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(71) Applicant: **CEDARS-SINAI MEDICAL CENTER** [US/US]; 8700 Beverly Boulevard, Los Angeles, CA 90048 (US).

(72) Inventors: **KORONYO, Maya**; 453 Alandele Avenue, Los Angeles, CA 90036 (US). **LEY, Eric**; 7018 Rindge Avenue, Playa Del Rey, CA 90293 (US). **KORONYO, Yosef**; 453 Alandele Avenue, Los Angeles, CA 90036 (US). **BLACK, Keith, L.**; 1233 Roberto Lane, Los Angeles, CA 90077 (US). **KATEB, Babak**; 11730 West Sunset #330, Brentwood, CA 90049 (US).

(74) Agents: **VAKHARIA-RAO, Hema** et al.; Nixon Peabody LLP, Gas Company Tower, 555 West Fifth Street, 46th Floor, Los Angeles, CA 90013 (US).

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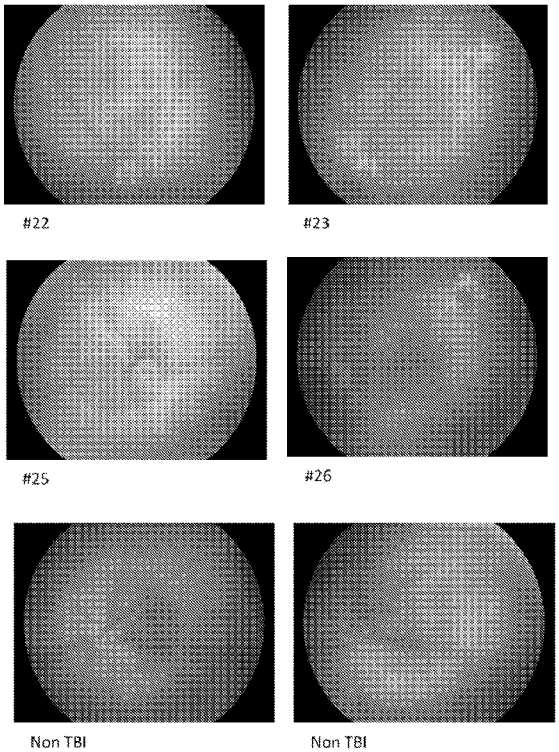
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(54) Title: DIAGNOSIS AND MONITORING OF TRAUMATIC BRAIN INJURY BY IMAGING RETINAL ABNORMALITIES

(57) Abstract: Described herein are methods for diagnosing and monitoring traumatic brain injury by imaging a subject retinal to determine the presence and/or extent of deviations from a normal retina.

FIG. 1



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DIAGNOSIS AND MONITORING OF TRAUMATIC BRAIN INJURY BY IMAGING RETINAL ABNORMALITIES

FIELD OF INVENTION

[0001] This invention relates to the diagnosis and monitoring of traumatic brain injury.

BACKGROUND

[0002] All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

[0003] Traumatic brain injury (TBI) is the most frequent and devastating damage resulting from the current wars in Iraq and Afghanistan. An estimate of 1.64 million U.S. troops have deployed to support Afghanistan and Iraq wars, in which 19.5 % (>300,000 U.S. veterans) have experienced a traumatic brain injury during deployment. Evidence shows a strong link between TBI and increased risk for Alzheimer's disease (AD). In one third of TBI cases, brain accumulation of the key pathological sign of AD, amyloid-β protein (Aβ) deposits (Aβ plaques), were found, and associated with a risk for AD. The presence of these plaques in the brain of the patients may also accelerate the onset of Alzheimer's symptoms in people who have already preexisting risk factors for developing AD. In spite of the extensive efforts to develop diagnosis and monitoring methods to follow individuals with TBI, there is no effective way to detect and monitor the outcome condition to assess prognosis and transformation to AD.

[0004] For individuals who suffer from TBI and develop signs of AD, the quality of life and economic costs are significant. A noninvasive diagnosis of Aβ plaque pathology and especially at early stage is essential for effective prevention and treatment, and presents an urgent unmet need. Since existing noninvasive brain imaging technologies cannot provide sufficient detail about micrometer-size changes, tissue evaluation is limited in both specificity and resolution.

[0005] Further, immediate diagnosis of traumatic brain injury is difficult due to the need for large imaging scans of the skull-shielded brain using computed tomography, PET or MRI. Also, existing imaging methods are severely limited by low resolution and specificity, and are less accessible and have high cost.

[0006] Accordingly there is an unmet need for noninvasive methods of diagnosing and monitoring TBI, as well as selecting a treatment for patients diagnosed with having TBI.

BRIEF DESCRIPTION OF THE FIGURES

[0007] Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[0008] Figure 1 depicts moderate to severe traumatic brain injury. Experiment #1 [right eye (R.E.) one day after injury]; n=4 mice, n=2 controls. Moderate to severe TBI (crush injury) was inflicted in the left hemisphere of the brain, in WT C57BL6 mice. Retinal abnormalities were detected via noninvasive retinal imaging especially in the right eyes.

[0009] Figure 2 depicts experiment #2 of a traumatic brain injury mouse #25. Moderate to severe TBI (crush injury) was inflicted in the left hemisphere of the brain, in WT C57BL6 mice. Longitudinal noninvasive retinal imaging reveals various retinal abnormalities (similar to those reported in TBI brains) that increased with time, especially in the opposite eye retinas (ex. Right) to the inflicted brain injury (left hemisphere) but also found in the retina in the same side where injury was inflicted in the brain.

[0010] Figure 3 depicts experiment #2 of a traumatic brain injury mouse #26. Moderate to severe TBI (crush injury) was inflicted in the left hemisphere of the brain, in WT C57BL6 mice. Longitudinal noninvasive retinal imaging reveals various retinal abnormalities (similar to those reported in the brain) especially in the right retinas and less in the left retinas.

[0011] Figure 4 depicts brain CT imaging for demonstration of the inflicted brain injury in the left hemisphere.

[0012] Figures 5A-D depicts another experiment of a traumatic brain injury, which further confirmed abnormalities in the retina following TBI. Moderate to severe TBI (crush injury) was inflicted in the left hemisphere of the brain, in WT C57BL6 mice. Longitudinal

noninvasive retinal imaging reveals various retinal abnormalities (similar to those reported in TBI brains) that increased with time, especially in the opposite eye retinas (ex. Right) to the inflicted brain injury (left hemisphere) but also found in the retina in the same side where injury was inflicted in the brain. (A) Mouse 1; (B) Mouse 2; (C) Mouse 5; (D) Mouse 6.

