様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

# 科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 １３ 日現在

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研究課題名（和文）Cellular resolution macro analysis tools for functional and structural brain imaging

研究課題名（英文）Cellular resolution macro analysis tools for functional and structural brain imaging

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：蛍光顕微鏡は、脳内のさまざまな空間スケールおよび時間スケールにわたる画像化を可能にする神経科学における重要なツールです。この研究プロジェクトは、三次元脳イメージングを行うにあたり深紫外線を用いた新規の蛍光顕微鏡を確立することであった。他の研究室が簡単に使用できるようにするための、オープンソースのハードウェアおよびソフトウェアのセットアップも課題であった。

研究成果の学術的意義や社会的意義
We developed and established a simple and cost-effective imaging tool (fluorescence microscopy set-up) for whole brain and volumetric imaging using novel light source - deep ultraviolet illumination. Open source tools were adopted and developed for data acquisition and image analysis.

研究成果の概要（英文）：Fluorescence microscopy is an important tool in neuroscience which enables imaging across different spatial and temporal scales to obtain the structural and functional organizational details of the brain. The cost and complex design of microscopes that can do whole brain imaging significantly affect the use of microscopy tool for scientific discoveries. This research project focused on developing and establishing a simple and cost-effective fluorescence microscopy for 3-dimensional brain imaging. Deep ultraviolet illumination was utilized which allows for simplified optical design and allows for optical sectioning on block-face imaging. A combination of optical sectioning and mechanical sectioning technique was utilized using open-source tools for whole brain imaging using conventional fluorescent dyes. Adopting and adapting open source solutions (hardware and software) for this microscopy allows for easy duplication of this tool by other labs.

研究分野：Biomedical Engineering

キーワード：Optical Imaging　Fluorescence Microscopy　Brain Imaging

## １．研究開始当初の背景 Background of the Research

Fluorescence microscopy is an important tool in neuroscience which enables imaging across different spatial and temporal scales to obtain the structural and functional organizational details of the brain. Fluorescence microscopy that combines mechanical and optical sectioning along with different tissue preparation procedures have been engineered for whole brain imaging in rodent studies. Existing 3-dimensional (3D) brain imaging microscopy tools like confocal, two-photon, structured illumination combines optical and mechanical sectioning capability for whole brain imaging. However, they are expensive, bulky and not easily affordable by smaller labs. The cost and complex design of microscopes that can do whole brain imaging can significantly affect the use of microscopy tool for scientific discoveries.

Previously we had developed a novel fluorescence microscope using deep ultra violet (DUV) illumination for brain imaging. We have shown prototypical application of this novel microscope for imaging tiny anatomical structures like habenula for quantitative evaluation (***Kasaragod et al., BioRxiv 2020***). The current research project focused on developing and establishing this DUV fluorescence microscope as a simple and cost-effective tool for 3-dimensional brain imaging. Deep ultraviolet illumination was utilized which allows for simplified optical design and provides optical sectioning capability on block-face imaging. A combination of optical sectioning and mechanical sectioning technique was utilized using open-source tools for whole brain imaging using conventional fluorescent dyes. Adopting and adapting open source solutions (hardware and software) for this microscopy allows for easy duplication of this tool by other labs.

## ２．研究の目的 Research Objective

The main objective was to develop and establish a novel fluorescence microscopy based on deep ultraviolet illumination for 3D brain imaging and develop image processing tools for volumetric analysis of brain structures in normal and diseased models. In addition, focus was also on developing open source microscopy tools for image acquisition and analysis for 3D brain imaging.

## ３．研究の方法 Research Methods

This research project was built upon our previous work (***Kasaragod et al., BioRxiv 2020***) in which we had developed a novel fluorescence microscope for applications in brain imaging for 2D imaging. We had previously shown the prototypical application of this microscope for 3D imaging especially with respect to smaller structures like habenula. In this project, we explicitly showed the optical sectioning capability of this fluorescence microscope and the microscope was established as a simple and cost-effective fluorescence microscope for whole brain imaging using rodent brains. This was shown specifically using in-situ fluorescence Nissl staining in ex-vivo rodent brains. Image acquisition and image analysis workflow was developed using open source tools based

on Python and ImageJ. Adapting and adopting open source tools for this microscopy set-up would allow for easy duplication of this set-up by other labs allows for more community based development in the field.

## ４．研究成果 Research Results

A technical journal article was published in a peer-reviewed journal (***Kasaragod and Aizawa, Scientific Reports 2023***). A couple of presentations in domestic and international conferences were also done. Development and dissemination of open source tools for 3D microscopy shall be carried out as expected. A manuscript is under preparation on open microscopy for 3D deep ultra violet illumination based brain imaging. Developmental work for complete automation of the microscopy tool for whole brain imaging are also ongoing. Overall the research has progressed as expected.

