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iFLUOR

"Simultaneous calcium imaging
of multiple nematodes using
iFLUOR's ultra-wide field for
studying neuronal activation
under controlled mechanical
stimuli"

D-TAILS
Disruptive Technological Advances In Life Science

Ultra-wide field acquisition of a population of *C. elegans* in a microfluidic chip.

At D-Tails, we developed an innovative acquisition system that boasts ultra-wide field of view coupled with high-resolution imaging capabilities, that enable researchers worldwide to acquire images across diverse applications in a groundbreaking way.

Our new product, the iFLUOR system, is based on a high-quality relay lens and a cutting-edge CMOS sensor able to acquire images with a field of view of $10 \times 10 \text{ mm}^2$, paired with an illumination system that includes two customizable channels (transmission and fluorescence). In addition to that, our device integrates a syringe pump system with up to four syringe pumps equipped with temperature and leakage controls, for managing microfluidic experiments, maintaining cell cultures, or preserving sample integrity during *in vivo* experiments.

For a better experimental control, iFLUOR is also compatible with Okolab incubators. Finally, its dedicated software allows for timelapse design for up to 3 days, fluidic delivery with customizable dynamics, acquisition controls, super resolution image acquisition and data analysis.

In this Application Note, we use the iFLUOR system to acquire full-field images of a population of *C. elegans* loaded in a microfluidic chip, using the transmission and fluorescence channel at 2.5 microns pixel resolution and 1.1x magnification.

Using iFLUOR to perform calcium imaging on *C. elegans* in a microfluidic chip to assess neuronal activation of targeted neurons.

We loaded a population of adult nematodes in a microfluidic chip especially designed to perform calcium imaging experiments with *C. elegans*^{1,2}.

The chip features a wide central arena filled with a forest of pillars that enable the animals to freely move inside the chip while providing support to the geometry. Such a device allows to keep under the field of view about 50 nematodes and perform high-throughput acquisitions, while giving the possibility to expose animals to chemicals in a timely controlled manner.

Genetically modified adult *C. elegans* hermaphrodites (PS6374 line) were synchronized and loaded onto the microfluidic chip.

The genetic modification drives the expression of a calcium indicator (GCaMP6) in a targeted chemosensory neuron (AWC) that has been demonstrated to respond to changes in fluidic shear stress as well³.

We used the iFLUOR system to record the activity of up to 50 nematodes in the fluorescence channel, while exposing the animals to changes in pressure and shear stress.

As Fig. 1 shows, iFLUOR's large field of view allows to record the activity of 50 nematodes. In almost all animals, its high resolution enables to identify not only as small details as the AWC neuronal soma (6 - 8 microns) with calcium imaging expression, but also its neurites (about 2 microns).

With such a setup, a single recording enables to collect data from multiple nematodes reaching statistic relevance in just one acquisition. In this case, we were able to assess the activation of AWC after applying a pressure change in the microfluidic device, confirming the mechanosensory nature of the targeted neuron along with its chemosensory abilities.

Such application shows the advantage of using iFLUOR to perform calcium imaging studies, which rely in the possibility of recording wide field images with a high level of detail, that allows to track the dynamics of the multiple neurons on different nematodes at once.