DESCRIPTION OF THE INVENTION

[0013] All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology 3rd ed., Revised*, J. Wiley & Sons (New York, NY 2006); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure 7th ed.*, J. Wiley & Sons (New York, NY 2013); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual 4th ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2012), provide one skilled in the art with a general guide to many of the terms used in the present application. For references on how to prepare antibodies, see D. Lane, *Antibodies: A Laboratory Manual 2nd ed.* (Cold Spring Harbor Press, Cold Spring Harbor NY, 2013); Kohler and Milstein, (1976) Eur. J. Immunol. 6: 511; Queen et al. U. S. Patent No. 5,585,089; and Riechmann et al., Nature 332: 323 (1988); U.S. Pat. No. 4,946,778; Bird, Science 242:423-42 (1988); Huston *et al.*, Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); Ward *et al.*, Nature 334:544-54 (1989); Tomlinson I. and Holliger P. (2000) Methods Enzymol, 326, 461-479; Holliger P. (2005) Nat. Biotechnol. Sep;23(9):1126-36).

[0014] One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. Indeed, the present invention is in no way limited to the methods and materials described.

[0015] The inventors' group previously identified amyloid-β protein (Aβ) plaques in postmortem and live human retina of AD patients, and possibly at early stages of the disease. Retinal plaque appears a faithful reflection of plaque pathology in the brain. The discovery of Aβ plaques in postmortem retinas of AD patients was demonstrated in a study by the inventors' group. Further development of a novel noninvasive retinal imaging approach by

the inventors' team to detect these plaques by curcumin labeling in live AD transgenic mouse models, allowed for the *in vivo* imaging of Aβ plaques at unprecedented high sensitivity and specificity.

[0016] Rapid identification of traumatic brain injury, follow up of long term changes after brain injury, or the effect of treatment or repeat mild traumatic brain injury can be possible by identifying the retinal hemorrhage, aggregates or neuronal cell death, and vascular changes observed as described herein.

[0017] The fundamental advantages of feasibility, cost and ease-of use of retinal imaging make it an attractive modality, as an early diagnostic tool for monitoring Aβ deposition pathology associated with TBI progression that may enable prevention and/or earlier effective treatment.

[0018] TBI patients exhibit Aβ-plaque neuropathology, which could be detected early in the retina using a state-of-the-art biomarker-specific retinal optical imaging technology developed by the inventors' group. The overexpression of APP and accumulation of Aβ deposits, similar to AD, are shown in posttraumatic brains. The retina as an extension of the brain shares many similarities with the brain, and thus can represent brain pathology.

[0019] For the first time the inventors have identified in mouse models subjected to traumatic brain injury (TBI) by noninvasive retinal imaging the following: 1. Retinal hemorrhages, in correlation to the injury site in the brain; 2. Possible degenerated retinal cells and/or aggregates; and 3. Vascular changes. Specifically, deviations from normal mouse eyes were observed in eyes of mice after TBI: white spots in abluminal/parenchyma (deposits and/or dead cells), hemorrhages in the peripheral arcades and expansion of the vessels next to the optic disc. Since these abnormalities can now be identified by imaging the retina, diagnosis of brain injury can be quickly and easily determined in soldiers, football players or any individuals subjected to brain injury.

[0020] Embodiments of the present invention provide for noninvasively imaging the retina to quickly determine if an injury is severe or minimal. Repeated brain injuries could be quantified by the changes observed in the retina.

[0021] Various embodiments of the present invention provide for a method of diagnosing traumatic brain injury in a subject in need thereof, comprising: imaging the subject's retina to determine the presence or absence of one or more deviations from a normal retina; and correlating the presence of the one or more deviations with the presence of traumatic brain injury, or correlating the absence of any deviations with the absence of traumatic brain injury.

[0022] In some embodiments, the method further comprises administering a fluorescent marker to the subject.

[0023] In some embodiments, the subject is suspected of having sustained a traumatic brain injury.

[0024] In some embodiments, imaging the subject's retina is performed with an optical imaging system (for example, fundus imaging). In some embodiments, imaging the subject's retina is performed using optical coherence tomorgraphy (OCT), including but not limited to three-dimensional OCT imaging or resolution, isotropic imaging. In additional embodiments, imaging the subject's retina is performed using a combination of an optical imaging system and OCT.

[0025] In some embodiments, the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal aggregates, vascular change, and combinations thereof. Particularly, the one or more deviations can be selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.

[0026] In particular embodiments, the one or more deviations comprise amyloid-β (Aβ) peptides. In these embodiments, the method can further comprise administering a fluorescent marker to the subject to stain the Aβ peptides.

[0027] In additional embodiments, the one or more deviations comprise tau protein accumulation. In these embodiments, the method can further comprise administering a marker (such as a fluorescent marker) to the subject to stain the tau protein.

[0028] In further embodiments, the one or more deviations comprise hemorrhaging of retinal blood vessels. In these embodiments, the method may further comprise administering indocyanine green (ICG) dye to the subject for imaging.

[0029] In additional embodiments, the one or more deviations comprise death of neurons in the retina. In these embodiments, the method can further detecting death of neurons using annexin V, for example, annexin v labeled with a fluorescent molecule.

[0030] In some embodiments, the deviations are any or more of amyloid-β, tau protein, hemorrhaging of retinal blood vessels, death of neurons in the retina or a combination thereof

[0031] In various embodiments, the one or more deviations that are evaluated for the presence, absence or extent in the retinal image are a combination of all of the above mentioned deviations.

[0032] Various embodiments provide for a method of monitoring traumatic brain injury in a subject in need thereof, comprising: imaging the subject's retina to identify one or more deviations from a normal retina; comparing the one or more deviations to a previously obtained retinal image of the subject; quantitating the increase or decrease of the one or more deviations; and determining a progression or improvement of the traumatic brain injury based on the comparison.

[0033] In some embodiments, the method further comprises administering a fluorescent marker to the subject.

[0034] In some embodiments, imaging the subject's retina is performed with an optical imaging system (for example, fundus imaging). In some embodiments, imaging the subject's retina is performed using optical coherence tomorgraphy (OCT), including but not limited to three-dimensional OCT imaging or resolution, isotropic imaging. In additional embodiments, imaging the subject's retina is performed using a combination of an optical imaging system and OCT.

[0035] In some embodiments, the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal aggregates, vascular change, and combinations thereof. In some particular embodiments, the one or more deviations is selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.

[0036] In particular embodiments, the one or more deviations comprise amyloid-β (Aβ) peptides. In these embodiments, the method can further comprise administering a fluorescent marker to the subject to stain the Aβ peptides.

[0037] In additional embodiments, the one or more deviations comprise tau protein accumulation. In these embodiments, the method can further comprise administering a marker (such as a fluorescent marker) to the subject to stain the tau protein.

[0038] In further embodiments, the one or more deviations comprise hemorrhaging of retinal blood vessels. In these embodiments, the method may further comprise administering indocyanine green (ICG) dye to the subject for imaging.

[0039] In additional embodiments, the one or more deviations comprise death of neurons in the retina. In these embodiments, the method can further detecting death of neurons using annexin V, for example, annexin v labeled with a fluorescent molecule.

[0040] In some embodiments, the deviations are any or more of amyloid-β, tau protein, hemorrhaging of retinal blood vessels, death of neurons in the retina or a combination thereof.

[0041] In various embodiments, the one or more deviations that are evaluated for the presence, absence or extent in the retinal image are a combination of all of the above mentioned deviations.

