

**LABORATORY FOR SPATIOTEMPORAL REGULATIONS**



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Our laboratory is currently pursuing two paths of scientific inquiry that are of interest to developmental biology. One goal is to clarify the mechanism of left-right (L-R) determination. The other is to develop imaging technologies for biological analyses.

**I. Initial step for left-right asymmetry**

In mammalian development, the initial left-right asymmetry first appears as cilia-driven leftward fluid flow on the ventral surface of the node, and this flow provides the initial cue for asymmetric gene expressions that actually determine asymmetric morphogenesis. The mechanisms that convert the flow to asymmetric gene expression, *i.e.* the flow sensing mechanism, remain controversial, with several models being proposed, and the involvement of  $Ca^{2+}$  being suggested.

We pursued this question by measuring  $Ca^{2+}$  dynamics in the node and found that the node cells apparently cause stochastic elevation of  $Ca^{2+}$ . The spatiotemporal distribution is equal on the left and right sides but becomes more prevalent on the left after the late headfold stage, when flow-induced commitment occurs. This asymmetry was disrupted in dynein mutant *iv/iv* and *pkd2<sup>-/-</sup>* mutants, in accordance to their left-right phenotypes.

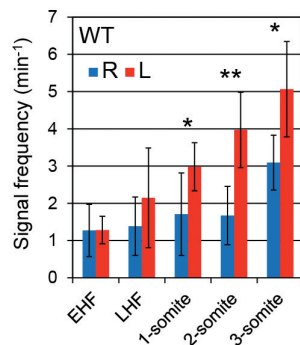
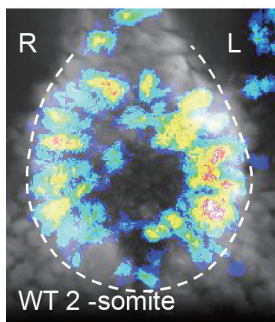


Figure 1. Left: Distribution of  $Ca^{2+}$  elevation in a 2-somite wild-type node. Right: Time course of  $Ca^{2+}$  elevation frequency at the left and the right sides.

Based on these findings, we are now examining the validity of the two major proposed models of flow sensing, mechanosensing and chemosensing, as well as a third novel mechanism.

**II. Development of light-sheet microscopy**

Light-sheet microscopy has become popular during this decade for benefits such as low photodamage and fast image acquisition. We are now running two light-sheet microscopes, one commercial and the other self-made, and are maintaining them for collaborations and our own research

interest (this being left-right asymmetry).

Over several years, we have developed a fast light-sheet microscope named ezDSLMS, in which the excitation light sheet and detection objective move instead of the specimen for z-scanning. We installed an electrically tunable lens (ETL) to achieve greater speed and the exclusion of mechanical vibrations that disturb continuous 4D imaging of floating or loosely held specimens.

Our light-sheet microscopes are available to other researchers via NIBB's Collaborative Research and MEXT's Advanced Bioimaging Support (ABIS) programs. Numerous collaborations are in progress including observation of moving *Amoeba proteus*, neuronal activity in *Drosophila* larvae, cell migration in zebrafish embryos, cleared mouse brains, and marine crustaceans, *etc.*

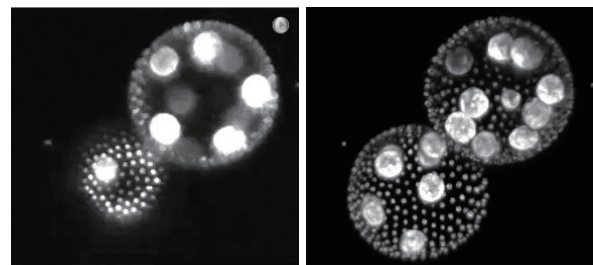


Figure 2. Images of floating volvox taken by ezDSLMS with ETL. Left: Single optical section. Right: Maximum intensity projection.

**Publication List:**

[Original papers]

- Nishigami, Y., Ohmura, T., Taniguchi, A., Nonaka, S., Manabe, J., Ishikawa, T., and Ichikawa, M. (2018). Influence of cellular shape on sliding behavior of ciliates. *Commun. Integr. Biol.* *11*, e1506666.
- Ohmura, T., Nishigami, Y., Taniguchi, A., Nonaka, S., Manabe, J., Ishikawa, T., and Ichikawa, M. (2018). Simple mechanosense and response of cilia motion reveal the intrinsic habits of ciliates. *Proc. Natl. Acad. Sci. USA* *115*, 3231-3236.
- Okimura, C., Taniguchi, A., Nonaka, S., and Iwadate, Y. (2018). Rotation of stress fibers as a single wheel in migrating fish keratocytes. *Sci. Rep.* *8*, 10615.