Unrestrained worms bridled by the light

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Two systems allow precise optogenetic stimulation of specific neurons in freely behaving nematodes.

Optogenetics, the use of genetically encoded probes to image and control cellular activity with light, has grown explosively in recent years. In neuroscience studies, this has meant an ever-increasing array of molecules with improved characteristics for detecting calcium and voltage changes and for silencing and activating neurons, often with carefully tailored kinetics and spectral properties[1]. Complementary hardware and software are critical for continued advances, and two teams now report an enabling milestone in this issue of *Nature Methods*[2,3].

By combining and improving several existing technologies, these groups independently developed two optogenetic illumination systems that allow real-time light delivery with high spatial resolution to specified targets in freely moving *Caenorhabditis elegans*. Using this technology, they could activate specific neurons in freely behaving nematodes with a flexibility and resolution that will open the door to new investigations into the integration of sensory information and the operation of defined neural circuits *in vivo*.

There are three elements at the core of the newly combined systems. First, there are the genetically modified worms, which are engineered to express a light-sensitive protein called channelrhodopsin in particular neurons. When these neurons are exposed to blue light, the channels open and depolarize their host cells (halorhodopsin or Mac can be used to hyperpolarize and inhibit neurons). In *C. elegans*, this approach rests on a deep foundation of knowledge that includes the position and connectivity of all of the worm's 302 neurons[4] as well as a wide range of promoters known to drive expression in single or small groups of cells. However, cell-specific promoters are not available for every neuron of interest, and in any case, it is desirable to rapidly target specific parts of the worm to investigate circuits involving more than one channelrhodopsin-expressing neuron. This has recently led to the development of the second element, the use of an illumination system, such as a digital micromirror device that allows illumination of specific pixel locations in the field of view[5]. A standard microscope provides the magnification that is required to resolve different subsections of an immobilized worm in which neurons of interest are located. However, keeping a freely moving worm in the resulting small field of view requires a third element, a system that automatically locates the worm body and a motorized stage that keeps it centered. Such systems have been used to record and quantify the behavior of freely behaving worms in the past[6,7], but in those experiments analysis could be carried out offline after completion of recording. In contrast, automated optogenetic stimulation of freely behaving worms requires the development of software that allows each movie frame to be segmented and the worm position to be determined in real time (**Fig. 1**).

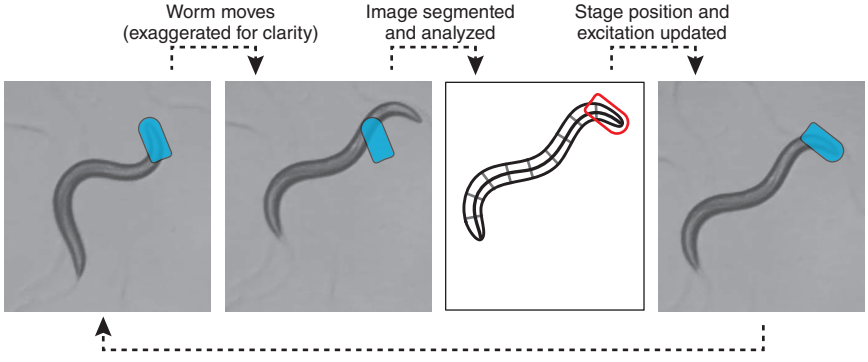

Figure 1 | Automated optogenetic stimulation of freely behaving worms. During stimulation, in this case with blue light at the head, the unrestrained worm crawls to a new position. Using appropriate software, the new worm image is then segmented and analyzed to determine the stage offset and new excitation coordinates, which are updated in real time.

Although the underlying concepts of the instruments described by the two teams in this issue are similar, there are some interesting differences in their implementation. The Leifer *et al.*[2] system uses diode-pumped solid-state lasers reflected off of a digital micromirror device to illuminate the sample, which can provide a high excitation intensity, and their tracking and targeting software can operate at 50 hertz including image aquisition, analysis and micromirror updating. In contrast, the Stirman *et al.*[3] system is somewhat slower at 25 hertz and has a maximum illumination intensity around 5 mW mm^{-2}, although, as the authors pointed out, this is sufficient for many optogenetic applications.

What makes the Stirman *et al.*[3] system noteworthy, however, is its light source: a slightly modified off-the-shelf LCD projector aimed through the epi-illumination port of an inverted microscope. In the long run, this is perhaps this system's most important feature because the Stirman *et al.*[3] approach should be affordable and technically accessible to researchers in a range of laboratories.

