

High-Throughput Neurotechnology

Academic and Research Staff

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Overview of group:

Our lab is developing high-throughput high-content technologies for investigating the complex development, function, reprogramming, degeneration and regeneration of the nervous system. We employ a variety of techniques including micromanipulation, microfluidics, ultrafast optics, advanced microscopy, quantum physics, genetics, and biochemistry. We also work with a variety of organisms and preparations ranging from *C. elegans*, zebrafish, primary rodent and human tissue to human embryonic stem cell derived neurons.

1. Refinement of our microfluidic high-throughput screening platform for *C. elegans*:

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NIH Director's Innovator Award
NSF Career Award

Project Staff

Christopher Rohde, Chrysanthi Samara, Cody Gilleland

We have significantly improved robustness of our microfluidic screening platforms such that we are now able to perform complex chemical screens on thousands of *C. elegans* without hardware and software failures. See Samara et. al. PNAS 2010 for details.

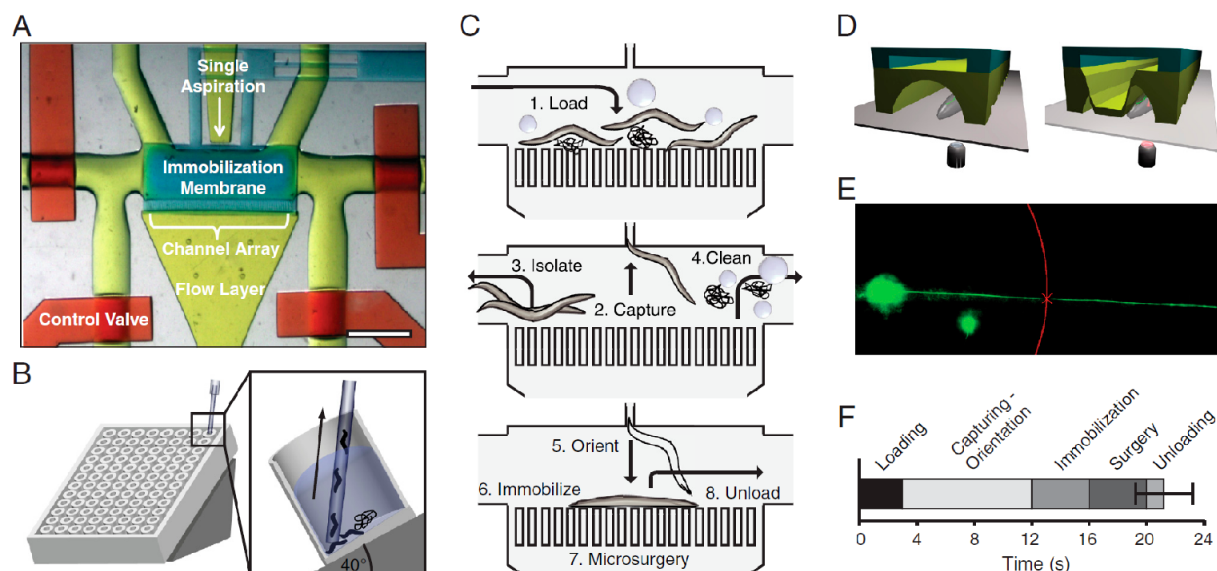


Fig. 1. Microfluidic *C. elegans* manipulation for subcellular laser microsurgery and chemical library screening. (A) Micrograph of dye-filled microfluidic chip. Red: Control (valve) layer, yellow: Flow layer, blue: Immobilization layer. Scale bar: 1 mm. (B) Animal loading from multiwell plates. The multiwell plate is held at a 40° angle and a stainless steel tube is inserted to the well bottom. (C) Microfluidic *C. elegans* manipulation steps. 1. Loading of nematodes. Dust, debris, air bubbles, and bacteria may also enter the chip. 2. Capture of a single animal by the single aspiration channel. 3. Isolation of a single animal within the chamber by low-pressure washing of the channels to remove and recycle the rest of the nematodes. 4. Cleaning of channels by high pressure washing to remove debris and bubbles. 5. Orientation of the single animal by releasing it from the single aspiration port and recapturing it by the channel array. 6. Immobilization by pressurizing a thin membrane (see part D). 7. Laser microsurgery (see part E). 8. Unloading of the animal from the chip after surgery. (D) Illustration of the final immobilization process. Once a single animal is captured and linearly oriented (*i*), a channel above the main chamber is pressurized pushing a thin membrane downwards (*ii*). The membrane wraps around the animal significantly increasing immobilization stability for imaging and surgery. Precise laser targeting of subcellular features is achieved using a femtosecond laser tightly focused inside the *C. elegans* body by a high numerical aperture objective lens (see Materials and Methods). (E) Software interface to accelerate axon targeting for laser axotomy. A right mouse click on the cell body is used to identify the portion of the axon a set distance from the soma, and a left mouse click moves this location to the laser focal point. (F) Average time per animal for screening steps. Total time per animal is from 3 independent experiments with 100 worms each. See Samara et. al. PNAS 2010.

