Title

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ISBN

978-1-5106-1151-1

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Publication Date

2017-08-25

DOI

10.1117/12.2274563

PROCEEDINGS OF SPIE

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Christopher Carmona, Daryl C. Preece, Veronica Gomez-Godinez, Linda Z. Shi, Michael W. Berns, "Probing mechanobiology with laser-induced shockwaves," Proc. SPIE 10347, Optical Trapping and Optical Micromanipulation XIV, 103470D (25 August 2017); doi: 10.1117/12.2274563

SPIE.

Event: SPIE Nanoscience + Engineering, 2017, San Diego, California, United States

Probing mechanobiology with laser-induced shockwaves

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ABSTRACT

Traumatic Brain Injury (TBI) occurs when an external force injures the brain. While clinical outcomes of TBI can vary widely in severity, few mechanisms of neurodegeneration following TBI have been identified for treatment. We propose a model for studying TBI using laser-induced shockwaves (LISs). An optical system was developed that allows single cells to be studied in response to LISs. Our system utilizes an optically-coupled force measurement component that allows for the visualization of shockwave dynamics. Here, the force measurement system is characterized by imaging stages over the period of violent expansion and collapse of microbubbles responsible for shockwave generation

Keywords: traumatic brain injury, TBI, in vitro, neurodegneration, laser-induced shockwaves, shockwaves, cavitation, neurodegenerative disorders, microbubbles

1. INTRODUCTION

1.1 Motivation

According to the Center of Disease Control and Prevention, TBI is a contributing factor to a third of all injury-related deaths in the United States. Each year 1.7 million TBIs occur in the United States and about 5.3 million Americans are living with TBI-related functional and cognitive impairments.[1] Those that survive TBI can face temporary or permanent effects that significantly impact thinking or memory, movement, sensation, or emotional functioning. TBI increases long-term mortality and reduces life expectancy while constituting a high-risk epigenetic factor for developing Parkinson's disease, Alzheimer's disease, and/or dementia of Alzheimer-type.[1] While clinical outcomes of TBI can vary widely in severity, few mechanisms of neurodegeneration following TBI have been identified for treatment. To further our understanding on the way that cells respond to shear stress, an optical system was developed that allows single cells to be studied in response to a sudden but short application of shear stress. This system can generate LISs at a specified time and location. A LIS can exert a shear stress between 0 - 50 kPa within a range that preserves cell viability and depends on the distance from the shockwave epicenter.[2] Our system utilizes an optically-coupled force measurement component that allows for the visualization of shockwave dynamics. Ultimately, this systems capacity to quantitatively study shockwave dynamics permits the study of the spatiotemporal details occurring in cells responding to shear stress. Thus, TBI can be further studied and subsequent results could reveal the molecular basis for neurodegeneration and potential therapeutic avenues for treatment.

1.2 Background

While TBI is triggered by an initial injury, it is a disease process that results in chronic neurodegenerative consequences.[1] The effects of brain injury on cellular processes have been assessed at both the cellular and organismal level. However, models of these two levels are incomplete and bridging the gap is not readily achieved with traditional models of traumatic brain injury. While it is easy to recreate the forces responsible for traumatic brain injury, animal experimentation and clinical research in many ways do not distinguish relevant physiological

Optical Trapping and Optical Micromanipulation XIV, edited by Kishan Dholakia, Gabriel C. Spalding,
Proc. of SPIE Vol. 10347, 103470D · © 2017 SPIE · CCC code: 0277-786X/17/$18 · doi: 10.1117/12.2274563

responses to brain injury.[3] Conversely, in vitro experiments simplify brain injury models such that individual pathways are more easily interrogated. This simplification, however, comes at the cost of less reliable damage models that include secondary injury mechanisms resulting from disruptions of the blood-brain barrier.[4] Most techniques used to recapitulate TBI in vitro do not achieve application of forces at single-cell resolution, which further restricts the in vitro study of primary injury mechanisms in single neurons within physiologically-relevant culture environments. By utilizing the precision of optical tools, we have begun to study the response of in vitro cultures of various cell-lines to early shockwave dynamics and to further validate the use of laser-induced shockwaves as a model for TBI.

Laser induced-breakdown occurs after irradiation of a fluid media with a high-energy, short-pulsed laser microbeam initially causes photoionization, yielding free seed electrons.[5] Photoionization allows for inverse Bremsstrahlung absorption and avalanche ionization depending on the availability of free electrons.[5] The result of these ionization events is the formation of plasma at the laser focus which, due to collision of high energy electrons with surrounding molecules, has high temperature and pressure.[5] While this plasma expands, it creates a bubble within the fluid media and an ensuing shock wave that extends up to several hundred microns in diameter. A shock wave pressure can either be measured directly using a hydrophone or calculated indirectly based off of the maximum cavitation bubble diameter.[5] The magnitude of the shock wave pressure is dependent on the laser pulse length, numerical aperture of the microscope objective, and energy density of the laser beam. Since laser power can be controlled using a half wave-plate and polarizer, modulation of the resultant pressure from shock waves can be readily achieved and allows for the creation of precise forces and impulses. Based on the Gilmore model for bubble expansion and collapse, the expected shear profiles for a given laser power can then be calculated.[6] This permits the study of the molecular biology of single cells in response to mechanical stimulation.[7,8]

