

July 22, 2020

National Institute of Information and Communications Technology  
Japan Science and Technology Agency  
Toin University of Yokohama  
Chiba University

**Press Release**

**Development of Instantaneous Color Holography System for Sensing Fluorescence and White Light**

– The group has realized single-shot color-multiplexed 3D fluorescence microscopy with holography –

**[Abstract]**

The National Institute of Information and Communications Technology (NICT), the Japan Science and Technology Agency (JST), Toin University of Yokohama, and Chiba University have succeeded in developing a color-multiplexed holography system by which 3D information of objects illuminated by a white-light lamp and self-luminous specimens are recorded as a single multicolor hologram by a specially designed and developed monochrome image sensor. Single-shot color-multiplexed fluorescence holographic microscopy is realized by exploiting digital holography and computational coherent superposition (CCS), with the latter having been proposed by NICT. The developed microscope acquires the color 3D information of self-luminous objects with a single-shot exposure and no color filter array. The developed system does not need a laser light source and has the capability to conduct multicolor 3D motion-picture sensing of biological samples and moving objects at video rate. Further development will be conducted toward multicolor 3D microscopy of extremely weak light such as autofluorescence light and nonlinear light.

This achievement was published in *Applied Physics Letters* as an open-access paper on July 22, 2020.

**[Achievements]**

Simultaneous color 3D sensing of multiple self-luminous objects was demonstrated by recording a single color-multiplexed hologram of fluorescence light. Multicolor 3D imaging with a white-light lamp was also demonstrated with a single-hologram recording. CCS, which is a holographic multiplexing technique, was exploited to record a color-multiplexed hologram on a monochrome image sensor without any color filter. Only a single-shot exposure is required to conduct color fluorescence holographic

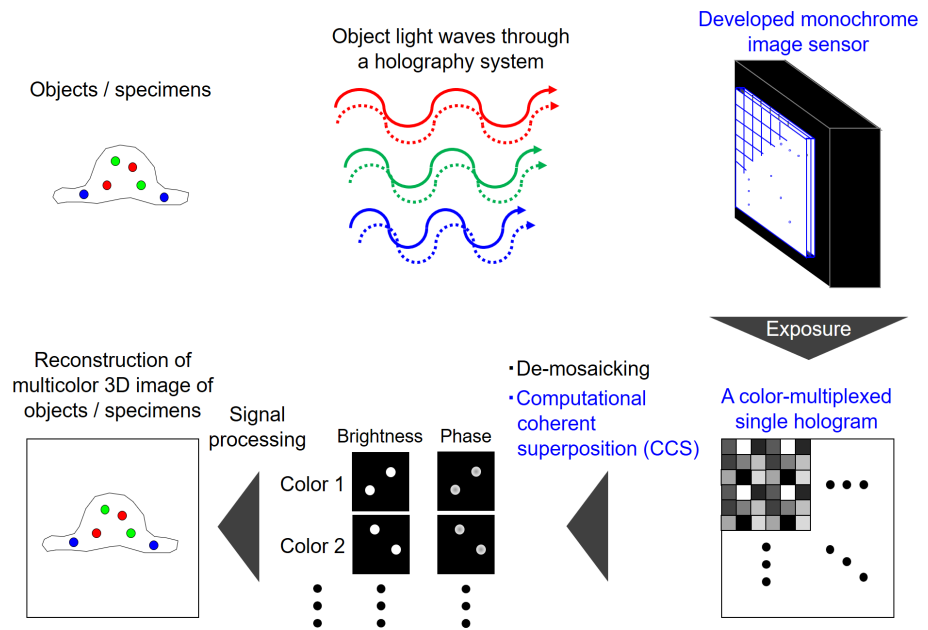


Figure 1 Overview of the proposed instantaneous color-multiplexed holographic sensing technique with a computational coherent superposition (CCS) scheme.

3D sensing using the developed system. The number of exposures required to acquire the information is less than 1/250 of that required for a conventional color-multiplexed fluorescence holographic microscope.

The developed system will be useful for applications to high-speed multicolor holographic 3D motion-

picture microscopy for spatially and temporally incoherent light and multicolor holographic 3D image sensing of ultimately weak light such as autofluorescence light, nonlinear light including spontaneous Raman scattering light, chemically activated light, and natural light.