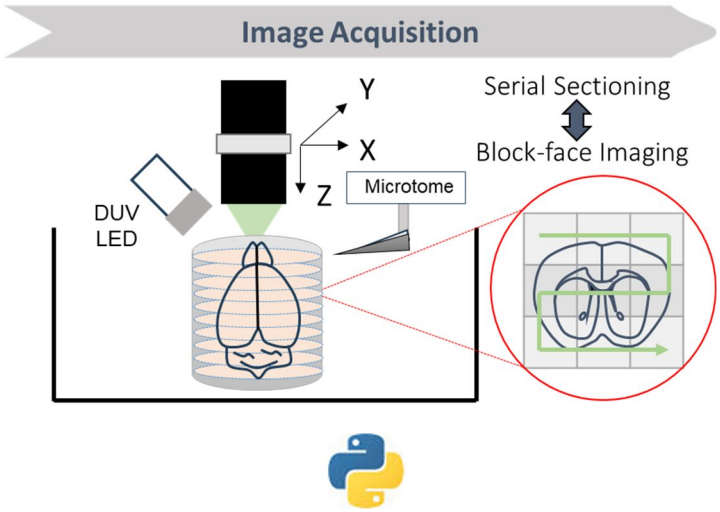


**Figure 1: A schematic of the microscopy set-up for serial block-face acquisition for whole brain imaging**

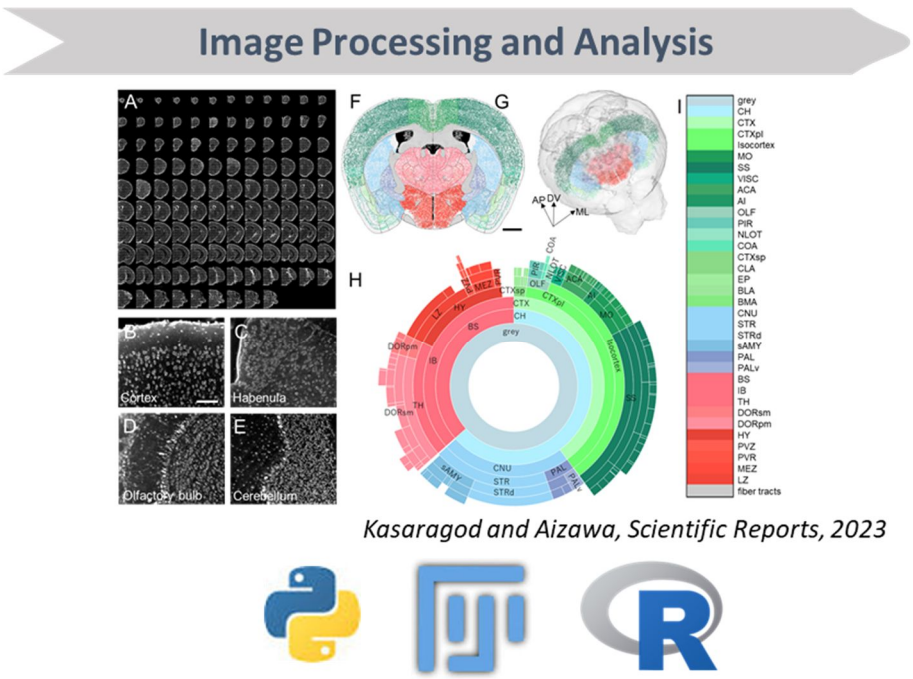


**Figure 2: Prototypical 3-dimensional dataset and analysis. Adapted from Kasaragod and Aizawa, Scientific Reports, 2023.**

５．主な発表論文等

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

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|---|---|
| １．著者名<br>Deepa Kamath Kasaragod and Hidenori Aizawa | ４．巻<br>13 |
| ２．論文標題<br>Deep ultraviolet fluorescence microscopy of three-dimensional structures in the mouse brain | ５．発行年<br>2023年 |
| ３．雑誌名<br>Scientific Reports | ６．最初と最後の頁<br>8553 |
| 掲載論文のDOI（デジタルオブジェクト識別子）<br>なし | 査読の有無<br>有 |
| オープンアクセス<br>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<br>該当する |

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　3件）

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| １．発表者名<br>Deepa Kamath Kasaragod |
| ２．発表標題<br>Kinetics of the extracellular dopamine in the mouse striatum with fiber photometry using biosensor |
| ３．学会等名<br>Neuro2023- Japan Neuroscience Society Annual Meeting 2023（国際学会） |
| ４．発表年<br>2023年 |

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| １．発表者名<br>Deepa Kamath Kasaragod |
| ２．発表標題<br>Open microscopy for serial block-face deep ultraviolet fluorescent imaging |
| ３．学会等名<br>Focus on Microscopy 2024, Genoa Italy（国際学会） |
| ４．発表年<br>2024年 |

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| １．発表者名<br>Deepa Kamath KASARAGOD |
| ２．発表標題<br>Deep ultraviolet based serial block-face imaging for 3-dimensional morphological assessment of the rodent brains |
| ３．学会等名<br>Optics and Photonics International Congress - BISC 2021（国際学会） |
| ４．発表年<br>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<br>（ローマ字氏名）<br>（研究者番号） | 所属研究機関・部局・職<br>（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 |
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