

## Application of a Multi-Photon High-Resolution Large-Scale Montage Imaging Technique to Characterize Transgenic Mouse Models of Human Neurodisorders

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The thorough characterization of transgenic mouse models of human CNS diseases is a necessary step in realizing the full benefit of using animal models to investigate disease processes and potential therapeutics. Because of the labor- and resource-intensive nature of high resolution imaging, detailed investigation of possible structural or biochemical alterations in brain sections has typically focused on specific regions of interest as determined by the researcher *a priori*. For example, Parkinson's disease researchers often focus imaging on regions of the brain expected to exhibit pathology such as the substantia nigra and striatum. Due to limitations in acquiring and storing high resolution imaging data, additional data contained in the specimen is not usually acquired or disseminated/reported to the research community. In the case of valuable transgenic animal models with limited availability, this approach is not the most desirable for realizing the short term and long term potential of images. Here we present a method of imaging large regions of brain at close to the resolution limit of light microscopy using a montage technique in conjunction with multi-photon microscopy. Using the described montage technique, our group is characterizing of distributions of immunolabeled proteins of interest in the CNS of a transgenic mouse model of Parkinsonism [1].

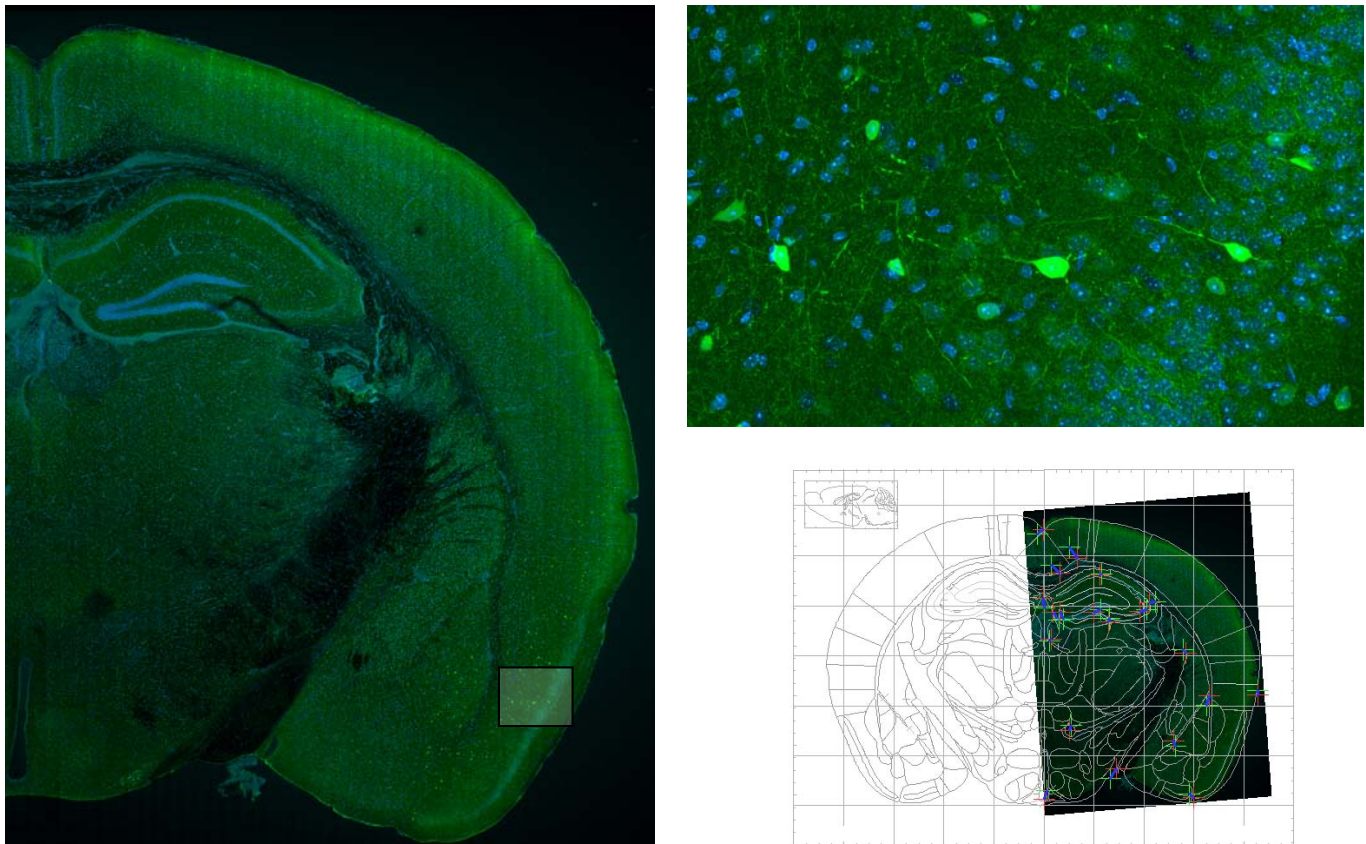
**Montaging method:** An RTS 2000 multi-photon microscope [2] at the NCMIR resource is equipped with a custom automated high precision montage stage (Applied Precision LLC), which allows for the automatic acquisition of ultra-large field image montages in 2 and 3 dimensions. The montages are typically acquired by rastering the specimen along the X, Y, and Z axes, introducing a prescribed amount of overlap between acquired images (in this case 10%) to aid alignment. Unprocessed montage data acquired on the RTS2000 microscope is subsequently stored as a single stack of images. The image stack is analyzed using the JAVA-based ImageJ, a freely available software package, using plugins developed at NCMIR for aligning the tiles with sub-pixel accuracy. Briefly, each file is separated into three separate .tiff stacks, one for each channel. Each tile is normalized to eliminate shading gradients, followed by the auto alignment of individual tiles to form a full size montage image of the data for each channel. The assembled montages are then combined into one full-scale color montage (Fig. 1). For 3D imaging, the process is repeated for each wide field image plane in Z.

