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Perspective

Imaging techniques for comprehensive understanding of scattering and fluctuation fields

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Scattering and fluctuation phenomena arise in various fields, from microscopic life activities on the nanometer scale to atmospheric fluctuations on the kilometer one. Understanding and overcoming these phenomena will facilitate breakthroughs in various academic fields, including life sciences, preventive medicine, information and communication engineering, and astronomy. This review article presents an overview of the imaging techniques applied to scattering and fluctuating fields, as part of a new approach that visualizes information inside or beyond scattering media or fluctuating fields by eliminating the effects of scattering and fluctuating fields. More specifically, we introduce imaging techniques to visualize the inside of scattering media and light propagation behaviors in scattering media, to facilitate modeling.

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1. Introduction

Imaging technology has revolutionized science, technology, and manufacturing. A representative example of this is the equipment used for observing multi-scale objects, from microscopes that magnify and visualize small objects to telescopes that reduce large celestial bodies. Furthermore, a super-resolution microscope that exceeded the diffraction limit (thought to be the minimum observable limit when using light) achieved a resolution of 100 nm and was awarded the 2014 Nobel Prize in Chemistry. However, scattering and fluctuation fields cannot be seen using these state-of-the-art imaging techniques. Scatterers (e.g., frosted glass) deflect light in many directions and render internal information inaccessible via multiple scattering. Fluctuations and scattering are phenomena that appear in various places, from the nanometer-scale microscopic life activities to kilometer-scale atmospheric fluctuations. Understanding and overcoming these phenomena will facilitate breakthroughs in various academic fields, including life sciences, preventive medicine, information and communication engineering, and astronomy. Biological tissues contain a dense mixture of large and small structures, from tissue-level structures (e.g., blood vessels, bones, and vascular bundles) to intracellular organelles (e.g., cell nuclei, mitochondria, and chloroplasts) and macromolecules (e.g., DNA and proteins). The light passing through these structures is diffracted, refracted, reflected, and so on; hence, it appears as scattering. Thus, the deeper the living tissue to be observed and manipulated, the stronger the scatterer influences the light passing through. This can significantly distort images and make it impossible to manipulate specific cells efficiently. Even with advanced imaging technologies such as multi-photon microscopy, it is difficult to achieve high resolutions at depths of 1 mm in the brain (which is relatively transparent) and depths exceeding 100 μm in the liver and aerial parts of plants (which are less transparent) [1,2]. Current research is also aiming to elucidate brain functions via optogenetics, which manipulates individually cell activity using light [3–5]. To manipulate the activity of a living mouse brain neuron (∼10 μm in size) via light irradiation, we require a technique to eliminate the effects of scattering and accurately produce a minute spot deep in the brain.

Light disturbance presents problems beyond bioimaging and photo-stimulation of cell activity. In cutting-edge fields such as spatial optical communication (e.g., large-scale ground-to-satellite optical communication and observational astronomy using ground-based telescopes), fluctuations in the atmosphere and air through which light pass have become a problem.

As a comprehensive methodology for the non-destructive and high-resolution visualization of the inside of such multi-scale scattered fields (as well as the scattered field itself), researchers are working toward the fabrication of "seeing through scattering fields (scattering clairvoyance)," which integrates optical imaging, mathematical, and information sciences. In this paper, we first introduce an overview of scattering clairvoyance. Next, we introduce the scattering clairvoyance imaging technique, as well as techniques for visualizing the state of light propagation in the scatterer. Mathematical modeling and applications are omitted for brevity.

2. Overview of scattering clairvoyance

Scattering and fluctuation phenomena are produced by clusters of molecules on a small scale and occur on a scale of 12 orders of magnitude (from nanometer to kilometer orders) up to large-scale atmospheric fluctuations (see Fig. 1). We aim to construct "scattering clairvoyance theory" to measure this multi-scale scattering/fluctuation field and to extract and manipulate the information it contains. In electromagnetism, Maxwell's equations describe the behavior of electromagnetic waves at all wavelengths, spatial scales, and time scales. Scattering clairvoyance theory does not describe scattering and fluctuation phenomena using a single equation (as Maxwell's equation does). However, we construct a comprehensive theory by integrating measurements, mathematical modeling, and prior knowledge (e.g., the structure of the target object) obtained from applications. For example, the scattering inverse problem is treated as a "multidimensional scattered field problem," and the partial differential equations that the scattered field should satisfy (as well as their analytical solutions) are derived [6].