2. Identification of small molecules that enhance regeneration of neurons *in vivo* (post femtosecond laser microsurgery):

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NIH Director's Innovator Award
NSF Career Award

Project Staff

Christopher Rohde, Chrysanthi Samara, Cody Gilleland

We have used our microfluidic technologies to perform the first large-scale *in vivo* neuronal injury screen. We have identified cellular targets and chemicals that enhance regeneration of sensory neurons *in vivo* in *C. elegans*. See Samara et. al. PNAS 2010 for details.

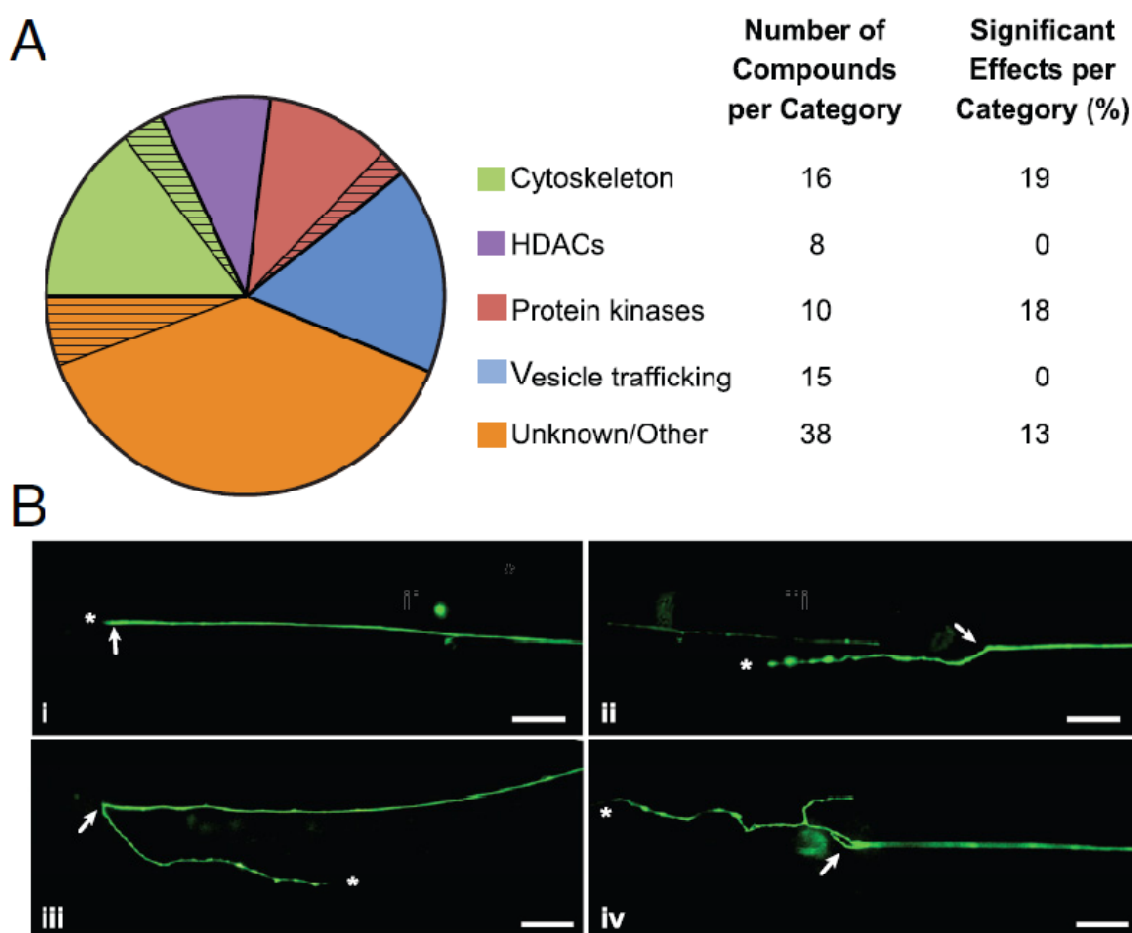


Fig. 2. *In vivo* chemical screen for small molecules affecting axonal regeneration. (A) Primary target-categories of the screened compound library. The dashed parts of the pie chart represent the percentage of compounds in each category affecting regeneration. The number of screened compounds and the percentage of the effective compounds of each category are denoted. (B) Common regeneration phenotypes observed 72 h following axotomy and compound exposure: (i) No axon regrowth, (ii) forward regrowth, (iii) backward regrowth, and (iv) regrowth with branching. Arrows and asterisks indicate start and end points of regenerated axons respectively. For regrowth with branching, indicated start and end points are for the longest regrown branch. Scale bars: 20 μ m. See Samara et. al. PNAS 2010.

3. Development of a detailed protocol on microfluidic *C. elegans* manipulation techniques:

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NIH Director's Innovator Award
NSF Career Award

Project Staff

Cody Gilleland, Christopher Rohde

We have developed a simplified method for building microfluidic chips to immobilize and image *C. elegans*. We published a step-by-step protocol for the research community to be able to implement this technology. See Gilleland et. al., Nature Protocols 2010 for details.

