20 microlenses in the x-direction and 20 microlenses in the y-direction, a pitch between adjacent microlenses, and a thickness. The microlens array **500** has four-hundred (400) microlenses and is capable of generating four-hundred (400) illumination beams from a single input beam. As depicted by the two-sided arrows, the microlens array **500** is scanned in the x-direction and the y-direction during data acquisition phase.

[0094] The illustrated example shows an enlarged portion of the microlens array **500** at the upper right corner with a microlens **510a**. It would be understood that the microlens **510a** is representative of the four-hundred (400) microlens shown in microlens array **500** and that in other implementations there may be fewer or greater numbers of microlenses implemented depending on the number of illumination beams desired.

III. MFOR-PAMER IMAGING METHODS

[0095] In various implementations, an MFOR-PAMER imaging method includes a multifocal data acquisition phase and an image reconstruction phase that include localization operations. During the data acquisition phase, photoacoustic signals are detected while the plurality of illumination beams is scanned through the field-of-view of the sample being imaged. During an image reconstruction phase, the photoacoustic image is reconstructed using data from the encoded photoacoustic signals and the system responses determined during a training/calibration process. In one aspect, the image reconstruction phase uses time-delay characteristics of the ergodic relay to map the measured photoacoustic signals to optical foci regions in the field-of-view. The time-delay characteristics are determined in a training or calibration process, which is generally performed prior to the multifocal data acquisition phase. The operations an MFOR-PAMER imaging method can be performed by various implementations of an MFOR-PAMER system such as the MFOR-PAMER system 100 shown in FIG. 1 or the MFOR-PAMER system 200 shown in FIG. 2.

A. Training/Calibration process

[0096] During the training or calibration process, a point-by-point determination of the MFOR-PAMER system response is conducted to quantify the impulse response for each pixel location across the field-of-view. During this phase, a tightly focused light beam of pulsed or modulated radiation is scanned over the field-of-view of the sample being imaged while photoacoustic signals are recorded for the various locations of the light beam to map the response characteristics of the system for the sample. For example, the system response being measured can include a signature time delay between the light beam being produced and the detection of the photoacoustic signal received at the ultrasound transducer device as a function of location within the field-of-view.

[0097] During a typical training or calibration process, the MFOR-PAMER system is configured to generate a tightly focused light beam of pulsed or modulated radiation that is scanned over the field-of-view of the sample. Via the photoacoustic effect, photoacoustic waves are generated by the focused beam excitation and propagated through the ergodic relay. Some examples of photoacoustic waves generated by focused laser excitation are described in Wang, L.V., Hu, S. "Photoacoustic Tomography: in vivo imaging from organelles to organs," *Science* **335**, 1458–1462 (2012) and Xia, J., Yao, J., Wang, L.V., "Photoacoustic tomography: principles and advances," *Electromagn. Waves* **147**, 1 (2014), which are hereby incorporated by reference in their entireties. In most aspects, the focal spot diameter and pulse width of the focused beam

are much narrower than the central wavelength and the reciprocal of the bandwidth of the ultrasonic transducer device, respectively. Consequently, the photoacoustic wave input to the MFOR-PAMER system can be approximated as a spatiotemporal delta function, and the detected photoacoustic signals can be used to quantify the impulse response of the linear system for each excitation position. The focal spot of the illumination is raster scanned over the entire field-of-view and the point-by-point impulse responses are recorded. In one aspect, the time delay between the production of the illumination beam at the light source and the detected photoacoustic signal is determined and the determined time delay of each photoacoustic signal is mapped to the known location of the illumination beam within the field-of-view to determine the signature impulse response characteristics of the system.

[0098] For example, prior to multifocal measurement, the MFOR-PAMER system **200** shown in **FIG. 2** can be modified to replace the pattern-generating device **222** (e.g., a microlens array such as the 64-479 microlens array made by Edmund Optic of Barrington, New Jersey) with a focusing lens configured to focus the light beam of pulsed or modulated radiation into a narrow beam delivered to the sample **20**. An example of a commercially-available focusing lens that can be used to focus the beam to a 5 μm spot is the LA1509 lens made by Thorlabs, Inc.® having a 25.4 mm diameter and 100 mm focal length) was used to focus the laser beam to a 5 μm spot. Via the photoacoustic effect, photoacoustic waves are generated by the focused beam excitation and propagated through ergodic relay **240**. The MFOR-PAMER system **200** can then be used to detect a photoacoustic signal from the ergodic relay **240** in response to illumination of an optical foci region of the field-of-view of the sample **20** for a plurality of optical foci regions. In this implementation, the focal diameter and pulse width of the illumination beam are much narrower than the central wavelength and the reciprocal of the bandwidth of the ultrasonic transducer device **250**, respectively. Consequently, the photoacoustic wave input to the MFOR-PAMER system **200** can be approximated as a spatiotemporal delta function, and the detected photoacoustic signals can be used to quantify the impulse response of the linear system for the excitation position. The focused laser spot is raster scanned over the entire field-of-view using the x-scanning motor **230a** and the y-scanning motor **230b** and the point-by-point impulse responses are detected by the ultrasonic transducer device **250**. For example, the impulse response may be measured as a time delay between the production of the illumination beam at the light source **210** and the detected photoacoustic signal. The one or more processors **260** can execute instructions to map the measured time delay of each photoacoustic signal recorded at each optical foci region within the field-of-view onto the location of the optical foci region. After

the calibration/training process, the focusing lens can be replaced with the pattern-generating device to generate multiple optical foci in a data acquisition phase.

[0099] FIG. 6 depicts a flowchart 600 depicting operations of an MFOR-PAMER method, according to certain aspects. The operations are performed by an MFOR-PAMER system such as the system 100 shown in FIG. 1 or the system 200 shown in FIG. 2. One or more of the depicted operations may be caused by executing instructions retrieved from memory.

[0100] In certain implementations, each photoacoustic signal detected by the ultrasonic transducer device after passing through the ergodic relay includes multiple acoustic modes in some cases. In these instances, to accommodate multiple modes in each photoacoustic signal, the root-mean-squared value of each photoacoustic signal in the calibration process is used to reconstruct the photoacoustic image. The photoacoustic signals from each of the calibration locations of the illumination beam can be measured sequentially as $C_i(t)$, where i = the index number of the illumination beam input $x_i(t)$ at location i . The measured calibration signal $C_i(t)$ may be stored as a calibration signal $C(n)$, where n is the number of samples obtained over time, t . The root-mean-square calibration value, $X_{i,rms}$, for each calibration location is the root-mean-squared value of the calibration signal $C(n)$, calculated as:

$$X_{i,rms} = X_{i,rms} = \sqrt{\frac{1}{N} \sum_{n=1}^N |C(n)|^2} \quad (\text{Eqn. 1})$$

wherein N is the number of samples taken during the calibration operation.

[0101] At operation 610, the MFOR-PAMER system being calibrated is arranged in a calibration setup to configure the system to generate a tightly focused light beam of pulsed or modulated radiation that is scanned over the field-of-view of the sample. An example of a tightly focused beam is a beam that generates a 5 μm focal spot. In one implementation, to arrange the the MFOR-PAMER system in a calibration setup, the pattern-generating device is replaced with a focusing lens that can focus the light beam to a narrow beam (e.g., beam with focal spot of about 5 μm , beam with a focal spot less than 10 μm , beam with focal spot of less than 5 μm , etc.) delivered to the sample. An example of a commercially-available focusing lens that can be used to focus the beam to a 5 μm spot is the LA1509 lens made by Thorlabs, Inc.® having a 25.4 mm diameter and 100 mm focal length) was used to focus the laser beam to a 5 μm spot. Via the photoacoustic effect, photoacoustic waves are generated by the focused beam excitation and propagated through ergodic relay. In most aspects, the focal spot diameter and pulse width of the focused beam are much narrower than the central wavelength and the reciprocal of the bandwidth of the ultrasonic transducer device, respectively. Consequently, the photoacoustic wave input to the MFOR-PAMER system can be approximated as a

spatiotemporal delta function, and the detected photoacoustic signals can be used to quantify the impulse response of the linear system for each excitation position.

[0102] At operation **620**, the focal spot of the illumination beam is scanned over the entire field-of-view and photoacoustic signals at each of a plurality of locations are detected. The optical absorbers within the focal spot absorb light energy from the illumination beam, e.g., absorb short-pulsed light energy from a laser pulse, and convert the light energy into heat, resulting in the photoacoustic signal emission from the focal spot due to thermoelastic expansion. The photoacoustic signals emitted are propagated through the ergodic relay and are detected by the ultrasound transducer device in acoustic communication with the ergodic relay. In this operation, the focal spot of the focused illumination beam is directed to each of a plurality of locations in the field-of-view while the ultrasonic transducer device detects photoacoustic signals from the ergodic relay. In some cases, the illumination beam is directed to one or more locations within each optical foci region that will be used during an image acquisition phase. In one case, the illumination beam is directed to a location within each optical foci region that will be used during an image acquisition phase. In another case, the illumination beam is directed to each of the plurality of locations within each optical foci region that will be used during an image acquisition phase.

[0103] At operation **630**, a mapping of the point-by-point responses of the system for the plurality of illumination locations is determined. In one aspect, the system response for each focal spot calibration location is converted to the root-mean squared value of the calibration signal as described by *Eqn. (1)*. The mapping is based on the RMS amplitude of the system responses. The determined RMS amplitudes for the plurality of focal spot locations are mapped to the known locations of the focal spots within the field-of-view of the sample being imaged to calibrate the reconstructed photoacoustic amplitude image.

B. Multifocal Data Acquisition Phase and Image Reconstruction Phase

[0104] According to one aspect, during a multifocal data acquisition phase, the pattern generating device (e.g., a microlens array such as the 64-479 microlens array made by Edmund Optics® with a 500 µm pitch and 1.2° divergence angle) of an MFOR-PAMER system generates a plurality of optical foci (also referred to herein as “focal spots”) at the sample plane. The focal plane of the pattern generating device is imaged to the imaging face of the ergodic relay directly or via a relay lens such as a camera lens (e.g., 272EN II camera lens made by Tamron® having a 0.29 m minimum focus distance and 1:1 maximum magnification ratio). A scanning mechanism causes the plurality of focal spots to be scanned across the field-of-view such that each focal spot

is scanned over one optical foci region of the field-of-view. For example, if the pattern – generating device is a microlens array, during the multifocal measurement, only a distance equal to the pitch between adjacent microlenses needs to be scanned to be able to reconstruct a two-dimensional MFOR-PAMER image of the field-of-view. While the plurality of focal spots is scanned, the photoacoustic signals generated by the multiple optical foci are detected by an ultrasonic transducer device coupled directly or via an acoustic material to the ergodic relay.

