Femtosecond laser dissection of neurons in *C. elegans*

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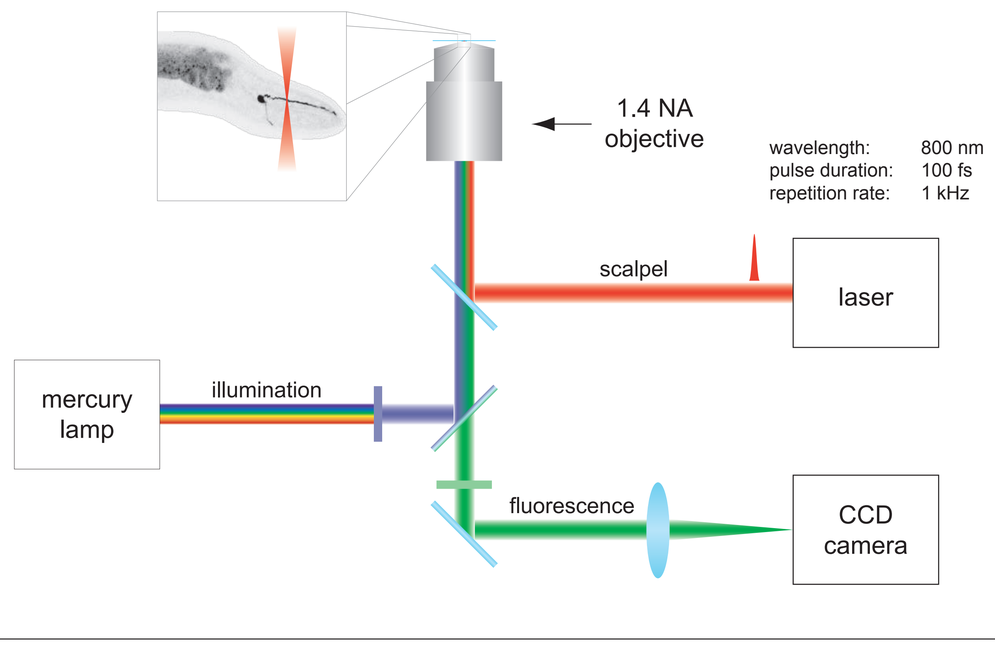
Introduction

Cell and developmental biologists continually search for new tools to manipulate cells and subcellular components to study their contribution and response to biological processes. Previously, research was limited by the insufficient resolution (tens of micrometers) of conventional tools such as microneedles and laser ablation. Femtosecond laser dissection has submicrometer resolution and causes almost no collateral damage, opening many processes and cellular structures to investigation.

Surgery and imaging setup



Characterization of dissection

Tight focusing of femtosecond pulses leads to extremely high intensities and nonlinear absorption, even with only nanojoule pulse energies. As a result:

1. Surgical effect localized to focal volume
2. Collateral damage (heating, shock waves) minimized

Figure shows the surgical precision with 3.2-nJ pulses on a bundle of DiO-stained amphid neurons of *C. elegans*.

Dissection precision

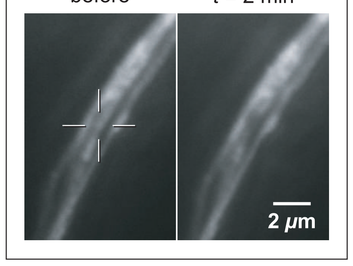


Figure shows retraction of the free ends following dissection of an AFD dendrite with 5.6-nJ pulses. This confirms a complete severing and indicates that dendrites are under tension. The AFD neurons express green fluorescent protein (GFP), which is used for imaging.

