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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Micro-scale, in-vivo structural and functional tomography of rodent habenula using polarization-sensitive optical coherence tomography

研究課題名（英文）Micro-scale, in-vivo structural and functional tomography of rodent habenula using polarization-sensitive optical coherence tomography

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,100,000円

研究成果の概要（和文）：Habenula is a tiny anatomical structure that links the forebrain to the midbrain which regulates pathways associated with a range of behaviors. The proposed research aimed at developing an imaging tool for volumetric assessment of small brain structures like habenula with high resolution.

研究成果の学術的意義や社会的意義
The proposed research developed a cost-effective 3-dimensional (3D) imaging tool that allows for high-throughput and high resolution imaging of brain structures. Serial imaging allows for easy 3D reconstruction for macroscopic quantitative analysis based on the high resolution brain imaging data.

研究成果の概要（英文）：Habenula is a tiny anatomical structure that links the forebrain to the midbrain which regulates pathways associated with a range of behaviors including reproductive behaviors, central pain processing, nutrition, sleep-wake cycles, stress responses, and learning with lateral and medial habenula showing differences in connectivity and function.
The proposed research aimed at developing an imaging tool for volumetric assessment of small brain structures like habenula with high resolution.

研究分野：Biomedical Engineering

キーワード：Optical Imaging

１．研究開始当初の背景

Habenula is a tiny anatomical structure that links the forebrain to the midbrain and acts as a critical neuroanatomical hub that connects and regulates pathways associated with a range of behaviors including reproductive behaviors, central pain processing, nutrition, sleep-wake cycles, stress responses, and learning with lateral and medial habenula showing differences in connectivity and function. Habenula is a small anatomical region but has heterogeneous substructure implicated in the psychiatric disorder of depression and addiction, etc. There are still open questions related to whether structural changes in habenula relate to functional seen in depressed subjects. Both human studies using magnetic resonance imaging and animal studies using histology and light microscopy have been inconclusive in terms of the heterogeneous changes seen in the habenular substructures and warrant more studies on micro-macro volumetric analysis.

The proposed research aimed at developing a deep tissue optical imaging tool based on polarization sensitive-optical coherence tomography (PS-OCT) for small animal brain imaging in-vivo. The focus was to develop an imaging tool for volumetric assessment of small brain structures like habenula with high resolution. As mentioned in the title the initial plan was to develop 1700 nm wavelength PS-OCT set up that provides only endogenous contrast but multi-contrast including birefringence (myelination), vasculature, cytoarchitecture to study rodent brains. However, due to initial technical difficulties in designing the PS-OCT system due to low light coupling efficiency, the focus was directed towards taking an alternative approach to develop a different optical tool towards which would provide better cellular resolution than existing technology for 1700 nm light source currently available for OCT. Moreover, unlike OCT, this tool is a fluorescence microscope that uses exogenous contrast agents like fluorescent probes with multitude of information.

２．研究の目的

Cell density is a fundamental information towards understanding the brain organization and function including the role of specific cell types in the specific brain regions and their associations in pathological situations. Imaging deeper brain structures and whole brain often involves optical clearing and whole tissue staining to allow for light to penetrate deeper layers. Moreover, optical sectioning ability that allows for reducing out of focus signal is required for imaging through thick block of tissue sample. This has been achieved through confocal, light sheet, two-photon microscope, etc, which are complex and expensive. Conventional epi-fluorescence microscopy design requires thin sectioning of tissue samples and do not provide sharp contrast on whole tissue block imaging. 3-dimensional reconstruction based on imaging of single thin-sectioned and mounted samples are prone to artefacts that do not allow for ease of reconstruction. In this research project, development of a novel fluorescence microscope that provides for optical sectioning ability on epi-fluorescence design was aimed at. The use of deep ultraviolet light for fluorescence imaging allows for surface excitation of fluorescent dyes. Hence, choice of shorter wavelength allows for block-face imaging using epi-fluorescence design of the microscope. Combining this microscope with a tissue slicer allows for easy registration for 3-dimensional reconstruction. Thus, a simpler and cost-effective optical design for optical imaging was developed that has the potential for high throughput analysis, especially applicable for behavioral studies and brain anatomy quantification to study the structural -functional associations.

