Directing growth cones of optic axons growing with laser scissors and laser tweezers

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ABSTRACT

We have combined a laser scissors and a laser tweezers to study, (1) the response of nerve fiber growth cones to laser-induced damage on single axons, and (2) localized microfluidic flow generated by laser-driven spinning birefringent particles. In the laser scissors study, sub-axotomy damage elicits a growth cone response whether damage is on the same or an adjacent axon. In laser tweezers study, the axon growth cones turn in response to the optically driven microfluidic flow. In summary, both the laser scissors and the laser tweezers studies elicit growth cone turning responses.

Keywords: laser tweezers, laser scissors, growth cone, axon, axonal growth

1. INTRODUCTION

To repair the neurological damage, it is very important to understand the mechanisms of nerve fiber growth cone behavior and axonal path finding. By exploiting laser micromanipulation technologies, such as laser scissors and laser tweezers, the whole neuronal cells or intracellular organelles can be studied with high spatial precision[1,2]. The ability to study the guided growth of growth cones responding to focused damage, and localized microfluidic flow applied on a single axon is novel and opens up opportunities not only for basic research on individual axons but also for studies on repair and regeneration that will impact our understanding of traumatic brain, spinal cord injury and retinal degeneration. The combination of laser scissors and laser tweezers with an *in vitro* or *in vivo* nerve system to study neuronal guidance, and the response of growth cones to this damage is a novel approach to probe important questions relating to nerve cell damage and repair.

The laser scissors[1,3-6] provides a unique opportunity to induce highly localized and controlled damage on various organelles of cells and different parts of the axon, thus allowing for the study of the response of the growth cone to the localized damage. In addition, laser tweezers generates localized microfluidic flow[2], which can guide the axonal growth. By generating controlled sub-axotomy laser microbeam injury and localized microfluidic flow on retinal ganglion axons derived from goldfish retina explants[1,7], we demonstrate the response of near-by axonal growth cones to the damage, in which filopodia growing towards and touching the localized damage induced by laser pulses, and to the microfluidic flow, in which the axon growth cones turn in response to the optically driven microfluidic flow.

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2. MATERIALS AND METHODS

2.1 Retinal Ganglion Cells

Goldfish retinal ganglion cells are used as samples in these studies[1,7]. The optic nerve of an adult goldfish was firstly crushed and the goldfish was then put back into the tank for the damaged axon to regenerate. After 7 days, the retinas were extracted, flatted, and cut into small pieces of square explants. These explants were placed into Petri dishes coated with poly-D-lysine and laminin and were incubated at room air and temperature in L15 medium supplemented with 10% fetal bovine serum. The axons were regenerating from the ganglion cells in the retina and growing out of the explants.

2.2 Experimental Setup

The system schematic is shown in Fig. 1. For the laser scissors system[1,6], the second harmonic (532 nm, 12 ns) of a Nd:YOV_4 laser was coupled to a Zeiss microscope through the epi-fluorescence port by scanning of a Fast Steering Mirror (FSM) and expanded to fill the back aperture of a 63X objective (NA=1.4). The number of the pulses irradiated on the axons is controlled by a mechanical shutter. For the laser tweezers system[2], a continuous wave ytterbium fiber laser (1064 nm) was the light source to build a dual-beam laser tweezers. Wave plates were inserted in the light paths to independently adjust the polarization of the two beams. The circularly polarized light beams rotated the birefringent vaterite beads to generate the microfluidic flow. Home-developed program, Robolase, was used to control the laser scissors and laser tweezers functions and take the images for later analysis. All the experiments were performed at room temperature.

