A simplified femtosecond laser repetition frequency divider for two-photon imaging

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We present a simplified femtosecond laser frequency divider designed to divide the repetition frequency of an 80 MHz laser pulse into a 40 MHz laser pulse using a resonant model Pockels cell. The simplified driving electronics include a commercially obtainable frequency divider, power amplifier and impedance transformer, which can be easily assembled in a laboratory. By simply integrating this device into a conventional two-photon imaging system, we observed a two-fold increase in two-photon excitation efficiency and imaging intensity, while maintaining the same average excitation laser power.

Two-photon imaging requires high excitation efficiency to achieve higher imaging intensity[1-3], or conversely, to utilize lower excitation power to reduce the risk of tissue damage[4]. One approach to achieve higher excitation efficiency is by using a low repetition rate femtosecond laser, which provides a higher peak power per pulse. Theoretically, using a femtosecond laser with half the repetition rate can result in a two-fold increase in two-photon imaging intensity at a given average power[3]. Moreover, longer interval in low repetition rate femtoseconds lasers also facilitate temporal multiplexing applications[6-8].

Commercial Ti:Sapphire femtosecond lasers used in conventional two-photon imaging microscope (2PM) typically operate at a repetition rate of 80 MHz. Lower repetition rate femtosecond lasers can be generated using an optical parametric amplifier (OPA)[3].

However, OPA devices are often complex and expensive. An alternative approach is to employ a commercial pulse picker, which selects one pulse from every four pulses of an 80 MHz femtosecond laser, resulting in a reduced 20 MHz repetition rate lase. In the pulse picker system, the 80 MHz femtosecond laser passes through an electro-optic modulator (Pockels cell). The voltage applied to the Pockels cell controls the polarization direction of the light. A polarizing cube beam splitter, positioned at the end of Pockels cells, divides the laser along transmission and reflection directions. The Pockels cell used in this setup has a capacitance of over 100 pF, and operates at high voltages in the range of hundreds (e.g. Conoptics Model 350-80-02 or Qioptiq LM 0202 P). To enable the Pockels cell to switch between fully ON and OFF within 10 ns, a fast and powerful driver with a high slew rate over 40,000 V/us is required, which makes the pulse picker system expensive.

In this study, we present a simplified femtosecond laser repetition frequency divider that effectively converts 80 MHz laser pulses to a 40 MHz repetition rate useing a Pockels cell operating in resonant mode. The crystal inside the Pockels cell can be considered as an equivalent electronic capacitance. By connecting it in parallel with an inductive coil (L1 in Fig. 1a and Extended Data Fig. 2) to form a resonant circle, the Pockels cell efficiently operates in resonance, requiring only 8 W (40 MHz) to achieve full switching. This radio frequency (RF) power can be readily obtained using affordable commercial RF amplifiers (Fig. 1a and Extended Data Fig. 3b). By synchronizing the phase of the 40 MHz driving signal with the 80 MHz laser pulses at the right phase, the Pockels cell can be switched fully ON and OFF precisely at the moments of each odd and even laser pulse, effectively dividing the 80 MHz laser pulses into two 40 MHz laser pulse trains (Fig. 1a).

To obtain a 40 MHz synchronizing signal, we utilized a commercial HMC432 frequency divider module or a simple circuitry assembled with an HMC432 chip to divide the 80 MHz synchronizing signal from the femtosecond laser pulses (Fig. 1a and Extended Data Fig. 1). In cases where a Spectra Physics Mai Tai or InSight serials femtosecond laser did not provide a direct 80 MHz synchronizing signal output, we employed a phototube detector module or a simple circuitry with S13773 photodiodes to obtained the 80 MHz synchronization signal (indicated by the gray part in Fig. 1a). The 40 MHz synchronization signal generated by the HMC432 divider had a relatively weak amplitude, below 1.0 Vpp. To address this, we utilized a commercial broadband amplifier (1-200 MHz) or a 40 MHz, 25 W RF power amplifier to significantly amplify the 40 MHz synchronizing signal up to near 8 W. The output power was adjusted using an adjustable 3-24 V DC supply. Additionally, we incorporated an experimentally designed 1:10 impedance transformer (B1) to match the impedance and deliver the RF power into the Pockels resonant circuitry (Fig. 1a and Extended Data Fig. 2).