３．研究の方法

This novel fluorescence microscope uses deep ultraviolet light (DUV) in the range of 270-290 nm to image the surface of the sample. Moving to the shorter wavelength provided good resolution than conventional wavelength used and allowed for block-face imaging on thick block of tissue samples owing to the lower depth penetration of the light into the biological sample. DUV light excites most of the conventional dyes with long Stokes shift and thus a simplified and easy to set up 3-dimensional (3D) fluorescence microscope that can be utilized for large-scale deployment in cost-effective manner was designed as part of this research project.

The research involved technical design including designing the optical, mechanical and software interface to allow for automated serial block-face imaging. High resolution wide-field imaging set up using custom designed microcontroller board-controlled translation stage and tissue slicer for serial sectioning in a side illumination epi-fluorescence design was

adopted for the 3D-microscopy design. Towards developing the project as open-source microscopy, custom written software using Python was used for controlling the microscope. Figure 1 highlights the overall design interface used for the microscope design and Figure 2 highlights the habenula imaging using 3D-DUV microscope for volumetric assessment. Towards development of framework to openly publish brain imaging data with tools to semantically link the information, a conceptual design was also proposed. As a future extension of this project, we are planning on using this 3D microscope for volumetric analysis of habenula in depression models of mouse as well as release the tool as an open-source project for benefit of the larger research community.

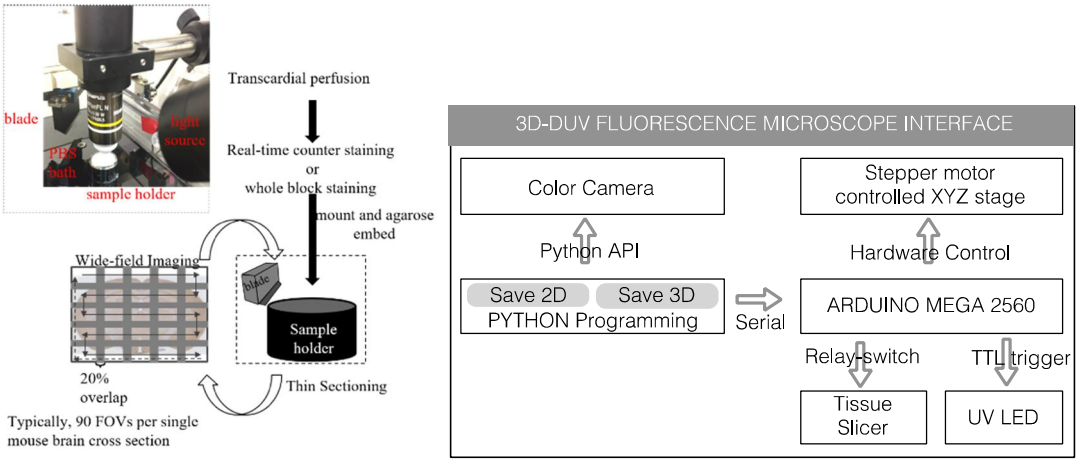

Figure1: A schematic of the 3D-DUV microscope imaging flow and the overall microscope hardware and software interface design [Kasaragod et al., BioRxiv 2020]

4．研究成果

A manuscript showcasing the various 2D imaging applications in brain imaging using this novel microscope was published on the preprint server. It also included prototypical fluorescence microscopy for 3D imaging of habenula. A peer reviewed submission is under preparation. A peer-reviewed conference proceeding in information science relating to the theoretical idea towards publishing findable brain imaging data was also submitted. In the course of the two years of this research project, a couple of presentations in domestic and international conferences was also done including technical conferences related to international optics society as well as domestic neuroscience society annual meetings. The publications and the conference presentations are listed in the research report in the later sections.