[0042] Various embodiments of the present invention provide for a method of selecting treatment for a subject suspected of having a traumatic brain injury, comprising: imaging the subject's retina to determine the presence or absence of one or more deviations from a normal retina; correlating the presence of the one or more deviations with the presence of traumatic brain injury, or correlating the absence of any deviations with the absence of traumatic brain injury; and selecting a traumatic brain injury treatment if the presence of traumatic brain injury is determined. In various embodiments, the method further comprises administering the selected treatment.

[0043] The traumatic brain injury treatment can include agents that reduce Aβ levels in the CNS. Other agents such as those that control Aβ toxicity are beneficial to reduce the detrimental outcomes of TBI. Such treatment approaches include immune-modulation

interventions, which can provide beneficial effects in removal of toxic compounds in the brain and retina, in controlling local inflammation, and in protecting neurons.

[0044] Other conventional treatments can also be selected. For example, in cases where the TBI injury is mild, rest and over-the-counter pain relievers to treat a headache can be selected, along with monitoring the person closely for any persistent, worsening or new symptoms.

[0045] In cases of moderate to severe traumatic brain injuries, the treatment can comprise ensuring that the person has an adequate oxygen and blood supply, maintaining blood pressure, and preventing any further injury to the head or neck.

[0046] Additional treatments may be to minimize secondary damage due to inflammation, bleeding or reduced oxygen supply to the brain. Medications that can be used to limit secondary damage to the brain can include: diuretics to help reduce pressure inside the brain; anti-seizure medications; and coma-inducing medications.

[0047] Further, surgeries can be selected to minimize additional damage to brain tissues. Surgeries for removing hematomas and be selected and performed. Surgeries for opening a window in the skull to relieve pressure inside the skull can also be selected and performed.

[0048] In some embodiments, the method further comprises administering a fluorescent marker to the subject.

[0049] In some embodiments, imaging the subject's retina is performed with an optical imaging system.

[0050] In some embodiments, the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal aggregates, vascular change, and combinations thereof. In some particular embodiments, the one or more deviations is selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.

[0051] In particular embodiments, the one or more deviations comprise amyloid-β (Aβ) peptides. In these embodiments, the method can further comprise administering a fluorescent marker to the subject to stain the Aβ peptides.

[0052] In various embodiments, the one or more deviations that are evaluated for the presence, absence or extent in the retinal image are a combination of all of the above mentioned deviations.

[0053] Various embodiments provide for a method of evaluating the effectiveness of a traumatic brain injury treatment in a subject in need thereof, comprising: imaging the subject's retina to identify one or more deviations from a normal retina; comparing the one or more deviations to a previously obtained retinal image of the subject; quantitating an increase or decrease of the one or more deviations; and evaluating the effectiveness of the traumatic brain injury treatment based on the level of the increase or decrease of the one or more deviations.

[0054] In some embodiments, the method further comprises administering a fluorescent marker to the subject.

[0055] In some embodiments, imaging the subject's retina is performed with an optical imaging system.

[0056] In some embodiments, the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal aggregates, vascular change, and combinations thereof. In some particular embodiments, the one or more deviations is selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.

[0057] In particular embodiments, the one or more deviations comprise amyloid-β (Aβ) peptides. In these embodiments, the method can further comprise administering a fluorescent marker to the subject to stain the Aβ peptides.

[0058] *Labeling compounds*

[0059] The fluorescent marker used in the methods of the present invention can be selected from the group consisting of curcumin, curcumin derivative, Thioflavin S, Thioflavin S derivative, Thioflavin T, Thioflavin T derivative, Congo Red, Congo Red derivative, methoxy-X04, Pittsburgh Compound-B (PiB), DDNP, Chrysamine-G, and combinations thereof.

[0060] Compounds are known in the art that can stain/label *in vivo* amyloid plaques, including, Thioflavin S and T and some derivatives, Congo Red and derivatives, methoxy-X04, Pittsburgh Compound-B (PiB), DDNP, Chrysamine-G and several more. However, curcumin and its derivatives are very appealing for in vivo optical imaging of amyloid plaques in animal models as well as humans, because of the following advantage. Curcumin generates specific and very bright signals in the commonly used optical spectrum, and is commercially available, exceptionally low cost. Safety issues related to curcumin are minimal (even at high dosages) and may even be considered beneficial to the patient's health as an antioxidant. Curcumin is an effective ligand with very good *in vitro* and *in vivo* binding characteristics to Aβ plaques and offers good initial brain uptake and washout rate from the brain (important properties for in vivo imaging agents).

[0061] Compounds are known in the art that can stain/label tau protein include but are not limited to phenyl/pyridinylbutadienly-benzothiazoles/benzothiazolimus (PBBs), 18F-T807, 18F-THK523 or combinations thereof.

[0062] Fluorescent molecules which are suitable for use according to the invention further include: cyamine dyes, including but not limited to Cy2, Cy3, Cy3.5, CY5, Cy5.5, Cy7 and FLUORX; BODIPY dyes including but not limited to BODIPY-FL, BODIPY-TR, BODIPY-TMR, BODIPY-630/650, and BODIPY-650/670; and ALEXA dyes, including but not limited to ALEXA-488, ALEXA-532, ALEXA-546, ALEXA-568, and ALEXA-594; as well as other fluorescent dyes which will be known to those who are skilled in the art. Electron rich indicator molecules suitable for the present invention include, but are not limited to, ferritin, hemocyanin, and colloidal gold.

[0063] *Imaging*

[0064] Spectral imaging provides digital images of an object at a large, sequential number of wavelengths generating precise optical signatures at every pixel. The fluorescence spectral signature of Aβ plaques, labeled in vivo with curcumin, was captured by our spectral imaging system using the following equipment: Nikon fluorescence microscopes (E800 and TE2000), mercury and xenon arc lamps, a CCD camera, an AOTF (acousto-optic tunable filters)-based spectral image acquisition system (ChromoDynamics, Inc) and post-analysis imaging software developed by our Minimally Invasive Surgical Technologies Institute. The final

images provided a visual pseudo-color representation of the spectral signature extracted from the raw images, representing the size and location of the analyzed objects. In multispectral imaging, fluorescence lifetime imaging, performed with a pulsed laser and a LaVision PicoStar HR gated camera, was supplementing the spectral acquisition.

[0065] The optical imaging system used in the methods of the present invention can be is selected from the group consisting of a spectrometer, a fluorescence microscope, a stereomicroscope, a mercury arc lamp, a variable wavelength light source, a xenon arc lamp, a CCD gated camera, a color digital camera, an acoustic-optic tunable filter–based spectral image acquisition system, adaptive optics, imaging software, and combinations thereof.

EXAMPLES

[0066] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

[0067] *Example 1*

[0068] First Retinal Imaging was conducted after TBI was inflicted in mouse models. Movies and images were selected and processed, and results were analyzed.

[0069] Retinal changes were seen. Deviations from normal mouse eyes were observed: white spots in abluminal/parenchyma (deposits and/or dead cells), hemorrhages in the peripheral arcades and expansion of the vessels next to the optic disc.

