Supplemental Document

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Femtosecond Diode Based Time Lens Laser for Multiphoton Microscopy: supplemental document

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1. Pulse Simulation

Simulations of the pulse evolution were used as a point of comparison for the operation of the time lens laser. These simulations were carried out using the generalized nonlinear Schrödinger equation (GNLSE) [1]. The input is a Gaussian pulse with a 70 ps full-width at half maximum (FWHM) and a ring down pulse (Fig. S1a) that reflects measurements of the gain switched diode with a fast photodiode. Next, the pulse was propagated through a multi-stage system matched to the parameters of the time lens laser system. First amplitude and phase modulation were applied (Fig. S1a) using values that matched the specifications of the electro-optic modulators and driving RF signals. The modulated pulses propagated through single mode fiber matching the length of the pre-amplification stage. The pulse was then propagated through double-clad (DC) fiber to match the DC amplification stage. In both stages, components manufacturer-reported losses and measured losses were included between fiber propagation steps and fiber gain was estimated from average power measurements from the system. The amplified pulses were compressed using the dispersion value of the volume Bragg grating (17 ps/nm, Fig. S1b). After applying losses for fiber coupling, the pulses were propagated using parameters from the photonic crystal fiber. Finally, the pulse was compressed using second and third-order dispersion values calculated from distance and angle measurement from the grating compressor (Fig. S1c).

This simulation is compared to the autocorrelation taken from the system (Fig. S1d). The central peak width of the autocorrelation (467 fs) matches the peak in the simulation well and the effects of the side pulses from the amplitude modulation are seen in both at 50 ps delay from the center as well. The experiment shows a broad pedestal that is not seen in the simulation. Nonlinear spectral chirp which will not be compressed to a transform limited pulse after compression from the quadratic phase correction provided by a grating compressor or chirped volume Bragg grating [2]. The pedestal of the peak matches autocorrelations of pulses with chirp that deviates from linear [2,3] and of pulses with random noise in amplitude and phase from one pulse to the next [4]. Deviations from linear chirp is a coherent effect that in that each pulse has the same autocorrelation, whereas autocorrelations of pulses with random noise represent an average of each individual pulse profile. We have identified two sources that can be a source nonlinear chirp in our pulses. Gain switching a diode produces pulses with chirp [5]; this was not experimentally measured or included in simulation. Self-phase modulation can also deviate from linear chirp on the edges or "wings" of the pulse spectrum [2]. For sources of random noise that would cause a coherent artifact, we look to

phase noise and timing jitter [4]. Based on simulations and measurements we believe this is not a major factor. While power fluctuations could also cause the pedestal, ours were less than 5%. Additionally, the 18 GHz modulation removes the effects of timing jitter in simulations. This leaves uncorrelated spectral phase from gain switching as a possible contributor. Spectral filtering of the pulse, as used in many pulse regeneration methods, would offer a likely way to clean up the pulses [2,6]. GNLSE simulations of these effects using Monte Carlo methods to simulate incoherent effects support these hypotheses.