[0105] In various implementations, each measurement of a photoacoustic signal propagating through the ergodic relay can be expressed as a linear combination of the impulse responses from all the illuminated calibration locations:

$$s(t) = \sum_{i=1}^{N_p} k_i(t) P_i, \quad (\text{Eqn. 2})$$

where $s(t)$ is the measured photoacoustic signal detected through the ergodic relay, i is the calibration location index, N_p the total number of calibration locations, $k_i(t)$ is the normalized impulse response, and P_i is the local photoacoustic amplitude at the i^{th} calibration location.

[0106] By discretizing time t according to the Nyquist criterion, **Eqn. 2** can be recast in matrix form as:

$$\mathbf{s} = \mathbf{K}\mathbf{P}, \quad (\text{Eqn. 3})$$

where $\mathbf{K} = [k_1, \dots, k_{N_p}]$ is the system impulse matrix and $\mathbf{P}$ is an amplitude photoacoustic image such as, e.g., a root-mean-squared amplitude photoacoustic image if the root-mean-square calibration values, $X_{i,rms}$ from **Eqn. 1** are used during reconstruction.

[0107] A photoacoustic image can be reconstructed by solving the inverse problem of **Eqn. 3** using various methods such as, for example, the two-step iterative shrinkage/thresholding (also referred to as “TwIST”) method. Examples of versions of the TwIST method that can be used are described in Li, Y., Li, L., Zhu, L., Maslov, K., Shi, J., Yao, J., Liang, J., Wang, L., and Wang L.V., “Snapshot photoacoustic topography through an ergodic relay for high-throughput imaging of optical absorption” and Bioucas-Dias, J.M. and Figueiredo, M.A.T. “A new TwIST: two-step iterative shrinkage/thresholding algorithms for image restoration,” *IEEE Trans. Image Process.* 16, 2992–3004 (2007), each of which is hereby incorporated by reference in its entirety.

[0108] In various aspects, during an image reconstruction phase of the MFOR-PAMER method, one or more photoacoustic images of a field-of-view of the sample are reconstructed using the data from the photoacoustic signals detected by the ultrasonic transducer device during the multifocal data acquisition phase and the calibrated system response values determined during the calibration phase. In one aspect, the root-mean-square calibration values, $X_{i,rms}$,

determined by *Eqn. 1* are used to calibrate the reconstructed root-mean-squared amplitude photoacoustic image.

[0109] In one aspect, an iterative shrinkage/thresholding method such as the method can be implemented to solve *Eqn. 3* for the root-mean-squared amplitude photoacoustic image, P , as a minimizer of the objective function:

$$\hat{P} = \arg \min_P \|s - KP\|^2 + 2\lambda \Phi_{TV}(P), \quad (\text{Eqn. 4})$$

where $\Phi_{TV}(P)$ is the total variation regularization term and λ is the regularization parameter.

[0110] In certain aspects, the reconstruction phase includes localization operations that improve image quality. For example, when localization operations are employed by the MFOR-PAMER system 200 in FIG. 2, the resolution is improved from acoustic resolution to optical resolution (from 220 μm to 13 μm). For each scanning location, an amplitude photoacoustic image (sometimes referred to herein as “reconstructed multifocal image”) is reconstructed based on the data from the photoacoustic signals and the calibrated system response values by solving the inverse problem of *Eqn. 3*. Each of the amplitude photoacoustic images is localized based on the known positions (e.g., center locations) of the focal spots at the sample plane in localization operations to generate a partial MFOR-PAMER image based on a single scan location. In one aspect, the localization operations include determining a maximum or a mean amplitude pixel value for the pixels within each focal spot and setting pixels within the focal spot to the maximum or mean amplitude value. For example, a maximum or a mean amplitude pixel value may be determined for the pixels in a circular area (e.g., 13 μm diameter) centered around the location of the focal spot. In one case, the localization operations also include zeroing out the pixels around the focal spot in the corresponding optical foci region of the amplitude photoacoustic image. To generate a two-dimensional MFOR-PAMER image, the pixel values for the localized amplitude photoacoustic images (partial MFOR-PAMER images) are summed.

[0111] During the multifocal measurement phase, only a distance equal to the pitch between optical elements in a pattern-generating device (e.g., microlenses in a microlens array) generating the illumination beams needs to be scanned to be able to measure photoacoustic signals needed to reconstruct a 2D MFOR-PAMER image over the field-of-view. For example, when using the microlens array that generates the four-hundred (400) optical foci regions in the field-of-view 400 shown in FIG. 4, each optical foci or focal spot 412a need only be scanned across an optical foci region 410a to be able to reconstruct a 2D MFOR-PAMER image of the of the total field-of-view 400. Thus, as compared to a system that does not have a pattern generating device (e.g., microlens array), the imaging speed of the MFOR-PAMER system is improved by a

factor of the number of optical elements (e.g., microlenses) generating the different illumination beams to cover and reconstruct an image of the same field-of-view.

[0112] After the training step, the focusing lens was replaced with a microlens array (64-479, Edmund Optics, 500 μm pitch, 1.2° divergence angle) to generate multiple optical foci. The focal plane of the microlens array was imaged to the imaging face of the ER by a camera lens (272EN II camera lens made by Tamron Inc.® with a 0.29 m minimum focus distance and 1:1 maximum magnification ratio). Photoacoustic signals generated from the multiple optical foci were then detected by the single-element ultrasonic transducer.

[0113] FIG. 7 depicts a flowchart 700 depicting operations of an MFOR-PAMER method, according to certain aspects. The operations are performed by an MFOR-PAMER system such as the system 100 shown in FIG. 7 or the system 200 shown in FIG. 700. One or more of the depicted operations may be performed by one or more processors executing instructions retrieved from memory. The depicted MFOR-PAMER method includes a multifocal data acquisition phase including operations 710, 720, and 730 and an image reconstruction phase including operations 730, 740, and 750, and 760 with localization operations.

[0114] At operation 710, the MFOR-PAMER system scans a plurality of illumination beams over a field-of-view being imaged such that each focal spot is scanned over a plurality of scan locations of a corresponding optical foci region in the field-of-view being imaged. For example, control signals from a processor or processors may be communicated to a light source to active the light source to provide a light beam, which is separated into a plurality of illumination beams by a pattern-generating device. In this example, control signals from the processor or processors may be communicated to the scanning mechanism coupled to one or more components of the MFOR-PAMER system to cause the plurality of illumination beams to be scanned over the field-of-view. In one aspect, each illumination beam is scanned in a first direction along the local x-axis of the optical foci region and/or a second direction along a local y-axis of the optical foci region. Via the photoacoustic effect, photoacoustic waves are generated at the illuminated areas of the sample at the focal spots and photoacoustic waves are propagated through the ergodic relay with characteristic time delays. Each focal spot may be scanned to any suitable number of locations within the corresponding optical foci region such as, e.g., 100 (10 x 10), 400 (20 x 20), 1000 (~33 x 33), 1600 (40 x 40), 2500 (50 x 50), 4225 (65 x 65), etc.

[0115] At operation 720, while the focal spots of the plurality of illumination beams are scanned over the field-of-view to N scan positions, photoacoustic signals are detected by the ultrasonic transducer device coupled directly or through acoustic material to the ergodic relay.

In one aspect, the ultrasonic transducer device is a single element transducer coupled to the ergodic relay. In many cases, the ultrasonic transducer device is coupled to the ergodic relay at a location or multiple locations (e.g. corner) that create an asymmetric relationship between the ultrasonic transducer device and ergodic relay to increase the distinctions among the

5 photoacoustic signals received from different input locations of the ergodic relay. In one aspect, the ultrasonic transducer device takes measurements at a rate of 50 MHz. In another aspect, the ultrasonic transducer device takes measurements at a rate of 75 MHz. In yet another aspect, the ultrasonic transducer device takes measurements at a rate of 100 MHz. In some implementations, control signals from a processor or processors may be communicated to digitizer (e.g., a two-
10 channel digitizer) in electronic communication with the ultrasonic transducer device to cause the digitizer to record the data from the encoded photoacoustic signals received from the ultrasonic transducer device while the illumination beams are illuminating the field-of-view in operation **710**. In some cases, the data from the encoded photoacoustic signals is stored to a computer readable medium.

15 **[0116]** At operation **730**, using the recorded data from the encoded photoacoustic signals and the system responses measured during a training/calibration process, a snapshot photoacoustic image is reconstructed for each scan position. In this operation, a plurality of N photoacoustic images is reconstructed where N is the number of scan positions. Each photoacoustic image can be reconstructed by solving the inverse problem of *Eqn. 3* using various methods such as, for
20 example, the two-step iterative shrinkage/thresholding (TwIST) method. In one aspect, the root-mean-square calibration values, $X_{i,rms}$, determined by *Eqn. 1* are used to calibrate the reconstructed root-mean-squared amplitude photoacoustic image.

[0117] The MFOR-PAMER method, depicted by the operations in the flowchart **700**, includes localization operations **740** and **750**. At operation **740**, for each optical foci region of each
25 photoacoustic image, a maximum or mean amplitude value of the pixels in the focal spot of the optical foci region is determined.

[0118] At operation **750**, a plurality N localized snapshot images is generated from the plurality of N photoacoustic images. Each localized snapshot image is generated by assigning the pixels within the focal spot of each optical foci region to the determined maximum or mean
30 amplitude value and assigning a zero value (zeroing) to the pixels outside the focal spot. This is done for each optical foci region to generate each localized snapshot image.

[0119] At operation **760**, an MFOR-PAMER image is constructed by summing the pixel values from the plurality of localized snapshot images.

IV. *Experimental and simulated results*

A. *Numerical simulation*

[0120] To demonstrate the capability of an MFOR-PAMER system according to certain implementations, the signals generated from a 2D microlens array were simulated with different detection parameters and results were analyzed. To synthesize the multifocal measurement, $P_i = 1$ was set at the pixel positions where optical foci were generated by a simulated 2D microlens array, and $P_i = 0$ at the other positions, as shown in **FIG. 8A**. **FIG. 8A** is an illustration of the actual positions of simulated microlens focal spots, according to an aspect. A zero-mean Gaussian random vector representing white noise was added to the synthesized signals.

[0121] **FIG. 8B** is a reconstructed image of the simulated microlens array pattern when the pitch is acoustically resolvable. **FIG. 8B** shows the reconstructed image from a synthesized multifocal measurement with a pitch of 500 μm . At this pitch, the photoacoustic signals from neighboring optical foci are clearly separated. Each reconstructed spot is larger than the optical focal spot size because the system resolution is acoustically defined and related to the central wavelength of the detector. Examples of systems with resolution that is acoustically defined and related to the central wavelength of the detector are described in Ing, R.K., Quieffin, N., Catheline, S., Fink, M., “In solid localization of finger impacts using acoustic time-reversal process,” Appl. Phys. Lett. 87, 204104 (2005) and Li, Y., Li, L., Zhu, L., Maslov, K., Shi, J., Yao, J., Liang, J., Wang, L., and Wang L.V., “Snapshot photoacoustic topography through an ergodic relay for high-throughput imaging of optical absorption,” which are hereby incorporated by reference in their entireties. Therefore, the reconstructed spots become smudged if the pitch is smaller than the system resolution, as shown in **FIG. 8C**. **FIG. 8C** is a reconstructed image of the simulated pattern when the pitch is not acoustically resolvable.

