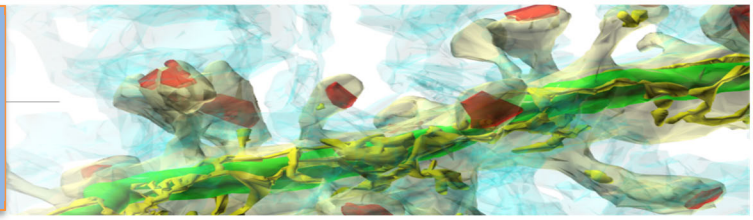
Connecting Neuronal Circuits to Subcellular Resources that Influence Synaptic Weight
June 3-5, 2019
NSF NeuroNex Award No. 1707356



2ND ANNUAL NSF NEURONEX WORKSHOP SPEAKER INFORMATION

Monday (3 June, 2019) – Keynote Speaker

Erik Jorgensen (University of Utah)

Title: Resolving fast events by electron microscopy

Abstract: Ultrafast endocytosis at synapses occurs as quickly as 30 - 300 ms. To image such rapid events using electron microscopy, we developed flash-and-freeze electron microscopy. In short, we expressed channelrhodopsin in neurons so that they could be stimulated with light. We then introduced a light path into the chamber of a high-pressure freezer. The sample was stimulated with a 10 ms light pulse and then frozen after fixed intervals to capture the morphology of the synapse. The limitation with this approach is that the action potential can occur at a random time point during the 10 ms light pulse, so our temporal resolution is limited.

The speed of synaptic vesicle fusion is less than 1 ms, thus to follow fusion accurately we need to improve our methods. To do this we built a specialized sample holder for the high pressure freezer, that is in fact a small printed circuit board. The device has a light-activated switch that discharges capacitors that have leads to the sample chamber. This generates an electric field that induces an action potential. Using this 'zap-and-freeze' device we have been able to capture synaptic vesicle fusions at synapses in hippocampal neurons.

Tuesday (4 June, 2019) – Workshop Speakers (In order of presentations)

Daniel Tward (Johns Hopkins University)

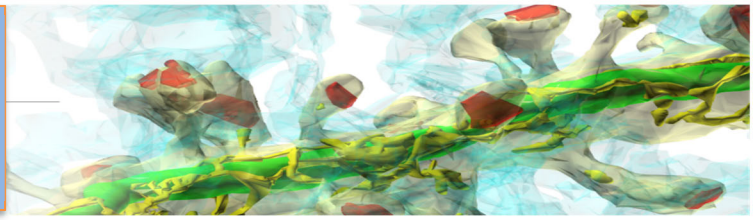
Title: Building deformable image registration tools for various image modalities

Abstract: Brain image registration to common coordinates is essential for combining and interpreting spatial data from multiple experiments and laboratories, enabling collaboration and effective statistical analysis. While there are well established methods for restiration of high quality brain MRI at the millimeter scale, many challenges remain for micron or smaller resolution data. These include working with multiple image modalities, a diverse set of model organisms, and tissue which is often sliced, incomplete, or damaged. By developing a statistical model of the imaging process we predict any missing information, including differences in image intensities, locations of missing tissue or artifacts, and any slicing planes. Given these predictions, registration can be performed in a well established setting. We use an expectation maximization algorithm to jointly predict incomplete information and registration parameters in a rigorous manner. These algorithms, together with example datasets and results, are being made publicly available through neurodata.io.

Linnaea Ostroff (University of Connecticut)

Title: Cell counts and circuit mapping: using Reconstruct for bigger things

Abstract: Presentation on light microscopy uses of Reconstruct. We're using it to analyze neuroanatomical tracing experiments and cell counts for estrus cycle staging. We're still using it for EM of course, so we'd want to participate in the EM focused content.



