Mesoscale Hub - Imaging Hub

1.0 Objective

Tissue histology has been a gold standard in biomedical research. However, sectioning the tissues results in analysis of restricted brain regions and fragmentation of long neuronal processes, thereby limiting the information on connectivity and reactivity. This hub therefore provides a unique combination of world-wide leading expertise in tracing and clearing technology. It combines novel clearing techniques (developed by hub members) allowing mapping neuronal, vascular and glial networks in whole rodent brains and spinal cords with novel monosynaptic tracing techniques (developed by hub members) allowing to monitor connectivity changes over time during disease progression. This scalable approach will strongly facilitate and standardize the study of neuronal circuits and their connectivity within the SyNergy cluster by allowing unbiased assessment within intact organs and even entire organisms. In addition, large human tissues (~1000 times thicker than standard histology) can also be labelled and cleared- making DISCO clearing a valuable tool also for translational SyNergy projects.

1.1 Organisation

Location and Coordinators

Name	**Function**	**Location**	**Mail**	**Phone**
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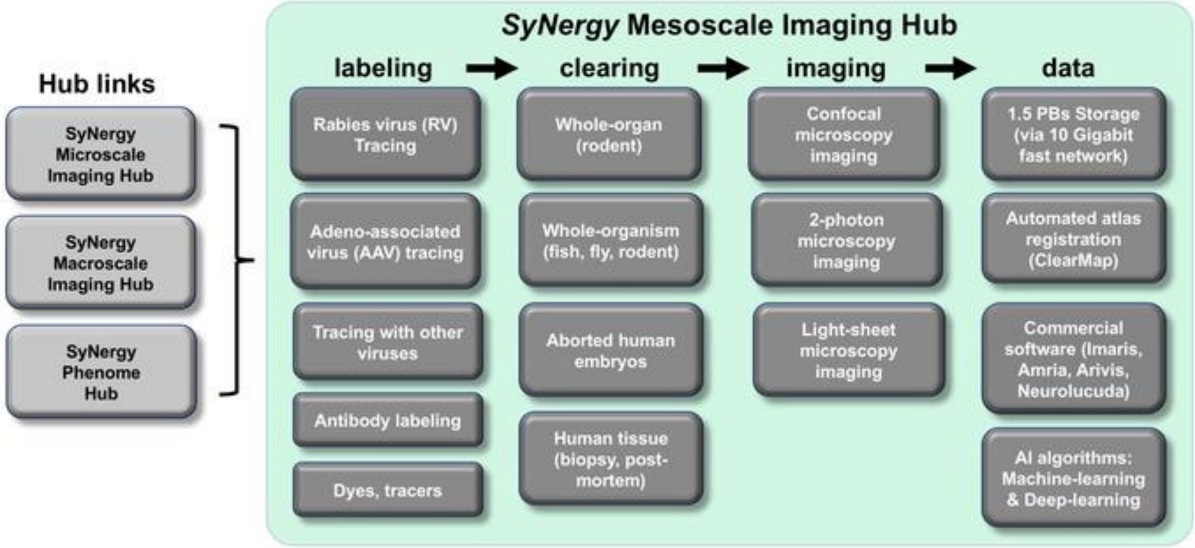
1.2 Technical portfolio

- Tissue clearing
- Light-sheet microscopy imaging



This hub offers 3D histology for experimental and clinical samples in combination with diverse labeling methods. In particular, RABV tracing of specific neuronal networks will be a powerful approach to dissect out neuronal circuits in health and disease. The hub will also provide a light-sheet microscope and a large data storage unit (recently purchased by the Synergy matching funds). Because many Synergy researchers rely on mouse, the hub will offer automated quantification of cells in whole mouse brains that are registered to Allen brain atlas (ClearMap technology) (Renier et al., Cell 2016).