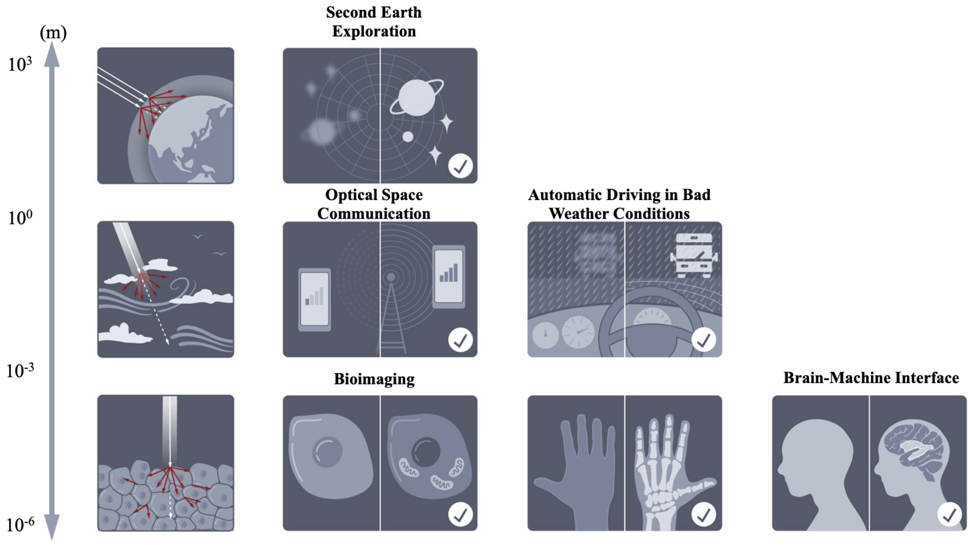

Fig. 1. Various problems of multiscale scattering and fluctuation fields.

3. Scattering clairvoyance imaging technology

The new approaches for scattering clairvoyance imaging techniques can be broadly classified into two types. One is an image restoration method that exploits a characteristic of thin scattering medium (the "memory effect") and applies a speckle correlation calculation and phase retrieval method [7]. We utilize the fact that the autocorrelation (speckle correlation) of the scattering image equates to the autocorrelation of the object intensity distribution when the scattering image is represented by the convolution of the point spread function with respect to the intensity distribution of the object and scattering medium. Furthermore, the phase distribution on the Fourier plane of the object intensity distribution is obtained via the iterative phase retrieval method. By taking the inverse Fourier transform of this, the original object intensity distribution can be restored. In the phase retrieval method, a non-negative real number constraint condition is used because the object is the intensity in the object plane. Another approach is to use a transmission matrix that assumes a linear input–output relationship for the scattering medium [8–10]. See Ref. 11 for several techniques.

We introduce three-dimensional (3D) fluorescence imaging using the transport of intensity equation. Here, we consider the case in which light passes through a weak scattering medium that does not diffuse it enough to produce speckles. At this time, the fluorescence emitted from the object passes through the scattering medium, which is described only by phase modulation. After passing through the scattering medium, the spatial frequency characteristics of the light wave (especially the amplitude spectrum) change with respect to the distance from the scattering medium. The authors have proposed a recovering method for weakly scattered images; it does not depend on the acquisition position of the scattered image [12]. The core of this method is the acquisition of a complex amplitude distribution for the diffused light wave of the fluorescence emitted from the micro-fluorophore via the transport of intensity equation (TIE) [13,14].

As mentioned in Sect. 1, light passing through the living tissues of plants and animals is scattered by structures such as intracellular organelles. In the following, we introduce the results obtained using tobacco cells cultured with the green fluorescent protein-tubulin (GFP-tubulin). Here, the cell itself is assumed to act as a weak scattering medium.

Figure 2 shows the experimental system. The light output from the light-emitting diode (LED) light source (wavelength: 470 nm) spreads the beam diameter via the lens L1 after passing through the bandpass filter BPF1. Thereafter, it is reflected by the dichroic mirror by condenser lens L2 and is focused on the pupil plane of the microscope objective lens MO. After the objective lens, the light becomes collimated light and illuminates the object. For BPF1, a bandpass filter centered at 457 nm with a bandwidth of 70 nm is used to narrow the bandwidth of the LED and prevents overlap with