When utilizing the Qioptiq LM 0202 P Pockels cell, we found that an 8 tunes coil with an outer diameter of 8 mm, constructed using 1 mm enameled copper wire, was suitable

for achieving a 40 MHz resonant frequency (L1 in Fig. 1A, Extended Data Fig. 2 and Fig. 3a). On the other hand, the Conoptics Model 350-80-02 Pockels cell required a 12 tunes coli for optical matching (Fig. 1A and Fig. 3b). The tightness of the coil could be slightly adjusted to achieve the best resonance, which could be estimated using a simple inductive coil connecting to oscilloscope. To compensate for the phase difference between the driving RF waveform and the laser pulses, the cable length between the HMC432 frequency divider and the RF power amplifier could be adjusted (Fig. 1b). When the resonant Pockels cell operates at the correct phase, the cube beam splitter could switch between transparent (ON) and reflection (OFF) precisely at the moments of 80 MHz laser pulses (Fig. 1b). Consequently, the laser frequency divider effectively divided the 80 MHz laser pulses into two separate 40 MHz laser pulse trains along direction A and B (Fig. 1a). For the Model 350-80-02 Pockels cell, the resonant current in the coil L1 was quite large, necessitating the use of a small fan for cooling purposes (Extended Data Fig. 3b).

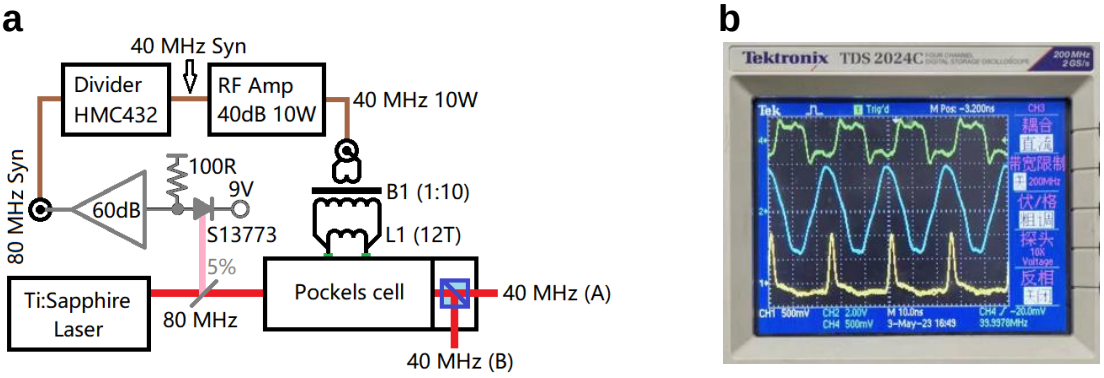

Fig. 1| The electronic circuitry and signals of a 40 MHz femtosecond laser repetition frequency divider. a, The divider utilizes a Pockels cell operating in 40MHz resonant mode. The simplified electronic driver circuitry consists of a frequency divider module, an RF power amplifier and an impedance transformer (B1). **b**, The signals of the electronic circuitry depicted. From top to bottom, the 40 MHz synchronizing signal (green track), the driving signal inducted from the resonant circuitry (blue track) and the 40 MHz femtosecond laser pulse measured by a photodiodes module.

To evaluate the performance of the 40 MHz femtosecond laser divider, we compared the 2PM images of macaque visual cortex expressing GCaMP5G, with and without the implementation of 40 MHz divider[9,10]. Remarkably, we observed over two-fold increases in both imaging intensity and SNR simply by adding the 40 MHz divider while maintaining the same average excitation power of 28 mW (Fig. 2, 4 frames averaged, SNR increases from 1.03 to 2.10 for single frame images). This enhancement can be attributed to the fact that a 40 MHz repetition rate laser delivers double the pulse power compared to an 80 MHz laser at the same average power. Importantly, throughout our long-term 2PM imaging

experiments, we did not encounter any tissue damage or bleaching (Fig. 3) , confirming that the pulse peak power remained within a safe range for cortical imaging[4]. Furthermore, the 40MHz divider has been employed in our laboratory for nearly one year, demonstrating consistent performance without any degradation, thus indicating its long-tern stability.