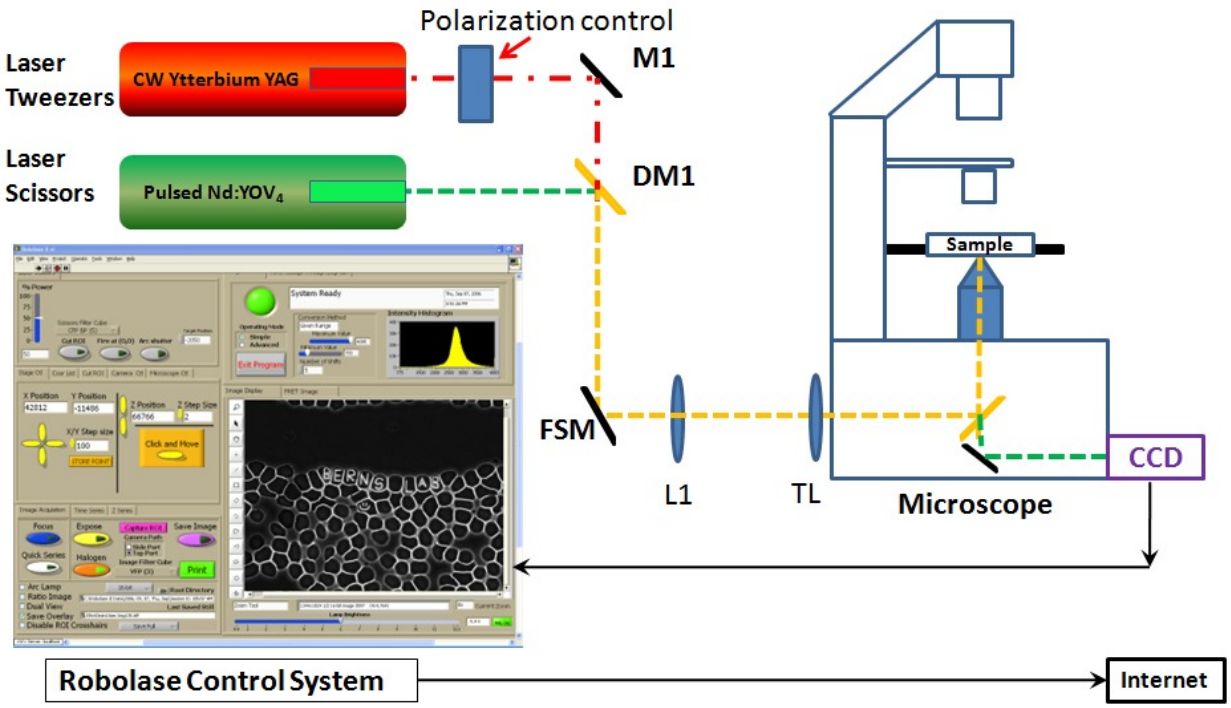

Figure 1. System schematic for laser scissors and laser tweezers. The second harmonic of a Nd:YOV_4 laser (532 nm, 12 ns) and CW Ytterbium laser (1064 nm) were coupled to a Zeiss microscope through the epi-fluorescence port and expanded to fill the back aperture of a 63X objective (NA=1.4). M1: Mirror 1; DM1: Dichroic Mirror 1; FSM: Fast Steering Mirror; L1: Lens 1; TL: Tube Lens.

3. RESULTS AND DISCUSSION

3.1 Laser Scissors

Fig. 2 shows the representative images of the damage of an axon after laser irradiation at a position marked by an arrow. The thinning of the axon was observed in Fig. 2(b-c) immediately after the laser irradiation. And Fig. 2(d) shows the recovery of the axon after 2 minutes of the irradiation. The thinning of the axon depends on the laser energy and is a transient phenomenon. The thickness can be recovered within a few minutes to its original thickness before the laser irradiation.