[0070] A second Retinal Imaging experiment was conducted after TBI was inflicted in mouse models. Movies and images were again selected and processed, and the results were analyzed. Again, retinal changes were seen and deviations from normal mouse eyes were observed.

[0071] *Example 2*

[0072] *Intravenous injections of curcumin for noninvasive in vivo retinal plaque imaging*

[0073] Retinas of live AD-Tg and wt mice are imaged following systemic administration of curcumin (7.5 mg/kg/day), for consecutive days or following a single i.v. injection 2 h prior to imaging. Mice are anesthetized with 70 mg/kg ketamine and 30 mg/kg xylazine. Mouse pupils are dilated to about 2 mm in diameter with 0.5% phenylephrine hydrochloride ophthalmic solution (Bausch & Lomb, Rochester, NY) combined with 0.5% tropicamide ophthalmic solution (Mydral, Bausch&Lomb). Mouse eyes are covered with a drop of Gonak (Hypromellose ophthalmic demulcent solution, 2.5%; Akorn, Lake Forest, IL), which served as an optical coupling medium, and the retinas are imaged in vivo using the Micron II retinal imaging microscope (Phoenix Research Laboratories, San Ramon, CA). TheMicron II is a retinal imaging microscope for rodents adjusted to visualize fluorescence signals at high resolution and is equipped with a 3-CCD camera (1000 to 1 dynamic range and 30 frames per second output at XGA resolution), and specific set of filters suitable to detect curcumin fluorescence (for the Zeiss Axio Imager Z1). Images are repeatedly captured at several angles of the retina in order to visualize a larger field and eliminate non-specific reflection signals. Care was taken to keep anesthetized animals warm throughout the procedure.

[0074] *Example 3*

[0075] *Microscopy*

[0076] Fluorescence and bright-field images are acquired using a Carl Zeiss Axio Imager Z1 fluorescence microscope equipped with ApoTome (Carl Zeiss MicroImaging, Inc.), and a Leica TCS SP5 double-spectral confocal microscope, using the same setting and exposure times for each experiment. For processing and analysis of the images, the AxioVision (Rel. 4.6.3) software (Carl Zeiss) is used. Fluorescence in the Zeiss Axio Imager Z1 is imaged using filter sets for excitation and emission at 365/50 and 445/50 nm for DAPI, 470/40 and 525/50 nm for ThioS, 550/25 and 605/70 nm for curcumin, and 640/30 and 690/50 nm for Cy5. In presented images, curcumin is assigned a green pseudocolor to differentiate from red Cy5.

[0077] *Example 4*

[0078] *Spectral imaging analyses*

[0079] Spectral imaging of mouse retinal tissues provides digital images of an object at a large, sequential number of wavelengths and generated precise optical signatures at every pixel. The fluorescence spectral signature of Aβ plaques labeled in vivo with curcumin is captured by our spectral imaging system using the following equipment: Nikon fluorescence microscopes (E800 and TE2000) with mercury and xenon arc lamps, a CCD camera, and an AOTF (acousto-optic tunable filters)-based spectral image acquisition system (~4 μm spectral resolution, commercialized by ChromoDynamics, Inc., Orlando, FL) (Wachman et al., 1997). Image acquisition is followed by image segmentation and classification using software that we previously developed (Burton et al., 2009). The final images provide a visual pseudocolor representation of the spectral signature extracted from the raw images, representing the size and location of the analyzed objects. Spectral analysis of individual Aβ plaques (regions of interest, ROI) as compared to background signals in human retinal tissues (single labeled with curcumin, single labeled with Cy5-conjugated Abs, or double labeled with both) is performed in quadruplicates using a Leica TCS SP5 double-spectral (excitation and emission) confocal microscope, with the same setting and exposure times for each ROI. Various excitation wavelengths and resulting emission spectra (at 5 nm intervals) are recorded, to determine the optimal wavelengths to excite and capture the fluorescence signal. GraphPad Prism 5 for Mac OS X version 5.0b is used to smooth curves by applying the fit spline/lowess method.

[0080] *Example 5*

[0081] *Noninvasive imaging of curcumin-labeled retinal plaques in live AD mice*

[0082] Following systemic administration of curcumin, in vivo imaging of the retina is performed in live mice utilizing Micron II rodent retinal imaging microscope. This in vivo optical imaging modality enabled the ability to identify individual plaques or plaque clusters at high resolution.

[0083] Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is

understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

[0084] The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

[0085] While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (*e.g.*, the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.).

WHAT IS CLAIMED IS:

1. A method of diagnosing traumatic brain injury in a subject in need thereof, comprising:
   imaging the subject's retina to determine the presence or absence of one or more deviations from a normal retina; and
   correlating the presence of the one or more deviations with the presence of traumatic brain injury, or
   correlating the absence of any deviations with the absence of traumatic brain injury.
2. The method of claim 1, further comprising administering a fluorescent marker to the subject.
3. The method of claim 1, wherein the subject is suspected of having sustained a traumatic brain injury.
4. The method of claim 1, wherein imaging the subject's retina is performed with an optical imaging system.
5. The method of claim 4, wherein the optical imaging system is selected from the group consisting of a spectrometer, a fluorescence microscope, a stereomicroscope, a mercury arc lamp, a variable wavelength light source, a xenon arc lamp, a CCD gated camera, a color digital camera, an acoustic-optic tunable filter–based spectral image acquisition system, adaptive optics, imaging software, and combinations thereof.
6. The method of claim 1, wherein the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal aggregates, vascular change, and combinations thereof.
7. The method of claim 1, wherein the one or more deviations is selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.
8. The method of claim 1, wherein the one or more deviations comprise amyloid-β (Aβ) peptides.
9. The method of claim 8, further comprising administering a fluorescent marker to the subject to stain the Aβ peptides.
10. The method of claims 2 or 9, wherein the fluorescent marker is selected from the group consisting of curcumin, curcumin derivative, Thioflavin S, Thioflavin S derivative, Thioflavin T, Thioflavin T derivative, Congo Red, Congo Red derivative,

methoxy-X04, Pittsburgh Compound-B (PiB), DDNP, Chrysamine-G, and combinations thereof.

11. A method of monitoring traumatic brain injury in a subject in need thereof, comprising:

imaging the subject's retina to identify one or more deviations from a normal retina;

comparing the one or more deviations to a previously obtained retinal image of the subject;

quantitating the increase or decrease of the one or more deviations; and

determining an progression or improvement of the traumatic brain injury based on the comparison.

12. The method of claim 11, further comprising administering a fluorescent marker to the subject.

13. The method of claim 11, wherein imaging the subject's retina is performed with an optical imaging system.

14. The method of claim 13, wherein the optical imaging system is selected from the group consisting of a spectrometer, a fluorescence microscope, a stereomicroscope, a mercury arc lamp, a variable wavelength light source, a xenon arc lamp, a CCD gated camera, a color digital camera, an acoustic-optic tunable filter–based spectral image acquisition system, adaptive optics, imaging software, and combinations thereof.

15. The method of claim 11, wherein the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal agreegates, vascular change, and combinations thereof.

16. The method of claim 11, wherein the one or more deviations is selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.