Labeling: Several labelling techniques will be implemented within our Mesoscale Hub:

1. RABV tracing: Modified RABVs can be used to trace monosynaptic inputs of defined neurons. Toxicity of RABV has been well documented over long expression time which would be counterproductive to the investigation of chronic circuit connectivity in health or disease. Therefore, within the Hub, we will utilize RABV vectors that are less toxic but still provide a strong transgene expression for effective tissue clearing. Because, less toxic RABV variants usually come with the price of weaker gene expression (Reardon et al., Neuron, 2016), the Hub will introduce controllable RABV vectors (Ciabatti et al., Cell 2017). RABV as an RNA virus cannot be regulated by different promoters or loxP-sites. Instead, regulation can occur at the protein level. Tools of choice are proteases, which can be inhibited by a variety of drugs, and thus, in combination with degrons, allow regulating viral protein levels. The hub has a high level of expertise to generate RABVs for the specific experimental setups (Falkner et al., Nature 2016; Ghanem and Conzelmann, Virus Res. 2016; Schnell et al., EMBO J 1994). In addition, the hub will develop new RABVs including less toxic, controllable and anterograde variants, as well as providing RABV with either genetic Ca-indicators or DREADDS allowing to manipulate the network connected to specific neuronal cell types in order to probe for disease progression upon silencing or activation.

2. AAVs: The hub will provide a platform for adeno-associated virus (AAV)-based tracing of defined cell populations, which can be delivered to the brain via injection (Jacobi et al., EMBO J 2015) or via non-invasive systemic delivery (Deverman et al., Nature Biotech. 2016).

3. In addition to transgene expression of fluorescent proteins, we will utilize deep tissue antibody labelling e.g., whole-mouse brain and thick human brain tissues using iDISCO and uDISCO methods (Pan et al., Nature Methods 2016; Renier et al., Cell 2014).

4. Finally, a variety of axonal tracers such as cholera toxin B (CTB) to label e.g., peripheral nerves and motor neurons, or nuclear dyes such as propidium iodine (PI) and TO-PRO-3 could be used in intact organs and whole organisms prior to DISCO clearing.

Clearing: The organic solvent-based DISCO clearing methods provide the highest transparency among all clearing methods (Hama et al., Nature Neurosci. 2015; Richardson and Lichtman, Cell 2015; Susaki et al., Nature Protocols 2015). The hub experts invented and improved the DISCO methods to a level that subcellular imaging of entire adult mouse bodies has become possible (Erturk et al., Nature Protocols 2012; Erturk et al., Nature Methods 2011; Pan et al., Nature Methods 2016). The hub will utilize an optimized DISCO clearing pipeline for adult mouse brains from antibody labelling to automated quantification, which will be a valuable tool as the majority of Synergy researchers work with mice.

For example, members will be able to conduct unbiased mapping/tracing experiments in intact mouse brains e.g., identification of neuronal circuitry and their changes in disease, discovery of glial reactivity throughout the brain and integration of transplanted or reprogrammed neurons in an unbiased way. It has only now become possible to image neuronal circuitry throughout the intact mouse central nervous system (CNS) using DISCO clearing (Pan et al., Nature Methods, 2016) and give the importance of inflammatory signals also beyond the brain aim to combine it with tools monitoring these processes also in other organs. Furthermore, DISCO clearing methods work well on other organisms including drosophila, adult zebrafish, rats and even on aborted human embryos and large human clinical tissues– making the hub valuable for each cluster member.

Imaging: The most ideal imaging method for the cleared tissues is the light-sheet microscopy, which scans an entire mouse brain at subcellular resolution within a couple of hours. The Synergy cluster already invested on the purchase of a light-sheet microscope during the first funding period (being installed late 2017). In addition, because the light-scattering is largely reduced after tissue clearing, usage of confocal or multi-photon microscopy within the Synergy members labs will complement the Hub light-sheet imaging and result in high-resolution imaging without the inherent decrease in quality due to tissue thickness (Erturk et al., Nature Medicine 2011).

Storage and analysis of large data: A typical light-sheet microscope scan produces ~40 GB data within a couple of hours. We estimate ~200 scans per month (~96 TBs data per year) for Synergy tandem projects, which will be performed by a dedicated hub technician. Synergy already invested on purchase of a data storage platform for 2017 and 2018 periods. This large-data storage unit works at 10 Gigabit network speed resulting in a transfer rate of 1TB/15min. The analysis of large data constitutes the major bottleneck of mesoscale hub, which ideally should be automated as much as possible. As a first step towards this goal, the hub will provide automated cell counting in the entire mouse brain with the annotations on Allen brain atlas, a method that is already established within the hub member labs. To meet diverse demands on data analysis especially for tracing neuronal circuits, segmenting glia cell morphologies (microglia and astrocytes) and vasculature, the hub will recruit scientific experts to develop machine-learning-based algorithms.

