シンポジウム

# [3S02m] 神経回路の多階層イメージングの将来

オーガナイザー：今吉 格（京都大学）、橋本 均（大阪大学 大学院薬学研究科）
2021年7月30日(金) 09:00 ～ 11:00 第2会場 (神戸国際会議場3F国際会議室)
Zoomはこちら

[3S02m-Introduction] Introduction
09:00 ～ 09:02

[3S02m-01] ゼブラフィッシュ前視蓋におけるオプティックフロー情報処理回路の機能と神経接続
Fabian Svara[2]、Winfried Denk[2]、Herwig Baier[2]、○久保 郁[1]（1.国立遺伝学研究所、2.マックスプランク研究所）
09:02 ～ 09:20

[3S02m-02] 相関顕微鏡技術による生体内ニューロンの微細構造観察
○平林 祐介[1]、柴山 光耀[1]、菅 翔吾[1]（1.東京大学大学院工学系研究科）
09:20 ～ 09:38

[3S02m-03] 神経回路自動再構築のための超多色標識法の開発
○今井 猛[1]（1.九州大学大学院医学研究院）
09:38 ～ 09:56

[3S02m-04] Whole-brain mapping with high-speed VISoR imaging: from mouse to monkey
○Guoqiang Bi[1,2]（1.University of Science and Technology of China, Hefei, China、2.Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, China）
09:56 ～ 10:21

[3S02m-05] 多細胞システムの理解に向けたセルオミクス技術の開発と応用
○洲崎 悦生[1]（1.順天堂大学大学院医学研究科）
10:21 ～ 10:39

[3S02m-06] 高精細全脳活動マッピングと活動依存的な全脳回路構造
○笠井 淳司[1]、勢力 薫[1,2]、橋本 均[1,3,4,5]（1.大阪大学大学院薬学研究科、2.大阪大学国際共創大学院、3.大阪大学データビリティフロンティア機構、4.大阪大学先導的学際研究機構、5.大阪大学大学院連合小児発達学研究科）
10:39 ～ 10:57

[3S02m-Closing] Closing Remarks
10:57 ～ 11:00

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## [3S02m-Introduction] Introduction

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## [3S02m-01] ゼブラフィッシュ前視蓋におけるオプティックフロー情報処理回路の機能と神経接続

Fabian Svara[2]、Winfried Denk[2]、Herwig Baier[2]、○久保 郁[1] (1.国立遺伝学研究所、2.マックスプランク研究所)

キーワード：Calcium imaging, visual processing, zebrafish, EM reconstruction

The pretectal area is responsible for processing visual motion information to drive compensatory eye and body movements in response to optic flow. Recent functional imaging studies identified a diverse array of functional neuron types that process optic flow information in the zebrafish pretectum, ranging from "simple" monocular and direction-selective cells to "complex" binocular cells that, on the other hand, respond selectively to either translational or rotational motion. These findings predicted a wiring diagram that could explain the underlying circuit for the binocular optic flow processing. However, this hypothesized connectivity of the pretecal circuit has not been experimentally verified. To test these anatomical predictions made based on the circuit model, we first established a novel optogenetic method, combining functional characterization with morphological analysis of single cells. In FuGiMA (Function-guided inducible morphological analysis), a cell of a specific response profile is labeled by activation of photoactivatable GFP, thus revealing its morphology. Our data show that cells of the same response profile tend to share a similar projection pattern. Furthermore, we combined the functional imaging with a connectivity analysis by electron microscopy (EM). Using a whole-brain EM stack of larval zebrafish from which we recorded the responses of pretectal neurons, we have reconstructed about 200 optic flow responsive cells and putative direction-selective retinal ganglion cell (RGC) axons. We are currently in the process of determining the connectivity of pretectal neurons as well as the RGC-pretectal circuit by their direct synaptic connections. A combination of the two complementary approaches will directly test and refine our circuit model for the binocular optic flow computation in the pretectum.