[0122] To quantify the relationship between the minimum separation pitch and the central wavelength, the 10 MHz transducer was replaced with other transducers having similar physical parameters but different central wavelengths. As **FIG. 8D** shows, the minimum separation pitch equals approximately 1/2 of the acoustic central wavelength in the ergodic relay, according to certain implementations. **FIG. 8D** is a quantification of the minimum separation distance required to separate two spots in the MFOR-PAMER system at various transducer central wavelengths, according to certain implementations.

B. Performance Improvements with Localization

[0123] The MFOR-PAMER system differs in several ways from a PAMER system with acoustically defined resolution (AR). For example, the MFOR-PAMER system includes a pattern-generating device such as, e.g., a microlens array, and a scanning mechanism, which can significantly reduce the imaging time as compared to a PAMER system imaging the same size field-of-view. For example, when employing a microlens array that can generate 400 illumination beams, an MFOR-PAMER system can image at least 400 times faster than an OR-PAM system at the same imaging resolution. The MFOR-PAMER system also differs from the PAMER system in that the MFOR-PAMER system uses optical focal spot localization operations in its reconstruction process. An example of a PAMER system is described in Li, Y., Li, L., Zhu, L., Maslov, K., Shi, J., Yao, J., Liang, J., Wang, L., and Wang L.V., "Snapshot photoacoustic topography through an ergodic relay for high-throughput imaging of optical absorption," and in International PCT application PCT/US2018/032007, titled "SNAPSHOT PHOTOACOUSTIC PHOTOGRAPHY USING AN ERGODIC RELAY" and filed on May 10, 2018, which are hereby incorporated by reference in their entireties.

[0124] Two physical objects were used to quantify the image resolution with and without optical focal spot localization. To determine the acoustic resolution without localization, a black acrylic sheet was placed on the imaging face of the ergodic relay to provide uniform absorption. Two 5 μm diameter laser spots were simultaneously shone on the black acrylic sheet. While one illumination beam was held stationary, the other illumination beam was translated linearly away from the first during photoacoustic measurements. The contrast-to-noise ratio (CNR) versus the distance between the two laser spots was then calculated and the results are shown in **FIG. 9A**.

[0125] **FIG. 9A** is an illustration of the quantification of the acoustical lateral resolution without localization. Two light spots shone on a black sheet were imaged to quantify the minimum distance needed to differentiate them in the reconstructed image. **FIG. 9B** is a graph of the contrast-to-noise ratio (CNR) versus the distance between the two light spots. The lateral resolution, measured as the distance with 6 dB CNR in **FIG. 9B**, was 220 μm , which agrees well with the simulation results.

[0126] To determine the optically-defined image resolution using multifocal localization, the edge of a sharp metal blade was imaged. The reconstructed MFOR-PAMER image of the blade edge is shown in **FIG. 9C**. **FIG. 9C** is an illustration of the quantification of the optical lateral resolution of an MFOR-PAMER system, according to one implementation. An RMS photoacoustic amplitude projection image of a sharp edge is reconstructed by summing the

partial MFOR images. The averaged amplitude measurement was fitted to an error function to obtain the edge-spread function (ESF). The line-spread function (LSF) of the system was calculated by taking the derivative of the ESF, which is shown in **FIG. 9D**. **FIG. 9D** is an illustration of the edge-spread function (ESF) used to calculate the line-spread function (LSF) of an MFOR-PAMER system, according to one implementation. The lateral resolution, measured as the full width at half maximum of the LSF, was approximately 13 μm , matching the diameter of the focused laser spot.

C. *In vitro experimental results*

[0127] The MFOR-PAMER system 200 shown in **FIG. 2** with a pattern-generating device 222 is in the form of a microlens array was used to image a leaf skeleton phantom *in vitro*. The imaged leaf skeleton phantom contained a rich network of vessel-like structure. The microlens array was replaced with a focusing lens during the calibration/training process. A piece of transparency film was cut to a size of 25 mm $\times$ 25 mm and painted with black ink on one side for the calibration/training process. Since the imaged sample is effectively a part of the ergodic relay system response, the leaf skeleton was attached to the film with ultrasonic gel to facilitate acoustic coupling. After the calibration/training process, the microlens array was switched back and the painted film was replaced with an unpainted film to image the leaf skeleton with the microlens array. The microlens array was raster scanned with a 500 μm $\times$ 500 μm range and a step size of 6.3 μm to image the entire field-of-view.

[0128] **FIG. 10A** is a single-frame reconstructed image from a single scanning step of the microlens array excitation pattern of an of an MFOR-PAMER system, according to one implementation. All the optical focal spots are well separated in the acoustically defined resolution pixels. Reconstructed images from all the scanning steps were summed directly to create an image without localization. The image created without localization is in **FIG. 10B**. This image is equivalent to an image obtained from wide-field light illumination. To enable a fair comparison, the summation procedure ensures identical total energy depositions and optical illumination profiles for the AR- and MFOR-PAMER images. The AR-PAMER image shows a blurry vessel skeleton in which many vessels cannot be visually separated and different vessel diameters cannot be differentiated.

[0129] **FIG. 10C** is an MFOR-PAMER image that was constructed by localizing the optical foci from the scanning steps, according to an aspect. By comparison, the 2D MFOR-PAM image with localization presents a detailed vascular skeletal network with different diameters and much finer image resolution.

D. *In vivo experimental results*

[0130] The MFOR-PAMER system 200 shown in FIG. 2 with a pattern-generating device 222 is in the form of a microlens array, according to one implementation, was tested *in vivo* by imaging blood vessels in the ear of a female ND4 Swiss Webster mouse (Envigo; 18–20 g and 6–8 weeks). For comparison, a AR-PAMER system was also used to image the blood vasculature in the mouse ear. The laboratory animal protocols were approved by the Institutional Animal Care and Use Committee of California Institute of Technology. The mouse was anesthetized with 5% vaporized isoflurane mixed with air to induce anesthesia, and then transferred to a customized animal mount allowing the mouse ear to be laid flat on the imaging face of the ER. The mouse was kept anesthetized with a continuous supply of 1.5% vaporized isoflurane during the experiment. The surface optical fluence at each optical focal spot through the microlens array was maintained at $< 20 \text{ mJ/cm}^2$ to comply with the ANSI safety limit per laser pulse described in Laser Institute of America, “American National Standard for the safe use of lasers,” *American National Standard Institute* (2000), which is hereby incorporated by reference in its entirety.

[0131] FIGS. 11A-B are experimental *in vivo* images of the blood vasculature in a mouse ear.

[0132] FIG. 11A is an AR-PAMER image of the ear vasculature with a zoomed in portion. The vasculature appears blurry due to the poorer acoustically defined resolution.

[0133] FIG. 11B is an MFOR-PAMER image of the ear vasculature with a zoomed in portion. In comparison with the AR-PAMER image in FIG. 11A, the 2D MFOR-PAMER image in FIG. 11B shows a much better-resolved vasculature. FIG. 12 is a graph of line profiles across the two white dashed lines in the boxed regions in the zoomed in views from FIG. 11A-B. The MFOR-PAMER image reveals small vascular structures that can hardly be seen in the AR-PAMER image.

[0134] Many capillary-level vessels in the mouse ear cannot be resolved in the MFOR-PAMER image, for two possible reasons. On the one hand, an optical resolution of $13 \text{ }\mu\text{m}$ cannot fully resolve vessels that are much smaller in size. This limitation could be overcome by implementing a microlens array with a higher numerical aperture (NA), or by using a relay lens with a compression ratio, to reduce the size of the optical focal spots. On the other hand, the current embodiment of the PAMER system suffers from low signal sensitivity due to signal attenuation inside the ER during propagation. As a result, signals from small vessels become too weak to be differentiated from background noise. This problem could potentially be solved by utilizing an ultrasonic detector with higher sensitivity and broader bandwidth, such as an optical

micro-resonator detector. An example of an optical micro-resonator detector is described in Guggenheim, J.A., Li, J., Allen, T.J., Colchester, R.J., Noimark, S., Ogunlade, O., Parkin, I.P., Papakonstantinou, I., Desjardins, A.E., Zhang, E.Z., Beard, P.C. , “Ultrasensitive plano-concave optical microresonators for ultrasound sensing,” *Nat. Photon.* **11**, 714-721 (2017), which is
 5 hereby incorporated by reference in its entirety.

[0135] In certain implementations, the 2D MFOR-PAMER system can form an MFOR-PAMER image with a 13 μm spatial resolution, 10 mm $\times$ 10 mm FOV, and 2500 scanning steps in less than 10 seconds, excluding the training time. An example of such an MFOR-PAMER system is the MFOR-PAMER system 200 described with respect to FIG. 2. Given sufficient
 10 SNR, the training step can also be done within 10 seconds, because only AR-quality training is required. These results significantly surpass those of previously demonstrated MFOR-PACT systems. Some examples of MFOR-PACT systems can be found in Li, G., Maslov, K.I., and Wang, L.V., “Reflection-mode multifocal optical-resolution photoacoustic microscopy,” *J. Biomed. Opt.* **18**, 030501 (2013) and Xia, J., Li, G., Wang, L., Nasiriavanaki, M., Maslov, K.,
 15 Engelbach, J.A., Garbow, J.R., Wang, L.V., “Wide-field two-dimensional multifocal optical-resolution photoacoustic-computed microscopy,” *Opt. Lett.* **38**, 5236–6239 (2013), which are hereby incorporated by reference in their entirety.

[0136] In certain implementations, an MFOR-PAMER system may have improved image resolution, scanning time, and system sensitivity by employing an ultrasonic detector with higher
 20 sensitivity and broader bandwidth, and a microlens array with a larger NA and smaller pitch (in accordance with the minimum AR separation distance). For example, for an ultrasonic transducer with 20 MHz central frequency, the expected AR resolution is 110 μm . In this case, for a microlens array with a physical dimension of 10 mm $\times$ 10 mm, the pitch can be reduced to 150 μm (150 μm > 110 μm) so that more optical foci ($\sim 65 \times 65$) can be packed into the field-of-view
 25 and scanning time can be reduced.

[0137] In certain aspects, an MFOR-PAMER system can reconstruct a photoacoustic image from the encoded photoacoustic signals where the acoustic spatial resolution of the photoacoustic image is less than 220 μm . In other aspects, an MFOR-PAMER system can reconstruct a photoacoustic image from the encoded photoacoustic signals where the acoustic spatial
 30 resolution of the photoacoustic image is less than 20 μm .

