achieving cellular-level imaging resolution, or due to their susceptibility to scatter. Random access scanning approaches, which may increase frame rate by limiting scanning to targeted locations, need prior knowledge on the location of neurons and are thus difficult for application with awake animals, as neurons move frame-by-frame.

SUMMARY

[0008] A tissue imaging system according to an aspect of the present disclosure includes a laser module for outputting a laser pulse, an optical delay module configured to split a laser pulse received from the laser module into a plurality of sub-pulses, a spectral dispersion element configured to spatially disperse the sub-pulses into spectral components, a telescope for delivering the sub-pulses from the optical delay module to a target volume and a photodetector configured to collect photons generated within the target volume in response to excitation of the target volume by the first and second sub-pulses. The sub-pulses include a first sub-pulse and a second sub-pulse. The telescope includes an objective lens, and the spectral components of the sub-pulses are geometrically dispersed on the objective lens outside of a focus of the objective lens.

[0009] The optical delay module preferably includes a beam-splitter configured to split the laser pulse into the plurality of sub-pulses, at least two optical paths for introducing the time delay between the first sub-pulse and the second sub-pulse and an optical combiner for combining the first and second sub-pulses to form a temporally multiplexed laser pulse comprising the first and second sub-pulses, wherein the telescope delivers the temporally multiplexed laser pulse to the target volume. The optical paths may introduce the time delay via free-space propagation or via an optical fiber. The optical delay module further preferably includes a first focusing lens with a first divergence for focusing the first sub-pulse received from the beam splitter to the first depth within the target volume, and a second focusing lens with a second divergence for focusing the second sub-pulse received from the beam splitter to the second depth within the target volume.

[0010] The tissue imaging system further preferably includes a spatial multiplexing module configured to receive the temporally multiplexed laser pulse from the optical delay module. The

spatial multiplexing module includes a beam splitter for splitting the temporally multiplexed laser pulse into a plurality of sub-beams including a first sub-beam and a second sub-beam. The first sub-beam and the second sub-beam are spatially separated with respect to a first image plane formed at the first depth within the target volume and with respect to a second image plane formed at the second depth within the target volume.

[0011] The spatial multiplexing module further preferably includes a scanner for angularly deflecting the first and second sub-beams, whereby the first and second sub-beams are respectively scanned on first and second focusing regions of the first and second image planes. In this aspect, the laser module is configured to output a laser beam comprising light pulses emitted at a first repetition rate and duration. The spatial multiplexing module further preferably includes a controller configured to move the scanner at a rate based on the first repetition rate, such that a focus spot within the target volume for light output by the laser module is deflected in a first direction by approximately a width of the focus spot in the first direction between emission of successive pulses by the laser module.

[0012] The system further preferably includes a temporal focusing grating for receiving the angularly deflected first and second sub-beams from the scanner and for dispersing light pulses in the angularly deflected first and second sub-beams into their respective spectral components.

[0013] In addition, the photodetector preferably includes a photomultiplier tube and a lens array for focusing the photons generated within the target volume. It is also conceivable that the present tissue imaging system is provided only with the spatial multiplexing module.

[0014] In another aspect of the present disclosure, a method for high-speed imaging of fluorophores within a target volume is provided. The method includes providing a pulsed laser beam comprising pulses each less than 10 ps in duration, splitting a pulse of the pulsed laser beam into a plurality of sub-pulses including a first sub-pulse and a second sub-pulse, introducing a time delay between the first sub-pulse entering the target volume and the second sub-pulse entering the target volume and collecting photons

generated within the target volume in response to excitation of the target volume by the first and second sub-pulses. Splitting the pulse of the pulsed laser beam into a plurality of sub-pulses includes geometrically dispersing spectral components of the sub-pulses such that the spectral components of the sub-pulses are geometrically dispersed on an objective lens outside of a focus of the objective lens. This method may further include focusing the first sub-pulse at a first depth within the target volume and focusing the second sub-pulse at a second depth within the target volume, wherein the second depth is different than the first depth.

[0015] The excitation of the target volume by the first and second sub-pulses according to the method preferably includes excitation of fluorophores within the target volume with a two-photon excitation scheme, a three-photon excitation scheme, or a combination thereof.

[0016] Also, the method further preferably includes combining the first and second pulses to form a temporally multiplexed laser pulse comprising the first and second sub-pulses, spatially separating the temporally multiplexed laser pulse into a plurality of sub-beams including a first sub-beam and a second sub-beam, and delivering the spatially separated and temporally multiplexed laser pulse to the target volume, wherein the first sub-beam and the second sub-beam are spatially separated with respect to a first image plane formed at the first depth within the target volume and with respect to a second image plane formed at the second depth within the target volume.

