LIGHT SHEET FLUORESCENCE MICROSCOPY TO STUDY NERVOUS SYSTEM WIDE NEURAL DYNAMICS IN *C. ELEGANS*

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The anatomical connectivity of the nematode *C. elegans'* nervous system has already been established [1]. However, it is still poorly understood how these neuronal networks engage in dynamical activity. Recently developed calcium indicators offer the opportunity to image neuronal activity with high temporal resolution. In our previous work we have used transgenic lines expressing a nuclear localized calcium reporter with imaging techniques that are either difficult to use [2], or that offer a high temporal resolution at the cost of image quality [3]. Light sheet fluorescence microscopy (LSFM), due to low phototoxicity and high spatial- and temporal resolution, is an attractive alternative approach.
We have built a tailor-made LSFM to perform high speed large volumetric imaging of the entire nervous system of *C. elegans* with single cell resolution. One key component has been the development of a sample holder that allows for the biologically relevant experiments to be performed while the animal is kept inside a previously developed microfluidic poly(dimethylsiloxane) (PDMS) chamber [2, 3]. We were able to further improve the stability of the setup by implementing an active laser-intensity and position stabilization. Here we present our preliminary imaging results and their quantification. Using the same transgenic lines as before [2], we acquired stroboscopic images of the neural network dynamics as the sample is repeatedly swept with a linear ramp through the light sheet at 5 volumes per second. A typical acquisition lasts 30 minutes, during which time the correspondence between each image and the position of the sample is recorded for the accurate reconstruction of each volume. We are in the process of modifying our image processing software [4] to segment and identify individual neurons to generate neural time series data. From this information we will use computational methods to identify and quantify behavioral relevant network dynamics.

[1] White, J. G., Southgate, E., Thomson, J. N., & Brenner, S. The structure of the nervous system of the nematode Caenorhabditis elegans. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* **314**, 1–340 (1986)
[2] Schrödel, T., Prevedel, R., Aumayr, K., Zimmer, M., and Vaziri, A., "Brain-wide 3D imaging of neuronal activity in Caenorhabditis elegans with sculpted light," *Nat. Methods* **10**, 1013–1020 (2013)
[3] Prevedel, R.*, Yoon, Y.*, Hoffmann, M., Pak, N., Wetzstein, G., Kato, S., Schrödel, T., Raskar, R., Zimmer, M., Boyden, E., and Vaziri, A.(*joined first authors) "Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy," *Nat. Methods* **11**, 727–730 (2014)
[4] Kato, S., Kaplan, HS., Schrödel, T., Skora, S., Lindsay, TH., Yemini, E., Lockery, S., Zimmer, M. "Global Brain Dynamics Embed the Motor Command Sequence of Caenorhabditis elegans", *Cell* **163**, 656-69 (2015)