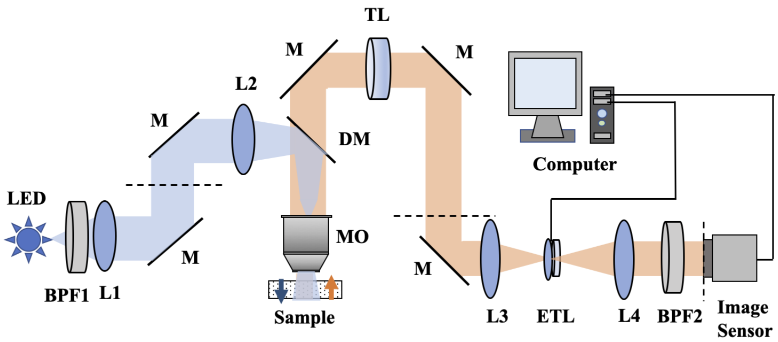

Fig. 2. Experimental setup of 3D fluorescence imaging for obtaining the amplitude and phase distributions by transport of intensity equation.

the fluorescence spectrum. The microscope objective lens has a magnification of 20 and a numerical aperture (NA) of 0.45. The samples were cultured tobacco cells; these were stained with GFP-tubulin in the nucleus. Fluorescence from the cultured tobacco cells was magnified by an objective lens and tube lens TL before being imaged on an image sensor via a $4f$ optical system consisting of two lenses L3 and L4. A variable focal length lens ETL placed on the Fourier plane of the $4f$ optical system was controlled by a personal computer, which varied the focal length. This made it possible to shift the depth imaging position of the object observed by the image sensor, without changing the imaging magnification. In the experiment described below, seven images were acquired at intervals of 0.81 µm. The image sensor has 2048 × 2048 pixels and a pixel pitch of 6.5 µm; 2 × 2 binning was used for image acquisition. A band-pass filter BPF2 with a central wavelength of 525 nm and a bandwidth of 78 nm was used to obtain fluorescence from GFP-tubulin.

Figure 3(a) shows an example of the acquired image. Cultured tobacco cells were placed in solution on the slide glass; however, they were also distributed in the depth direction. Furthermore, because the image was taken at a location shifted away from the focal position, it was a little blurred. Figure 3(b) shows a phase image extracted via the TIE. The phase sign for yellow is on the positive side; that for blue is on the negative side. In other words, the yellow and blue areas indicate that in-focus images can be reconstructed from the observation plane via propagation in opposite directions. Figure 4 shows a focused image obtained by Fresnel propagation calculations using the TIE-obtained amplitude and phase distributions. Figures 4(a) and 4(b) are the intensity images when propagated +24.3 µm and −24.3 µm, respectively. Focusing on the area surrounded by squares, we can see that the image is focused on the object. Thus, high-speed 3D fluorescence imaging can be achieved by reconstructing in-focus images from the obtained complex amplitude distribution by TIE. We also confirm that reconstruction is possible even when a weak scattering medium is placed between the object and microscope objective lens. See Ref. 12 for more information.

4. Visualization of light propagating in scattering medium

We can use the relationship between the input and output (e.g., the intensity correlation and scattering matrix) to depict the image in the scattering medium [7,10]. It is also important to observe and clarify how light propagates in and through scattering medium. By doing so, the state of the scattering medium can be examined from the image obtained. On the other hand, light travels at 300,000 km/s in a vacuum (the maximum speed possible). Therefore, it is difficult to obtain a video of the propagation, even with the typical imaging methods or the world's fastest cameras. Hence, the authors are currently researching technologies and applications that record and replay the behavior of light propagation in a scattering medium as a video. In this section, we introduce these techniques and the results obtained thus far [15,16].

Light-in-flight holography [17,18] is a technique for recording and obtaining video of light propagation. This technique uses a low-coherence light source to record holograms. Holography is known as a 3D imaging technique. This technique makes it possible to record a video comprising more

