IMAGING PROPOSALS

Indiana University Bloomington Center for Cannabis, Cannabinoids, and Addiction

Multi-Scale Imaging Microscopy – Multi-Photon Microscopy

Open Applications

Eligibility:

1. Trainees and PIs working in or moving into the substance use disorder field.
2. Well-developed research plan designed to address significant questions related to substance use disorders and provide preliminary data for grant applications.
3. Clear rationale on how the experimental data generated by the imaging core will answer a research question.
4. For proposals proposing an in vivo MP experiment, identification of a qualified scientist from the PI's lab who can physically be in Bloomington for the duration of the funded experiment.
5. Clear plan to analyze and summarize data in timely manner.
6. Agreement to share the data generated with the greater scientific community once results are published.

ScaleS-2P Proposals:

In the application, it is imperative for investigators to provide a compelling rationale for choosing ScaleS-2P imaging over conventional imaging procedures. Of note the ScaleS clearing method effectively preserves the tdTomato fluorescent signal, while regrettably, GFP signals are diminished. Consequently, it is more practical to utilize tdTomato labeling whenever feasible.

Before sending samples to the Imaging Core, it is imperative that the labeled target undergoes thorough validation at the investigator's home institute. This validation process ensures the accuracy and reliability of the labeled target, contributing to the integrity of the subsequent imaging procedure.

Investigators are encouraged to visit C3A core in person for one or two-weeks for hands-on data analysis utilizing the Core's Imaris software, especially if they intend to use Imaris for analysis. This arrangement not only expedites the data analysis process but also provides investigators with the opportunity to gain first-hand experience in setting up samples and acquiring images using our system during their stay. Such an arrangement would not only expedite the data analysis process, but also afford investigators the opportunity to familiarize themselves with sample setup and image acquisition procedures using our system.

Typical ScaleS-2P Workflow:

1. Generate a testable hypothesis with investigators, design strategy to label the circuit of interest, and troubleshoot experimental challenges (40hrs)
2. Confirm the labeling strategy with data acquired by the investigator at their home institution using immunostaining and conventional imaging (12hrs)

3. Investigator ships 8-10 labeled brains to the C3A Imaging Core. The core will conduct ScaleS clearing and 2P imaging (120hrs)
4. Original 2P image data will be deposited into database (8hrs)
5. Investigator will be trained to conduct data analysis with Imaris (16hrs)
6. Investigator will summarize results and upload all Imaris analysis files into the C3A database.

In vivo 2P Imaging Proposals:

In the application, investigators must provide rationale for choosing in vivo two-photon sensor imaging over one-photon imaging. It is essential for an investigator to be present on-site for a minimum of 12 weeks to conduct the full range of experimental procedures. Investigators will receive comprehensive training in tasks such as AAV virus injection (if required), cranial window installation, animal training (if necessary), simultaneous sensor imaging alongside behavioral monitoring and video recording, as well as basic data analysis. *We strongly recommend that investigators possess substantial experience in small animal brain surgery, including skills in stereotaxic intracranial injection and probe implantation, among other skills.*

The exact duration of the investigator's stay on site will be determined by the specific experimental design. For instance, conducting imaging in neonatal mice and adult mice involves significantly different timelines. Given the multifaceted nature of the tasks involved in completing the experiment, we offer the option to accommodate two investigators from the same application. This allows for the distribution of tasks to the second investigator should the primary investigator face an overwhelming workload. For instance, the second investigator could possess basic coding skills to handle the intensive data analysis process.

Typical *in vivo* 2P service and hours supporting C3A investigators, affiliates, and pilot projects:

1. Form testable hypothesis with investigators, design experimental strategies, and consult/troubleshoot to carry out region/cell type-specific sensor labeling in investigator's own lab (80-120hrs).
2. Confirm desired sensor expression with data acquired at the investigator's home institution using conventional imaging (20hrs).
3. The Imaging Core will receive or order the experimental mice and conduct rAAV injections if needed (24-72hrs).
4. 1st six-week trip, the visiting investigator will receive hands-on training with detailed protocols to conduct surgical installation of the cranial window (80hrs) and 2P imaging (24hrs).
5. Trained investigator will install cranial windows with assistance (20hrs) and then install cranial windows independently.
6. 2nd 6-week trip, the visiting investigator will receive a second round of training for 2P imaging and data analysis (40hrs) and receive ~160 hrs of 2P time to conduct the proposed experiments.
7. All imaging data will be deposited into C3A data storage site.
8. Ensure appropriate data deposition, discussion, and interpretation (40 hrs). Estimated time to complete one project: ~6-12 months.

Imaging Workflow:

Specific training plans, timing of specific experimental steps, and milestones will be jointly established together with PIs of accepted proposals and Dr. Huang, the technical expert for Multiphoton and ScaleS imaging of the MSIC, before projects are begun.