[0138] In certain aspects, an MFOR-PAMER system can reconstruct a photoacoustic image of a field-of-view of a 100 mm² area in less than 10 seconds. In certain aspects, an MFOR-PAMER

system can reconstruct a photoacoustic image of a field-of-view of a 100 mm² area in less than 1000 seconds.

[0139] In certain aspects, the MFOR-PAMER system design can image at least 400 times faster than OR-PAM systems at the same imaging resolution. The MFOR-PAMER system has promising potential for many biomedical applications, such as utilizing ultra-violet (UV) illumination for high-speed, label-free histological study of biological tissues. An example of a label-free histological study of biological tissues is described in Wong, T.T.W., Zhang, R., Hai, P., Zhang, C., Pleitez, M.A., Aft, R.L, Novack, D.V., and Wang, L.V., “Fast label-free multilayered histology-like imaging of human breast cancer by photoacoustic microscopy,” *Sci. Adv.* **3**, 1602168 (2017), which is hereby incorporated by reference in its entirety. The MFOR-PAMER system can reduce the imaging time from several hours (with UV OR-PAM systems) to less than a minute, significantly improving the efficiency of clinical histology and diagnostics.

[0140] Certain implementations pertains to a multifocal photoacoustic imaging method, comprising: (a) receiving encoded photoacoustic signals detected by an ultrasound transducer device in acoustic communication with an ergodic relay, the photoacoustic signals detected while a plurality of illumination beams is scanned over a field-of-view being imaged, wherein a focal spot of each illumination beam is scanned to a plurality of locations within an optical foci region of a plurality of optical foci regions in the field-of-view; (b) reconstructing a plurality of snapshot photoacoustic images, each snapshot image reconstructed using calibrated system responses and encoded photoacoustic signals detected by the ultrasound transducer device while the plurality of illumination beams is at one of a plurality of scan locations; (c) for each of the plurality of snapshot photoacoustic images, generating a localized photoacoustic image by assigning pixels within the focal spot of each optical foci region to a calculated value (e.g., a maximum value or a mean value of pixels within the focal spot of each optical foci region); and (d) combining the plurality of localized photoacoustic images to generate a multifocal optical resolution photoacoustic image of the field-of-view. In one implementation, operation (c) further comprises zeroing pixels outside of the focal spot of each optical foci region.

[0141] Although most implementations of MFOR-PAMER systems described herein are depicted as the ergodic relay projecting a plurality of illumination beams from the pattern-generating device to the sample being imaged, other implementations may not project the plurality of illumination beams through the ergodic relay. For example, in one implementation, an MFOR-PAMER system may have the plurality of illumination beams projected to the sample directly from the pattern-generating device or via one or more other optical elements (e.g., one or

more mirrors) from the pattern-generating device. In one case, the sample may be mounted to a surface of the ergodic relay and the illumination beams are directed toward the surface. In one instance, the illumination beams may be projected onto the sample using a mirror.

[0142] In one implementation, an MFOR-PAMER system may implement an ultrasound

transducer device with multiple transducer elements. This MFOR-PAMER system may have the technical advantage of improved frame rate or imaging resolution over a single-element transducer element system by providing multiple parallel channels by which the acoustic waves from multiple photoacoustic source locations may be detected and signals recorded. However, this implementation could be more costly and add complexity.

[0143] In one embodiment, an MFOR-PAMER system does not implement scanning mechanism to move the illumination beams to different locations in the region. In this example, each of the illumination beams illuminates a single corresponding optical foci region in the field-of-view and the system uses these signals to construct a photoacoustic image.

[0144] Modifications, additions, or omissions may be made to any of the above-described

embodiments without departing from the scope of the disclosure. Any of the embodiments described above may include more, fewer, or other features without departing from the scope of the disclosure. Additionally, the steps of the described features may be performed in any suitable order without departing from the scope of the disclosure.

[0145] 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.

[0146] 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, 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.

[0147] Although the foregoing disclosed embodiments have been described in some detail to facilitate understanding, the described embodiments are to be considered illustrative and not

limiting. It will be apparent to one of ordinary skill in the art that certain changes and modifications can be practiced within the scope of the appended claims.

[0148] 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, 5 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.

CLAIMS

WHAT IS CLAIMED IS:

1. A multifocal photoacoustic imaging system comprising:
a pattern-generating device configured to generate a plurality of illumination
5 beams, the plurality of illumination beams having focal spots located in corresponding optical
foci regions of a field-of-view of a sample being imaged;
an ergodic relay in optical communication with the pattern-generating device and
acoustically coupled to the sample, the ergodic relay configured to encode photoacoustic signals
received from the illuminated optical foci regions; and
10 an ultrasonic transducer device in acoustic communication with the ergodic relay.
2. The multifocal photoacoustic imaging system of claim 1, further
comprising one or more optical elements configured to direct the plurality of illumination beams
from the pattern-generating device to the field-of-view.
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3. The multifocal photoacoustic imaging system of claim 1, wherein the
ergodic relay is further configured to reflect the plurality of illumination beams to the field-of
view.
- 20 4. The multifocal photoacoustic imaging system of claim 3, further
comprising one or more optical elements configured to propagate the plurality of illumination
beams from the pattern-generating device to the ergodic relay.
- 25 5. The multifocal photoacoustic imaging system of claim 4, wherein the one
or more optical elements comprise a relay lens.
6. The multifocal photoacoustic imaging system of claim 1, further
comprising a scanning mechanism configured to scan the plurality of illumination beams in the
field-of-view, wherein the focal spot of each illumination beam is scanned to a plurality of scan
30 locations in the corresponding optical foci region.
7. The multifocal photoacoustic imaging system of claim 6, wherein the
scanning mechanism comprises a scanning mirror.

8. The multifocal photoacoustic imaging system of claim 6, wherein the scanning mechanism comprises a first motor configured to move the plurality of illumination beams in a first direction and a second motor configured to move the plurality of illumination beams in a second direction orthogonal to the first direction.

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9. The multifocal photoacoustic imaging system of claim 1, further comprising one or more processors and memory configured to reconstruct a photoacoustic image from the encoded photoacoustic signals.

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10. The multifocal photoacoustic imaging system of claim 6, further comprising one or more processors and memory configured to:

generate a plurality of snapshot photoacoustic images from the encoded photoacoustic signals, each snapshot photoacoustic image corresponding to one of the plurality of scan locations;

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for each of the plurality of snapshot photoacoustic images, localize pixel values within each focal spot of each optical foci region to generate a localized snapshot image; and

combine the localized snapshot images to generate a multifocal optical resolution photoacoustic image.

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11. The multifocal photoacoustic imaging system of claim 1, wherein the ultrasonic transducer device is a single-element transducer.

12. The multifocal photoacoustic imaging system of claim 9, wherein an acoustic spatial resolution of the photoacoustic image is less than 220 μm .

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13. The multifocal photoacoustic imaging system of claim 9, wherein an optical spatial resolution of the photoacoustic image is less than 20 μm .

30

14. The multifocal photoacoustic imaging system of claim 1, wherein:
an area of the field-of-view is 100 mm^2 ; and
the photoacoustic imaging system is configured to reconstruct a photoacoustic image from the encoded photoacoustic signals in less than 10 seconds.

15. The multifocal photoacoustic imaging system of claim 1, wherein:
an area of the field-of-view is 100 mm^2 ; and
the photoacoustic imaging system is configured to reconstruct a photoacoustic
image from the encoded photoacoustic signals in less than 1000 seconds.

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16. The multifocal photoacoustic imaging system of claim 1, wherein the
photoacoustic imaging system is configured to reconstruct a photoacoustic image with an optical
spatial resolution of less than $20 \text{ }\mu\text{m}$ from the encoded photoacoustic signals.

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17. The multifocal photoacoustic imaging system of claim 1, wherein the
photoacoustic imaging system is configured to reconstruct a photoacoustic image with an optical
spatial resolution of less than $220 \text{ }\mu\text{m}$ from the encoded photoacoustic signals.

18. The multifocal photoacoustic imaging system of claim 1, wherein the
pattern-generating device is configured to generate the plurality of illumination beams from one
or more light beams.

19. The multifocal photoacoustic imaging system of claim 1, wherein the
pattern-generating device is in optical communication with a light source configured to produce
a light beam of pulsed and/or modulated radiation, wherein the pattern-generating device is
configured to generate the plurality of illumination beams from the light beam.

20. The multifocal photoacoustic imaging system of claim 19, wherein the
light source is one of a pulsed laser and one or more light emitting diodes.

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21. The multifocal photoacoustic imaging system of claim 20, further
comprising one or more optical elements configured to spatially filter and expand the light beam
generated by the light source from a source of pulsed or modulated radiation and configured to
propagate the first beam to the pattern-generating device.

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22. The multifocal photoacoustic imaging system of claim 1, wherein the
pattern-generating device is a microlens array.

23. The multifocal photoacoustic imaging system of claim 1, wherein the pattern-generating device is a digital mirror or a spatial light modulator.

24. The multifocal photoacoustic imaging system of claim 1, wherein the
5 ultrasonic transducer device is asymmetrically coupled to the ergodic relay.

25. The multifocal photoacoustic imaging system of claim 1, wherein the ergodic relay is a prism.

10 26. The multifocal photoacoustic imaging system of claim 1, wherein the ergodic relay is a glass or quartz plate or a silicon wafer.

27. A multifocal photoacoustic imaging method, comprising:

15 (a) controlling a pattern-generating device to generate a plurality of illumination beams with focal spots illuminating a corresponding plurality of optical foci regions in a field-of-view of a sample being imaged;

(b) receiving encoded photoacoustic signals detected by an ultrasound transducer device in acoustic communication with an ergodic relay, wherein the ergodic relay is in acoustic communication with the sample being imaged; and

20 (c) reconstructing a snapshot photoacoustic image using calibrated system responses and the encoded photoacoustic signals.

28. The multifocal photoacoustic imaging method of claim 26, further comprising generating a localized photoacoustic image by assigning a calculated value to pixels
25 within the focal spot of each optical foci region in the snapshot photoacoustic image.

29. The multifocal photoacoustic imaging method of claim 27, wherein the calculated value is a maximum value or a mean value of pixels within the focal spot.

30 30. The multifocal photoacoustic imaging method of claim 27, further comprising zeroing pixels outside of the focal spot of each optical foci region.

31. The multifocal photoacoustic imaging method of claim 26, further comprising communicating control signals to a scanning mechanism to cause scanning of the plurality of illumination beams, each illumination beam scanned to a plurality of scan locations within a corresponding optical foci region.

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32. The multifocal photoacoustic imaging method of claim 30, further comprising repeating (c) for each of the plurality of scan locations to reconstruct a plurality of snapshot photoacoustic images.

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33. The multifocal photoacoustic imaging method of claim 31, further comprising for each of the plurality of snapshot photoacoustic images, generating a localized photoacoustic image by assigning a calculated value to pixels within the focal spot of each optical foci region in the snapshot photoacoustic image.