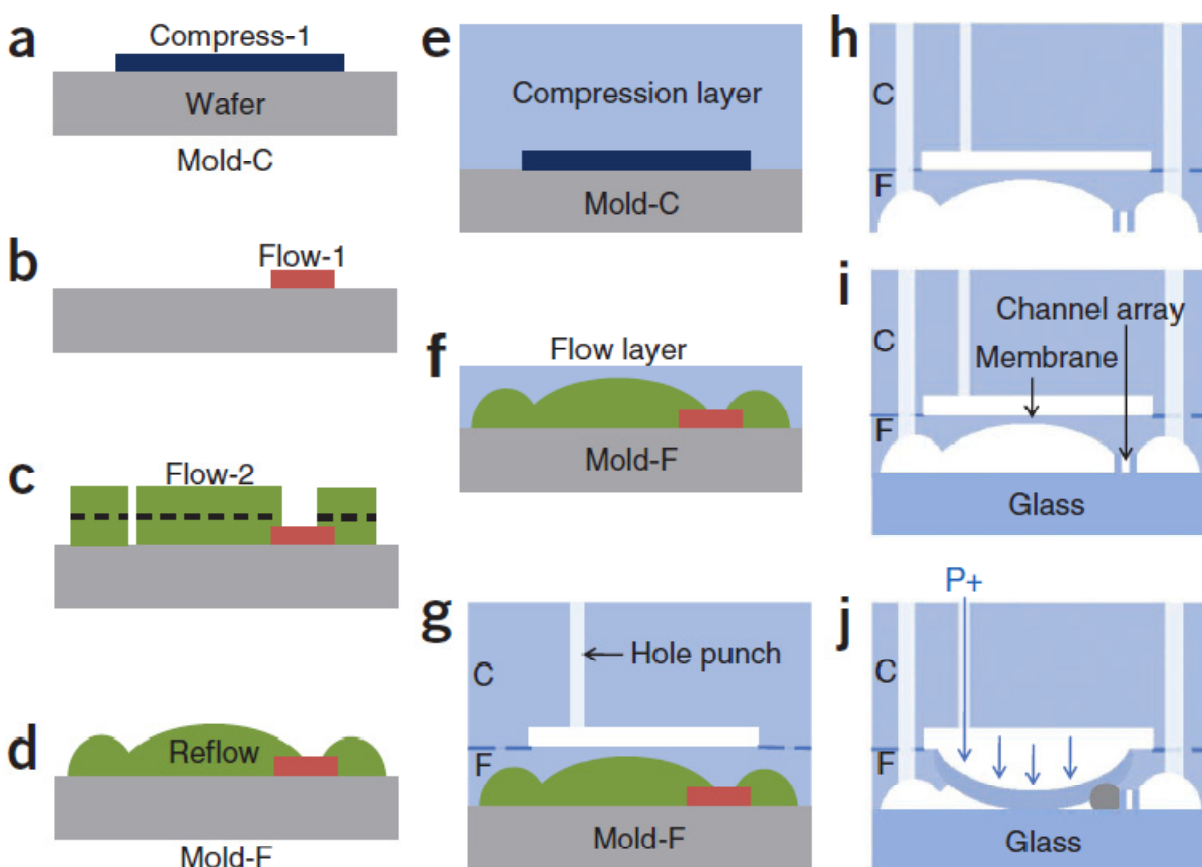


Fig. 3. Fabrication of photoresist molds and PDMS layers. The patterning of mold-C (a) and mold-F (b–d). Molding (e–f) and bonding (g) of PDMS layers. Bonding of the released PDMS layer to glass (h–i). Actuation of the immobilization membrane when pressurized (j). These illustrations are referenced throughout the fabrication procedure. See Gilleland et. al., Nature Protocols 2010.

4. Development of a high-throughput screening platform for vertebrates (zebrafish) with capability to do *in vivo* neuronal degeneration and regeneration studies:

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NIH Transformative Research Award

NIH Director's New Innovator Award

Project Staff

Carlos Pardo-Martin, Tsung-Yao Chang, Bryan Koo, Cody Gilleland, Steven Wasserman

We have developed the first platform that can perform large-scale genetic and drug screens on vertebrate organisms at cellular-resolution. See Pardo-Martin et.al., *Nature Methods* 2010 for details. This work was selected as the cover of *Nature Methods* in August 2010.

