funakoshi
FRONTIERS IN LIFE SCIENCE

9-7 Hongo 2-Chome, Bunkyo-Ku
Tokyo 113-0033, Japan

HistoBright <Tissue Structure Fluorescent Dye>

Catalog NO. FDV-0051

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Product Background

Tissue structures are historically visualized by staining with various colorimetric dyes such as Hematoxylin and Eosin (HE) stains. These conventional colorimetric stains generally require thinly sliced tissue sections and are unsuitable for thick tissue blocks. However, the preparation of thinly sliced tissues loses the 3D structural information. To reconstitute 3D information, a lot of additional thin slices and stainings are required. HistoBright (original compound name; PC in Ref.1) is originally synthesized by Dr. Yosuke Niko, Kochi University, and Dr. Takeshi Imamura, Dr. Masamoto Murakami, and Dr. Ryosuke Kawakami, Ehime University, promotes its biological 3D imaging application. HistoBright is a novel solvatochromic fluorescent dye that changes fluorescent colors in response to solvents or microenvironments and has a high affinity to various biological membranes. The combination of two features of HistoBright contributes to clear and bright imaging of tissue structures.

As HistoBright can be excited by not only one-photon but also two-photon, HistoBright is compatible with two-photon microscopy and useful for tissue imaging. Furthermore, HistoBright, coupled with several tissue-clearing methods such as LUCID and RapiClear, which do not require lipid removal, contributes to analyzing 3D deep tissue imaging of thick tissue slices or blocks, etc., without breaking pathophysiological tissue structures. HistoBright is an innovative fluorescent dye for the visualization of 3D tissue structures.

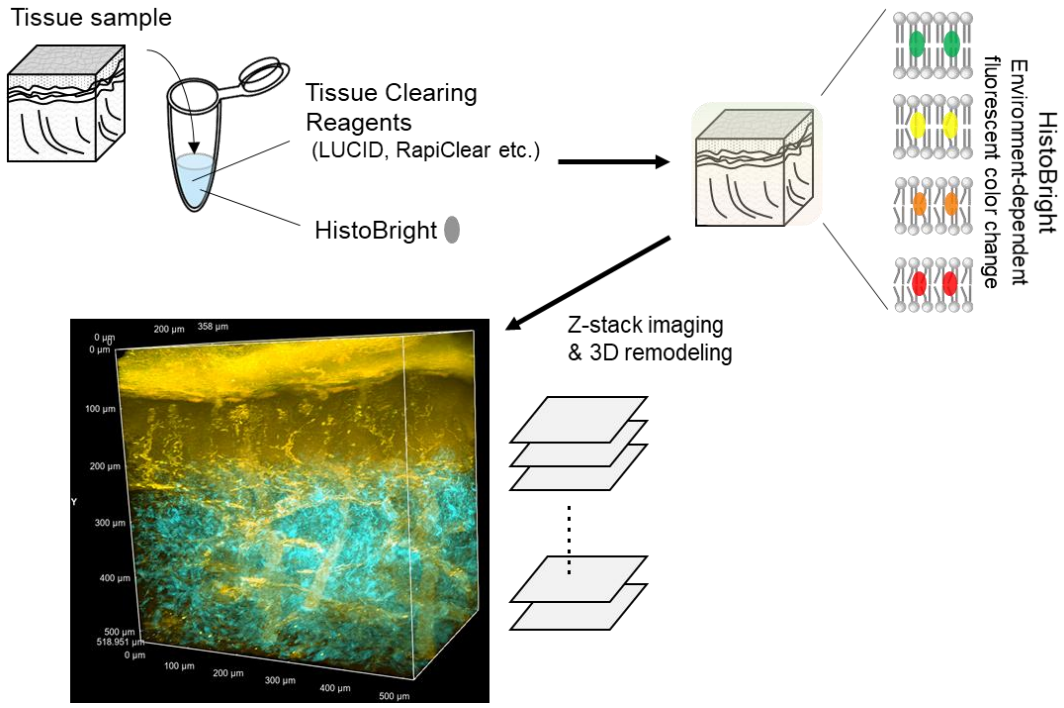


Figure 1 Overview of HistoBright staining

Description

Catalog Number: FDV-0051
Size: 0.1 mg
Formulation: $C_{27}H_{27}NO$
Molecular weight: 381.5 g/mol
Solubility: Soluble in DMSO
Fluorescent characteristics:
Ex. 350-500 nm (maximum ~410 nm)
Compatible with 405-488 nm laser (confocal microscopy), 960-1100 nm laser (two-photon microscopy)
Em. 500-800 nm (depending on solvents)

Reconstitution and Storage

Reconstitution: Stock solution recommended concentration 1 mM in 100% DMSO.
Storage (powder): Store powder at -20°C.
Storage (solution): After reconstitution in DMSO, aliquot and store at -20°C.
Avoid repeated freeze-thaw cycles.

How to use

General procedure for tissue staining

*This procedure is an example of HistoBright

1. Tissue samples were excised and then fixed in freshly prepared paraformaldehyde.
2. Tissue blocks or thick slices prepared by any appropriate methods.

