Macro Photography with Lightsheet Illumination Enables Whole Expanded Brain Imaging with Single-cell Resolution

Chia-Ming Lee1, #, Xuejiao Tian1, 2, #, Chieh Tsao1, #, Peilin Chen1, Tzyy-Nan Huang3, Yi-Ping Hsueh3, Bi-Chang Chen1, 2, 4, * # Corresponding authors: Bi-Chang Chen, Research Center for Applied Sciences, Academia Sinica, 128 Sec. 2, Academia Rd., Nankang, Taipei 11529, Taiwan; Phone: 886-2-27873133; Fax: 886-2-27873122; Email: chenb10@gate.sinica.edu.tw

ABSTRACT

Macro photography allows direct visualization of the enlarged whole mouse brain by a combination of lightsheet illumination and expansion microscopy with single-cell resolution. Taking advantage of the long working distance of a camera lens, we imaged a 3.7 cm thick, transparent, fluorescently-labeled expanded brain. In order to improve 3D sectioning capability, we used lightsheet excitation confined as the depth of field of the camera lens. Using 4x sample expansion and 5x optical magnification, macro photography enables imaging of expanded whole mouse brain with an effective resolution of 300 nm, which provides the subcellular structural information at the organ level.

Keywords
Macro photography, expansion microscopy, lightsheet microscopy, tissue clearing, whole-brain imaging.

Volumetric imaging of whole organ structures at subcellular resolution helps scientists to understand the relationship between cell composition and organ function. Therefore, it is desirable to develop a microscopic tool to image large tissue samples with subcellular resolution. With recent advances in hydrogel-based tissue-clearing and tissue-expansion methods, it is now possible to obtain super-resolution images of tissue samples with sizes of hundreds of microns through conventional diffraction-limited microscopic techniques, such as lightsheet fluorescence microscope - a rapid volumetric technique with low photobleaching1-5, where microscopic objectives are used. In order to obtain brain-wide cellular distributions in an expanded brain6 with thickness of several centimeters, macro photography, a common close-up photography feature in the camera, is integrated with lightsheet illumination to image the whole brain with good optical sectioning capabilities.

To visualize mouse brain structure, mice expressing nuclear tdTomato by crossing Ai75D mice and E2a-Cre mice (Jackson Laboratory, JAX stock #025106 and #003724, respectively) were used in this experiment. Mouse brain was fixed and collected at postnatal Day 14 (P14) (Figure 1A, inset). Through a combination of the passive CLARITY7 clearing method and a swellable...
hydrogel formulation\textsuperscript{8}, we successfully expanded linearly the whole mouse brain by \~4.5-fold (\~100-fold volumetric expansion) (Figure 1A), where the sample volume was expanded from $1.25 \times 1 \times 0.6$ cm$^3$ to $5 \times 3.75 \times 2.4$ cm$^3$. Without mechanical sectioning, it is very challenging to image an expanded whole brain at single-cell resolution due to the limited working distance of the detection objective. To overcome this problem, we used a camera lens with sufficient working distance (~36 mm in the air) to allow optical sectioning via lightsheet illumination\textsuperscript{9,10} where a virtual thin lightsheet was generated by scanning a laser beam through a TL2X-SAP objective (Thorlabs, USA).
The wide-field fluorescence images were captured through a reverse-mounted Single-lens Reflex Camera lens (SLR lens; EF 50 mm f/1.2L USM Canon Inc., Japan) with a 112 - 7 mm² field-of-view at 1.25X to 5X magnification using a 4-megapixel sCMOS camera (ORCA-Flash4.0 V2, Hamamatsu, Japan) (Figure 1B). To image the whole intact brain, a glass chamber containing the expanded mouse brain with hydrogel was translated via a triple-axis stage. To facilitate high-magnification imaging, reverse-lens macro photography was used. With this setup, we were able to reconstruct the expanded whole brain in 3D in which all nuclei were labeled with red fluorescent tdTomato protein (Figure 1C), encompassing ~1 million 2K×2K images at a voxel size of ~1.3 x 1.3 x 5 μm. The effective resolution for an expanded mouse brain in our system is ~0.3 x 0.3 x 1 μm due to the ~4-fold expansion ratio. In Figure 1C, the green box represents a sub-volume along the axial direction of the camera lens, where individual cells can be resolved as shown in the white box in the inset. The selected optical slices of the expanded mouse brain are shown in Figure 1D, with an interval of 700 μm between contiguous sections. The zoomed-in image of the red box in Figure 1D of the hippocampus area is shown in Figure 1E, where a single-cell resolution was achieved laterally (blue box in Figure 1E) by macro photography with lightsheet illumination.