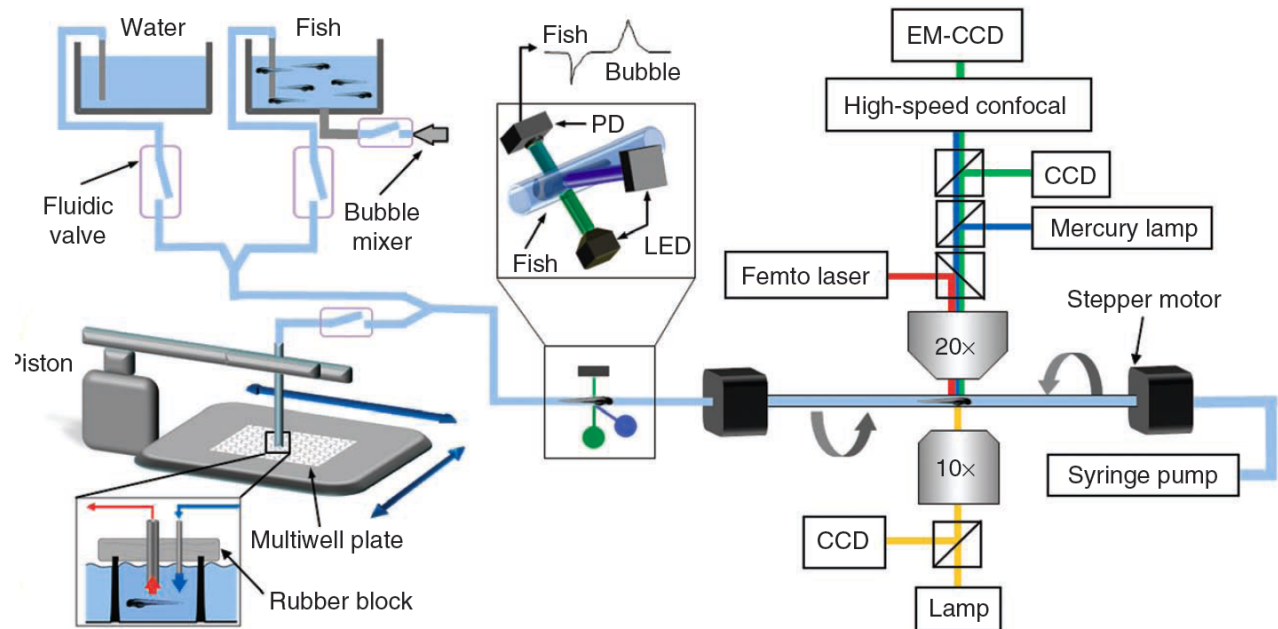


Fig. 4. Schematic of zebrafish manipulation and imaging platform. Larvae are automatically loaded to the system from either reservoirs or multiwell plates. Reservoirs are connected to the system via fluidic valves and a bubble mixer prevents the larvae from settling. The multiwell plate is located on a motorized x–y stage, which positions individual wells below larva-loading (red arrow) and water-supply (blue arrow) tubes, both held by a silicone rubber block. The block seals the well surfaces as a piston moves the tubes into the wells. A photodetection system including two LEDs and one high-speed photodiode (PD) discriminates the passage of a larva from air bubbles and debris. Two stepper motors hold a capillary along its axis of rotation; this assembly is mounted on a three-axis position stage (not shown) and held between an upright microscope for confocal imaging and an inverted microscope for brightfield imaging. A multifocal confocal head with a cooled electron-multiplying charge-coupled device (CCD) camera and a second large-area CCD connected to the upright port are used for high-speed confocal and widefield fluorescence imaging. A high-speed CCD camera connected to the inverted port allows rapid brightfield detection and positioning of larvae. A femtosecond laser beam used for microsurgery is directed to the upper beam path by a dichroic filter and focused on the sample through the objective. See Pardo-Martin et.al., *Nature Methods* 2010

5. Development of a detailed protocol on femtosecond laser neurosurgery:

Sponsors

NIH Director's New Innovator Award
NSF Career Award

Project Staff

Joseph Steinmeyer, Christopher Rohde, Cody Gilleland, Matthew Angel, Carlos Pardo-Martin, Tsung-Yao Chang

We have developed a simplified method for building systems to perform femtosecond laser microsurgery on small organisms. We published a step-by-step protocol for the research community to be able to implement this technology. See Steinmeyer et. al., Nature Protocols 2010 for details.