2. METHODS

For our studies, we utilize Robolase II (RBII): a system configured for laser-induced shockwave generation and measurements depicted schematically in Figure 1. The pump or ablation laser used to generate shockwaves is a Coherent Flare 532 nm, 100 Hz repetition rate system with a 2 ns pulse width and 450 μJ pulse energy (Spectra-Physics, Mountain View, CA). For each shockwave event, two-pulses are generated by the 532 nm laser as depicted in Figure 1. While the second pulse is pumped into fluid media at the sample stage, the first is detected by a photodiode and triggers a signal generator. The triggered signal generator produces a 5 V square-wave pulse that opens a mechanical shutter (Vincent Assosciates, Rochester, NY) and, upon its fall, triggers an exposure on the Orca-Flash 4.0 V2 (C11440-22CU, Hamamatsu Photonics K.K., Japan). The imaging system is mounted on an Zeiss Axiovert 200M microscope(Carl Zeiss, Thornwood, NY).

The source of illumination used to visualize bubble-cavitation events is produced via an optically-coupled dye laser module (DUO-210, Laser Innovations, Santa Paula, CA). The dye laser module utilizes 532 nm laser light as an excitation source for the fluorescent dye (Rhodamine 590, Exciton, Dayton, OH), which has a emissions peak measured at 557 nm. The dye used was chosen for its rapid decay rate[9] and high quantum-yield in order to maximize the intensity of light produced from short pulses of excitation light while minimizing the duration of the illumination pulse. The fluorescent dye is dissolved in methanol and relative fluorescence intensities were measured at various concentrations in order to optimize emissions intensity. Relative fluorescence intensities at 557 nm for various dye concentrations are shown in Figure 2. The dye laser light is delivered from the output of the module through the microscope condenser to illuminate the sample of interest via a fiber optic bundle. Delays of the dye laser illumination source relative to the shockwave pump source is adjusted by varying the beam path length using a translatable mirror-rail system. The light produced by plasma formation, which precedes bubble cavitation, emits a broad spectrum of bright light that competes with our dye laser illumination source. Therefore, a bandpass filter selected for the transmission of the dye laser light is utilized to minimize the contributions of light from plasma formation in the final image. In this manner, we acquire time-integrated images of laser-induced bubble cavitation coincident with our illumination source.

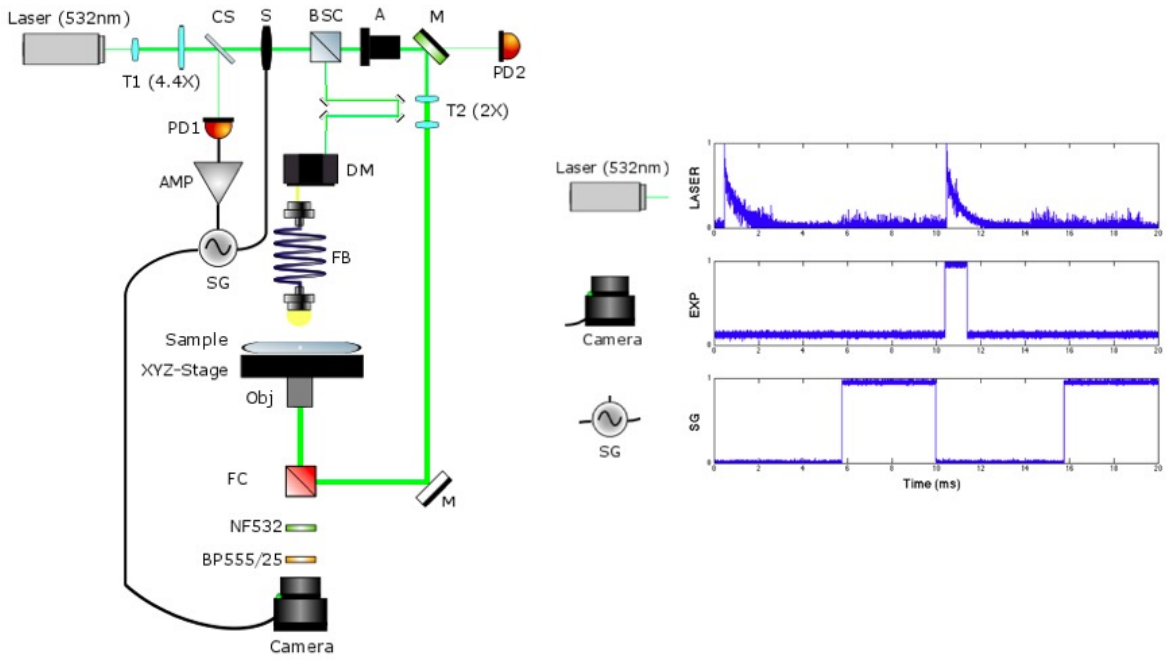

Figure 1: System schematic (left) and timing schematic (right) for operation of Robolase II. The first laser pulse triggers the signal generator, which in turn triggers a camera exposure coincident with the second pulse.