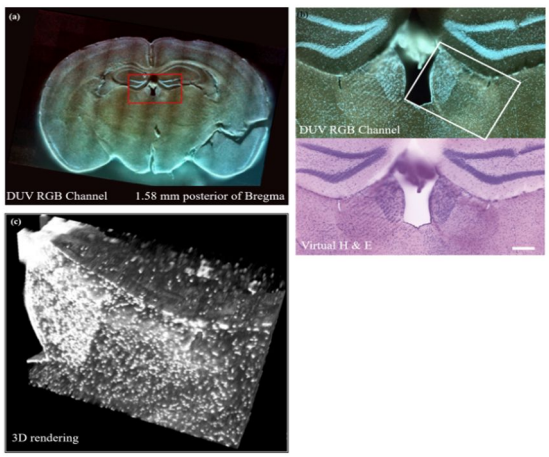

Figure2: Serial block-face imaging of habenula of the whole brain-stained sample (using Hoechst- 33258) imaged using 3D-DUV fluorescence microscope is shown. Virtual H & E image refers to the pseudocolor image obtained from DUV image to resemble the conventional histology stain. [Kasaragod et al., BioRxiv 2020]

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　1件／うち国際共著　2件／うちオープンアクセス　1件）

１．著者名 D Kasaragod, M Zhu, H Terai, K Kawakami, H Aizawa	４．巻 -
２．論文標題 Deep ultraviolet light based wide-field fluorescence microscope for brain imaging	５．発行年 2020年
３．雑誌名 bioRxiv	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1101/2020.10.27.342345	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 N Thalhath, M Nagamori, T Sakaguchi, D Kasaragod, S Sugimoto	４．巻 -
２．論文標題 Semantic Web Oriented Approaches for Smaller Communities in Publishing Findable Datasets	５．発行年 2021年
３．雑誌名 Metadata and Semantic Research	６．最初と最後の頁 234-242
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/978-3-030-71903-6_23	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計7件（うち招待講演　0件／うち国際学会　4件）

１．発表者名 Deepa Kamath Kasaragod
２．発表標題 Wide-field surface emission deep ultraviolet fluorescence microscope for whole brain imaging
３．学会等名 Neuro2019-Niigata Japan Neuroscience Society Annual Meeting
４．発表年 2019年

１．発表者名 Deepa Kamath Kasaragod
２．発表標題 Whole-brain imaging using wide-field fluorescence microscope with deep ultraviolet surface excitation
３．学会等名 Neural Imaging and Sensing 2020, SPIE BIOS Photonics West- 2020, San Francisco（国際学会）
４．発表年 2020年

１．発表者名
Deepa Kamath Kasaragod

２．発表標題
Wide-field deep ultraviolet surface excitation microscope for 3-dimensional brain imaging

３．学会等名
Focus on Microscopy-2020-Osaka (accepted for oral presentation, cancelled due to COVID-19)（国際学会）

４．発表年
2020年

１．発表者名
D Kasaragod, M Zhu, H Takemoto, H Aizawa

２．発表標題
Wide-field serial block-face fluorescence microscope using deep ultraviolet light for whole-brain imaging

３．学会等名
Neuro2020- Japan Neuroscience Society Annual Meeting (Online)（国際学会）

４．発表年
2020年

１．発表者名
N Thalhath, M Nagamori, T Sakaguchi, D Kasaragod, S Sugimoto

２．発表標題
Semantic Web oriented approaches for smaller communities in publishing findable datasets

３．学会等名
Metadata and Semantic Research - 2020 (Online)

４．発表年
2020年

１．発表者名
D Kasaragod, M Zhu, H Aizawa

２．発表標題
A simplified optical scheme for high resolution serial block-face fluorescence microscopy for 3-dimensional morphometry of rodent brains

３．学会等名
Focus on Microscopy - 2021 (Online)

４．発表年
2021年

１．発表者名 D Kasaragod, M Zhu, H Aizawa
２．発表標題 Deep ultraviolet based serial block-face imaging for 3-dimensional morphological assessment of the rodent brains
３．学会等名 Optics and Photonics International Congress -The 7th Biomedical Imaging and Sensing Conference (Online)（国際学会）
４．発表年 2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関