Tom Kazimiers (Janelia, HHMI)

Title: Federation tools for connectome analysis with multiple CATMAID instances

Abstract: CATMAID is a web-based analysis tool for connectomics data, including neuron reconstruction workflows and ontology based image classification. A focus on collaborative work and parallel use allows broad and parallel access to the data, supported by fine grained access control. With growing userbases and datasets including more and better segmentations, it becomes useful to store and edit data in a decentralized fashion: allowing for fast local access, more direct control and easier maintenance. This doesn't need to happen in a locked-down data silo though: CATMAID learned to talk to other CATMAID instances and---given permission---access remote data. Neurons accessed that way, possibly with a transformation into local space, can be visually compared to local neurons and used in analysis tools like NBLAST to rank similarity to local data. More tools will follow that can benefit from shared or published remote data.

Gregg Wildenberg (University of Chicago)

Title: Industrial Brain Mapping at the National Labs

Abstract: The promise of industrial scale brain mapping creates several challenges that we hope to solve by developing neuroscience tools for distribution at the National Labs. I will present three topics related to this effort: 1) high throughput x-ray tomography, 2) genetic labeling techniques for serial electron microscopy and 3) scalable algorithms for connectomics. These tools serve as an example of the advantages of supporting brain mapping at the National Lab which can be distributed widely to the scientific community to advance connectomics.

Daniel Berger (Harvard University)

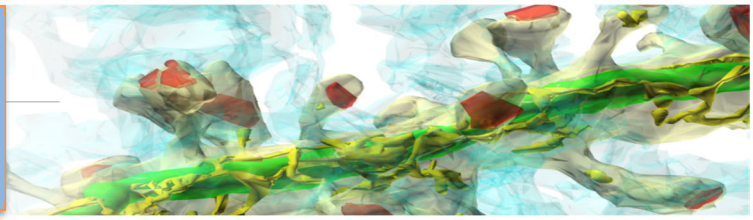
Title: VAST (Volume Annotation and Segmentation Tool): Efficient Manual and Semi-Automatic Labeling of Large 3D Image Stacks

Abstract: Recent developments in serial-section electron microscopy allow the efficient generation of very large image data sets but analyzing such data poses challenges for software tools. This talk introduces VAST (Volume Annotation and Segmentation Tool), a freely available Windows program for generating and editing annotations and segmentations of large volumetric image (voxel) data sets. It provides a simple yet powerful user interface for real-time exploration and analysis of large data sets even in the Petabyte range. VAST can be downloaded here: https://software.rc.fas.harvard.edu/lichtman/vast/

Sven Dorkenwald (Princeton University)

Title: A framework for proofreading and analyzing dynamic segmentations and annotations

Abstract: Ongoing improvements in volume electron microscopy and automatic segmentation have created large, fully reconstructed datasets with cubic-millimeter sized datasets in reach. However, present segmentations still contain errors prohibiting immediate analysis and requiring proofreading. Proofreading such datasets is a large, ongoing effort and, hence, we view these datasets as dynamic with constantly changing segmentation.



Furthermore, maturing scientific inquiries rely on diverse annotation types extending beyond synapse annotations. Combined, this poses problems for any analysis infrastructure supporting concurrent annotation, proofreading, and analysis by many users. Here, we present a framework that allows scientists to collaboratively proofread segmentations and create annotations of arbitrary types with little restrictions on their workflow. We maintain consistency by queuing changes to the same neuron in our centralized database and making changes immediately visible to all users. Rapid analysis is made possible in a timestamped manner allowing researchers to run their analysis at different stages of proofreading. Our proofreading infrastructure is designed to scale to peta-byte sized datasets by decoupling the local nature of proofreading changes from the spatially distributed nature of neurons through implementing a hierarchical, octree-like, view of the segmentation. Annotations are handled independently from the segmentation and are only bound to segmented neurons at specific timestamps.