NOTE: As HistoBright is a membrane staining dye, any de-lipid procedures including organic solvents or high concetration of detergents such as Triton X100, Tween 20 etc. do not recommened. If you need to use detergents for permealization etc., please optimize experimental condition.

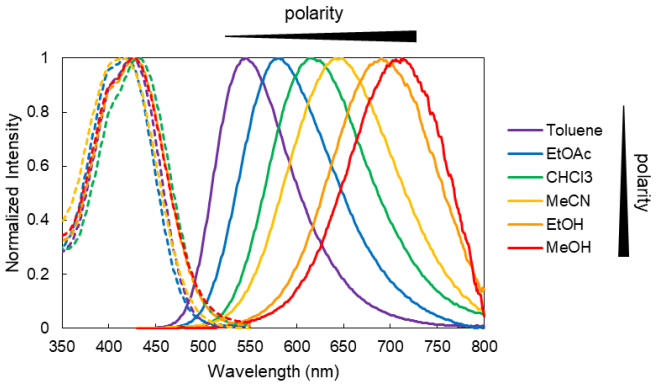
3. Stock solution of HistoBright (1 mM) was mixed in tissue clearing solution (LUCID or RapiClear etc.) to prepare the staining and clearing solution (hereafter called Master mix). The initial recommendation of HistoBright concentration is 10 μM.
4. Immerse tissue blocks/sections in the Master mix for over 48 hours at room temperature.
5. After staining tissue blocks/sections, image by confocal laser microscopy or two-photon microscopy.

NOTE: Empirically optimize staining conditions (tissue sample size, pre-treatment of tissues, the concentration of HistoBright, clearing methods, staining time, etc.) for your experiments.

Reference data

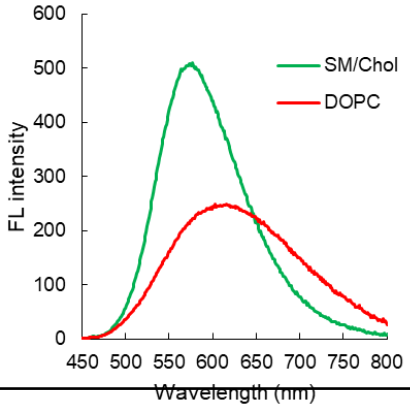
Solvatochromic property of HistoBright

Absorbance and fluorescent spectrum of HistoBright in various solvents. While the absorbance spectrum of HistoBright is not sensitive to solvent polarity, HistoBright changes fluorescent color from green to far-red depending on solvent polarity. Under a low polarity environment, HistoBright emits green color. On the other hand, under a high polarity environment, HistoBright emits red to far-red with low quantum yield. Quantum yields are 0.82 (Toluene), 0.77 (EtOAc), 0.83 ($CHCl_3$), 0.65 (MeCN), 0.20 (EtOH), and 0.07 (MeOH).



Fluorescent spectrum on a liposome membrane model

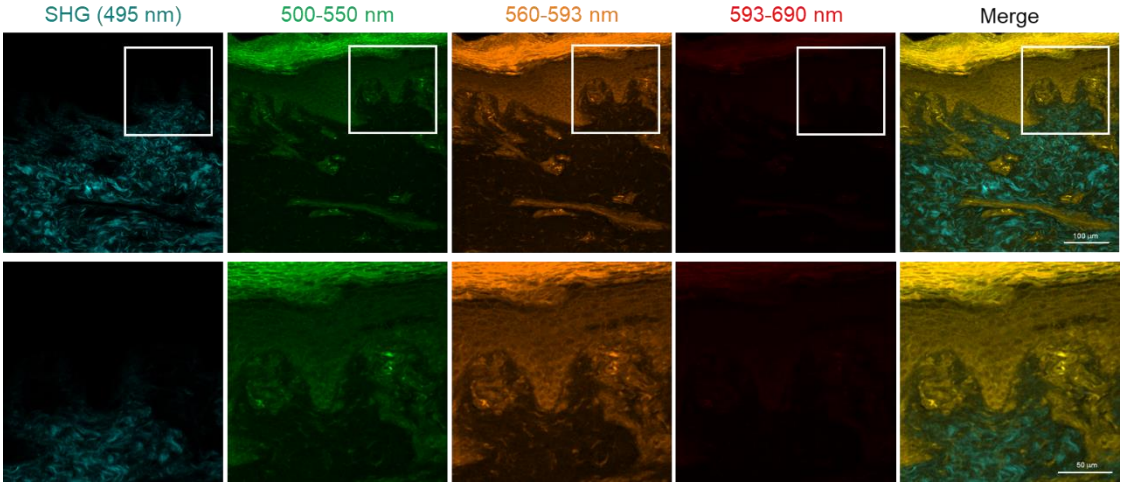
HistoBright rapidly accumulates in lipid membranes and emits different fluorescent colors in response to the membrane environments. On the liquid order (Lo) model liposome (sphingomyelin (SM)/cholesterol (Chol)), HistoBright emits a maximum of around 575 nm fluorescence with a high quantum yield value (0.72). On the other hand, HistoBright on the liquid disorder (Ld) model liposome (DOPC) exhibits significantly broad and relatively weak fluorescence (quantum yield 0.37), and the maximum wavelength is red-shifted by approximately 30 nm compared to the Lo model.