17. The method of claim 11, wherein the one or more deviations comprise amyloid-β (Aβ) peptides.

18. The method of claim 17, further comprising administering a fluorescent marker to the subject to stain the Aβ peptides.

19. The method of claims 12 or 18, wherein the fluorescent marker is selected from the group consisting of curcumin, curcumin derivative, Thioflavin S, Thioflavin S

derivative, Thioflavin T, Thioflavin T derivative, Congo Red, Congo Red derivative, methoxy-X04, Pittsburgh Compound-B (PiB), DDNP, Chrysamine-G, and combinations thereof.

20. A method of selecting treatment for a subject suspected of having a traumatic brain injury, comprising:

imaging the subject's retina to determine the presence or absence of one or more deviations from a normal retina;

correlating the presence of the one or more deviations with the presence of traumatic brain injury, or

correlating the absence of any deviations with the absence of traumatic brain injury; and

selecting a traumatic brain injury treatment if the presence of traumatic brain injury is determined.

21. The method of claim 20, further comprising administering a fluorescent marker to the subject.

22. The method of claim 20, wherein imaging the subject's retina is performed with an optical imaging system.

23. The method of claim 22, wherein the optical imaging system is selected from the group consisting of a spectrometer, a fluorescence microscope, a stereomicroscope, a mercury arc lamp, a variable wavelength light source, a xenon arc lamp, a CCD gated camera, a color digital camera, an acoustic-optic tunable filter–based spectral image acquisition system, adaptive optics, imaging software, and combinations thereof.

24. The method of claim 20, wherein the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal aggregates, vascular change, and combinations thereof.

25. The method of claim 20, wherein the one or more deviations is selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.

26. The method of claim 20, wherein the one or more deviations comprise amyloid-β (Aβ) peptides.

27. The method of claim 26, further comprising administering a fluorescent marker to the subject to stain the Aβ peptides.

28. The method of claims 21 or 27, wherein the fluorescent marker is selected from the group consisting of curcumin, curcumin derivative, Thioflavin S, Thioflavin S derivative, Thioflavin T, Thioflavin T derivative, Congo Red, Congo Red derivative, methoxy-X04, Pittsburgh Compound-B (PiB), DDNP, Chrysamine-G, and combinations thereof.

29. A method of evaluating the effectiveness of a traumatic brain injury treatment in a subject in need thereof, comprising:

imaging the subject's retina to identify one or more deviations from a normal retina;

comparing the one or more deviations to a previously obtained retinal image of the subject;

quantitating an increase or decrease of the one or more deviations; and

evaluating the effectiveness of the traumatic brain injury treatment based on the level of the increase or decrease of the one or more deviations.

30. The method of claim 29, further comprising administering a fluorescent marker to the subject.

31. The method of claim 29, wherein imaging the subject's retina is performed with an optical imaging system.

32. The method of claim 31, wherein the optical imaging system is selected from the group consisting of a spectrometer, a fluorescence microscope, a stereomicroscope, a mercury arc lamp, a variable wavelength light source, a xenon arc lamp, a CCD gated camera, a color digital camera, an acoustic-optic tunable filter–based spectral image acquisition system, adaptive optics, imaging software, and combinations thereof.

33. The method of claim 29, wherein the one or more deviations is selected from the group consisting of retinal hemorrhage, degenerated retinal cells, degenerated retinal aggregates, vascular change, and combinations thereof.

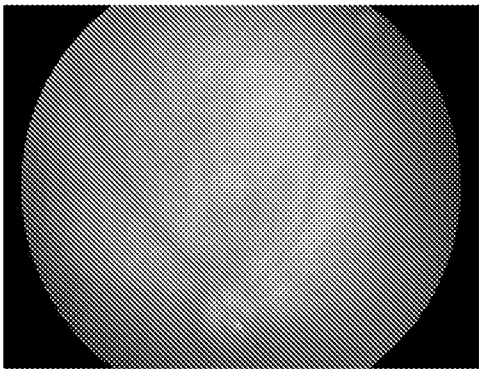
34. The method of claim 29, wherein the one or more deviations is selected from the group consisting of white spot in abluminal or parenchyma, hemorrhage in the peripheral arcade, expansion of vessels next to the optic disc, and combinations thereof.

35. The method of claim 29, wherein the one or more deviations comprise amyloid-β (Aβ) peptides.

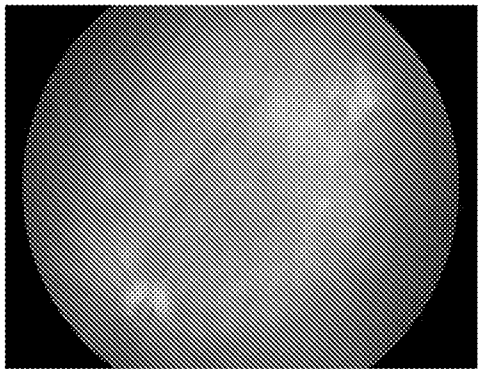
36. The method of claim 35, further comprising administering a fluorescent marker to the subject to stain the Aβ peptides.

37. The method of claims 30 or 36, wherein the fluorescent marker is selected from the group consisting of curcumin, curcumin derivatives, Thioflavin S and derivatives, Thioflavin T and derivatives, Congo Red and derivatives, methoxy-X04, Pittsburgh Compound-B (PiB), DDNP, Chrysamine-G, and combinations thereof.

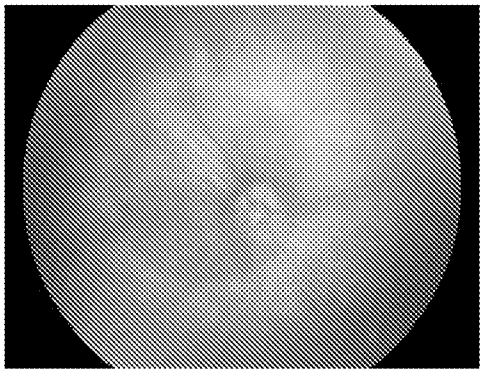
FIG. 1



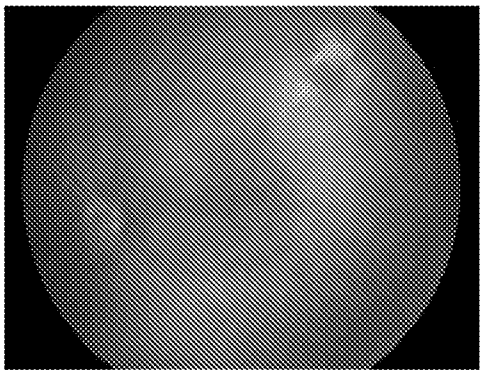
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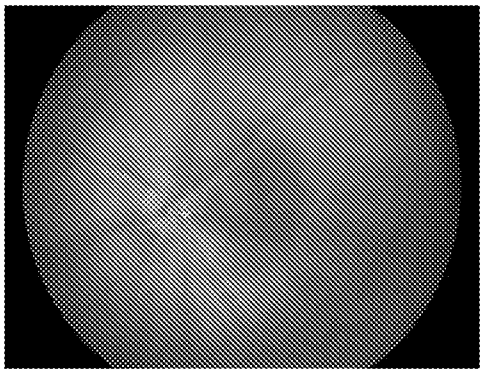
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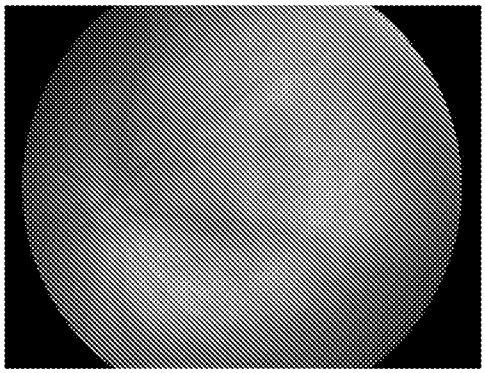
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#26