Forrest Collman (Allen Brain Institute)

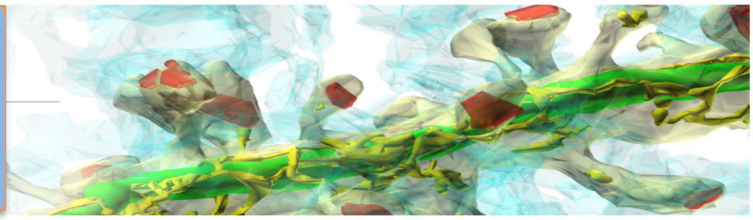
Title: What you see is what I see: sharing dynamic perspectives on connectomics data with web based tools

Abstract: Over the past 5 years, connectomics research has undergone a dramatic phase transition. Dataset size has exploded and machine learning based segmentation has advanced to the point where the amount of data and questions one can answer with a dataset now explodes beyond the capability of an individual researcher or even research lab. For the impact of these data to be maximized, effort must be made to make these data available, and more importantly, USEFUL to those both inside and outside the connectomics research community. To achieve this goal, we need an ecosystem of community minded tools, where labs can collaborate on proofreading, analysis, and easily query datasets for answers to the questions that will help them in their research. CATMAID, is an excellent example of such tool that allows sharing of manually traced skeletons, connectivity and annotations. I'll demonstrate another example, neuroglancer (developed primarily by Jeremy Maiten-Shepard at Google) but subsequently extended by groups at Princeton, Allen Institute, Janelia, and Johns Hopkins can facilitate inter-person and inter-group communication and collaboration on large complex datasets. I'll provide some demonstration about how one can quickly prototype simple python applications that use neuroglancer and share both the perspectives on the data and the application with non-programmers. Finally, I'll provide some example use cases that demonstrate how data scientists, neuroscientists and computer scientists can all benefit from being able to quickly and seamlessly share a perspective into a complex dataset, not only as static images but as dynamic objects.

Jae Hoon Jung (Texas A&M University)

Title: EM3D, an integrated software application for electron microscope tomography

Abstract: An electron microscope (EM) tomography application called EM3D was created in McMahan lab at Stanford University in the late 1990s. It was designed to provide convenient, general tools for processing EM tomography data to facilitate their 3-dimensional (3D) visualization and analysis for cellular and molecular biologists. EM3D allows a user to proceed from a set of two-dimensional (2D) gray scale transmission electron



microscope images/projections of a tissue section, which are taken at many tilt angles, to 3D models of tissue components within the section that are amenable to visualization and quantitative analysis. It includes five functional stages: projection alignment, 3D reconstruction of the section, segmentation of specific structures from the reconstruction, generation of surface models of the structures based on gray scale, and analysis tools of these models. As a freely available application, it has long been used to study 3D structures of synapses at macromolecular scale providing novel information about the subcellular structure and function in synapses and also applied for structural studies in materials science.

James Carson (The University of Texas, Austin – Texas Advanced Computing Center (TACC))

Title: 3DEM.org - a resource for sharing and collaboration linked to high performance computing

Abstract: In addition to dissemination information and datasets associated with our Neurotechnology Hub, 3DEM.org facilitates leveraging powerful NSF cyberinfrastructure. Demonstrated capabilities include web-based data sharing and launching applications to run on high performance computing.

Tom Bartol (The Salk Institute)

Title: Computational Tools for Quantitative 3DEM: An In-Silico Ultramicrotome and Neuropil Tools

Abstract: A brief introduction and overview will be given to highlight the capabilities and features of a suite of tools being developed for quantitative 3DEM at the nanoscale. First, an In-Silico Ultramicrotome designed to answer the question: How precise and accurate is manual and automated segmentation of subcellular structures in neuropil? And second, Neuropil Tools, a pipeline for intake, processing, computational quality meshing, annotation, management, visualization, and analysis of nanoscale subcellular structures in neuropil. An in-depth hands-on session will follow the introduction for interested participants.

Stephan Saalfeld (Janelia, HHMI)

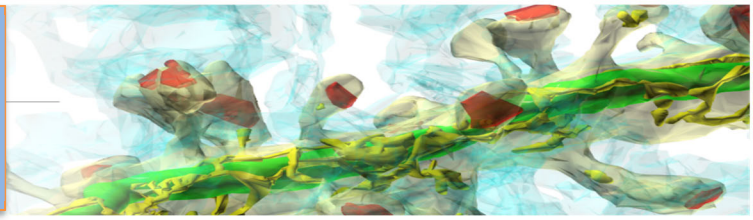
Title: Synapses and cellular organelles in FIB-SEM

Abstract: Modern sample preparation protocols for EM connectomics excellently visualize cellular organelles and subcellular components such as synapses, mitochondria, the endoplasmic reticulum, vesicles, microtubules, ribosomes et. We are developing scalable methods and tools for machine learning based automatic reconstruction of these structures in large isotropic volumes imaged with FIB-SEM.