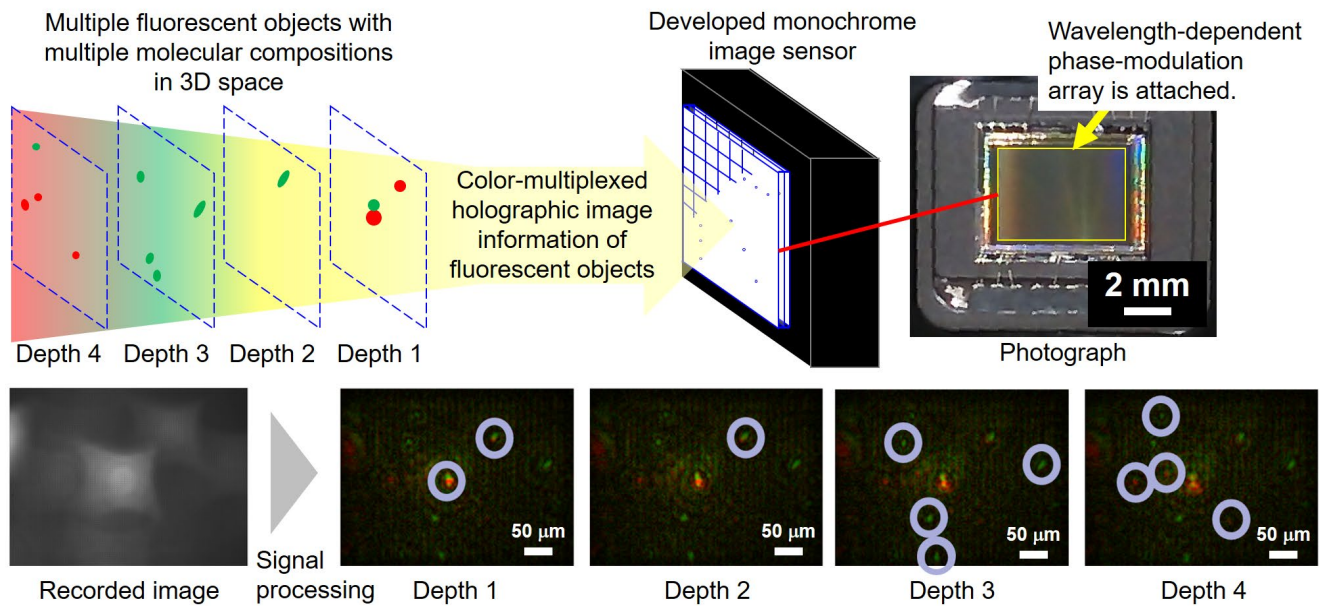


Figure 2 Experimental results for the application to single-shot color-multiplexed fluorescence holographic microscopy.

### [Future prospects]

- Increasing the recording speed in holographic multidimensional sensing of ultimately weak self-luminous light sources
- Applications to multicolor holographic 3D motion-picture image sensing for spatially incoherent light

### [Information of the article]

Journal: *Applied Physics Letters*

DOI: 10.1063/5.0011075

URL: <https://aip.scitation.org/journal/apl>

Title: Single-shot wavelength-multiplexed digital holography for 3D fluorescent microscopy and other imaging modalities

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This work was supported by the Japan Science and Technology Agency (JST) Precursory Research for Embryonic Science and Technology (PRESTO) (JPMJPR16P8 and JPMJPR17P2), Japan Society for the Promotion of Science (JSPS) (18H01456), Research Foundation for Opt-Science and Technology, and Konica Minolta Science and Technology Foundation.

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**Computational coherent superposition (CCS)**

CCS is an in-line holographic multiplexing scheme. Figure 3 illustrates the recording and reconstruction parts of CCS. In CCS, we construct a wavelength-multiplexed interferometer in the real world to record wavelength-multiplexed phase-shifted holograms and assume an interferometer in a computer to reconstruct a color holographic image from the recorded holograms. By setting a phase modulator to sequentially generate wavelength-dependent phase shifts, multiple wavelength-multiplexed phase-shifted holograms are obtained with a monochrome image sensor. These phase shifts are regarded as the code to demultiplex object waves at multiple wavelengths from the recorded holograms. After recording, a virtual interferometer, recorded wavelength-multiplexed images, and complex amplitude modulators to introduce the decode key are assumed in a computer. Light intensity distributions generated from these recorded holograms interfere with each other in the computer and only the object wave at the desired wavelength is reconstructed by adjusting the complex amplitude modulators. Numerical interference in the computer means computational coherent superposition (CCS). By changing the wavelength-multiplexed holograms and the complex amplitude modulations in the interferometer in the computer, another object wave at another wavelength is reconstructed. As a result, a multicolor 3D image is reconstructed from wavelength-multiplexed phase-shifted holograms.