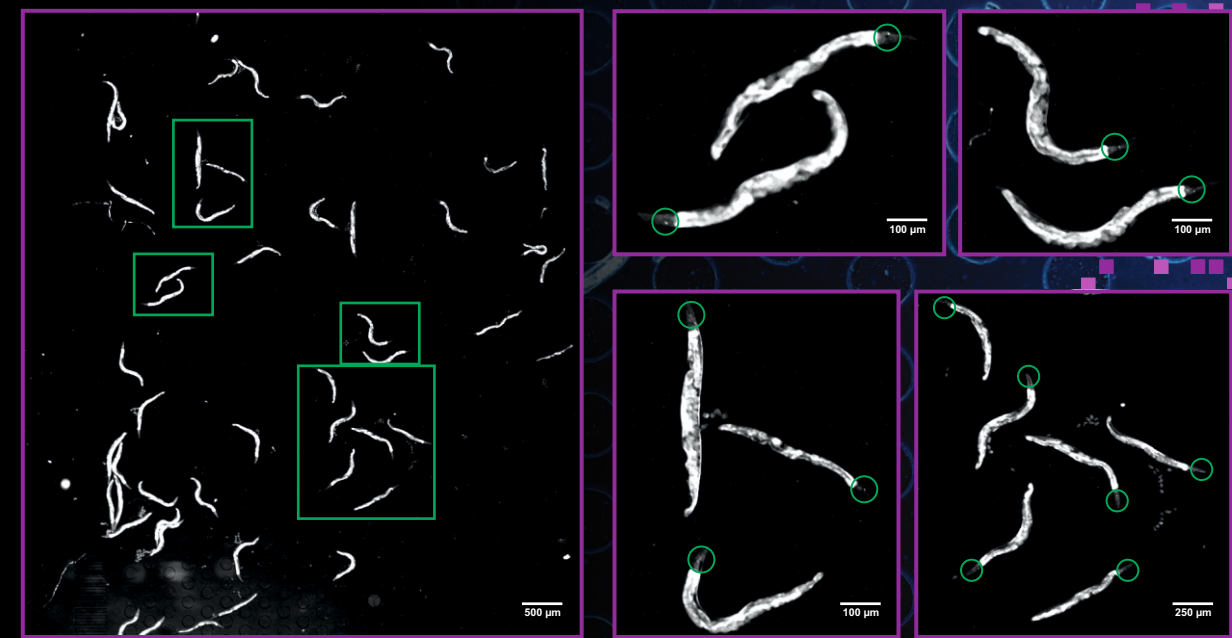


Fig. 1. Calcium imaging recording of a population of *C. elegans* with calcium indicator expression in the AWC chemosensory neurons.

With its large field of view and its high resolution, the iFLUOR system allows to record the activity of multiple nematodes at once (50 in this case, see left panel), with such a level of detail that allows to identify as tiny details as somas and neurites of neurons with calcium indicators expression (panels on the right, green circles).

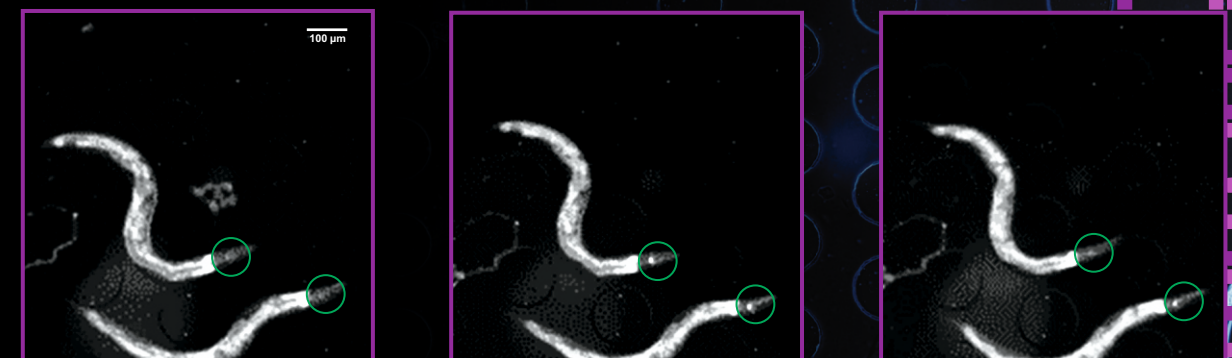


Fig. 2. Activation of *C. elegans* AWC chemosensory neurons in response to mechanical stimuli.

iFLUOR's high resolution, allows to appreciate the differences in activation levels of multiple nematodes at once in response to mechanical stimuli.

1. Larsch, J., Ventimiglia, D., Bargmann, C. I. & Albrecht, D. R. High-throughput imaging of neuronal activity in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 110, (2013).
2. Lanza, E., Di Rocco, M., Scwhartz, S., Caprini, D., Milanetti, E., Ferrarese, G., Ruocco, G., Martinelli, S., Folli, V. A *C. elegans* based strategy for the early detection of cancer metabolites in urine samples: a proof of principle study. *Submitt. to ELife* (2021).
3. Caprini, D. u.c. A Shearless Microfluidic Device Detects a Role in Mechanosensitivity for AWC Neuron in *Caenorhabditis elegans*. *Adv. Biol.* 5, (2021).