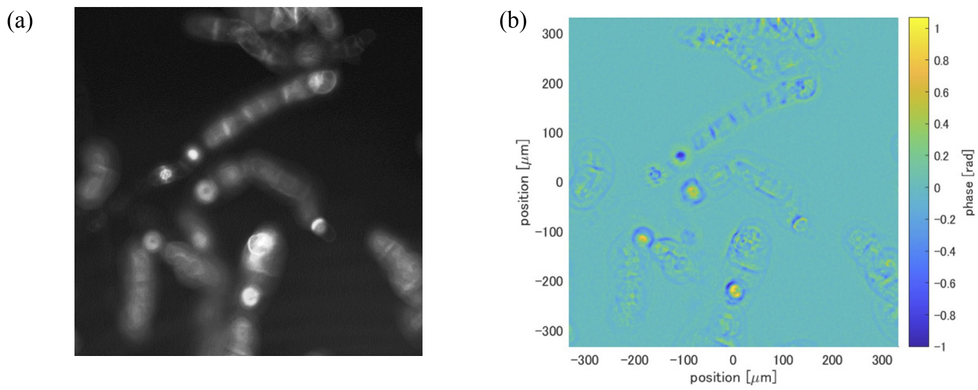

Fig. 3. (a) Fluorescence image of tobacco cultured cells with GFP tubulin and (b) phase image by TIE.

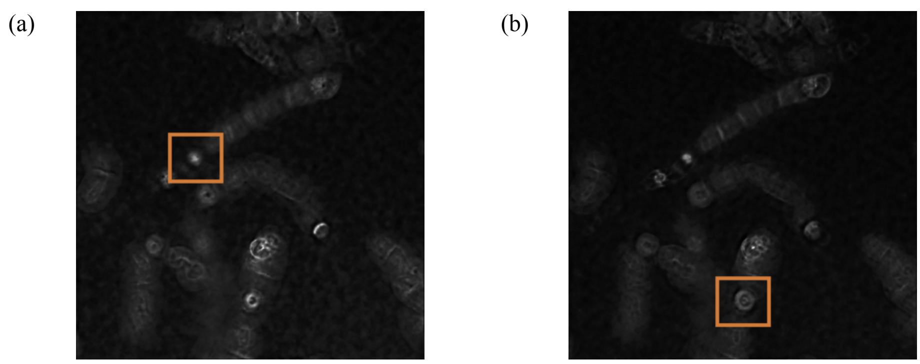

Fig. 4. Recovered images after propagation calculation using complex amplitude distribution obtained by TIE; (a) after +24.3 µm propagation, (b) after −24.3 µm propagation.

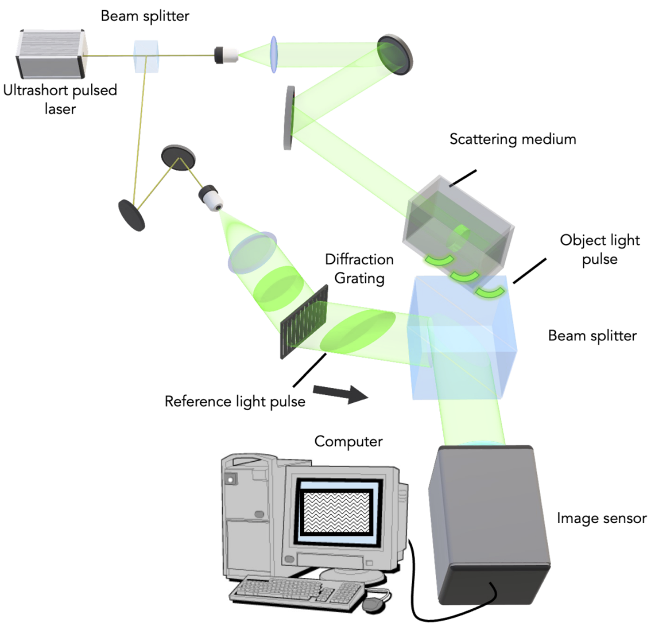

Fig. 5. Optical configuration for video recording of ultrashort light pulses propagating in a scattering medium.

than several thousand frames (with ultra-high time resolution) in a single shot [19]. The authors used an ultrashort pulsed laser as the light source [20–23]. Figure 5 shows a schematic diagram of the optical system used for light-in-flight holography, which records a video of light propagating through a scattering medium. A light pulse emitted from an ultra-short pulsed laser is split into two by a beam splitter. One pulse is incident on the scattering medium whose propagation state is to be observed. The incident light pulse is scattered whilst passing through the scattering medium, and the light pulse that separates from the scattering medium is recorded. This light pulse is called the object light pulse. The other light pulse is collimated and obliquely impinged on the image sensor as a reference light pulse. Because the reference light pulse is obliquely incident on the image sensor, it is illuminated obliquely across the image sensor's surface (from left to right in Fig. 5). On the other hand, the object light pulse is generated at different times and locations, depending on the shape of the scattering medium; hence, it reaches the image sensor at different times. Because the temporal coherence length of these two light pulses is extremely short, the interference fringes are formed only in a very narrow area where the object and reference light pulses arrive simultaneously on the image sensor surface. As a result, the image sensor records interference fringes that include ultrashort light pulses at different times (from left to right in Fig. 5).