Research funds: KAKENHI JP 17K20147

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## [3S02m-02] 相関顕微鏡技術による生体内ニューロンの微細構造観察

○平林 祐介[1]、柴山 光耀[1]、菅 翔吾[1] (1.東京大学大学院工学系研究科)

キーワード：Light-Electron Correlative Microscopy

Revealing the intracellular ultrastructural features of neurons, which give rise to the diverse intrinsic properties of neurons, is critical for understanding the molecular basis for the neural computation (Hirabayashi et al., *Science,* 2017). However, it has been challenging to connect the synaptic connections

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within neural circuits and the intracellular ultrastructural features over scales spanning several orders of magnitude (nanometers to meters). Although the recent development of serial section electron microscopy (SSEM) technologies allowed us to obtain large volume detailed structures of the brain, several major roadblocks have impaired its general applicability to the study of mammalian neural circuits.
In the present study, we introduce new approaches that circumvent some of these roadblocks. First, we adapted a genetically-encoded ascorbate peroxidase (APEX2) as a fusion protein to a nuclear-targeted fluorescent reporter for labeling neurons of interest both for the light and electron microscopy. This approach allows us to perform Correlated Light-SSEM (CoLSSEM), a variant of Correlated Light-EM (CLEM), on individual neurons, reconstructing their ultrastructures (Hirabayashi et al., *Scientific Reports,* 2018). Second, to circumvent the highly labor intensive segmentation of serial electron microscopy images, we introduced deep learning-based automatic segmentation of the intracellular structure. We developed a new tool called Philis (Python-based human-in-the-loop iterative segmentation platform) for integrating the steps required for deep learning-based segmentation. Armed with these new tools, we will investigate the structural bases of neuronal functions.

Research funds: AMED JP20dm0207082

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## [3S02m-03] 神経回路自動再構築のための超多色標識法の開発

○今井 猛[1] (1.九州大学大学院医学研究院)

キーワード：connectomics, fluorescence imaging

Stochastic multicolor labeling is a powerful strategy to dissect and reconstruct densely labeled neuronal circuits. We previously developed a stochastic multicolor labeling method with enhanced expression levels that uses a tetracycline-operator system (Tetbow) (Sakaguchi et al., *eLife*, 2018). In Tetbow, three fluorescent protein (XFP) genes were expressed stochastically in neurons to generate various color hues. We optimized Tetbow for either plasmid or virus vector-mediated multicolor labeling. When combined with the tissue clearing method, SeeDB2, Tetbow was powerful enough to visualize the three-dimensional architecture of individual neurons. For example, we were able to visualize the axonal projection patterns of individual mitral and tufted cells along several millimeters in the mouse olfactory system. However, the number of color hues generated by the combination of three XFPs was limited. Therefore, we still had to trace the multicolor-labeled neurites manually, which was the rate-limiting step in large-scale circuit reconstructions. Here we developed the "super-multicolor labeling" method, in which the number of possible color combinations was massively expanded. As trichromatic human eyes can only recognize the combination of red, green, and blue, we also developed a fully automated pipeline for the quantitative analysis of the color combinations generated by the super-multicolor labeling. Using this strategy, we have successfully reconstructed neuronal circuits in 3D "without manual neurite tracing". Our strategy should facilitate fully automated light microscopy-based connectomics.

Research funds: JP20dm0207055h

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## [3S02m-04] Whole-brain mapping with high-speed VISoR imaging: from mouse to monkey

〇Guoqiang Bi[1,2] (1.University of Science and Technology of China, Hefei, China、2.Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, China)

キーワード：Mesoscopic brain mapping, 3D imaging, mouse, rhesus macaque

Whole-brain mesoscale mapping of rodents has provided crucial information regarding the wiring and function of the brain. Similar mapping for the primate brain has been hindered by large brain size and the relatively low throughput of available microscopy methods. We have recently developed an integrative approach that combines tissue sectioning and clearing with ultrahigh-speed, large-scale, volumetric fluorescence microscopy technique that we termed VISoR, capable of completing whole-brain imaging of a mouse at 1 μ m × 1 μ m × 2.5 μ m voxel resolution within 1 hour, and rhesus monkey within 100 hours. For the monkey brain, we have also developed a progressive strategy for high-efficiency, long-range tracing of individual axonal fibers through the petabyte-sized dataset. The VISoR technique thus supports effective connectomic-scale brain mapping of large primates and reveals distinct features of thalamocortical projections of the rhesus brain at the level of individual axonal fibers.