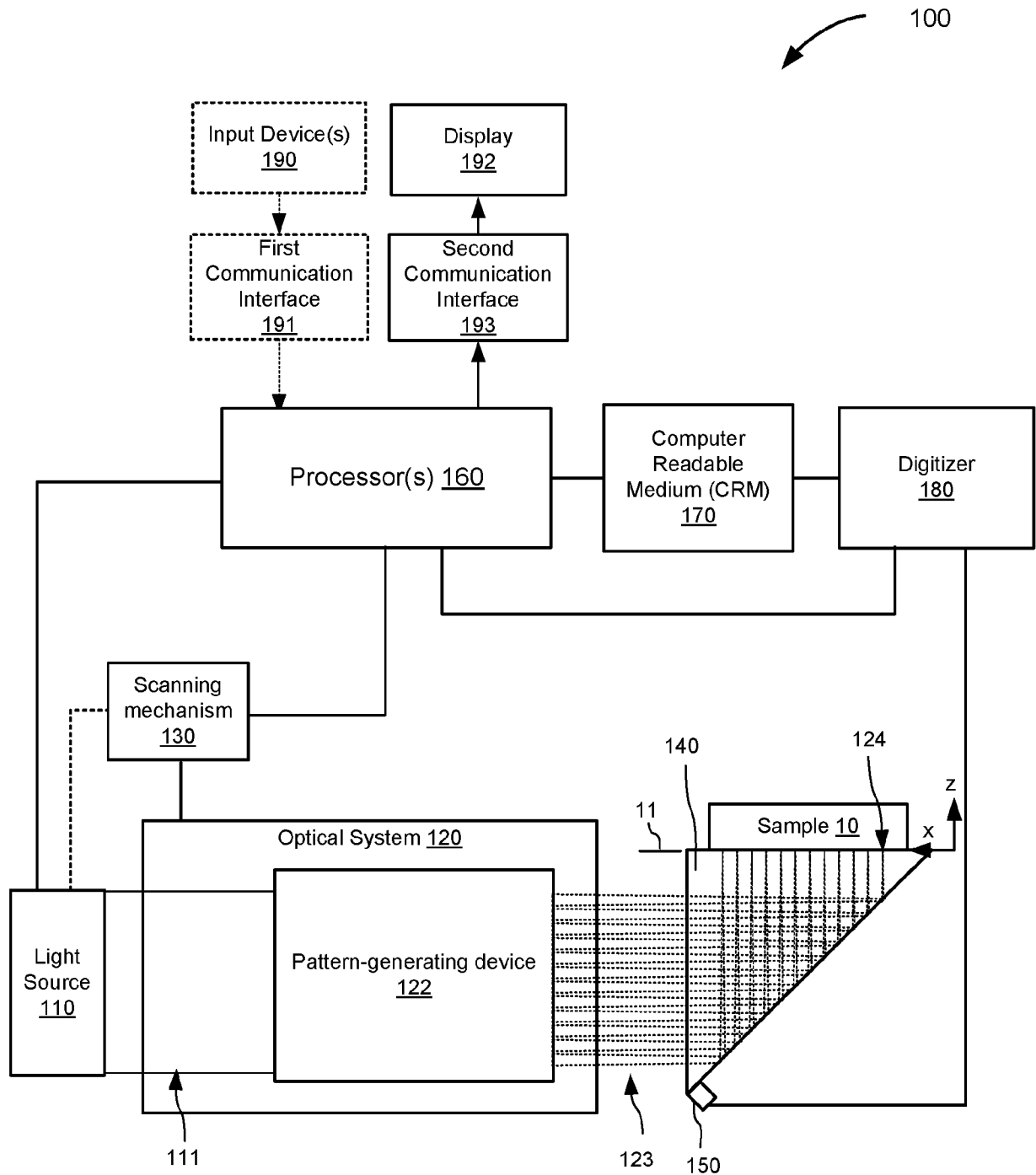
15

34. The multifocal photoacoustic imaging method of claim 32, wherein the calculated value is a maximum value or a mean value of pixels within the focal spot.

35. The multifocal photoacoustic imaging method of claim 33, further comprising zeroing pixels outside of the focal spot of each optical foci region.

20