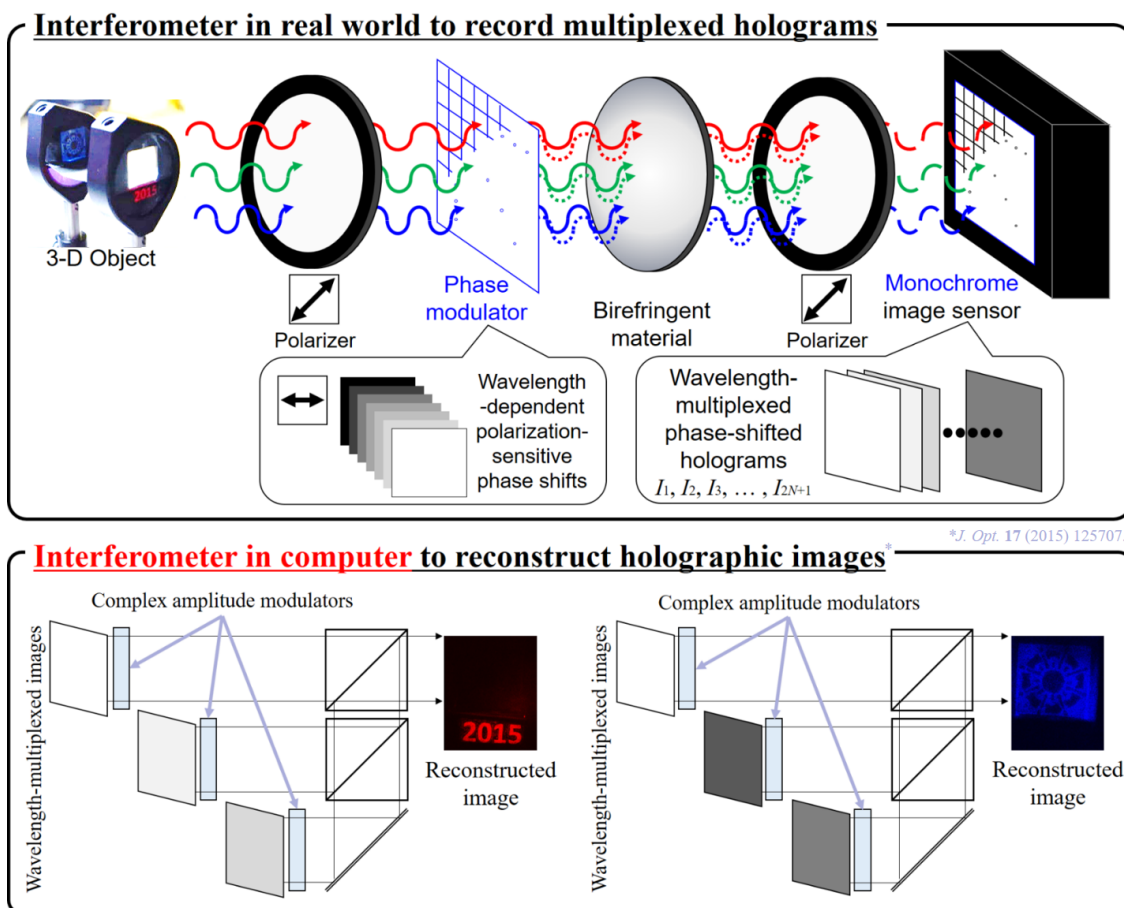


Figure 3 Recording and reconstruction parts of CCS.

**Optical components and constructed microscope**

The focus of this development is the single-shot recording of multiple incoherent holograms required for CCS. Instead of using a phase modulator, we have used a wavelength-dependent phase-modulation array and space-division multiplexing of holograms. We have developed a single-shot self-interference color-multiplexed incoherent digital holography system exploiting CCS.

Figure 4 shows the optical components required for the system. A polarizer and birefringent materials generate two light waves to form a wavelength-multiplexed hologram of objects/specimens. The specially designed and developed image sensor records multiple wavelength-multiplexed phase-shifted holograms simultaneously with a wavelength-dependent phase-modulation array on the basis of space-division multiplexing of holograms. Figure 5 shows a photograph and schematic of the constructed instantaneous color-multiplexed fluorescence holographic microscope based on CCS. The microscope consists of a commercially available inverted optical microscope and the incoherent digital holography system shown in Fig. 4. Color-multiplexed fluorescence light illuminates the incoherent digital holography system without wavelength division. The developed monochrome image sensor records color-multiplexed holograms simultaneously with a single-shot exposure. The temporal resolution and frame rate of color 3D imaging are respectively determined by the exposure time and the frame rate of the specially designed and developed image sensor.