[0017] The method further preferably includes angularly deflecting the first and second sub-beams with a scanner, whereby the first and second sub-beams are respectively scanned on first and second focusing regions of the first and second image planes. In addition, the scanner is preferably moved at a rate based on a repetition rate of the pulsed laser beam, such that a focus spot within the target volume for light output by the laser module is deflected in a first direction by approximately a width of the focus spot in the first direction between emission of successive pulses of the pulsed laser beam.

[0018] Here too, it is conceivable that the method only includes the steps of spatially multiplexing the beam.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] The drawing figures depict one or more implementations in accord with the present teachings, by way of example only, not by way of limitation. In the figures, like reference numerals refer to the same or similar elements.

CLAIMS

What is claimed is:

1. An imaging system comprising:

a laser module for outputting a laser pulse;

an optical delay module configured to split a laser pulse received from the laser module into a plurality of sub-pulses including a first sub-pulse and a second sub-pulse and to introduce a time delay between the first sub-pulse and the second sub-pulse;

a spectral dispersion element configured to spatially disperse the sub-pulses into spectral components;

a telescope for delivering the sub-pulses from the optical delay module to a target volume, the telescope including an objective lens, and wherein the spectral components of the sub-pulses are geometrically dispersed on the objective lens outside of a focus of the objective lens; and

a photodetector configured to collect photons generated within the target volume in response to excitation of the target volume by the first and second sub-pulses.

2. The imaging system as defined in Claim 1, wherein the optical delay module comprises:

a beam-splitter configured to split the laser pulse into the plurality of sub-pulses; and

an optical combiner for combining the first and second sub-pulses to form a temporally multiplexed laser pulse comprising the first and second sub-pulses,

wherein the telescope delivers the temporally multiplexed laser pulse to the target volume.

3. The imaging system as defined in Claim 2, wherein the optical delay module further includes at least two optical paths for introducing the time delay between the first sub-pulse and the second sub-pulse via free-space propagation or via an optical fiber.

4. The imaging system as defined in Claim 2, wherein the optical delay module further comprises:

a first focusing lens with a first divergence for focusing the first sub-pulse received from the beam splitter to a first depth within the target volume; and

a second focusing lens with a second divergence for focusing the second sub-pulse received from the beam splitter to a second depth within the target volume.

5. The imaging system as defined in Claim 4, further comprising a spatial multiplexing module configured to receive the temporally multiplexed laser pulse from the optical delay module, the spatial multiplexing module including a beam splitter for splitting the temporally multiplexed laser pulse into a plurality of sub-beams including a first sub-beam and a second sub-beam, the first sub-beam and the second sub-beam being spatially separated with respect to a first image plane formed at the first depth within the target volume and with respect to a second image plane formed at the second depth within the target volume.

6. The imaging system as defined in Claim 5, wherein the spatial multiplexing module further comprises a scanner for angularly deflecting the first and second sub-beams, whereby the first and second sub-beams are respectively scanned on first and second focusing regions of the first and second image planes.

7. The imaging system as defined in Claim 6, wherein the laser module is configured to output a laser beam comprising light pulses emitted at a first repetition rate and duration, and wherein the spatial multiplexing module further comprises a controller configured to move the scanner at a rate based on the first repetition rate, such that a focus spot within the target volume for light output by the laser module is deflected in a first direction by approximately a width of the focus spot in the first direction between emission of successive pulses by the laser module.

8. The imaging system as defined in Claim 6, further comprising a temporal focusing grating for receiving the angularly deflected first and second sub-beams from the scanner and for dispersing light pulses in the angularly deflected first and second sub-beams into their respective spectral components.

9. The imaging system as defined in Claim 1, wherein the photodetector includes:

a photomultiplier tube; and

a lens array for focusing the photons generated within the target volume.

10. An imaging system comprising:

a laser module for outputting a laser pulse;

a spatial multiplexing module configured to receive the laser pulse from the laser module, the spatial multiplexing module including a beam splitter for splitting the laser pulse into a plurality of sub-beams including a first sub-beam and a second sub-beam, the first sub-beam and the second sub-beam being spatially separated with respect to a first image plane formed at a first depth within a target volume;

a spectral dispersion element configured to spatially disperse the sub-beams into spectral components;

a telescope for delivering the first and second sub-beams from the spatial multiplexing module to the target volume, the telescope including an objective lens, and wherein the spectral components of the sub-beams are geometrically dispersed on the objective lens outside of a focus of the objective lens; and

a photodetector configured to collect photons generated within the target volume in response to excitation of the target volume by the first and second sub-beams.