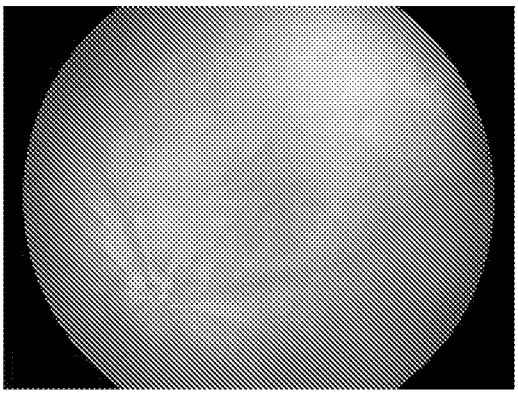


Non TBI

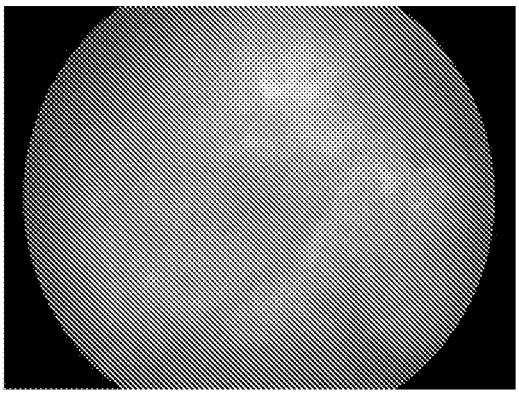


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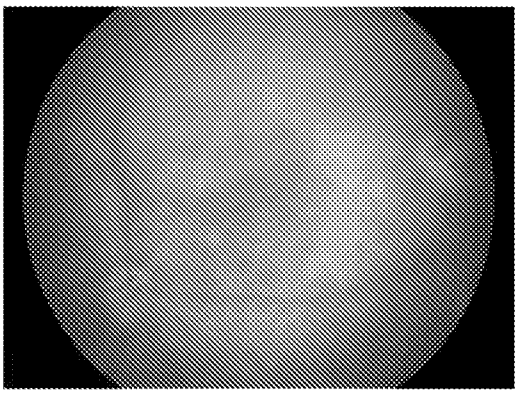
FIG. 2