In summary, we have constructed a macro photographic system integrated with lightsheet illumination to reveal the three-dimensional cellular distribution in the whole brain with an effective resolution of 300 nm, where the samples were cleared and expanded.

Acknowledgements
This work was supported by Academia Sinica’s Career Development Award (CDA-107-M08) and Ministry of Science and Technology (109-2628-M-001 -001 -MY4) to B.C.C., Academia Sinica’s Thematic Research Project (AS-110-TP-L10) to Y.P.H. and the Higher Education Sprout Project. We thank Dr. John O’Brien for the English editing.

Conflict of Interest
The authors declare no competing interests.

References
1. Ueda HR, Dodt H-U, Osten P, Economo MN, et al. Whole-brain profiling of cells and circuits in mammals by tissue clearing and light-sheet microscopy. Neuron. 2020 May 6; 106(3):369-387. doi: 10.1016/j.neuron.2020.03.004
2. Zhao Y, Bucur O, Irshad H, et al. Nanoscale imaging of clinical specimens using pathology-optimized expansion microscopy. Nat Biotechnol. 2017 Aug;35(8):757-764. doi: 10.1038/nbt.3892
3. Bucur O, Fu F, Calderon M, et al. Nanoscale imaging of clinical specimens using conventional and rapid-expansion pathology. Nat Protoc. 2020 May;15(5):1649-1672. doi:10.1038/s41596-020-0300-1
4. Gallagher B, Zhao Y. Nanoscale Imaging of Synaptic Connections with Expansion Microscopy. Discoveries. 2019 Sep 30;7(3):e101. doi: 10.15190/d.2019.14
5. Tillberg PW, Chen F, Piatkevich KD, et al. Protein-retention expansion microscopy of cells and tissues labeled using standard fluorescent proteins and antibodies. Nat Biotechnol. 2016 Sep;34(9):987-92. doi: 10.1038/nbt.3625
6. Ku T, Swaney J, Park JY, et al. Multiplexed and scalable super-resolution imaging of three-dimensional protein localization in size-adjustable tissues. Nat Biotechnol. 2016 Sep; 34(9):973-81. doi: 10.1038/nbt.3641
7. Chung K, Wallace J, Kim SY, et al. Structural and molecular interrogation of intact biological systems. Nature. 2013 May 16; 497(7449):332-7. doi:10.1038/nature12107.
8. Chen F, Tillberg PW, Boyden ES. Expansion Microscopy. Science. 2015 Jan 30; 347(6221):543-8. doi:10.1126/science.1260088
9. Ratzlaff EH, Grinvald A. A tandem-lens epifluorescence macroscope: Hundred-fold brightness advantage for wide-field imaging. J Neurosci Methods. 1991; 36(2–3):127–137. doi:10.1016/0165-0270(91)90038-2
10. Lu CH, Tang WC, Liu YT, et al. Lightsheet localization microscopy enables fast, large-scale, and three-dimensional super-resolution imaging. Commun Biol. 2019 May 9; 2, 177. doi:10.1038/s42003-019-0403-9

This article is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited and it is not used for commercial purposes; 2021, Lee CM et al., Applied Systems and Discoveries Journals.