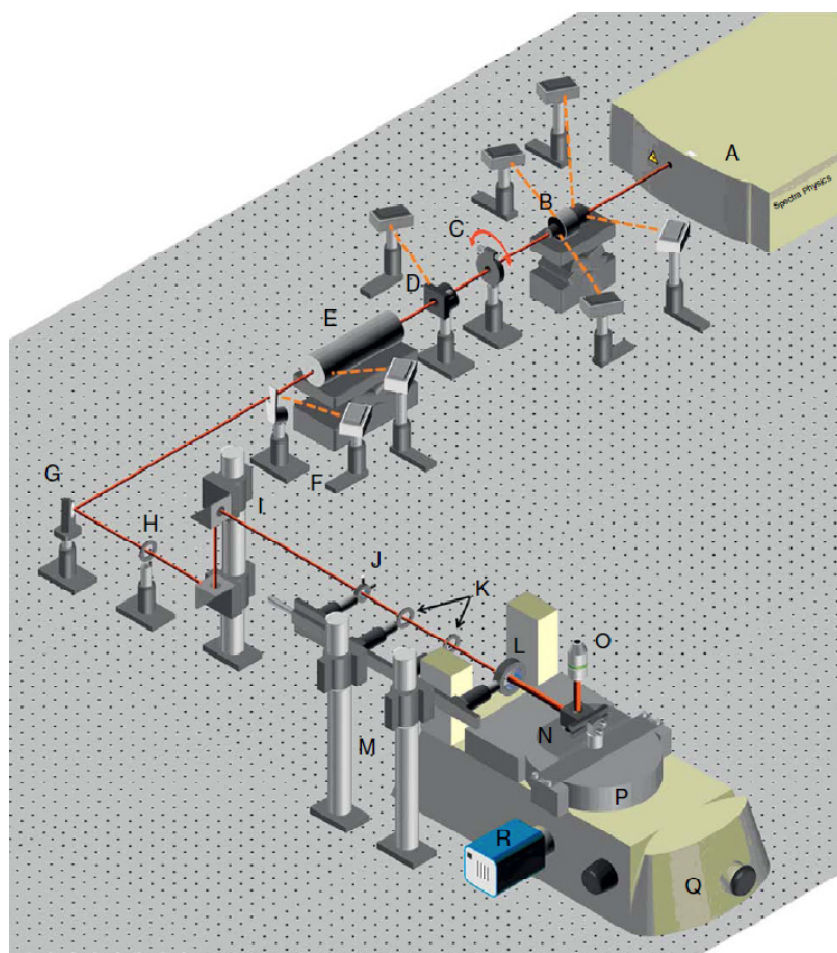


Fig. 5. Femtosecond laser microsurgery is a powerful method for studying cellular function, neural circuits, neuronal injury and neuronal regeneration because of its capability to selectively ablate sub-micron targets in vitro and in vivo with minimal damage to the surrounding tissue. We developed a step-by-step protocol for constructing a femtosecond laser microsurgery setup for use with a widely available compound fluorescence microscope. The protocol begins with the assembly and alignment of beam-conditioning optics at the output of a femtosecond laser. Then a dichroic mount is assembled and installed to direct the laser beam into the objective lens of a standard inverted microscope. Finally, the laser is focused on the image plane of the microscope to allow simultaneous surgery and fluorescence imaging. We illustrate the use of this setup by presenting axotomy in *Caenorhabditis elegans* as an example. This protocol can be completed in 2 d. See Steinmeyer et. al., Nature Protocols 2010.

6. High-throughput single-neuron-resolution genetic and pharmacological screens using sub-micron-scale combinatorial protein patterns

Sponsors

NIH EUREKA (Exceptional Unconventional Research Enabling Knowledge Acceleration) Award

Project Staff

Zachary Wissner-Gross, Mark Scott, Peng Shi

We have been developing techniques that enable highly complex and large-scale assays on single neurons using substrates patterned by combinatorial proteins using femtosecond lasers and microfluidics. See Wissner-Gross et. al., Integrative Biology 2010 for details.