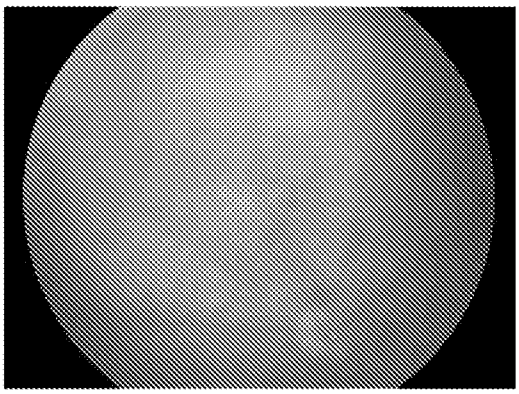
#5 RE



#7 RE

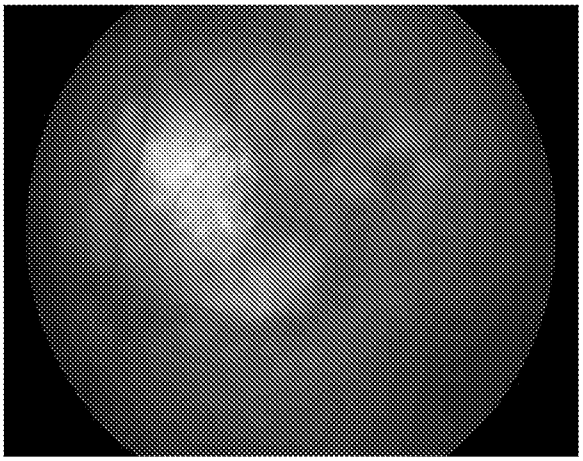


#12 RE

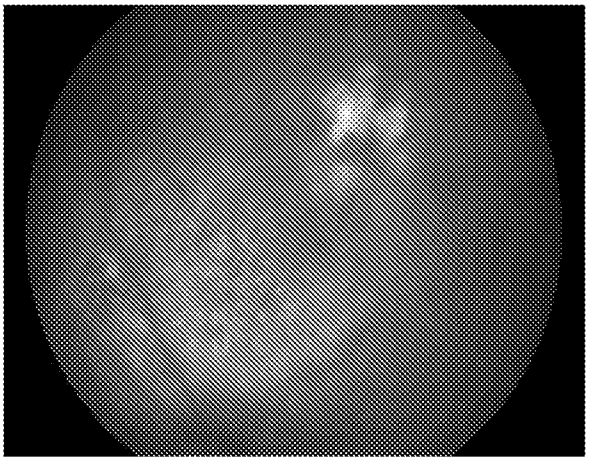


#12 LE

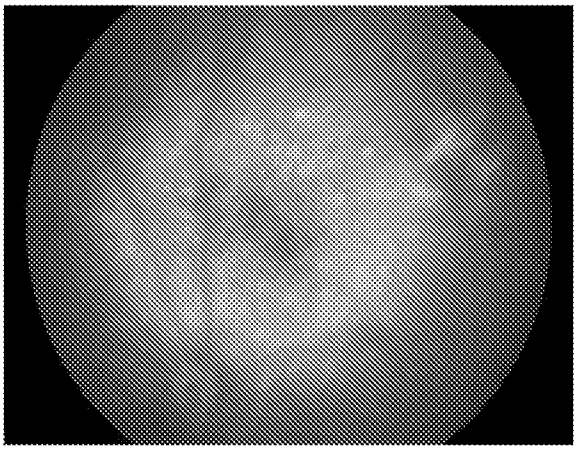
FIG. 3



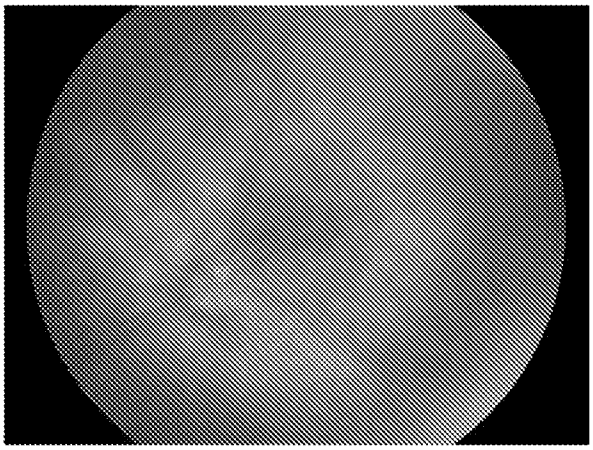
#5 RE



#7 RE



#12 RE



#12 LE

FIG. 4

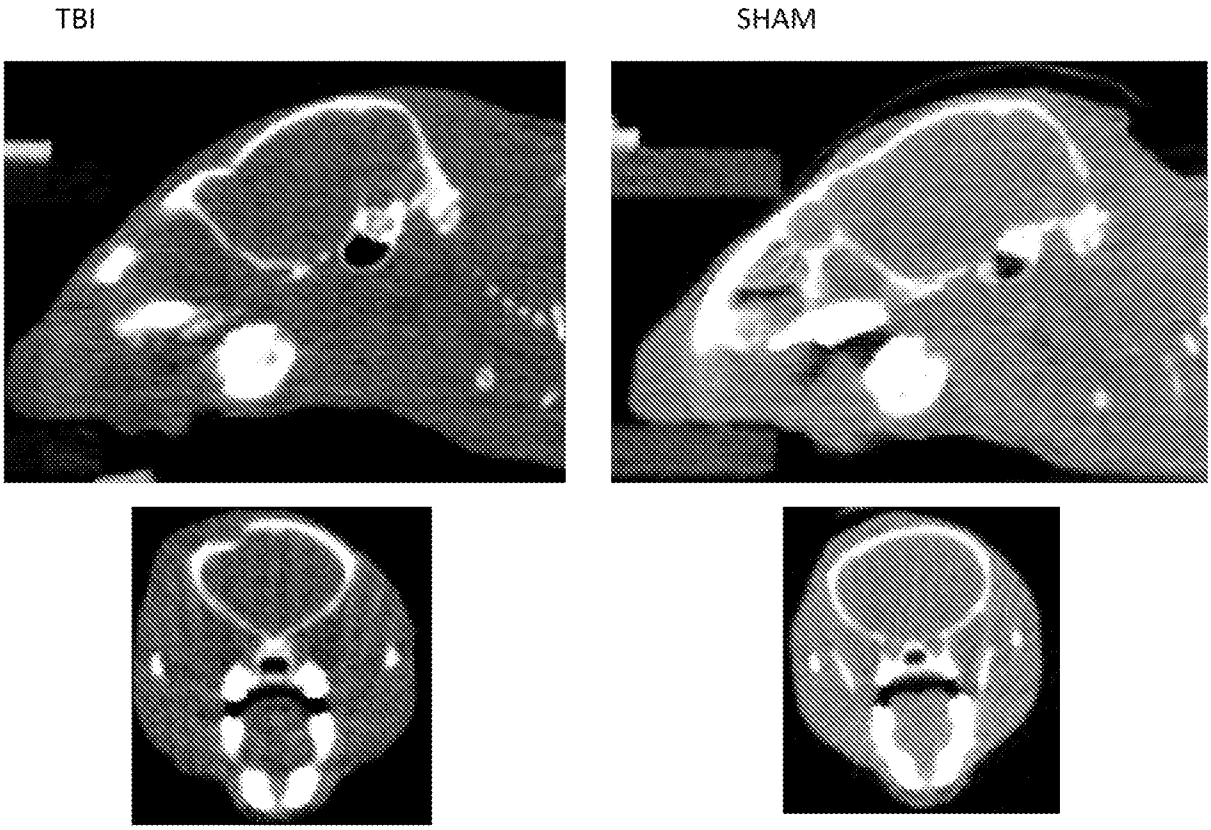
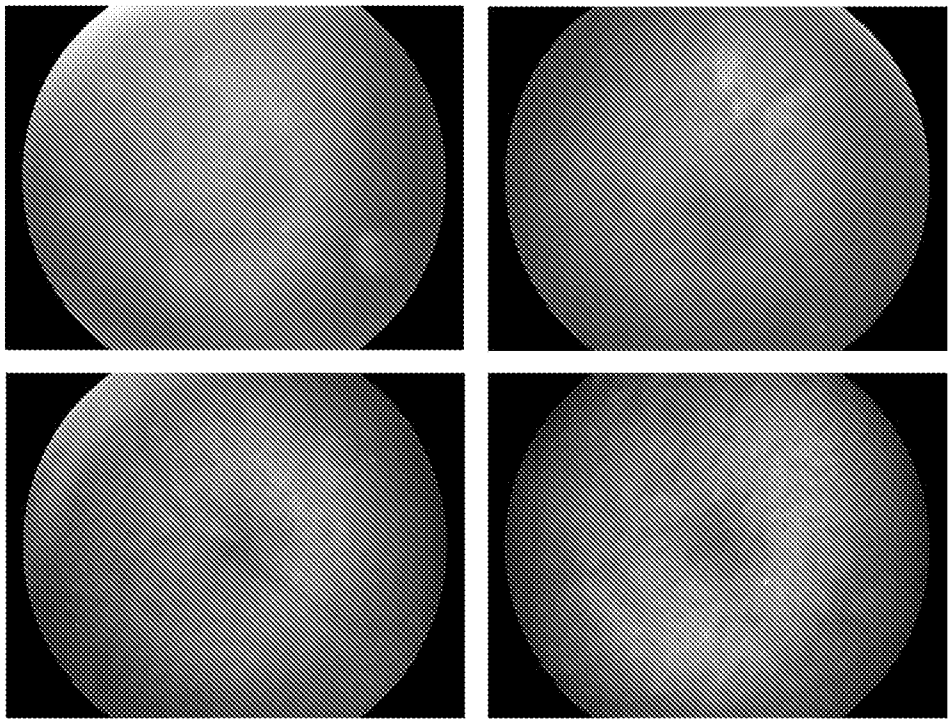
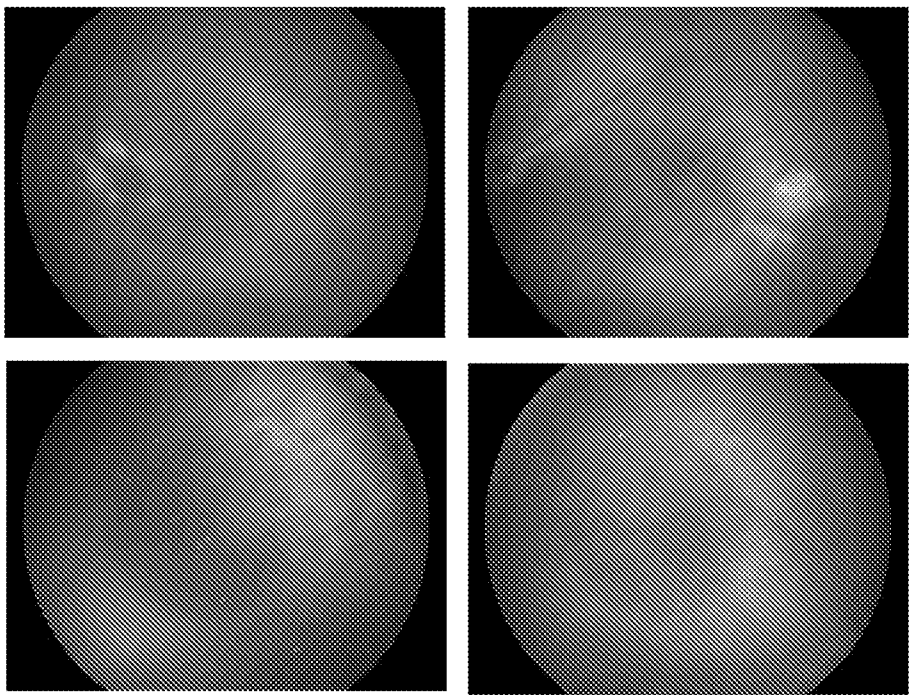


