



**Workshop 2:** Sample preparation in mammalian whole-brain connectomics  
*February 17, 2021*

**Overall Goal:** To identify current capabilities and open issues in sample preparation for (1) whole mouse brain (WMB) electron microscopy (EM) connectomics at synaptic resolution; and (2) complementary imaging at lower resolution, in mouse brains and larger (including human) brains.

**Agenda:**

11:00 am ET

**Welcome and Series Overview**

*John Ngai, Ph.D.*  
*Harriet Kung, Ph.D.*

11:09 am ET

**Welcome and Introduction**

*Davi Bock, Ph.D. (Workshop Co-Lead)*  
*Hongkui Zeng, Ph.D. (Workshop Co-Lead)*

11:25 am ET

**Workshop Logistics**

*Ruben Alvarez, Ed.D.*

11:30 am ET

**Session 1 - Continuous whole mouse brain EM connectomes**

Each speaker will address four questions:

- a) What current sample preparation methods for WMB EM connectomics maximally conserve structural continuity across the entire brain? What are the pros and cons for each approach?
- b) All currently available imaging methods suitable for WMB connectomics require subdivision of the sample prior to imaging. What amount of loss is expected with current subdivision methods? Can continuity at single axon (~50 nm) level across the entire brain be maintained?
- c) What are the prospects for improved methods to reduce loss during subdivision, either prior to resin embedding (e.g. vibratome sectioning) or after (e.g. hot knife)?
- d) Prior to imaging, how to assess the quality of the sample preparation? How to predict and/or validate continuity before, during and after imaging?

11:30 am ET

**EM Staining for Whole Mouse Connectome**

*Xiaotang Lu, Ph.D.*

11:45 am ET

**Sample preparation in the context of a large scale connectomics pipeline**

*Nuno Maçarico da Costa, Ph.D.*

12:00 pm ET Specimen Preparation and Screening for Volume EM  
*Eric Bushong, Ph.D.*

12:15 pm ET Ideas for thick sectioning brain tissue  
*Kenneth Hayworth, Ph.D.*

12:30 pm ET **Q & A session**

1:00 pm ET **Break**

1:10 pm ET **Session 2 - Complementary whole-brain imaging in mouse and larger species**

Each speaker will address four questions:

- a) What types of complementary light microscopy (LM) data should be collected on the same brain and/or on different brains to help with processing and/or interpretation of the WMB EM connectome? E.g. projectomes at population or single cell level; functional imaging in behaving animals before EM; LM/X-ray/EM of CNS, PNS, and/or whole body; cell type specific labeling/tagging; immunolabeling.
- b) Why is such information useful, what questions can it be used to address? What is the priority and order of such data generation in conjunction with the WMB EM connectome data generation?
- c) Which techniques can be extended/scaled to larger brains (e.g. human and non-human primate brains)? Will these techniques require pre-labeling in live tissues?
- d) What considerations are needed in sample selection (e.g. sex, strain or race, age, individual variation, health status, life history) and sample preparation (e.g. technical requirements, desired data types from the living brains)?

1:10 pm ET Scalable approaches for functional and structural light microscopy of the mammalian brain  
*Elizabeth Hillman, Ph.D.*

1:20 pm ET Brain connectivity in primates  
*Helen Barbas, Ph.D.*

1:30 pm ET Towards holistic phenotyping and understanding of the human brain  
*Kwanghun Chung, Ph.D.*

1:40 pm ET Engineered gene delivery vectors for high-precision broad coverage of the mammalian brain  
*Viviana Gradinaru, Ph.D.*

1:50 pm ET Projectomes and connectomes from mice to primates  
*Bobby Kasthuri, M.D., Ph.D.*

2:00 pm ET Chemical Tools for CLEM and Color EM  
*Stephen Adams, Ph.D.*

2:10 pm ET Nanoscale map of whole-cells and tissue using genetic probes technologies and 3D electron microscopy  
*Daniela Boassa, Ph.D.*

2:20 pm ET **Q & A session**

2:50 pm ET **Break**

3:00 pm ET **Discussion Panel**

Panel Discussion topic: *Charting a roadmap for whole mouse brain connectomes and larger brain projectomes – sample preparation feasibility as a prerequisite for choosing imaging platforms, sample selection criteria for scaling to multiple brains.*

Further charges:

- Summarize current state of tissue preparation
- Identify key issues to be resolved in sample prep for WMB EM connectome in conjunction with different EM imaging platforms
- Articulate needs and advantages of complementary data types for WMB EM connectomes
- Define the limitations, potentials and greatest opportunities in moving to larger brains

Panel chairs: *Davi Bock, Ph.D.; Hongkui Zeng, Ph.D.*

Discussants: *JoAnn Buchanan, M.S.; Jeff Lichtman, M.D., Ph.D.; Lisa Miller, Ph.D.; Linnaea Ostroff, Ph.D.; Clay Reid, M.D., Ph.D.*

4:00 pm ET **Closing remarks / End of workshop**