This optical system records a hologram using an image sensor for light-in-flight holography. Thereafter, it is essentially the same as the recording system for digital light-in-flight holography [24–26], which replays the recorded light video on a computer. The video-recordable time is the time it takes for the reference light pulse to pass through the image sensor surface. This time is determined by the incident angle of the reference light pulse with respect to the image sensor, as well as the horizontal length of the image sensor. The larger the incident angle, the longer the video-recordable time. However, because the resolution of the image sensor is generally several micrometers (too coarse to record interference fringes), the incident angle of the reference light pulse can only be set to a few degrees or less. Therefore, to obtain a longer recordable time, the diffracted light emitted from a diffraction grating is used as the reference light pulse. Because this reference light pulse is incident upon the imaging device with a tilted optical pulse plane, the time it takes to cross the image sensor plane can be extended when no diffraction grating is used. As a result, the video-recordable time for light propagation can be extended [27–29].

Please refer to Ref. 15 for the video playback processing of this technique. We introduce the results of recording and replaying ultra-short optical pulses propagating through a scattering medium. A jelly made of gelatin in a transparent container was set as a scattering medium, and ultra-short light pulses propagating therein were recorded. An Yb:YVO_4 laser with a pulse width of 178 fs and a central wavelength of 522 nm was used as the recording light source. To easily understand how the image of the optical pulse varied as it propagated, we recorded the propagation of an ultra-short optical pulse whilst concentrating it in a scattering medium with a convex lens. Figure 6 shows six frames extracted from the obtained video. The interval between each frame was 10.1 ps. The ultrashort optical pulse propagated from left to right. The duration of the recorded video was 59 ps, and the video recording speed was ∼58 trillion frames per second.

In the videos and images obtained, the scattered light emitted from the light propagating in the scattering medium can be recorded as though by a camera. This does not mean that we can observe the light propagation inside of the scattering medium in high fidelity; however, we can sketch the behavior of light in the scattering medium. The following methods can achieve a more faithful observation of light propagating in the scattering medium: (i) Numerical corrections are made to the videos played back with this technique.

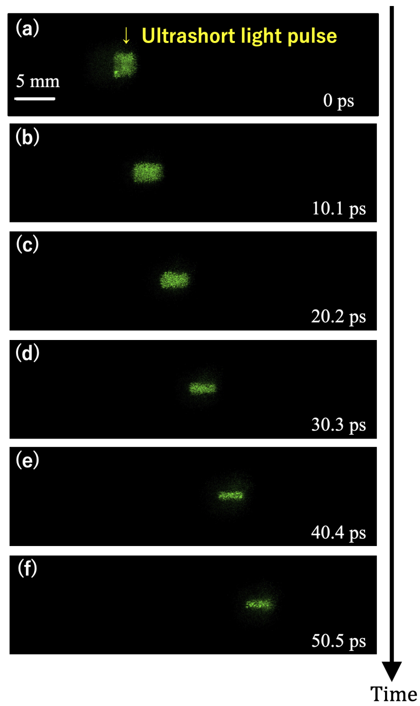

Fig. 6. Video recording of ultrashort light pulses propagating while focused in scattering medium.

(ii) The light pulse or reference light pulse that illuminates the object during recording is modulated with a spatial light modulator (or similar device) to record a hologram in a prior-calculated pattern, to obtain a corrected images in replayed video. The proposed techniques allow the spatio-temporal characteristics of light's behavior in the scattering medium to be observed. By exploiting this feature and fusing the proposed technique with other techniques for viewing images in scattering medium [7,10], we believe that a more faithful technique for observing image and light propagation in a scattering medium can be realized.

5. Conclusion

In this paper, we presented an overview of imaging techniques in scattering medium as well as techniques for visualizing the state of light propagation in a scattering medium, to serve as a measurement technique for modeling such media. To realize deep imaging of the scattering medium (which has not been achieved so far), it is necessary to model the structure of the scattering medium from the surface to the inside. This modeling allows illumination technology to deliver the desired light waves and restore the images degraded by scattering. Another approach is to use a guide star in astronomy as a landmark inside the scattering medium. Hence, it is necessary for making the breakthrough the imaging techniques in scattering medium by co-creation and cooperation of researchers in optics, mathematical science, information science, computer science, and applied research to work together.

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Profile

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