Michael Morehead (spyglass / University of South Florida)

Title: New 3D Virtual Reality Tools for Nanoscale Image Volumes

Abstract: We have developed software, called syGlass, for visualization, annotation, quantification and documentation of 3D image volumes and movies, using direct volume rendering in a virtual reality environment. Many tools are designed to work across a range of



imaging modalities and resolutions. We are currently optimizing tools for the special needs of electron microscopy image volumes, including manual and autosegmentation, correction of segmentation, visualization and quantification of objects and their spatial relationships, discussion of data by multiple viewers, and documentation for publication and scientific communication. We will present the status of these tools and encourage feedback for their continued development.

Eva Dyer (Georgia Institute of Technology)

Title: Towards interpretable data-driven models of brain organization and variability

Abstract: Deep neural networks provide flexible frameworks for learning relevant features from data rather than requiring features to be pre-specified. However, when using these frameworks to make predictions - especially when analyzing data from complex biological systems - it is important to understand the reasons why certain decisions are made. In this talk, I will describe ways in which we are trying to open up the "black box" and interpret the representations learned by deep neural network architectures trained to decode brain imagery. I will show examples of how we are using deep learning approaches to navigate through large-scale brain volumes, automatically identify brain areas, and learn signatures of disease and variability.

Davi Bock (University of Vermont)

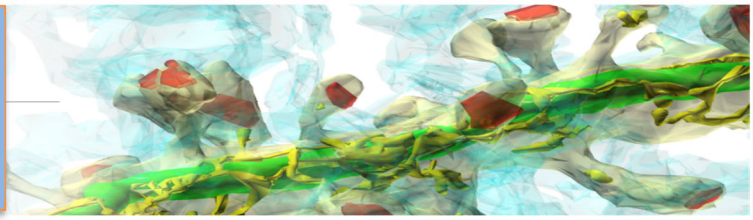
Title: Neuronal circuit reconstruction using a whole-brain segmentation of an adult fruit fly EM volume

Abstract: Recently, collaborators at Google have segmented the entire whole fly brain EM dataset generated by the Bock lab and collaborating labs (Zheng, Lauritzen et al. 2018). This segmentation generates fragments of neuronal arbors rather than complete neurons. Here we preview various approaches to best use this class of automatic segmentation, with an emphasis on CATMAID-based workflow. Example applications will include automatic tract identification, rapid sketching of binary connectivity in the mushroom body calyx, and principled sampling of pre- and postsynaptic partners.

John Edwards (Utah State University)

Title: Quantitative 3D Geometric Analysis using VolRoverN

Abstract: Quantitative geometric analysis of neuronal ultrastructure in 3D is challenging on a number of fronts, including model construction and the analyses themselves. We will present VolRoverN that can assist researchers in answering geometric questions about their data. Current functionality of VolRoverN includes 3D surface model generation from segmented EM imagery, model cleaning, smoothing and decimation, and 3D segmentation with export to NEURON HOC files. Several functionalities are proposed, including general area/volume analysis, modeling of extracellular space, and proximity analysis of neuronal processes.



Bryan Jones (University of Utah)

Title: Retinal connectomics for circuit reconstruction in health and disease

Abstract: I will briefly discuss our goals of complete circuit reconstruction in the mammalian retina and the basis for understanding how circuits go awry in neurodegenerative disease, and the approach that we've taken with serial transmission electron microscopy at 2nm/px resolution. James Anderson can discuss the architecture of our Viking annotation application and the database, as well as our build environment. Crystal and Becca can discuss some of the analytical tools we use for our environments.