11. The imaging system as defined in Claim 10, wherein the spatial multiplexing module further comprises a scanner for angularly deflecting the first and second sub-beams, whereby the first and second sub-beams are respectively scanned on first and second focusing regions of the first image plane.

12. The imaging system as defined in Claim 11, wherein the laser module is configured to output a laser beam comprising light pulses emitted at a first repetition rate and duration, and wherein the spatial multiplexing module further comprises a controller configured to move the scanner at a rate based on the first repetition rate, such that a focus spot within the target volume for light output by the laser module is deflected in a first direction by

approximately a width of the focus spot in the first direction between emission of successive pulses by the laser module.

13. The imaging system as defined in Claim 11, wherein the spatial multiplexing module further comprises a temporal focusing grating for receiving the angularly deflected first and second sub-beams from the scanner and for dispersing light pulses in the angularly deflected first and second sub-beams into their respective spectral components.

14. The imaging system as defined in Claim 10, wherein the photodetector includes:

a photomultiplier tube; and

a lens array for focusing the photons generated within the target volume.

15. A method for high-speed imaging of fluorophores within a target volume, the method comprising:

providing a pulsed laser beam comprising pulses each less than 10 ps in duration;

splitting a pulse of the pulsed laser beam into a plurality of sub-pulses including a first sub-pulse and a second sub-pulse, wherein splitting the pulse includes geometrically dispersing spectral components of the sub-pulses such that the spectral components of the sub-pulses are geometrically dispersed on an objective lens outside of a focus of the objective lens;

introducing a time delay between the first sub-pulse entering the target volume and the second sub-pulse entering the target volume; and

collecting photons generated within the target volume in response to excitation of the target volume by the first and second sub-pulses.

16. The method as defined in Claim 15, wherein the excitation of the target volume by the first and second sub-pulses includes excitation of fluorophores within the target volume with a two-photon excitation scheme, a three-photon excitation scheme, or a combination thereof.

17. The method as defined in Claim 15, wherein the time delay between the first sub-pulse and the second sub-pulse is introduced via free-space propagation or via an optical fiber.

18. The method as defined in Claim 15, further comprising focusing the first and second sub-pulses at a common plane within the target volume.

19. The method as defined in Claim 15, further comprising:

focusing the first sub-pulse at a first depth within the target volume; and

focusing the second sub-pulse at a second depth within the target volume, the second depth being different than the first depth.

20. The method as defined in Claim 19, further comprising:

combining the first and second pulses to form a temporally multiplexed laser pulse comprising the first and second sub-pulses;

spatially separating the temporally multiplexed laser pulse into a plurality of sub-beams including a first sub-beam and a second sub-beam, the first sub-beam and the second sub-beam being spatially separated with respect to a first image plane formed at the first depth within the target volume and with respect to a second image plane formed at the second depth within the target volume; and

delivering the spatially separated and temporally multiplexed laser pulse to the target volume.

21. The method as defined in Claim 20, further comprising angularly deflecting the first and second sub-beams with a scanner, whereby the first and second sub-beams are respectively scanned on first and second focusing regions of the first and second image planes.

22. The method as defined in Claim 21, further comprising moving the scanner at a rate based on a repetition rate of the pulsed laser beam, such that a focus spot within the target volume for light output by the laser module is deflected in a first direction by approximately a width of the focus spot in the first direction between emission of successive pulses of the pulsed laser beam.

23. A method for high-speed imaging of fluorophores within a target volume, the method comprising:

providing a pulsed laser beam comprising pulses each less than 10 ps in duration;

splitting the pulsed laser beam into a plurality of sub-beams including a first sub-beam and a second sub-beam, the first sub-beam and the second sub-beam being spatially separated with respect to a first image plane formed at a first depth within a target volume, wherein splitting the pulsed laser beam includes geometrically dispersing spectral components of the sub-beams such that the spectral components of the sub-beams are geometrically dispersed on an objective lens outside of a focus of the objective lens;

delivering the first and second sub-beams to the target volume; and

collecting photons generated within the target volume in response to excitation of the target volume by the first and second sub-beams.

24. The method as defined in Claim 23, further comprising angularly deflecting the first and second sub-beams with a scanner, whereby the first and second sub-beams are respectively scanned on first and second focusing regions of the first image plane.

25. The method as defined in Claim 24, further comprising moving the scanner at a rate based on a repetition rate of the pulsed laser beam, such that a focus spot within the target volume for light output by the laser module is deflected in a first direction by approximately a width of the focus spot in the first direction between emission of successive pulses of the pulsed laser beam.