FIG. 5A



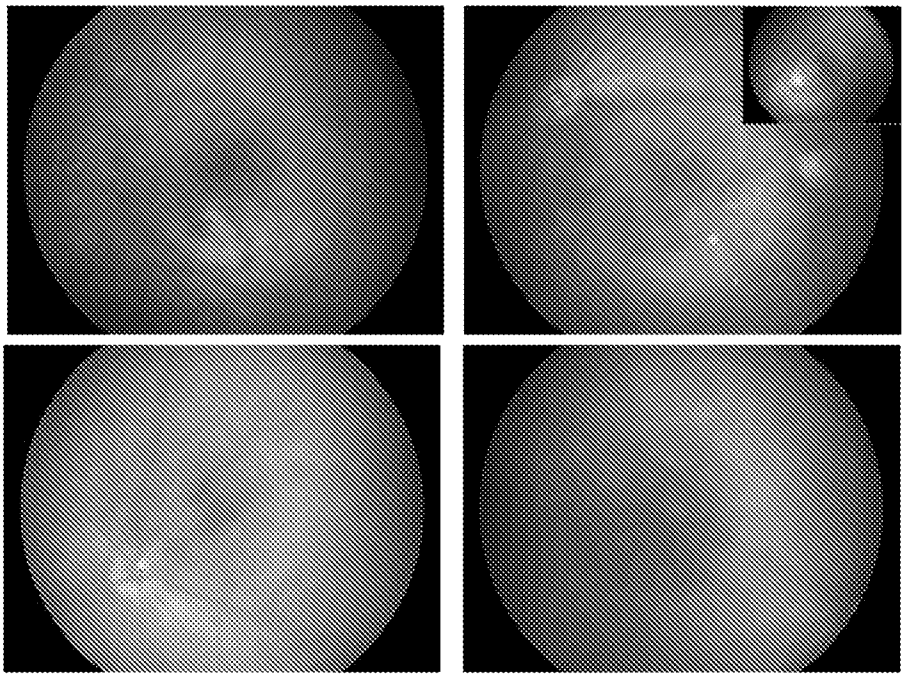
Mouse #1 Left Eye Mouse #1 Right Eye

FIG. 5B



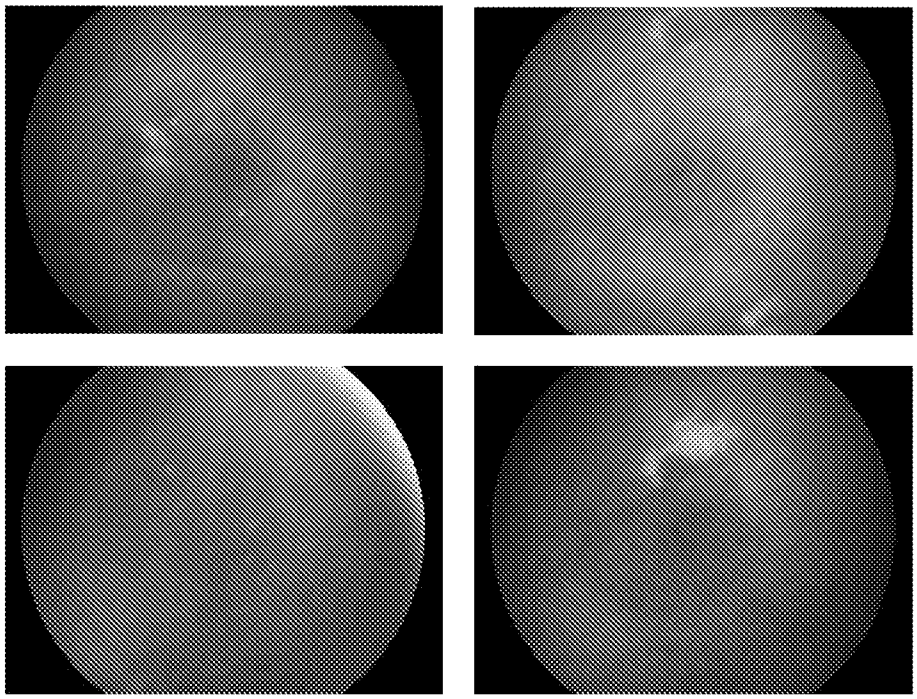
Mouse #2 Left Eye Mouse #2 Right Eye

FIG. 5C



Mouse #5 Left Eye Mouse #5 Right Eye

FIG. 5D



Mouse #6 Left Eye Mouse #6 Right Eye

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/34553

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00, A61P 25/28, A61B 3/00, A61B 3/14, A61B 5/00 (2015.01)
CPC - A61B 5/0042, A61B 5/0059, A61B 5/4064
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)
IPC(8) - A61K 49/00, A61P 25/28, A61B 3/00, A61B 3/14, A61B 5/00 (2015.01)
CPC - A61B 5/0042, A61B 5/0059, A61B 5/4064

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61B 5/0071, A61B 5/0075, A61B 5/0075, A61B 3/10, A61B 3/13, A61B 3/14
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar
Search terms: retina, retinal, traumatic brain injury, imaging, camera, microscope, spectrometer, fluorescent, curcumin, Thioflavin, Congo Red, methoxy-X04, Pittsburgh Compound-B, PiB, DDNP, Chrysamine-G, white spot, abluminal, parenchyma, hemorrhage

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0200531 A1 (TAN) 18 August 2011 (18.08.2011) para [0011], [0112], [0127], [0152]	1-5, 8-14, 17-23, 26-32, 35-37
X --- Y	US 20130296709 A1 (ZUZAK et al.) 07 November 2013 (07.11.2013) para [0241], [0244], [0409], [0411], [0412], [0414]	1, 6, 11, 15, 20, 24, 29, 33 --- 7, 16, 25, 34
Y	VINCENT et al., Retinal haemorrhages in inflicted traumatic brain injury: the ophthalmologist in court. Clin Experiment Ophthalmol. July 2010, Vol 38, No 5, pp 521-32. Especially p 522, left col, para 3	7, 16, 25, 34

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"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	Date of mailing of the international search report
20 August 2015 (20.08.2015)	11 SEP 2015
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774