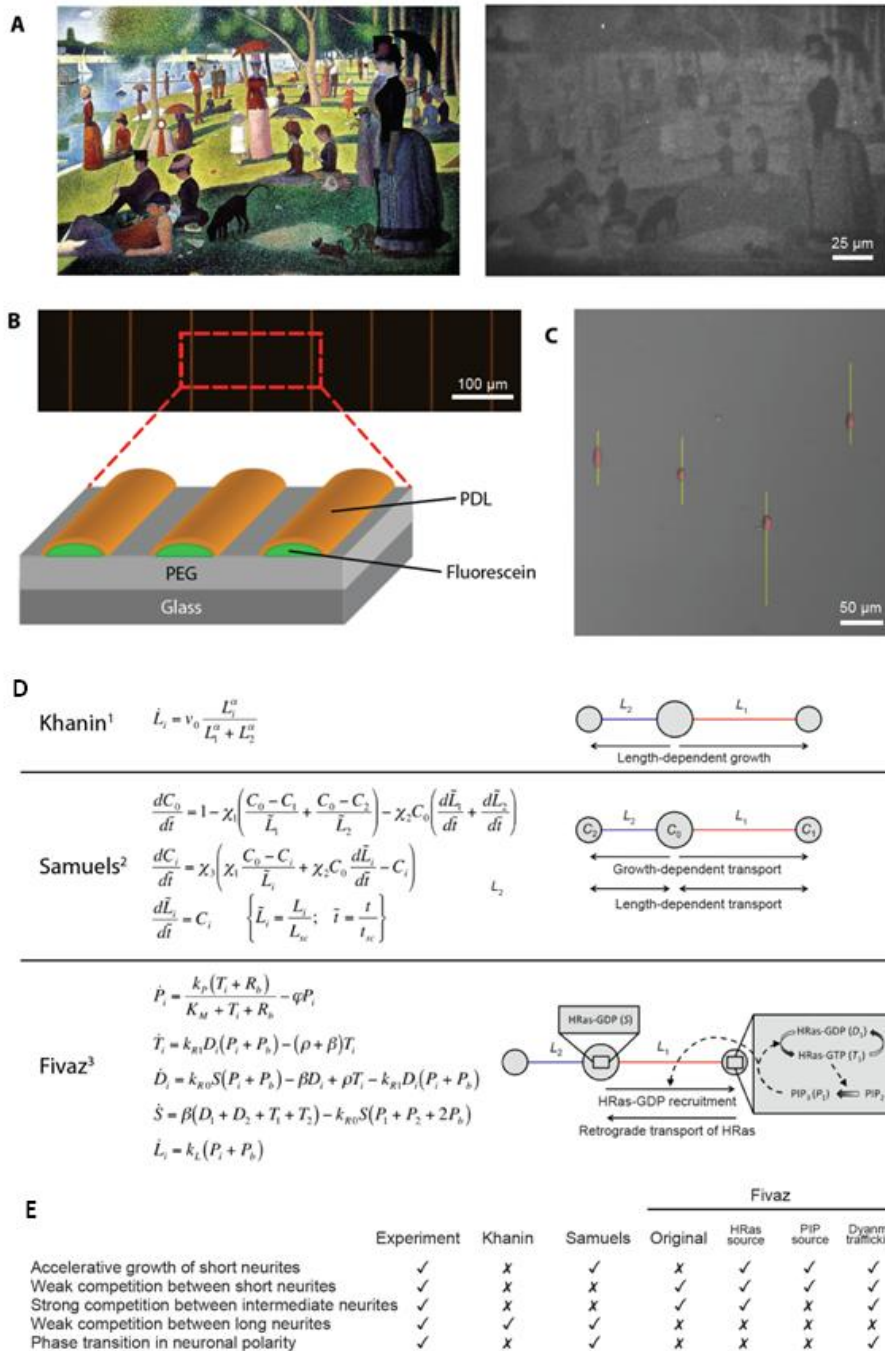


Fig. 6. Large-scale analysis of neurite growth dynamics on protein micropatterned substrates. (A) Reprint of Seurat's "A Sunday Afternoon on the Island of La Grande Jatte" (left), using laserprinted streptavidin-Cy3 (right). (B) Laser-printed micron-wide lines of poly-D-lysine (PDL) on a non-adherent poly(ethylene glycol) (PEG) substrate. (C) Four E18 rat hippocampal neurons growing two neurites each on the PDL substrate (soma in red, neurites in yellow). (D) Molecular models of neurite growth that we studied using our technology. See Wissner-Gross et. al., Integrative Biology 2010.

7. Generation of transplantable human neurons using mRNA reprogramming:

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Packard Award in Science and Engineering
Sloan Award in Neuroscience

Project Staff

Matthew Angel, Luigi Warren

We are developing technologies to generate transplantable neurons from human embryonic stem cells (via delivery of master transcription factor mRNAs). To be able to deliver mRNA to the cells, we have developed a technique to turn off cells' innate immune response to mRNA. See Angel and Yanik, PLoS ONE 2010 for details. This technology is now licensed to Stemgent Inc.