Research funds: Strategic Priority Research Program of Chinese Academy of Science (XDB32030200), the National Natural Science Foundation of China (91732304)

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## [3S02m-05] 多細胞システムの理解に向けたセルオミクス技術の開発と応用

〇洲崎 悦生[1] (1.順天堂大学大学院医学研究科)

キーワード：Cell omics, Tissue clearing, 3D imaging, 3D staining

The recent advent of various tissue clearing and three-dimensional (3D) imaging methods allows comprehensive observation of the whole organ/body with cellular resolution or more. This presentation will introduce the basics of our CUBIC framework for conducting cell-omics analysis and the latest CUBIC-HistoVIsion method for versatile whole-organ/body staining and imaging [1-3]. In the long history of histology, whole-organ/body 3D staining and imaging have been challenging due to the difficulty of adequate penetration of stains and antibodies. Even a small dye occasionally exhibits resistance to penetration, implying a complex physicochemical environment in the staining system. To dissect the complex physicochemical environment, we first conducted a precise characterization of biological tissue as an electrolyte gel. Then, we experimentally evaluated a broad range of 3D-staining conditions by using a simplified tissue-mimicking artificial electrolyte gel. The combination of essential conditions allowed a bottom-up design of an efficient 3D-staining protocol that could uniformly label adult whole mouse brains, an adult marmoset hemisphere, a ~1 cm3 tissue block of adult human postmortem cerebellum, and an infant whole marmoset body with dozens of antibodies and cell-impermeant nucleic acid stains. We also demonstrate that our protocol enabled structural and functional neural circuit identification and analysis with Rabies virus tracing and whole-brain c-Fos immunostaining. The CUBIC and CUBIC-HistoVIsion offer advanced opportunities for organ- and organism-scale histological analysis of multicellular systems in the brain and body.

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Reference:

1. Susaki et al. Cell 157: 726-739 (2014)

2. Susaki et al. Nature Protocols 10: 1709-1727 (2015)

3. Susaki et al. Nature Communications 11: 1982 (2020)

Research funds: KAKENHI JP (19H03413, 17H06328), AMED-PRIME (JP21gm6210027), Grant-in-aid from Takeda science foundation

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## [3S02m-06] 高精細全脳活動マッピングと活動依存的な全脳回路構造

○笠井 淳司[1]、勢力 薫[1,2]、橋本 均[1,3,4,5] (1.大阪大学大学院薬学研究科、2.大阪大学国際共創大学院、3.大阪大学データビリティフロンティア機構、4.大阪大学先導的学際研究機構、5.大阪大学大学院連合小児発達学研究科)
キーワード：whole brain imaging , activation mapping, activity-dependent labeling, stress

Brain functions are controlled by the complicated networks consisting of tens millions of brain cells, even in mice. In order to understand how the brain functions, we need to know the structural and functional basis of all networks. To this end, subcellular resolution imaging of the whole brain and precise activity-dependent labeling techniques are extremely useful tools. We have developed a high-speed imaging apparatus based on automated mechanical sectioning and confocal imaging, named FAST (block-FAce Serial microscopy Tomography) (Seiriki, Kasai et al, Neuron, 2017; Seiriki, et al, Nat Protoc, 2019). FAST acquires submicrometer-resolution images of a whole mouse brain in 2.4 hours. Then, we investigated the whole-brain neuronal activation patterns following acute stressors using an Arc-dVenus mouse that is a transgenic immediate early gene reporter mouse to visualize the neuronal activation history. Hypothesis-free analysis using machine learning of brain-wide activation mapping revealed a population of neurons that is previously overlooked due to a small brain area, which is important for stress responses. In addition, combining targeted recombination in active populations mouse by expressing an activity-dependent and tamoxifen-inducible iCreER$^{T2}$ recombinase with an adeno-associated virus vector that is retrogradely transported and expressed fluorescent protein in the soma of presynaptic neurons in a Cre-dependent manner, we identified neural circuit structures of the entire brain that inputs to the small brain area related to stress response. Thus, latest genetic labeling techniques and whole-brain imaging system provide new opportunities for global approaches to gain a better understanding of cellular and circuit-level brain systems.

Research funds: KAKENHI JP20H00492, JP20H03391, JP20H05065, JP19H05218, JP18H05416

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