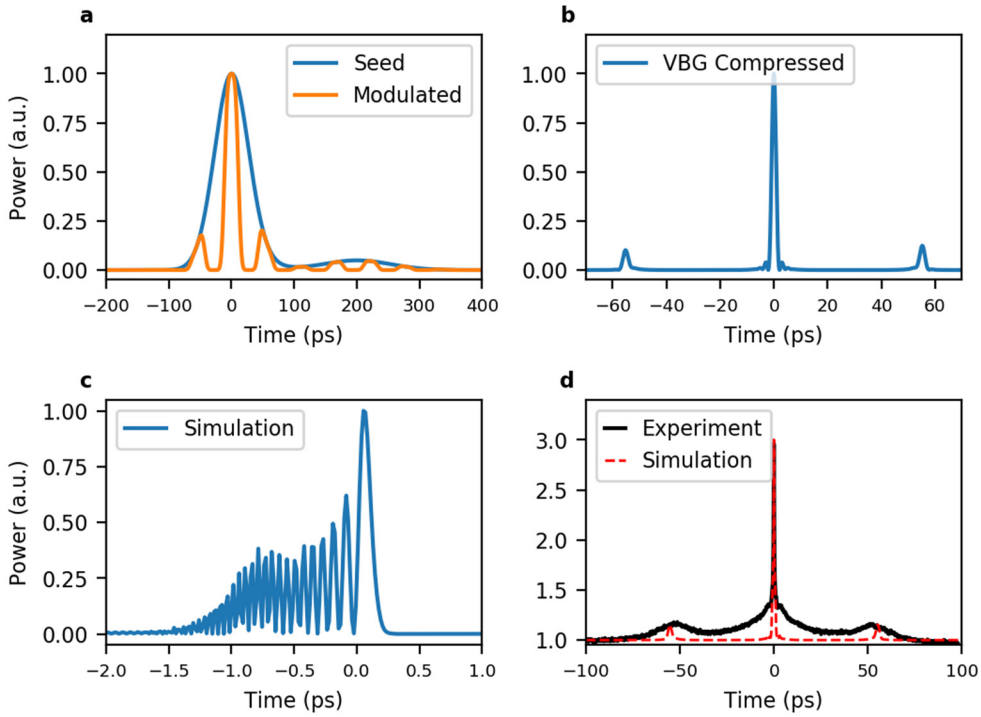

Fig. S1. Simulation results using the generalized nonlinear Schrödinger equation (GNLSE). **a** Estimated seed pulse intensity with a FWHM of 70 ps with a ring-down artifact (blue) and pulse after amplitude modulation from an EOM (orange). **b** Simulation pulse intensity profile after single mode fiber amplification and after the volume Bragg grating, which compresses the pulse due to the phase modulation applied to the seed. **c** Pulse intensity after spectral broadening in the photonic crystal fiber and compression from the grating pair compressor. The pulse shows significant uncompressed chirp of third and higher order. **d** Autocorrelation of the simulated pulse (dashed red) from **c** with the autocorrelation of the pulses taken from experiment. The central peak shows close agreement and both show evidence of side pulses at 50 ps delays from the center. The autocorrelation from experiment has a pedestal not seen in the simulated autocorrelation.

2. Imaging Comparison Using Ti:Sapphire Excitation

As a comparison point for the images acquired with the time lens system, imaging was performed using a Mai Tai DeepSee Laser (MAI TAI HP 1040 DS, Spectra Physics). The same sample (fixed, PLP-eGFP mouse brain slices of oligodendrocyte cell bodies in the neocortex region) was imaged. We operated the Mai Tai at 976 nm (to match the time lens source), but in a different microscope, using the same objective lens (Olympus 20x Water Objective - XLUMPLFLN20XW). For a volumetric image, the laser was scanned in a 196 µm x 196 µm square at 0.9 µm increments in the z-direction. The dwell time was kept constant at 2 µs for a 1024 pixel x 1024 pixel frame acquisition time of 5 seconds. Laser power was increased with depth in the sample, from 10 mW to 50 mW, measured at the sample. The pulses from the Ti:Sapphire had a pulse width >100 fs and a repetition rate of 80 MHz. These images serve to

demonstrate the similar performance of our system to a commercial laser, despite not being a one-to-one comparison. Slices at equal depths taken using both systems can be seen in Fig. S2. Both the systems could image at a depth of 900 µm into the sample and the resulting images are similar at equal depths. See Visualization 1 for the full image stack.

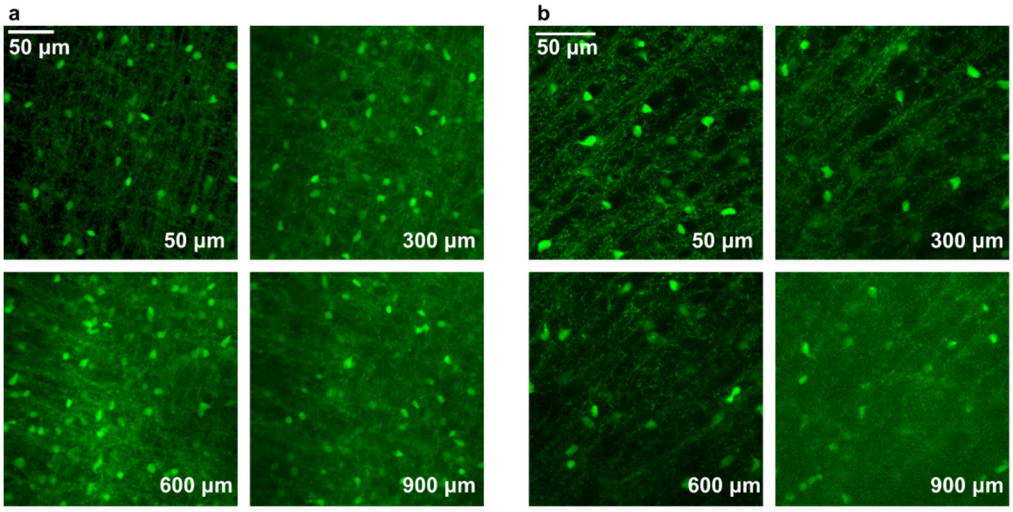

Fig. S2. Images acquired using **a** our time lens laser and **b** a Mai Tai DeepSee Laser at a matching wavelength of 976 nm. Both image the same sample of fixed, PLP-eGFP mouse brain slices of oligodendrocyte cell bodies in the neocortex region. Both systems were able to excite fluorescence at depths of 900 µm into the sample, with cell bodies and processes distinguishable in both. Note that the field of view is smaller in **b**.

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