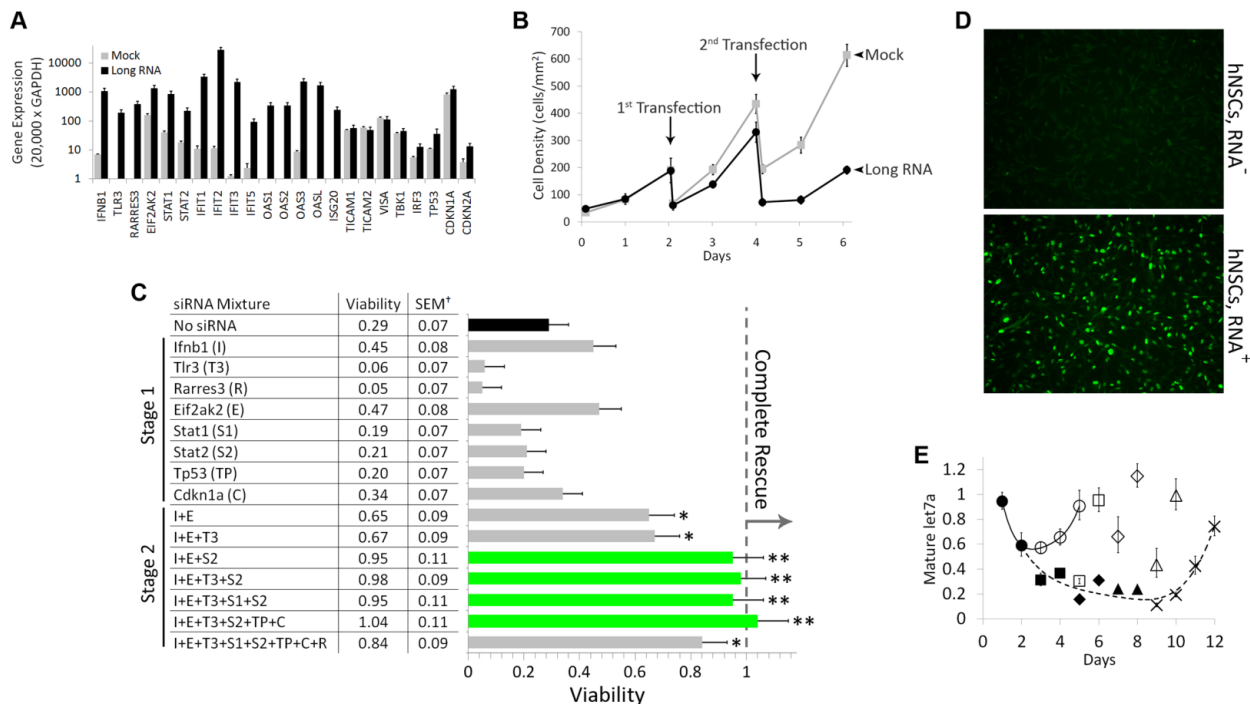


Fig. 7. Frequent RNA transfection enabled by innate immune suppression. Combinatorial siRNA screening identifies siRNA cocktails that rescue cells from the innate immune response triggered by in vitro-transcribed RNA transfection. (A) Upregulation of innate immune genes following RNA transfection. (B) Repeated RNA transfection causes cell death in human cells. (C) Combined knockdown of Ifnb1, Elf2ak2, and Stat2 rescues cells from the innate immune response triggered by frequent RNA transfection, showing that inhibition of interferon signaling and PKR activation enables sustained delivery of RNA encoding reprogramming factors. (D) Human neural stem cells are efficiently transfected with RNA encoding transcription factors. (E) Example: Sustained expression of an early neuronal transcription factor Lin28 downregulates its target, mature let7 miRNA. Solid line connects data points corresponding to cells transfected once; Dashed line connects data points corresponding to cells transfected five times (dark symbols). See Angel and Yanik, PLoS ONE 2010.

Publications:

1. Rohde, C., Zeng, F., Gonzalez, R., Angel, M., Yanik, M. F., "Microfluidic system for on-chip high-throughput whole-animal sorting and screening at subcellular resolution", **PNAS** 104, 13891 (2007).