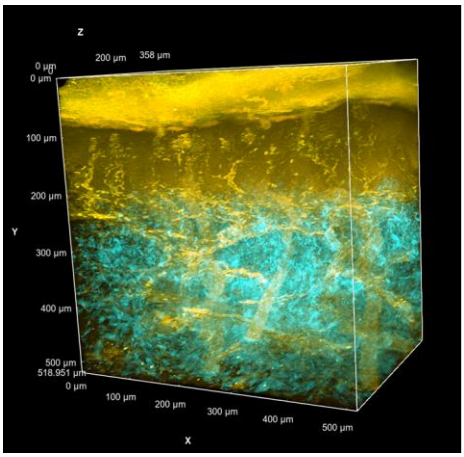
Application data

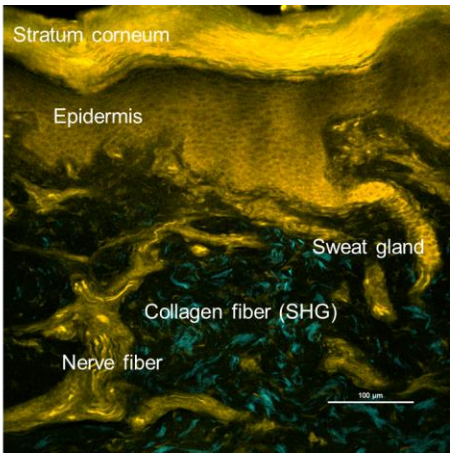
Two-photon fluorescence microscopy imaging of human normal skin tissue

After fixation of human skin tissue blocks with 4% paraformaldehyde/PBS, 500 µm thick slides were prepared and treated with the LUCID clearing reagent and 10 µM HistoBright for 76 hours. The tissue slides were observed by two-photon fluorescence microscopy (Ex. 960 nm, Em. cyan channel: 492 nm as SHG, green channel: 500-550 nm, orange channel: 560-593 nm, and red channel: 593-690 nm). The merged image was obtained from four channels.



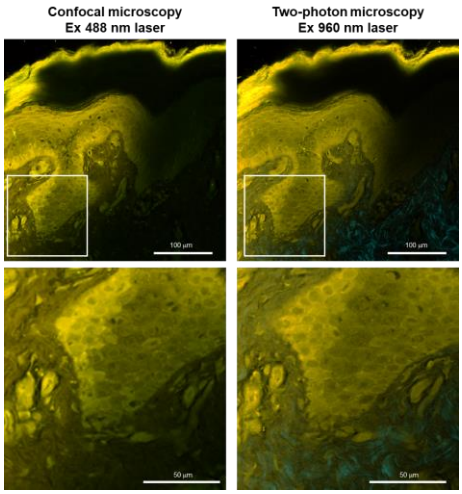
3D images were reconstructed from Z-stack sequences (step size, 5 µm) of 2D images.





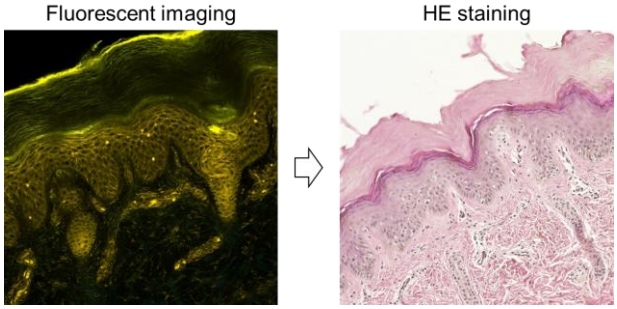
Comparison between confocal microscopy and two-photon microscopy imaging

After fixation of human skin tissue blocks with 4% paraformaldehyde/PBS, 500 µm thick slides were prepared and treated with the RapiClear clearing reagent and 10 µM HistoBright for 72 hours without any permealization treatments. Tissues were observed by confocal microscopy (488 nm laser) and two-photon microscopy (960 nm laser).



HE staining after HistoBright fluorescent imaging

After fluorescent imaging, the stained tissue block was washed with PBS. Thin tissue slices were prepared from the tissue block and re-stained with the conventional HE staining procedure.



Notes

All spectrum data were obtained by Dr. Yosuke Niko, Kochi University.

All cell imaging data were obtained by Dr. Ryosuke Kawakami, Ehime University.

Reference

1. Inoue *et al.,J. Mater. Chem. B*, 10, 1641-1649 (2022) Synthesis and photophysical properties of a new push-pull pyrene dye with green-to-far-red emission and its application to human cellular and skin tissue imaging.