

CLAIMS

We claim:

1. A method for determining a depth of a concentration of fluorophore lying beneath a surface of an optically absorbing and scattering medium comprising:

illuminating a surface of the medium with a broad beam of light at an excitation wavelength of the fluorophore using a first spatially modulated pattern comprising alternating high and low intensity regions at a first spatial frequency between 0.1 and 1 mm⁻¹, the first spatially modulated pattern projected onto the surface of the medium at a plurality of different phases;

imaging diffusely reflected light emitted by the surface of the medium to form an excitation wavelength image for each phase of the plurality of different phases of the first spatially modulated pattern;

illuminating the surface of the medium with a broad beam of light at the excitation wavelength of the fluorophore using a second spatially modulated pattern comprising alternating high and low intensity regions at a second spatial frequency between 0.1 and 1 mm⁻¹, the second spatially modulated pattern projected onto the surface of the medium at a plurality of different phases, the second spatially modulated pattern having a different spatial frequency than the first spatially modulated pattern;

imaging a pattern of diffusely reflected light emitted by the surface of the medium to form an excitation wavelength image for each phase the plurality of different phases of the second spatially modulated pattern;

applying a model of light propagation in an optically absorbing and scattering medium to calculate an absorption coefficient and transport scattering coefficient of the medium at the excitation wavelength of the fluorophore at pixels of the excitation wavelength images;

determining emissions wavelength optical coefficients at pixels;

while illuminating the surface of the medium with spatially modulated patterns at the excitation wavelength, recording fluorescent emissions wavelength images of the surface of the medium at a third spatial frequency;

while illuminating the surface of the medium with spatially modulated patterns at the excitation wavelength, recording fluorescent emissions wavelength images of the surface of the medium at a fourth spatial frequency;

extracting measured modulated amplitude at each of the third and fourth spatial frequencies for pixel regions in the fluorescent emissions wavelength images recorded at the third and fourth spatial frequencies;

predicting modulated amplitude in images for concentrations of fluorophore located at a plurality of depths below the surface of the medium for each of the third and fourth spatial frequencies;

fitting the measured modulated amplitude at the third and fourth spatial frequencies for the pixel regions to corresponding predicted modulated amplitudes to determine depth of the concentration of fluorophore;

where the third and fourth spatial frequencies comprise at least two spatial frequencies between 0.1 and 1 mm⁻¹.

2. The method of claim 1 where the excitation and emissions wavelength are both red or infrared light of wavelength greater than 630 nanometers.

3. The method of claim 2 wherein the spatially modulated patterns comprise a plurality of alternating light and dark bars.

4. The method of claim 2 wherein determining emissions wavelength optical parameters at pixels in the images is performed by a method comprising:

irradiating a surface of the medium with a broad beam of light at the emissions wavelength of the fluorophore using a spatially illuminated pattern comprising alternating high and low intensity regions at a fifth spatial frequency between 0.1 and 1 lines per millimeter at each of a plurality of phases;

imaging a pattern of diffusely reflected light emitted by the surface of the medium to form an emissions-wavelength, spatially-illuminated, image for the fifth spatial frequency at each of the plurality of phases;

irradiating a surface of the medium with a broad beam of light at the emissions wavelength of the fluorophore using a spatially illuminated pattern comprising alternating high and low intensity regions at a sixth spatial frequency between 0.1 and 1 lines per millimeter at each of a plurality of phases;

imaging a pattern of diffusely reflected light emitted by the surface of the medium to form an emissions-wavelength, spatially-illuminated, image for the sixth spatial frequency at each of the plurality of phases;

applying a model of light propagation in an optically absorbing and scattering medium to calculate an absorption coefficient and transport scattering coefficient of the medium at the emissions wavelength of the fluorophore at each pixel in the images.

5. The method of claim 1, 2, 3, or 4 further comprising using the depth of the concentration of fluorophore and at least the absorption coefficient at each pixel to quantify the concentration of fluorophore.

6. The method of claim 5 in which the fluorescent material is protoporphyrin IX.

7. The method of claim 6 further comprising preparing an image with fluorophore concentration encoded as brightness and fluorophore depth encoded as color.

8. The method of claim 7 in which the absorbing and scattering medium is mammalian tissue, the protoporphyrin IX being generated within the tissue upon administration of 5-aminolevulinic acid.

9. The method of any of claims 1-4 in which the concentration of fluorophore comprises a tumor.

10. The method of claim 5 in which the concentration of fluorophore comprises a molecular material or nanomaterial that fluoresces in the red or near-infrared spectral range from about 600 nm to 1300 nm.

11. The method of claims 1-4 wherein the first, third, and fifth spatial frequencies are the same, the second, fourth, and sixth spatial frequencies are the same, and first and second spatial frequencies differ.

12. A device for quantifying and determining depth of concentrations of fluorophores comprising:

an excitation-wavelength light source configurable to excite the fluorophores in the medium using a spatially-modulated light pattern, the spatially-modulated light pattern selectable from a plurality of light patterns with spatial frequencies between 0.2 and 1 lines per millimeter;

at least one optical element configured to transfer diffusely-reflected images and fluorescence emission images from the medium to an imaging subsystem;

the imaging subsystem comprising at least one filter insertable into the light path to reject light at the excitation wavelength, and at least one electronic camera configured to convert received light into a matrix of digital values according to an intensity of light striking detector pixels; and

at least one computer configured to control the spatially-modulated light pattern, to capture images, and configured with machine readable instructions to determine a depth of the concentration of fluorophore and determine a concentration of the fluorophore for image pixels of the camera; and

apparatus configured to display fluorophore concentration depth and concentration.

13. The device of claim 12 wherein the excitation wavelength is within 100 nanometers of a wavelength peak of fluorescent emissions of the concentration of fluorophore.

14. The device of claim 12 wherein the machine readable instructions to determine a depth of a concentration of fluorophore are configured for a method comprising:

extracting measured modulated amplitude at the plurality of spatial frequencies for pixel regions in fluorescent emissions images recorded at a plurality of spatial frequencies;

predicting fluorescence modulation for a concentration of fluorophore located at a plurality of depths below the surface of the medium for each spatial frequency of the plurality of spatial frequencies; and

fitting the measured modulated amplitude at the plurality of spatial frequencies for pixel regions at the plurality of spatial frequencies to corresponding predicted fluorescence modulation to determine depth of the concentration of fluorophore.

15. The device of claim 14 in which the absorbing and scattering medium is mammalian tissue.

16. The device of claim 13 wherein the excitation wavelength is between 620 and 640 nanometers.

17. The device of claim 12, 13, 14, 15, or 16 wherein the fluorescent emissions wavelength has a peak lying between 700 and 720 nanometers.