2. Pardo-Martin, C., Chang, T.-Y., Koo, B., Gilleland, C., Wasserman, S., Yanik, M. F., "High-throughput in vivo vertebrate screening", **Nature Methods**, July 19th (2010). *Cover of August issue*. Commentary by Owen T. J. and Zon L. I., "Fishing at cellular level", **Nature Methods**, Aug 2010.
3. Yanik M. F. *et al.*, "Neurosurgery: Functional Regeneration after Laser Axotomy," **Nature** 432, 822 (2004).
4. Samara, C., Rohde, C. B., Gilleland, C., Norton, S., Haggarty, S., Yanik, M. F., "Large-scale in vivo femtosecond laser neurosurgery screen reveals small-molecule enhancer of regeneration", **PNAS**, Oct. 2010.
5. Steinmeyer, J., Gilleland, C., Pardo, C., Angel, M., and M. F. Yanik, "Construction of a femtosecond laser microsurgery system", **Nature Protocols** 5, 395 (2010).
6. Gilleland C., Rohde C., Zeng F., Yanik M. F., "Microfluidic immobilization of physiologically active *Caenorhabditis elegans*", **Nature Protocols**, Nov 4th (2010).
7. Angel, M. and Yanik, M. F., "Innate Immune Suppression Enables Frequent Transfection with RNA Encoding Reprogramming Proteins", **PLoS ONE**, 5, e11756 (2010).
8. Wissner-Gross, Z. D., Scott, M. A., Ku, D., Ramaswamy, P., Yanik, M. F., "Large-scale analysis of neurite growth dynamics on micropatterned substrates", **Integrative Biology**, Oct 2010.
9. Chrysanthi Samara, Christopher B. Rohde, Cody L. Gilleland, Stephanie Norton, Stephen J. Haggarty, and Mehmet Fatih Yanik, "Large-scale in vivo femtosecond laser neurosurgery screen reveals small-molecule enhancer of regeneration", **PNAS Early Edition**, Aug. 2010,