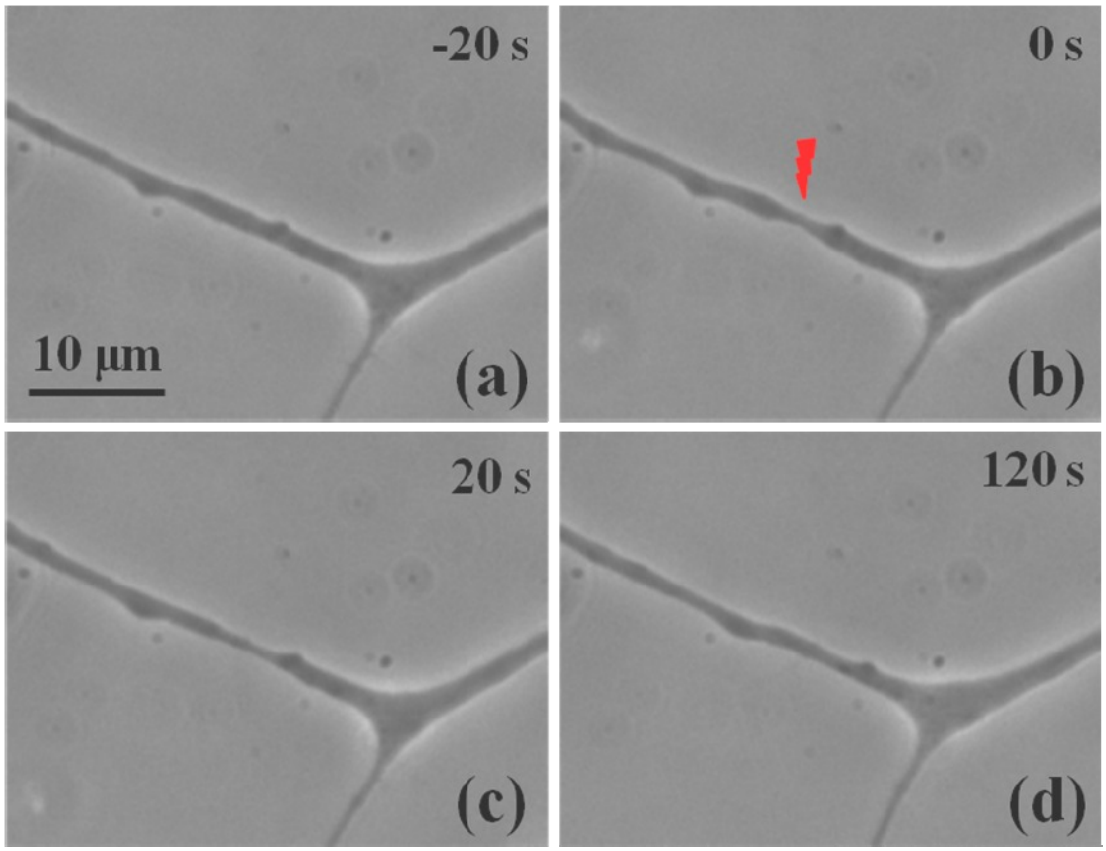

Figure 2. Representative phase contrast images of damage and repair of an axon subjected to mild laser injury. (a) the axon before laser irradiation; (b) axonal damage was induced at the position marked by the arrow; (c) the immediately thinning of the axon; (d) recovery of the mild damaged axon. Solid Bar: 10 μm.

Fig. 3 shows that a growth cone filopodia responded to the laser irradiation at the same axon. A bunch of filopodia extended to the damaged site and touched it, as shown in Fig. 3(c&d). During this period, the growth of axon stopped. However, the filopodia shrunk afterwards, and the whole growth cone continued to grow in its original direction before laser irradiation. This response is a temporary movement, which could be due to that there is a guidance cue released from the damaged site and it stimulates the growth of the filopodia.

Fig. 4 shows another example of the extension of the filopodia to a nearby damaged site on a different axon. Similarly, the growth cone can respond to the nearby damaged axon. After the axon was damaged as shown in Fig. 4(b), the nearby growth cone extended filopodia and touched the damaged axon. Moreover, the growth direction of the axon changed 30 degrees, Fig. 4(d), when compared to the growing direction before laser irradiation.