36. The multifocal photoacoustic imaging method of claim 32, further comprising combining the plurality of localized photoacoustic images to generate a multifocal optical resolution photoacoustic image of the field-of-view.

**FIG. 1**

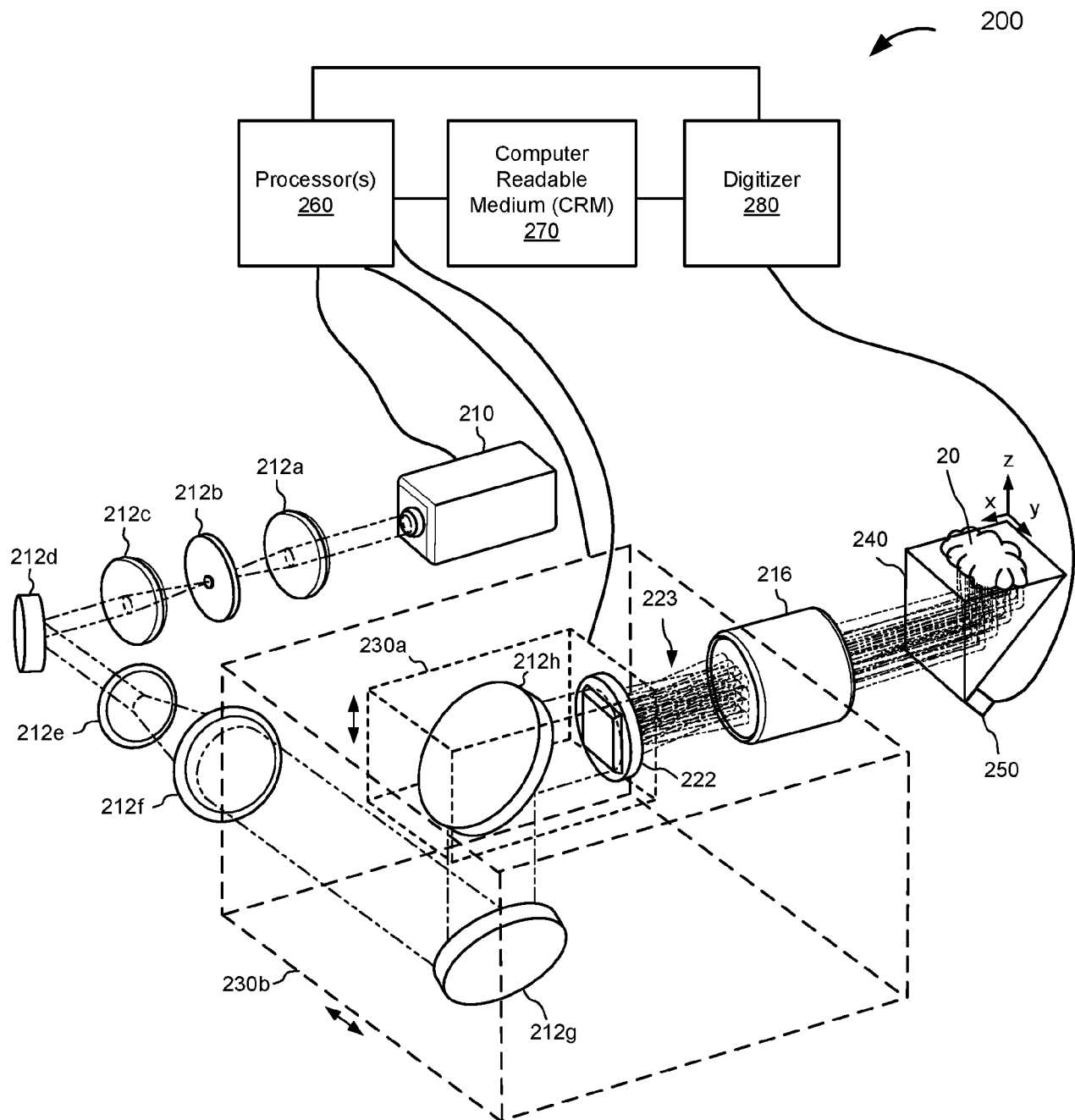
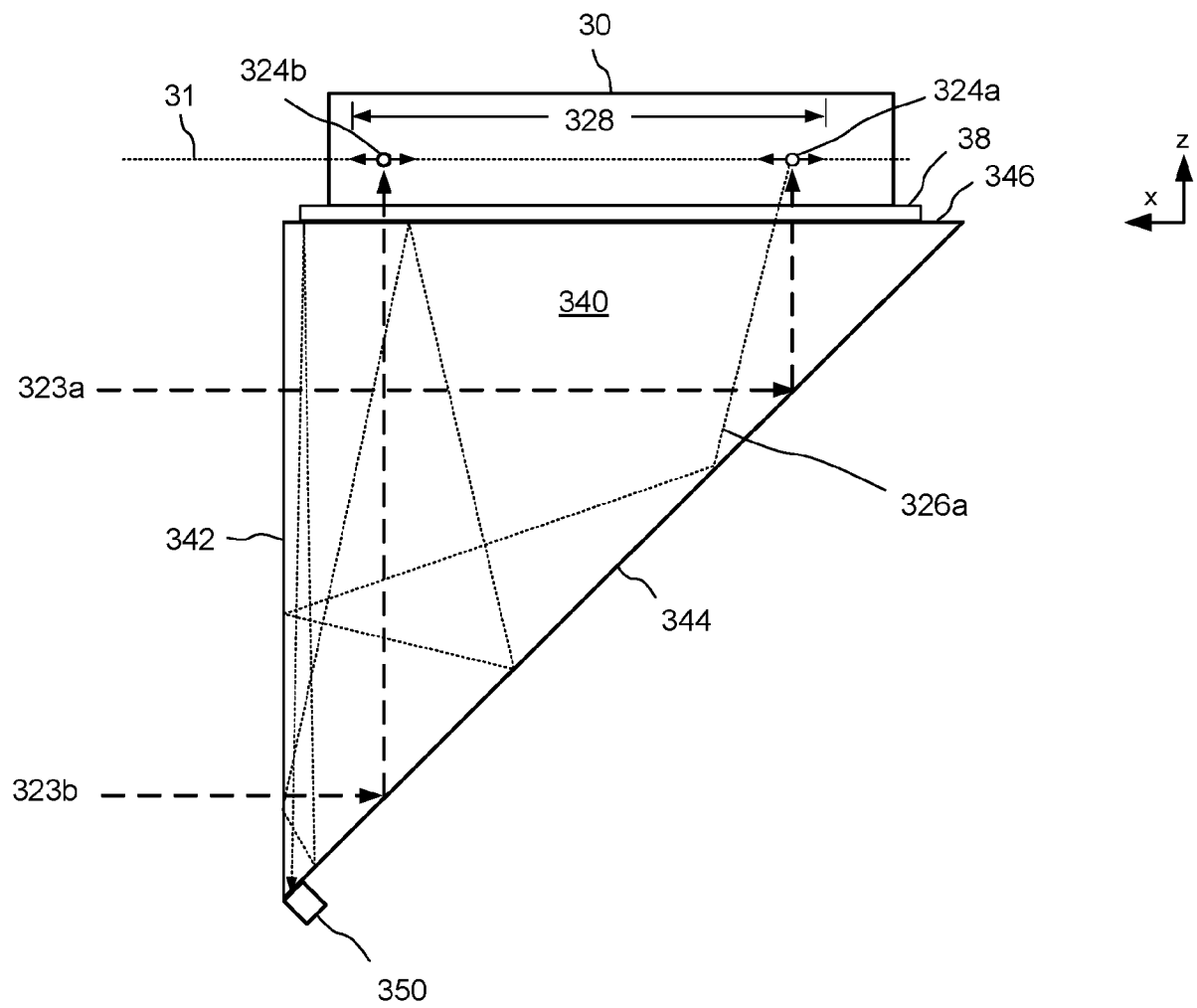
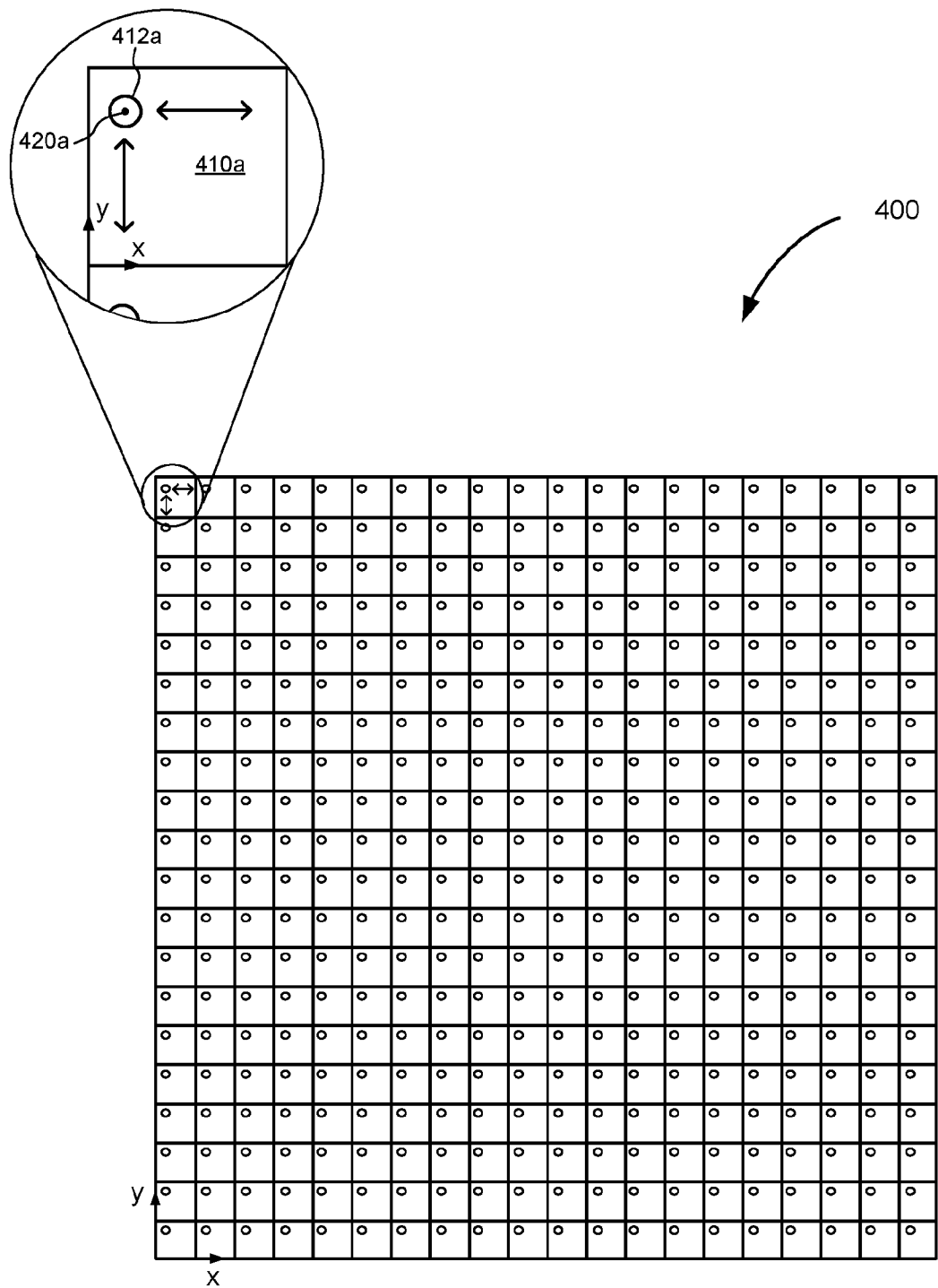