Post-surgery dynamics

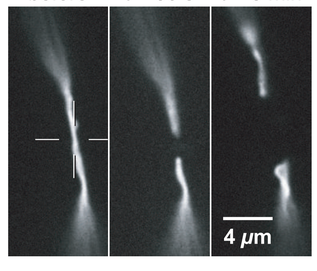
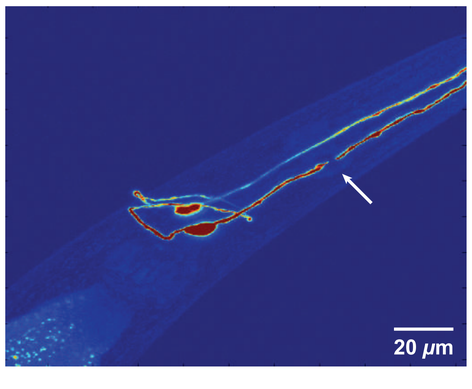


Figure shows GFP-fluorescent ASI neuron dissected with 14-nJ pulses at indicated location. Worm was revived and imaged on a confoclal microscope one day after surgery.

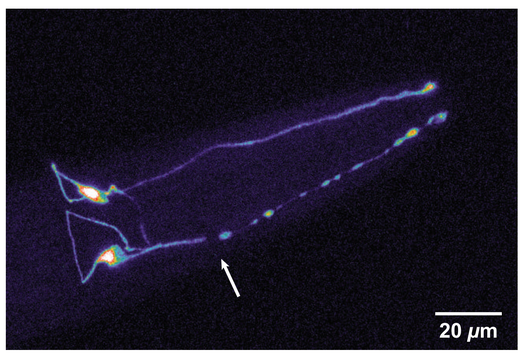


Application: thermotactic behavior

The neural network of the nematode worm *Caenorhabditis elegans* comprises 302 neurons that interconnect in the same way in every adult animal. The invariance implies that each neural structure has a stereotyped role in producing behavior, and the relative simplicity suggests it might be possible to completely map this behavior to the worm's neural pathways. Conventionally, a neuron's role is illuminated by linking a deficit in behavior to a defect in the neural network (mutant); however, such mutants may not exist or lack identification. Femtosecond laser dissection can remove a neuron or neuronal part from adult wild-type background worms, producing the desired defect that leads to the aberrant phenotype.

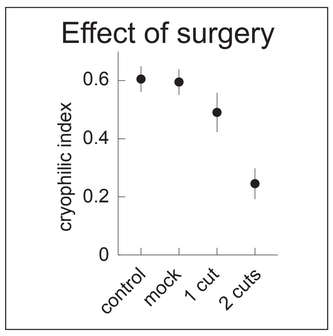
Mapping behavior to neural pathways

Confocal image of the AFD neurons expressing GFP two hours after surgery. One dendrite was dissected with 3.2-nJ pulses at the indicated location. Dissection blocks information from propagating down the dendrite.



Behavioral assays after severing dendrites of the AFD neuron show that a thermosensory measurement by the AFD dendrites contributes to cryophilic movement. Severing both dendrites is required to turn off cryophilic movement. Further experiments on mutants showed that the dendrite measures absolute temperature but not temperature changes.

Effect of surgery



Future application: neurodegeneration

Wallerian degeneration occurs along a neuronal fiber when it is severed from the cell body (see confocal image above). The discovery of a mutation in mice that slows Wallerian degeneration has produced reseach *in vitro* showing that sirtuins (a family of proteins present in many organisms) are involved in protecting axons from self-destruction*. Moreover, sirtuin activators delay aging in animals**.

Femtosecond laser dissection will sever neuronal fibers of *C. elegans* under various sirtuin-activating compounds, permitting a study of neurodegeneration *in vivo*. Such research will have applications to diseases such as Parkinson's and Alzheimers.

Brain shrinkage in Alzheimer's patient

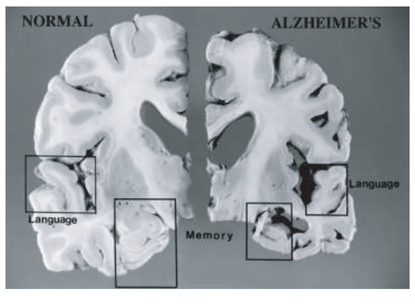


Image courtesy of Ennaceur Lab, University of Sunderland, UK

*T. Araki, *et al.*, Science **305**, 1010 (2004).

J. G. Wood, *et al.*, Nature **430, 686 (2004).

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