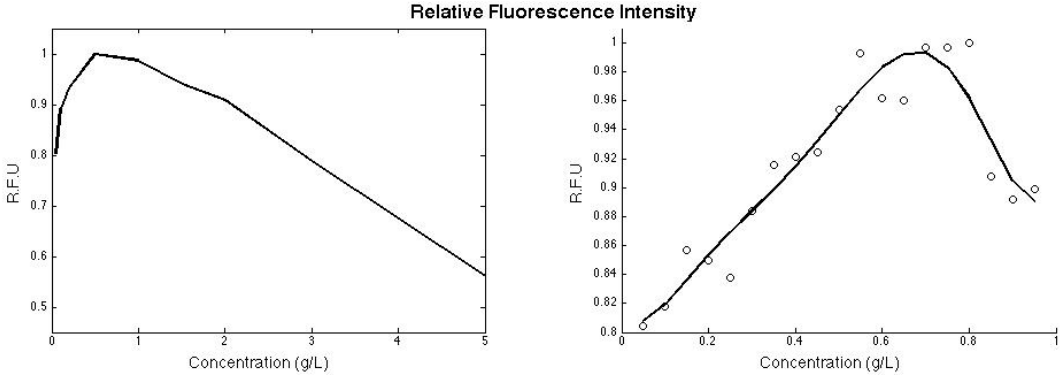

Figure 2: Relative fluorescence intensity is measured at 557 nm and compared using normalized units (R.F.U) across varied concentrations of Rhodamine Cl prepared in methanol. R.F.U for concentrations ranging from 0.05 g/L to 5 g/L is plotted on the left. A scatter plot of R.F.U for concentrations ranging from 0.05 g/L to 0.95 g/L is shown to the right with a polynomial fitted trendline. Peak emissions intensity is measured at 0.8 g/L.

3. RESULTS

The shockwave generation system, RBII, has thus far been capable of resolving nanosecond scale events that are characteristic of early LIS dynamics. The illumination intensity as measured at the dye laser module output indicates a conversion efficiency of 55%. The intensity measured at the sample stage reflects an effective conversion efficiency of 28.6% from a flux of 123.4 μW/cm^2. The uncertainty between the coincidence of an initiated camera exposure and the shockwave generating pulse is 147 μs and is determined by measuring the uncertainty associated with the delay time between each aforementioned event across ten trials.

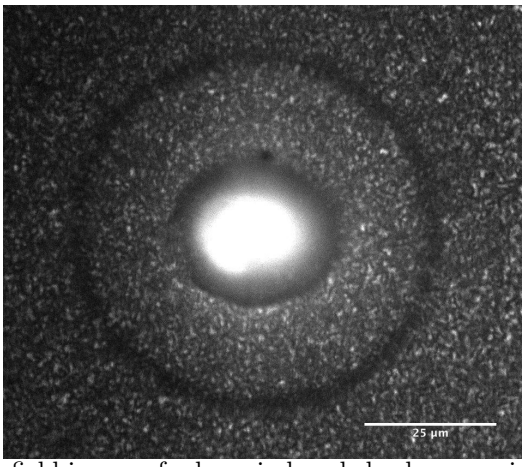

Figure 3: Time-integrated, bright-field image of a laser-induced shockwave using a 63X/1.4 oil immersion objective on RBII.

In Figure 3, the time-integrated image captured using RBII reveals early plasma formation evident by the bright, central region and bubble cavitation indicated by the dark, circular ring surrounding the plasma. Approximately 30 μm radially outwards from the epicenter of the shockwave shown in Figure 3 there is a dark ring which indicates the position of the wavefront of a shockwave generated by bubble cavitation.

4. FUTURE DIRECTION

While early shockwave dynamics are readily visualized in the current configuration of RBII, there is great interest in examining the subsequent evolution of the bubble generated via laser-induced cavitation. Further modifications will be made to the system in order to image the maximum bubble radius produced at selected laser powers. The speed and duration of laser-induced shockwave dynamics requires that the existing temporal resolution of the system be minimized while extending the range of image acquisition from a few nanoseconds to tens of microseconds following laser-induced breakdown, which encompasses at least three orders of magnitude with respect to the current illumination pulse-width. Subsequently, further studies on the molecular biology of single-cells will be performed in order to understand the consequences of exposure to varying degrees of stress on cell physiology and pathogenesis. Future studies will aim to elucidate mechanisms of chronic neurodegeneration in order to discover potential targets for developing therapeutic avenues that might mitigate or reverse observed neuropathology.

ACKNOWLEDGMENTS

This work was supported by grants from the Beckman Laser Institute Foundation, and from the Air Force Office of Scientific Research under FA9550-16-1-0342. Research reported in this paper was also supported, in part, by the National Institute for Neurological Disorders and Stroke of the National Institutes of Health under award number 1P01NS092525-01A1.

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