William Silversmith (Princeton University)

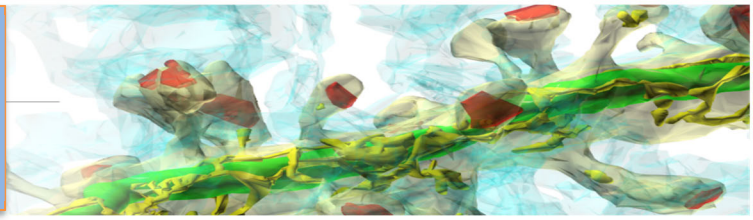
Title: Representing, Processing, and Visualizing Terascale Biomedical Images with CloudVolume

Abstract: Electron and confocal microscopy neuroscience datasets have become much larger than single workstations can handle. At the scale of hundreds of gigabytes, let alone modern datasets at the teravoxel and petavoxel scales, the basic tasks of accessing and visualizing your data become challenging. Neuroglancer, a browser based tool for visualizing large datasets and developed by Jeremy Maitin-Shepard at Google, has become popular due to its high performance and ease of sharing links that work on Linux, Mac OS, and Windows without installing special software. However, Neuroglancer does not come with a client that can read and write the Precomputed format it uses natively. CloudVolume is a Python library presenting a Numpy interface that reads and writes Neuroglancer compatible formats both to the cloud and on local filesystems. It scales from experimenting on workstations to cluster of tens of thousands of CPUs and includes a parallel mode of operation. CloudVolume comes with tools for creating new datasets and visualizing 3D cutouts even without Neuroglancer. It also has facilities for handling meshes and skeletons. In this talk, I will discuss the high level design of CloudVolume, how to get started, how to use it, and how it enables scalable computing for the connectomics community.

Art Wetzel (Pittsburgh Supercomputing Center)

Title: Overview of serial-section registration principles and expectations

Abstract: There are numerous approaches to image image matching for applications in astronomy, photogrammetry, target tracking, medical imaging and, our primary concern here, 3D reconstruction from serial EM image stacks. This presentation will briefly review principles commonly used by registration tool sets and the particular properties which have led to development of Signal Whitening Fourier Transform Image Registration (SWiFT-IR). SWiFT-IR has been successfully used for large scale reconstructions up to 100Tvoxels. Ongoing work in collaboration with the Salk Institute and with the UT-Austin team is implementing a new graphical user interface for the SWiFT-IR tools to make them more accessible to the research community. The initial version of that interface will be shown in demonstration sessions at this workshop.



Wednesday (5 June, 2019) – Workshop Speakers (In order of presentations)

Terry Sejnowski (The Salk Institute)

Title: While you were sleeping: Memory consolidation

Abstract: Your brain is active during sleep as it cycles between deep, slow wave sleep and rapid eye movement (REM), or dream sleep. Evidence is accumulating that experiences during the day are integrated into long-term memories during sleep. Synaptic mechanisms that may be responsible depend on accurate spike timing and spatially clustered synapses.

Eric Perlman (Independent)

Title: Programmatic interaction with Neuroglancer to visualize and interact with data

Abstract: Neuroglancer's python interface provide a powerful set of tools to visualize and interact with data. We will show how to rapidly assembly a simple yet functional proof-reading environment. Features utilized include local and dynamic data sources, custom shaders, synchronized annotations, and custom key bindings.

Ting Zhao (Janelia, HHMI)

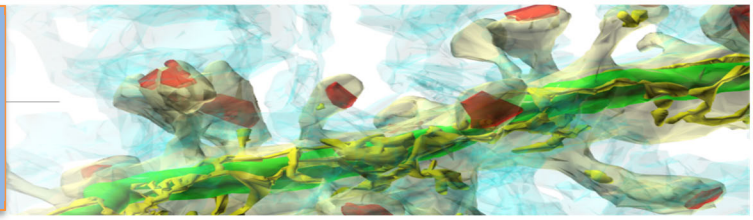
Title: From NeuTu to Neu3: Connectome Proofreading Software Shaped by Growing Data, High-Quality Segmentations and Ambitious Goals

Abstract: Rapid advances in imaging and segmentation technology are crucial to scaling connnectomics but require new proofreading tools to maximally exploit them. For example, with better segmentation, a neuron can be correctly segmented over thousands of images yet still requires multiple proofreaders due to the vast dataset. Previous tools do not allow collaborative or merge editing of segmentation, or require significant scrolling through the image volume. In the last few years, we developed NeuTu -- a powerful tool to collaboratively proofread massive segmented datasets and includes novel workflows to handle large, complicated splits. We recently introduced an even more 3D-centric client, Neu3, which combines 3D visualization and semi-automated segmentation to accelerate split (cleave) and merge operations in 3D. Both tools have been essential in generating the largest dense connectome in the world.