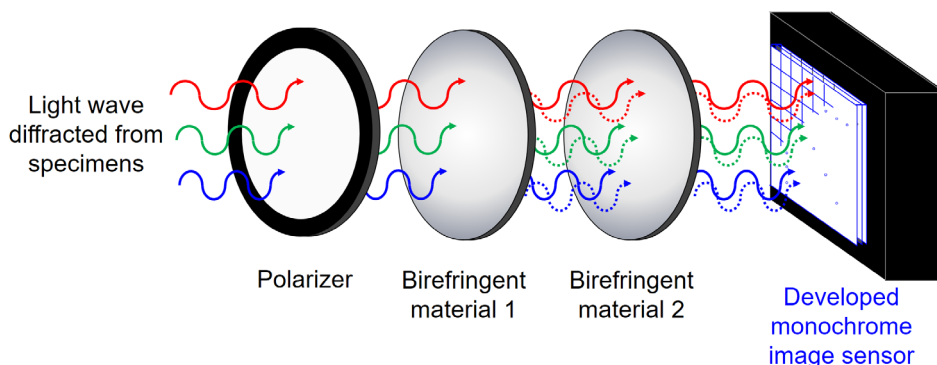


Figure 4 Schematic of single-shot digital holography system exploiting CCS for sensing a 3D space illuminated by a white-light lamp and a self-luminous 3D object. (a) Optical components and (b) composition of the developed image sensor.

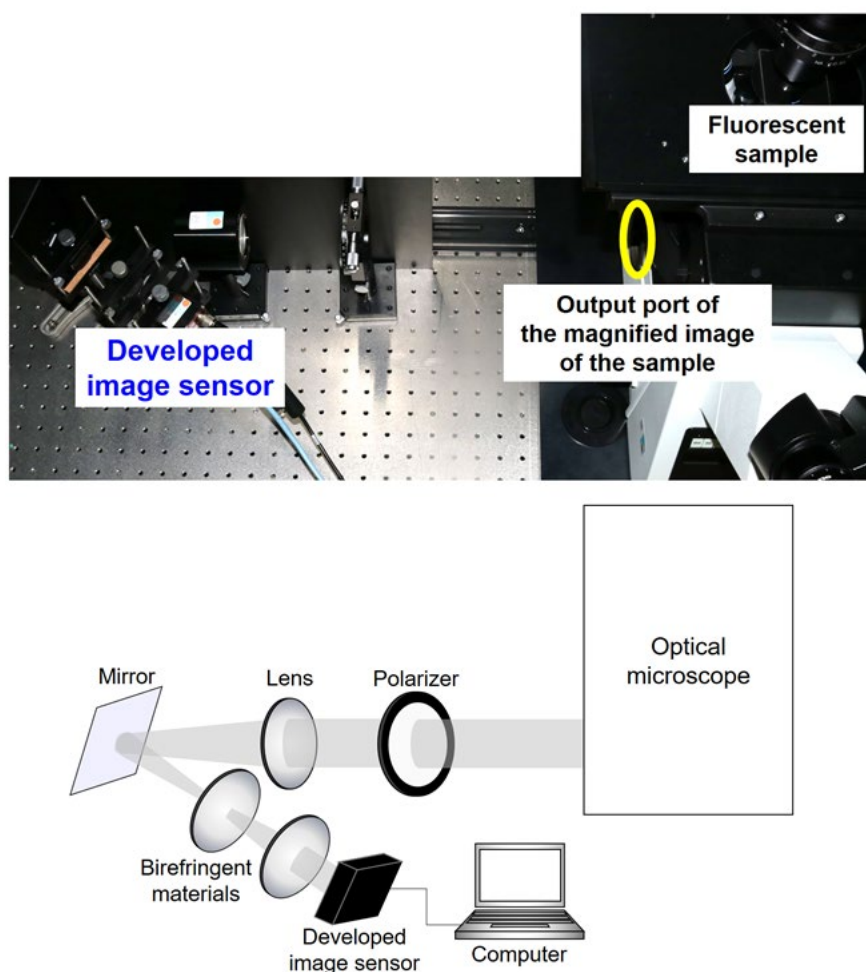


Figure 5 Photograph and schematic of the constructed instantaneous color-multiplexed fluorescence holographic microscope based on CCS.