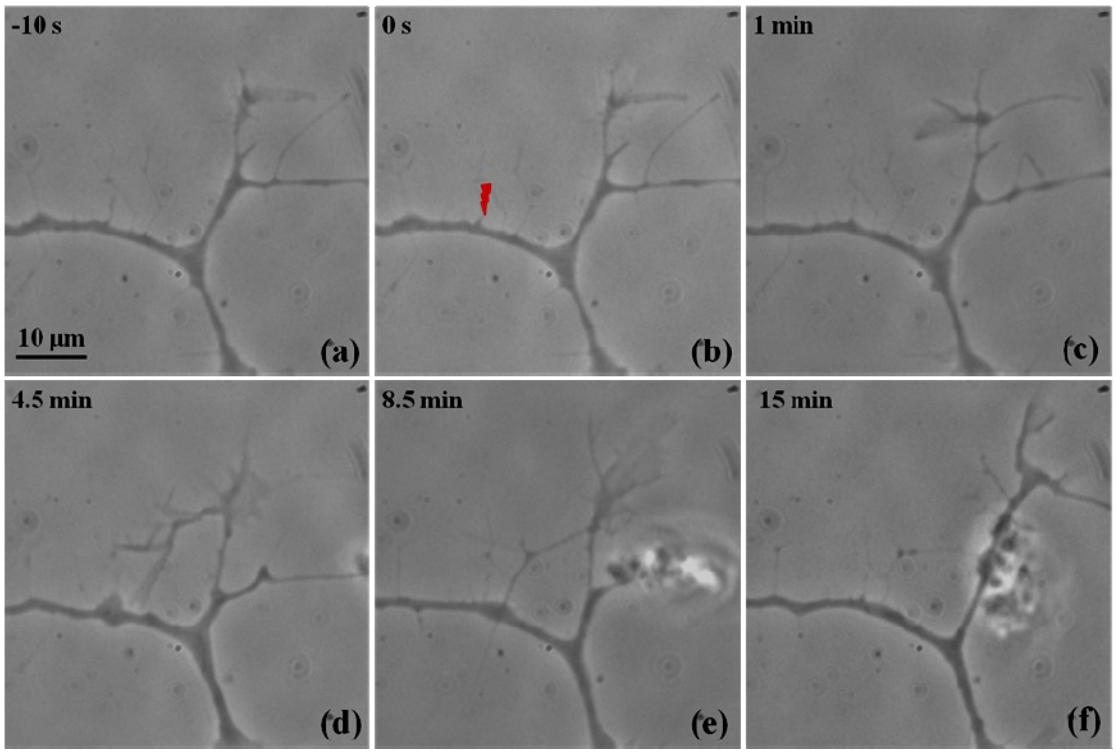

Figure 3. Growth cone response stimulated by the laser irradiation. (a) An axon before the laser irradiation; (b) mild damage of the axon marked by the arrow; (c-d) the extension of the nearby growth cones filopodia on the same axon towards the localized damaged site and touching the damaged site; (e-f) retraction of the extended filopodia. Solid Bar: 10 μm.

3.2 Laser Tweezers

The laser beam can be used as a tweezers to trap small particles to interact with axons. Traditional laser tweezers can only pick up and move particles, however, in this study, we developed a laser tweezers can also spin the particles to generate microfluidic flow. We applied these spinning particles close to the growth cones of axons and controlled the growth direction of axons using these optical micro-motors. In the control case, the growth cone ignored the particle and grew straightly. However in the Fig. 5, when spinning the particle in a clockwise direction and the growth cone responded to the generated microfluidic flow. The turning angles of the axon could be as large as 76 degrees after 8 minutes.

There is a relationship between the spin direction and the direction of the growth cone response. We also can change the position and rotation direction of the spinning particle to guide the axonal growth. The birefringent particle can either rotate in clockwise direction on the right side of the axon (anterograde case), or rotate in counterclockwise (retrograde case). In anterograde case, the nerve turns towards the spinning particle, and in the retrograde case, the nerve turns away from the spinning particle. In addition, we can use the dual laser tweezers system to trap two beads to create more complicated flow. The microfluidic flow created by two counter rotating particles can guide the axons to grow between the two rotating beads[2].

We developed a physical model to calculate the force density applied on the growth cone[2]. The average force density is 1.2 fN/um^2. If we multiple this value by the growth cone area, we can get the average force applied on the growth cone, which is smaller than 1 pN.