Both groups present a variety of proof-of-principle experiments that involve activating

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and inhibiting muscles and neurons, and show how controlled spatial and temporal stimuli will advance understanding even in some well-described neural circuits. For example, it is well known that anterior touch causes reversals in *C. elegans,* whereas posterior touch causes forward acceleration, consistent with a simple escape response. Until now, it has been difficult to quantitatively assess the competition between the two, but by varying the intensity between anterior and posterior illumination, Stirman *et al.*[3] provide some insight into how the two stimuli are integrated into a behavioral output.

Given that *C. elegans* locomotion is thought to have an important proprioceptive component, experiments on restrained worms are unlikely to provide a complete picture of how the motor circuit generates (and modulates) the undulatory waves necessary for crawling on surfaces or swimming through fluids. Experiments using traditional electrophysiology in freely moving worms are extremely challenging, but optogenetics presents two alternatives. Using calcium imaging to monitor muscle and motor neuron activity in freely behaving worms could provide critical data on how the circuit operates during normal locomotion. Even in the absence of knowledge of the underlying neural activity, targeted interventions in freely moving worms can also be revealing, as Leifer *et al.*[2] demonstrate by silencing only a portion of the ventral nerve cord motor neurons or body-wall muscles, which does not change the anterior body wave but prevents its posterior propagation past the silenced region. More extensive data and their direct comparison with competing theories of the motor circuit will be an important advance.

These two papers[2,3] highlight how genetics-based tools are revolutionizing the study of behavioral neuroscience in simple animal models. Engineered proteins targeted to specific neurons have been used to map neural circuits[8], monitor neural activity during behavior[9,10] and now to precisely manipulate neuronal function at the single-cell level. With the development of automated tools to effectively exploit these reagents, the possibility of understanding the complete neural circuitry of *C. elegans* has become a realistic goal.

The lessons learned from these studies, as well as the technologies used to obtain them, should provide important stepping stones toward a similar mechanistic understanding of more complicated brains, including those of flies, fish and ultimately humans.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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New approaches to modeling complex biochemistry

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Combining rule-based descriptions of biochemical reactions with agent-based computer simulation opens new avenues for exploring complex cellular processes.

A major challenge in modeling complex genetic and biochemical circuits is turning imprecise 'word models' (text and drawings) into precise mathematical statements suitable for computational analysis. Many biochemical processes common to eukaryotic signaling networks are remarkably difficult to describe and simulate with rigor and precision. Assembly of multiprotein complexes or multisite post-translational modification can create a very large number of distinct molecular species from interactions involving only a handful of gene products. Such 'combinatorial complexity' exceeds the ability of conventional modeling methods to enumerate species and track their dynamics, leading to *ad hoc* assumptions about which types of interactions matter and which do not.

The network-free stochastic simulator (NFSim), described in this issue of *Nature Methods*[1], is a new entry in an evolving class of general-purpose computational tools that addresses the challenge of combinatorial complexity by combining a rule-based representation with agent-based simulation. In a rule-based representation, patterns of interaction among proteins and other biomolecules are specified using a specialized computer language. By analogy to organic chemistry, only information important for a specific reaction is included in a rule; all nonparticipating structures are left unspecified. A rule-based approach differs from conventional reaction-centric models in which equations (commonly ordinary differential equations (ODEs)), one for every possible molecular species or complex, are enumerated to describe the time dynamics of the system. For combinatorially complex systems, equation-based models are hard to error-check, extend and reuse, in contrast to rule-based models, which are concise, comprehensible and easily extended. Research to date suggests that rule-based approaches enable simulation and analysis of classes of complex reactions that would otherwise be intractable[2].

Available rule-based modeling tools are differentiated by the way they perform simulations (**Fig. 1**). In BioNetGen[3], the technology on which NFSim is built, a rule set is typically used to generate the full network of all possible chemical reactions, which are then simulated using ODEs or the stochastic simulation algorithm of Doob-Gillespie[4]. However, for systems with a sufficiently high degree of combinatorial complexity, enumeration of all chemical reactions becomes prohibitively costly (as the authors[1] of NFSim demonstrate, this can occur in simulations of multisite phosphorylation with as few as six to eight sites).

To overcome this limitation, NFSim represents the system as a finite pool of interacting molecules, or 'agents', and the simulation unfolds stochastically by the repeated action of the rules on the pool of molecules. Because the rules themselves are used to direct the simulation, generation of the full reaction network is unnecessary. In this respect, NFSim adopts

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