**Results & conclusions:** We have acquired multiple datasets of transgenic and wildtype brain sections at different levels of the brain including the striatum, hippocampus and cerebellum. These datasets range in size from 800MB to 5 GB and allow the examination of the final images at a resolution of 0.24 microns/pixel (at 60X magnification). The fine detail contained in these large scale images allows the examination of immunolabeling patterns at the subcellular level across multiple brain regions. Examination of these images has revealed additional neuropathology (i.e. protein aggregate-filled cells in cortical and hippocampal regions). This technique is part of a multi-scale effort to characterize animal models using, ranging from whole brain imaging using MRI to ultrastructural characterization using electron tomography, as part of the Biomedical Informatics Research Network (BIRN) project. BIRN is a National Institutes of Health-sponsored initiative that enables large-scale biomedical science collaborations by utilizing emerging cyberinfrastructure (high speed networks, distributed high-performance computing and the necessary software and data integration capabilities). As part of the BIRN, the multi-scale data sets acquired on transgenic

animals will be used to create a persistent archive of macroscopic and microscopic imaging data through a federated database system. As part of NCMIR's contribution, montaging data such as the data sets shown here will be placed in the Cell Centered Database, a web-accessible database of 3D structural and protein localization data ([www.ncmir.ucsd.edu/CCDB](http://www.ncmir.ucsd.edu/CCDB)).

**References:**

- [1] D.L. Price et al., *Society for Neuroscience Abstract*, (2003) 297.10.
- [2] G.Y. Fan et al., *Biophys J*, 76(1999) 2412-20.
- [3] This work is supported by NIH NCRR RR04050, NIH NIDA DA016602 (CCDB), and The Branfman Family Foundation.



**Figure 1:** Montage of a section through one hemisphere of a transgenic mouse overexpressing alpha-synuclein (*left*) labeled with a nuclear marker (*blue*) and a protein known to form aggregates in the human Parkinson's disease state (*green*). A full resolution view of the region indicated by the gray box (*upper right*) reveals immunopositive cells in the piriform cortex at the level of the anterior hippocampal region. These images are being placed into a common coordinate system by warping each section to a standard brain atlas (*lower right*) for inclusion in the Cell Centered Database.