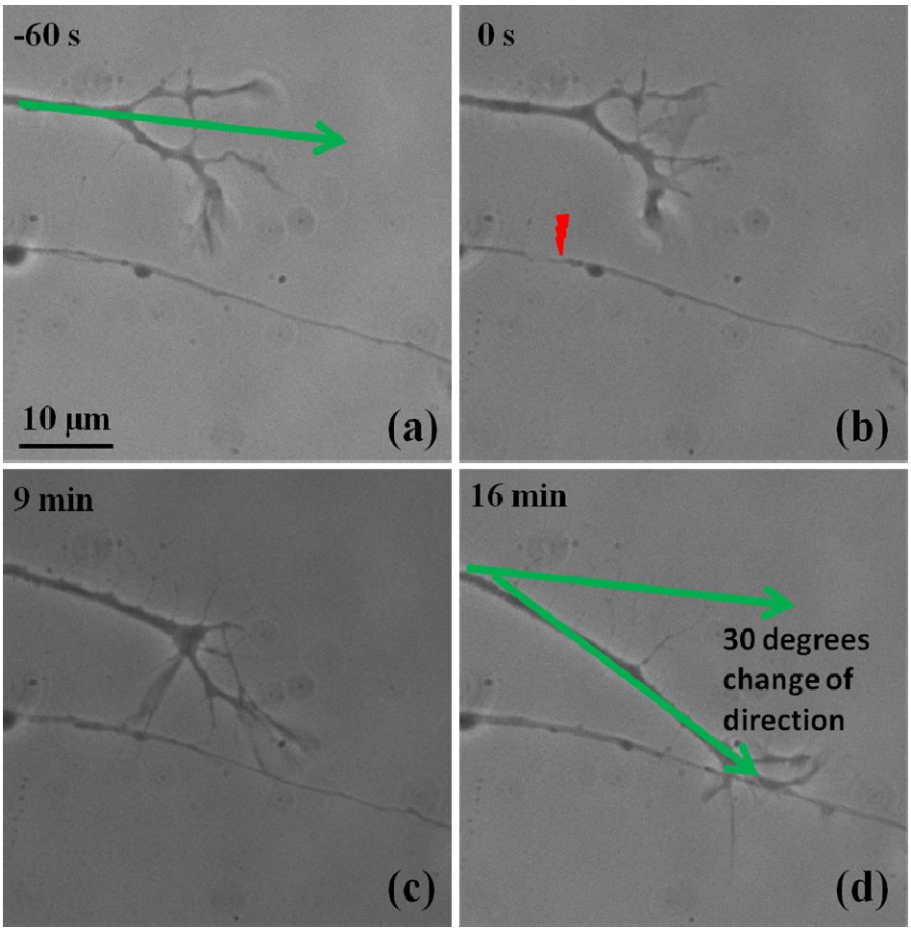

Figure 4. Growth cone response stimulated by the laser irradiation. (a) An axon and a growth cone before the laser irradiation; (b) mild damage of the axon marked by the arrow; (c) the extension of the nearby growth cones filopodia towards the localized damaged site and touching the damaged site on a different axon; (d) retraction of the extended filopodia and change of the growth direction of 30 degrees. Solid Bar: 10 μm.

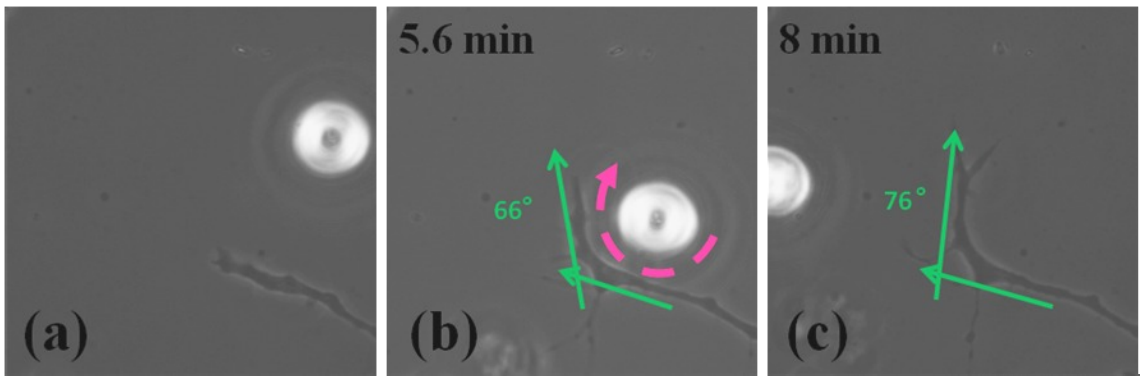

Figure 5. Spinning particle besides the growth cone of an axon can control the growth direction of the axon. (a) Before put the spinning particle close to the growth cone; (b) after put the clockwise spinning particle close to the growth cone, the growth cone responded to the generated microfluidic flow; (c) after 8 minutes, the turning angles of the axon is 76 degrees.

4. CONCLUSION

In a laser scissors study, a green laser with nanosecond pulse is used to induce mild damage on the axon near its own growth cone or a growth cone of an adjacent axon. After laser damage, the axon appears to morphologically "thin" but returns to pre-laser thickness. Sub-axotomy damage can elicit a growth cone response whether damage is on the same or an adjacent axon. In response to the damage, the growth cones extend filopodia to the damaged site and in some cases actually touch the damage region. In a laser tweezers study, a dual-beam laser tweezers is used to cause the rotation of birefringent vaterite particles, through the transfer of angular momentum, which generates a weak microfluidic flow against the growth cone. The response of the growth cone to the microfluidic flow is to turn in a specific direction related to the direction of the flow. The axonal growth direction can be controlled by changing the rotational direction and position of the birefringent particle. In summary, both the laser scissors and the laser tweezers studies elicit growth cone turning responses.

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