FIG. 2

**FIG. 3**

**FIG. 4**

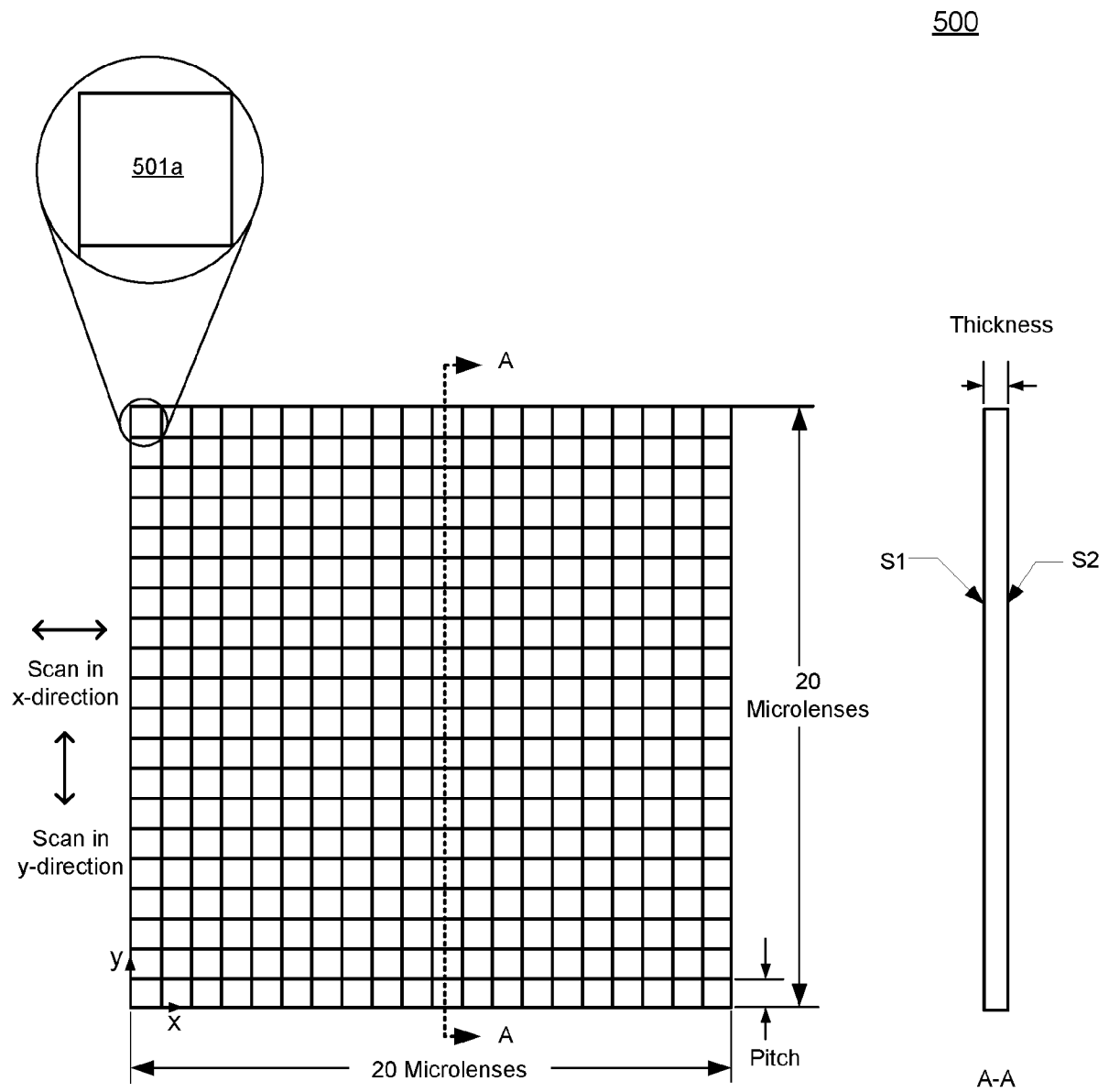
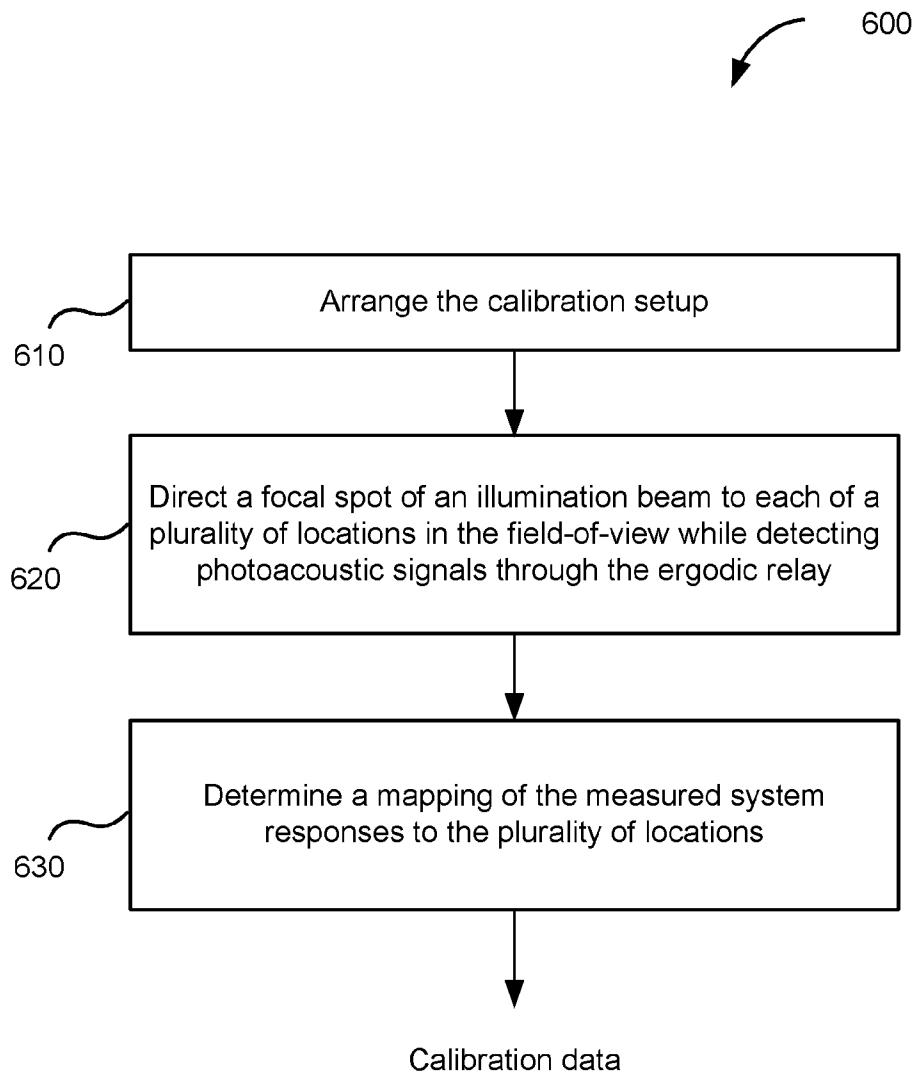
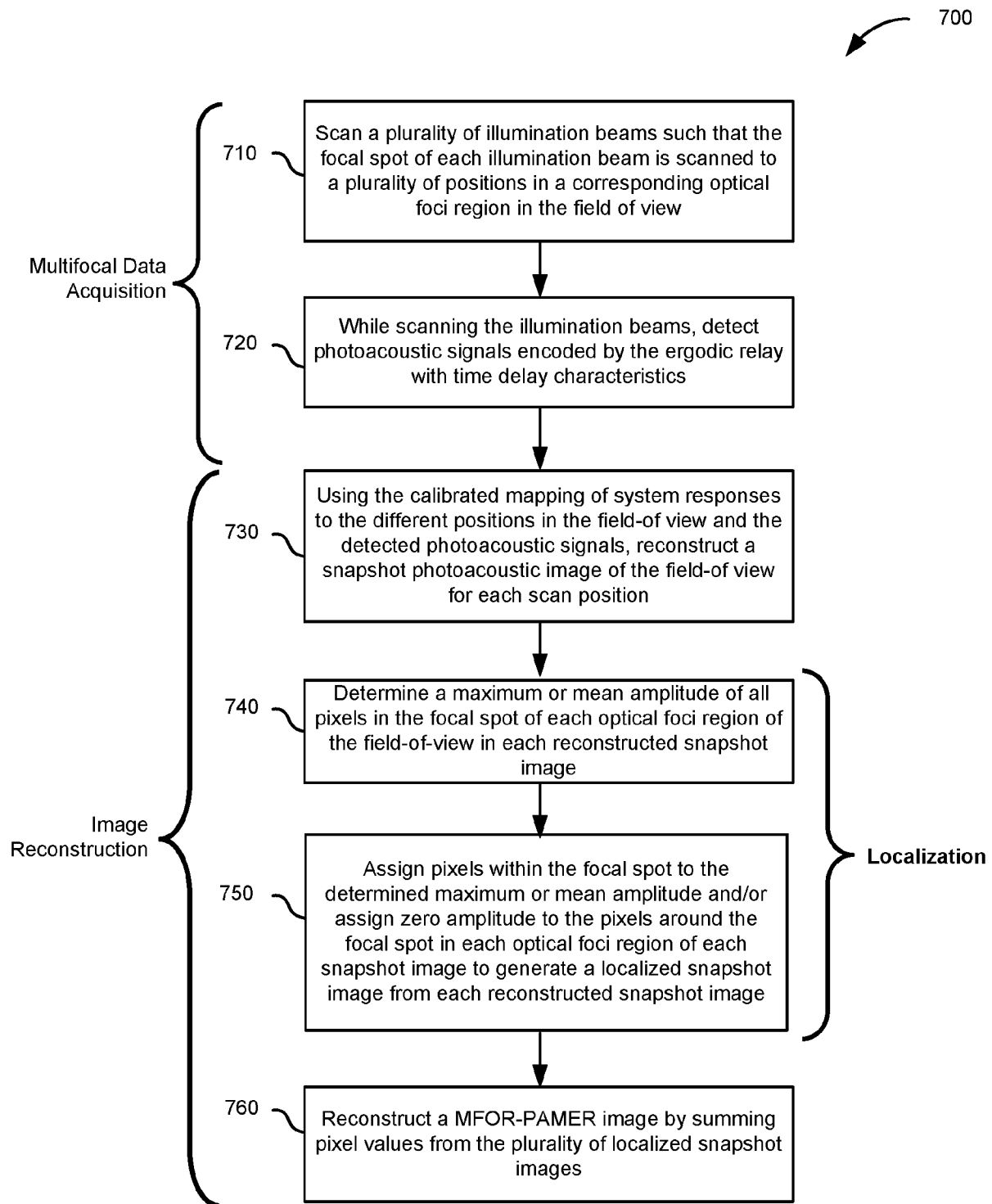
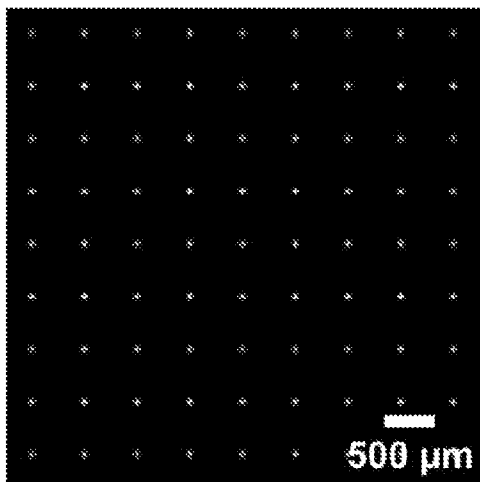
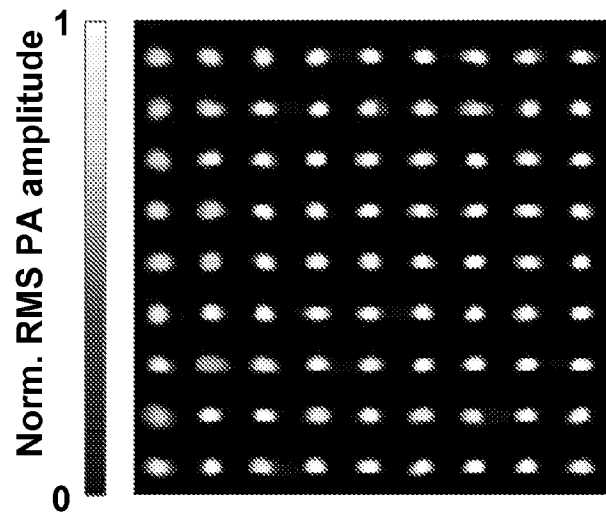
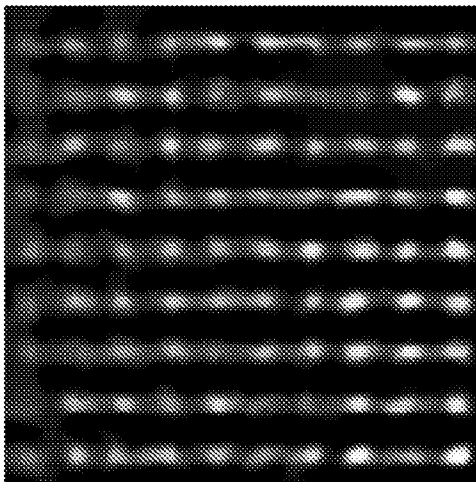
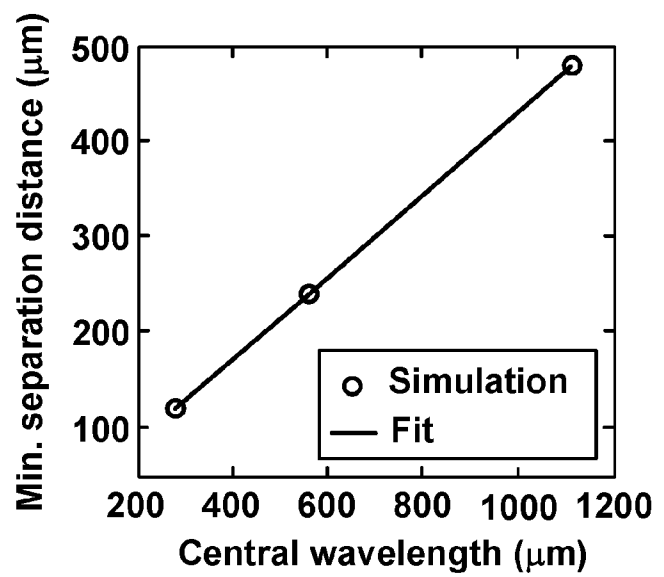