William Katz (Janelia, HHMI)

Title: 3DEM Data Management and Analysis

Abstract: As the number and size of EM-based datasets increases, the connectomics community will need tools to collaboratively edit, distribute, and analyze the flood of data. In this presentation, I'll describe the Janelia FlyEM team's approach to this problem. The central resource is DVID, a dataservice that provides a high-level connectomics API to a variety of clients and also records activity and changes to the dataset in a robust logging system (Kafka). I'll describe DVID and our use of Kafka to power tools critical to our project, particularly neuPrint, which lets us manage uncertainty in the reconstruction and explore/analyze large connectomics data.



Daniel Wagenaar (California Institute of Technology)

Title: GVox - Interactive gigavoxel scale volume visualization

Abstract: The combination of 3DEM with light microscopy is quickly gaining popularity. X-ray tomography (also called "micro-CT") is a powerful technique to locate a region to be imaged within a larger resin block. Unlike full 3DEM datasets, which often comprise many teravoxels (10^{12} voxels), x-ray volumes typically measure fewer than 1,000 pixels in each dimension, leading to a volume just one one gigavoxel, which comfortably fits in a modern laptop's memory in its entirety. Accordingly, I have produced a tool that can rapidly navigate such volumes without any need for precomputation. GVox allows the user to view slices of the volume with arbitrary instantaneous 3D rotation. It also allows for basic annotation and reporting of precise 3D coordinates. Our research group uses GVox to locate somata and trace thick primary neurites, which helps us to drastically reduce the volume that needs to be imaged with 3DEM. I am very much interested in discussing other potential use cases and augment the program's functionality to support those.

Qixing Huang (The University of Texas, Austin)

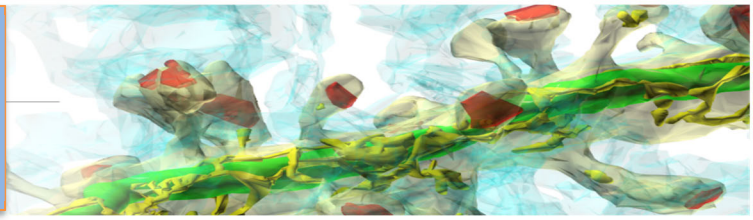
Title: Learning Transformation Synchronization

Abstract: Reconstructing the 3D model of a physical object typically requires us to align the depth scans obtained from different camera poses into the same coordinate system. Solutions to this global alignment problem usually proceed in two steps. The first step estimates relative transformations between pairs of scans using an off-the-shelf technique. Due to limited information presented between pairs of scans, the resulting relative transformations are generally noisy. The second step then jointly optimizes the relative transformations among all input depth scans. A natural constraint used in this step is the cycle-consistency constraint, which allows us to prune incorrect relative transformations by detecting inconsistent cycles. The performance of such approaches,however, heavily relies on the quality of the input relative transformations. Instead of merely using the relative transformations as the input to perform transformation synchronization, we propose to use a neural network to learn the weights associated with each relative transformation. Our approach alternates between transformation synchronization using weighted relative transformations and predicting new weights of the input relative transformations using a neural network. We demonstrate the usefulness of this approach across a wide range of datasets.

Jason Osborne (Ector County ISD Chief InnovatioChin Officer)

Title: Igniting Curiosity and Discovery Through Big Data Analysis in the K-12 Classroom

Abstract: Discovering scientifically significant fossil specimens, contributing to stem cell research, and mapping neurons in a brain are just a few ways students in K-12 classrooms are engaging with real data. Each experience is driven by a unique partnership with universities and institutes from around the nation. Learn the methods behind a novel educational model that utilizes real world research in the classroom while aligning with standards of learning.