The mesoscale Hub will be available for all tandem projects, supported by a technician for labelling techniques, a technician for tissue clearing and imaging experiments, one staff scientist for tackling large-data analysis and one staff scientist for RABV tracing

1.3 Project request procedure

See additional form for project requests.

1.4 Fees

User fees may apply to cover consumables and expenses over the allocated budget.

1.5 Publications

Ciabatti, E., Gonzalez-Rueda, A., Mariotti, L., Morgese, F., and Tripodi, M. (2017). **Life-Long Genetic and Functional Access to Neural Circuits Using Self-Inactivating Rabies Virus.** Cell 170, 382-392 e314.

Deverman, B.E., Pravdo, P.L., Simpson, B.P., Kumar, S.R., Chan, K.Y., Banerjee, A., Wu, W.L., Yang, B., Huber, N., Pasca, S.P., et al. (2016). **Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain.** Nat Biotechnol 34, 204-209.

Erturk, A., Becker, K., Jahrling, N., Mauch, C.P., Hojer, C.D., Egen, J.G., Hellal, F., Bradke, F., Sheng, M., and Dodt, H.U. (2012). **Three-dimensional imaging of solvent-cleared organs using 3DISCO.** Nat Protoc 7, 1983-1995.

Erturk, A., Mauch, C.P., Hellal, F., Forstner, F., Keck, T., Becker, K., Jahrling, N., Steffens, H., Richter, M., Hubener, M., et al. (2011). **Three-dimensional imaging of the unsectioned adult spinal cord to assess axon regeneration and glial responses after injury.** Nat Med 18, 166-171.

Falkner, S., Grade, S., Dimou, L., Conzelmann, K.K., Bonhoeffer, T., Gotz, M., and Hubener, M. (2016). **Transplanted embryonic neurons integrate into adult neocortical circuits.** Nature 539, 248-253.

Ghanem, A., and Conzelmann, K.K. (2016). **G gene-deficient single-round rabies viruses for neuronal circuit analysis.** Virus Res 216, 41-54.

Hama, H., Hioki, H., Namiki, K., Hoshida, T., Kurokawa, H., Ishidate, F., Kaneko, T., Akagi, T., Saito, T., Saido, T., et al. (2015). **ScaleS: an optical clearing palette for biological imaging.** Nat Neurosci 18, 1518-1529.

Jacobi, A., Loy, K., Schmalz, A.M., Hellsten, M., Umemori, H., Kerschensteiner, M., and Bareyre, F.M. (2015). **FGF22 signaling regulates synapse formation during post-injury remodeling of the spinal cord.** EMBO J 34, 1231-1243.

Pan, C., Cai, R., Quacquarelli, F.P., Ghasemigharagoz, A., Lourbopoulos, A., Matryba, P., Plesnila, N., Dichgans, M., Hellal, F., and Erturk, A. (2016). **Shrinkage-mediated imaging of entire organs and organisms using uDISCO.** Nat Methods 13, 859-867.

Reardon, T.R., Murray, A.J., Turi, G.F., Wirblich, C., Croce, K.R., Schnell, M.J., Jessell, T.M., and Losonczy, A. (2016). **Rabies Virus CVS-N2c(DeltaG) Strain Enhances Retrograde Synaptic Transfer and Neuronal Viability.** Neuron 89, 711-724.

Renier, N., Adams, E.L., Kirst, C., Wu, Z., Azevedo, R., Kohl, J., Autry, A.E., Kadiri, L., Umadevi Venkataraju, K., Zhou, Y., et al. (2016). **Mapping of Brain Activity by Automated Volume Analysis of Immediate Early Genes.** Cell 165, 1789-1802.

Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., and Tessier-Lavigne, M. (2014). **iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging.** Cell 159, 896-910.

Richardson, D.S., and Lichtman, J.W. (2015). **Clarifying Tissue Clearing.** Cell 162, 246-257.

Schnell, M.J., Mebatsion, T., and Conzelmann, K.K. (1994). **Infectious rabies viruses from cloned cDNA.** EMBO J 13, 4195-4203.

Susaki, E.A., Tainaka, K., Perrin, D., Yukinaga, H., Kuno, A., and Ueda, H.R. (2015). **Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging.** Nat Protoc 10, 1709-1727.