The investigator of a selected project will complete all necessary compliance-related training and be added to C3A-approved IBC/IACUC protocols in consultation with Dr. Franco. All MSIC members will be involved in designing experimental approaches and data interpretation with C3A investigators, affiliates, and trainees. Drs. Huang and Franco will train them for surgery, imaging, and data analysis required for their projects. Dr. Franco will schedule visits, conduct Scale-S cleaning, order/transfer mice, and assist in rAAV injections

when needed. Project investigators are responsible for in vivo 2P imaging and image data analysis. If a project requires modification of our established data analysis pipelines or requires a new data analysis pipeline, the investigator is responsible to establish their own data analyses pipeline (DataJoint may be able to support with contracts). Upon completion of the proposed projects/training, the project investigator will submit a detailed report to Dr. Lu and the advisory board members, as described above.

The Imaging Core will provide the required tools, equipment, and comprehensive training for surgical, imaging, data uploading, and Cloud-based data analysis using the pipeline established by DataJoint in collaboration with the Lu lab.

Application Requirements:

Applications with the following sections must be submitted electronically as a single PDF file via the C3A website. Applicants are strongly encouraged to consult with Dr. Hui-Chen Lu (hclu@iu.edu), MSIC-2P Core Director.

Section 1. Abstract (300 words):

State the short-term objectives of the proposed experiments and the specific aims for future grant proposals with the data acquired from the MSIC-2P core. Describe the approaches for achieving the proposal's objectives. The abstract should be informative for non-expert readers. Do not include any confidential information as it will be published on the C3A website if chosen for support.

Section 2. Research Plan (3-page maximum):

Research Plans should not exceed three single-spaced pages with ½ inch margins on all sides. The front must be 11 points or larger, and Arial is preferred. An additional page listing all citations can be added. The following sections should be included:

> *Significance:* Briefly review relevant prior work and identify the gaps in the knowledge related to the project. Provide rationale for the planned investigations and state the overall objective.

> *Hypothesis:* List the testable hypothesis and relate it to long-term aims of the project.

> *Approach:* Provide preliminary data that support the proposed investigation and available mouse lines and related tools. Briefly, describe the research design, concept, and procedures that will be used to achieve the specific aims of the project. Provide details on data interpretation.

> *Future Funding:* Provide a detailed explanation of how the proposed project can receive further support from external funding sources. Identify the specific funding agencies.

> *References:* Not included in the 3-page limit.

Section 3. Personnel (1/2-page maximum):

Provide details on the research/animal surgical experience of the proposed visiting investigator.

Section 4. Biosketch (5-page maximum in NIH style):

A biosketch should be provided for the principal investigator and the proposed visiting investigator.

Proposal Review:

Each proposal will first be reviewed by the core PI for suitability. Proposals deemed suitable will be reviewed by the C3A steering committee. Dr Lu will work with steering committee to select projects based on scientific value, projected impact on the substance use disorder field, and feasibility. All applicants will receive feedback on their proposal and will be offered the opportunity to be mentored by a C3A PI or Affiliate

in subsequent applications to the C3A or external agencies. One or two ScaleS-2P projects and/or one in vivo 2P project will be served per year.

<u>Proposals will be ranked by the following criteria:</u>
1. A clear rationale on how the proposed research project will benefit from access to the MSIC core and will address substance use disorders.
2. A well-thought-out experimental plan on how data generated from MSIC core will benefit a grant proposal and/or provide key data for a manuscript in preparation.
3. The impact of data generated by the MSIC core on the applicant's ongoing research program. Low priority will be given to proposals that merely supplement ongoing research.
4. Data from MSIC core will be critical for the PI's NIDA (or other relevant funding agency) grant application.
5. Readiness to conduct the proposed experiment, such as the availability of essential mouse lines and/or key reagents for the proposed experiment.
6. A clear plan and commitment to execute the proposed experiment. For in vivo 2P imaging experiments, it is required to have a designated senior researcher who is committed to conduct experiments in the C3A core at a specific time. This person should have demonstrated expertise in mouse surgical procedures.

Other Important Information:

- *It's important to note that the data analysis for in vivo sensor imaging is highly variable and contingent on factors like sensor type and experimental hypothesis. This makes it impractical to establish a standardized data analysis workflow applicable to all sensors. Nonetheless, we will aid with sensor imaging pre-processing (leveraging our established pipeline with DataJoint) and machine learning-supported 2D/3D behavioral annotation (using Deeplabcut). The core is continuously refining imaging protocols and data analysis techniques to support investigators in acquiring high-quality data and analyzing it in the most efficient manner.*
- Awardees must agree to give a short presentation of the project at least three times (typically, before the project starts, in the middle of the project, and at the project's completion). The presentations will be held at the monthly C3A Steering Committee meetings, and once to the external advisory board (EAB) at the annual EAB meeting.
- A summary report will be required upon project completion.