Uri Manor (The Salk Institute)

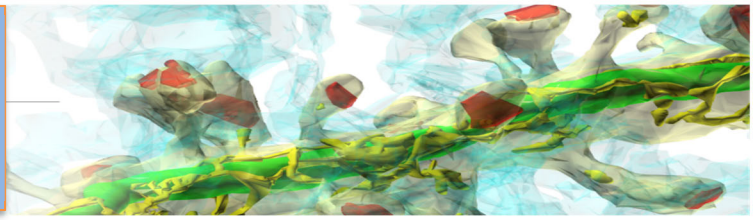
Title: Deep Learning-Based Point Scanning Super-Resolution Imaging

Abstract: Point scanning imaging systems are among the most widely used tools for cellular and tissue imaging. Like many other imaging modalities, their utility can be heavily constrained by sample damage and imaging speed. One method to deal with these issues is compressed sensing, which involves structured subsampled acquisition and post-acquisition image processing, which can be laborious, requires access to hardware settings not always available on the most widely used commercial systems, may result in undesirable artifacts, and has limited capabilities for matching full resolution acquisitions. We have employed a "compressed sensing" approach to increase image resolution by applying Deep Convolutional Neural Networks (DNNs) to upsample subsampled images, which effectively enables higher acquisition speeds as well as lower light doses. Oversampled "ground truth" images that were acquired from an Airyscan laser scanning confocal system or a scanning electron microscope, together with digitally downsampled as well as manually acquired undersampled images, formed image pairs for training and testing. In order to increase the efficiency of generating low vs high resolution training data, we used manually acquired image pairs to generate a model for downsampling large amounts of high resolution data. Testing was performed by comparing the resolution of the upsampled output from the DNN with its corresponding "ground truth" high-resolution image. Qualitative results through perceptual evaluation showed high fidelity between the network output of processed undersampled images and the oversampled high-resolution images, demonstrating the feasibility of this approach, which was further substantiated by the state-of-the-art quantitative DNN image superresolution metrics: Peak Signal-to-Noise Ratio (PSNR) and Structural Similarity (SSIM). The ability to undersample images allow us to generate higher resolution and SNR datasets on the 3View serial block face SEM system than would otherwise be possible, facilitating higher quality and higher throughput 3DEM imaging. Current efforts are underway to expand the application of DNNs to other imaging and processing modalities including correlative imaging.

Jingpeng Wu (Princeton University)

Title: Chunkflow: Distributed Hybrid Cloud Processing of Large 3D Images by Convolutional Nets

Abstract: It is now common to process volumetric biomedical images using 3D convolutional net, but it is challenging to scale them to the teravoxel and even petavoxel images that are being acquired today by light and electron microscopy. Here we introduce chunkflow, a software framework for robustly distributing ConvNet processing over local and cloud GPUs and CPUs. Chunkflow divides an image volume into overlapping chunks, each represented by an enqueued cloud task, which are then processed by a ConvNet running on any mixture of available internet connected hardware. The results are then blended and stitched together to yield the output image. The fault-tolerant architecture of chunkflow reduces the cost greatly by utilizing cheap unstable cloud instances. Chunkflow currently supports PyTorch for GPUs and PZnet for CPUs. To illustrate its usage, a large 3D brain image from serial section electron microscopy was processed by a 3D ConvNet with a U-Net



style architecture. Furthermore, chunkflow also provides a flexible command line interface for composing chunk operations that can be applied and extended to other image processing jobs.

John Mendenhall (The University of Texas, Austin)

Title: Toward tSEM Tomography: Conical Acquisition with Transmission Detection

Abstract: Conical scheme acquisition of high lateral resolution STEM/SEM images through thicker EM sections of brain tissue may eventually produce calculated virtual sections with axial resolution better than ultrasection thickness. With interval serial rotation at a one tilt angle, a single scan correction is needed for tilt and dynamic focus. Multiple images are needed per field, but with thicker sections and thinner calculated Z possible, may not be excessive considering fewer and more robust sections needed per volume.