FIG. 5

**FIG. 6**

**FIG. 7**

**FIG. 8A****FIG. 8B****FIG. 8C****FIG. 8D**

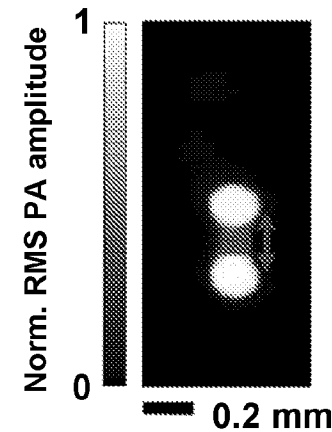


FIG. 9A

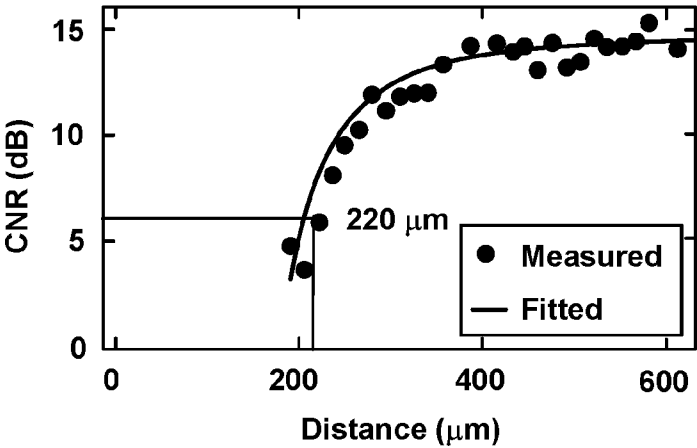


FIG. 9B

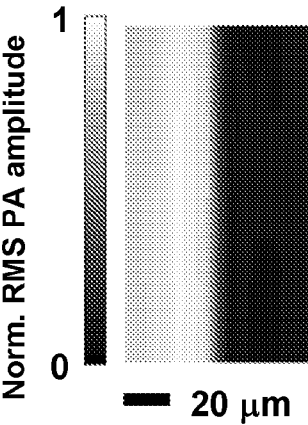


FIG. 9C

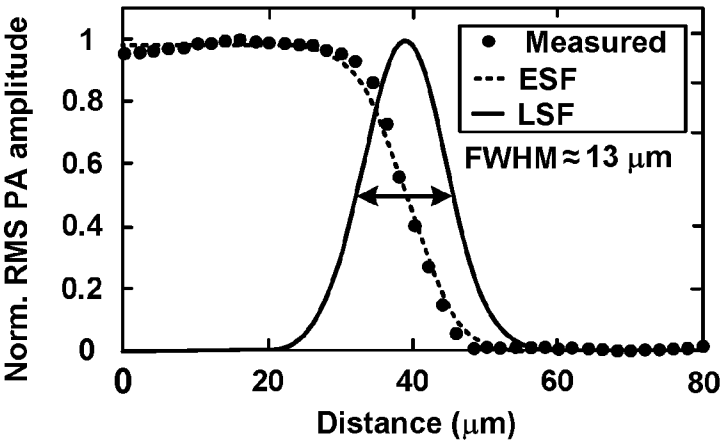
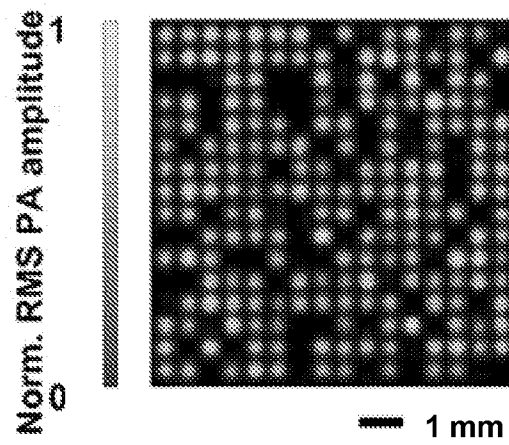
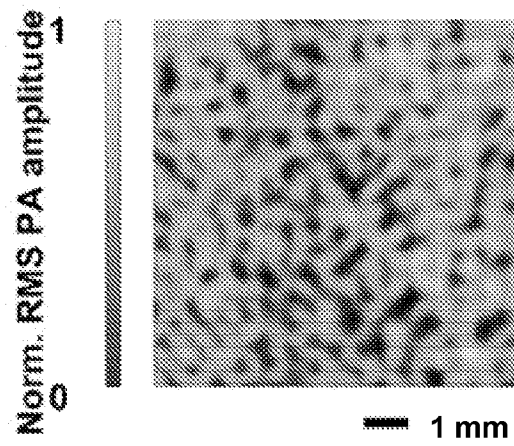
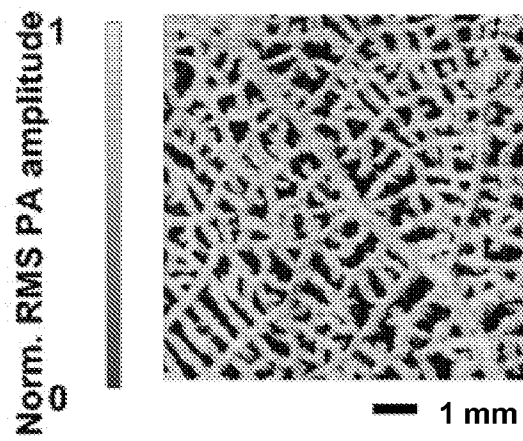


FIG. 9D

FIG. 10A**FIG. 10B****FIG. 10C**

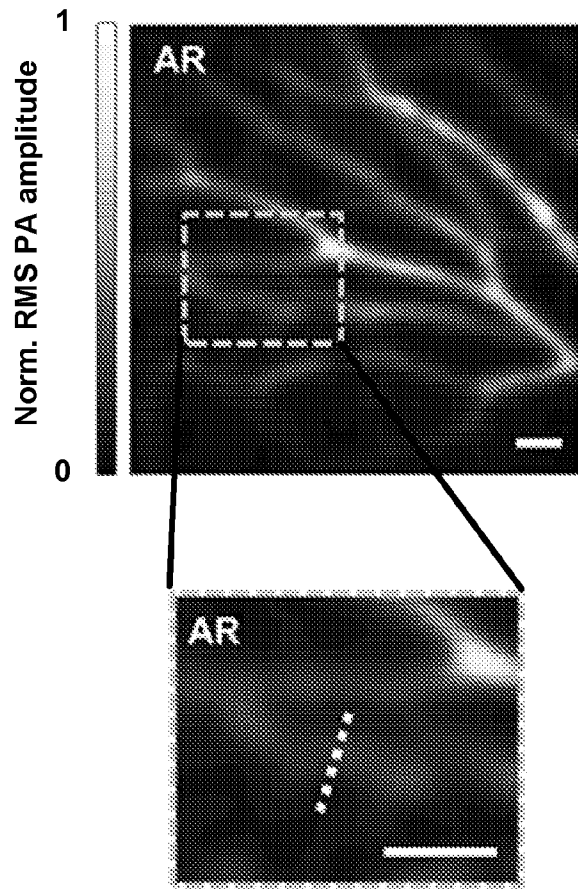


FIG. 11A

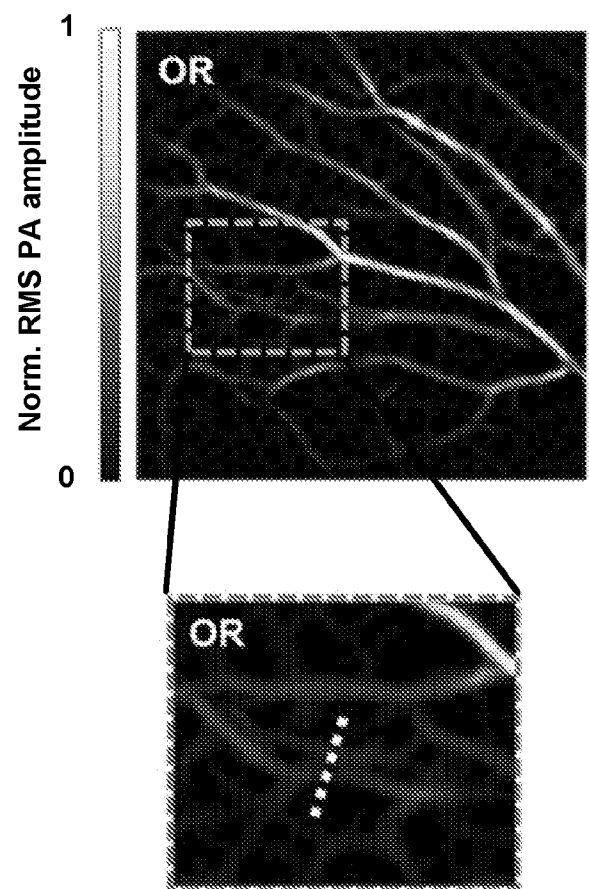
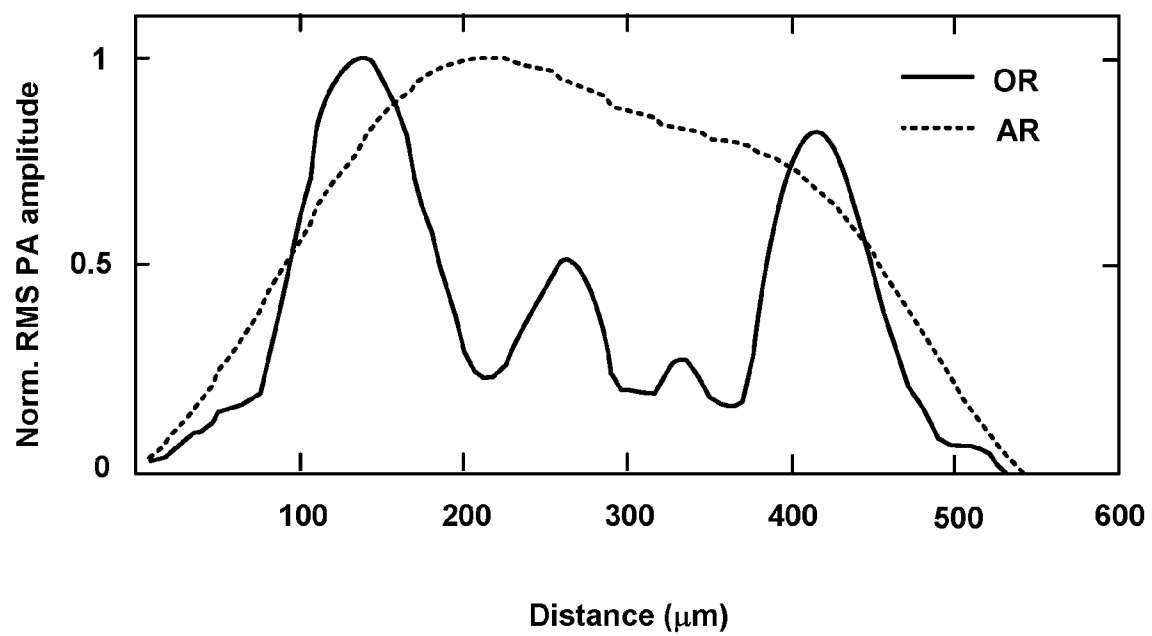


FIG. 11B

**FIG. 12**

A. CLASSIFICATION OF SUBJECT MATTER**A61B 5/00(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61B 5/00; A61B 5/145; A61B 8/00; A61B 8/13; G01N 29/24; G02F 1/29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: multifocal, photoacoustic, ultrasonic, transducer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIANG SONG et al., Multi-focal optical-resolution photoacoustic microscopy in vivo. NIH Public Access Author Manuscript, 13 May 2011. pages 1-7. See pages 2-3, 5; and figures 1, 3.	1-36
A	US 8997572 B2 (LIHONG WANG et al.) 07 April 2015 See column 5, line 59 - column 6, line 14; and figure 1.	1-36
A	JP 6086718 B2 (OLYMPUS CORP.) 01 March 2017 See paragraphs [0014]-[0027]; and figures 1-5.	1-36
A	JP 2016-101260 A (CANON INC.) 02 June 2016 See paragraphs [0017]-[0027]; and figure 1.	1-36
A	US 9618445 B2 (NATIONAL TAIWAN UNIVERSITY) 11 April 2017 See column 8, line 1 - column 9, line 60; and figures 3A-3B.	1-36



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

02 December 2019 (02.12.2019)

Date of mailing of the international search report

02 December 2019 (02.12.2019)

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2019/046574

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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JP 2016-101260 A	02/06/2016	JP 6587385 B2 US 2016-0150968 A1	09/10/2019 02/06/2016
US 9618445 B2	11/04/2017	US 2015-160120 A1	11/06/2015