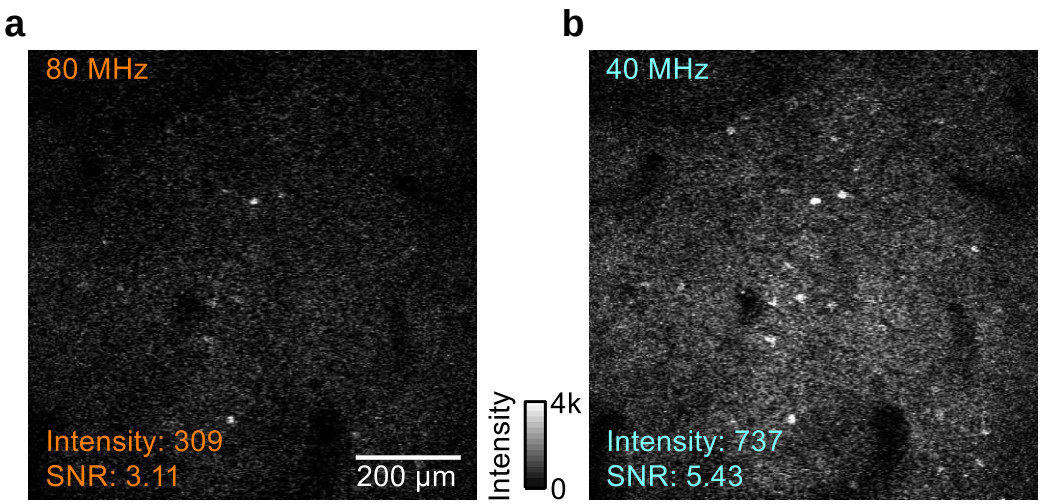

Fig. 2| Two-photon microscopy (2PM) images of macaque V1 with an 80 MHz and 40 MHz femtosecond laser. a, A 2PM averaged image (4 frames) acquired with the raw 80 MHz repetition rate laser. **b**, A 2PM image obtained using the 40 MHz repetition rate laser at the same average excitation power.

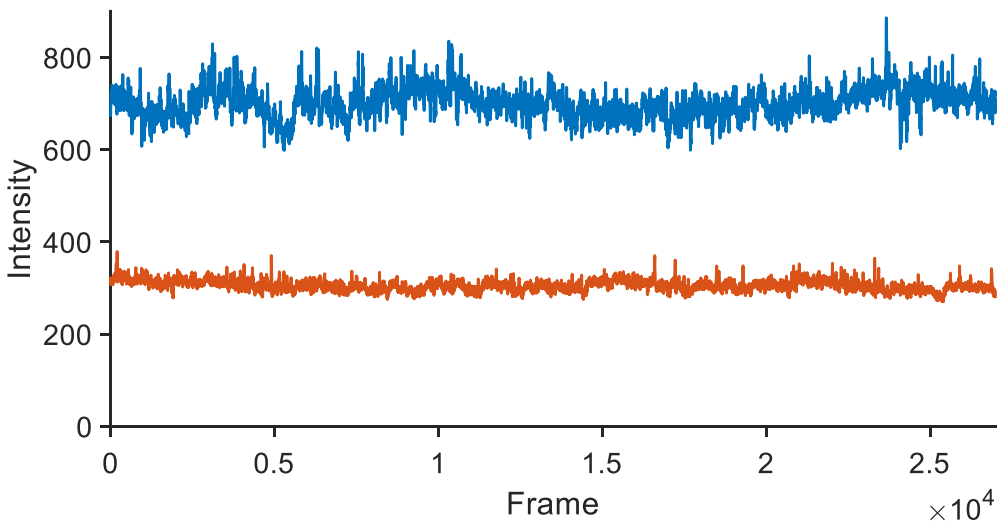

Fig. 3| Stability of two-photon images with 40 MHz and 80 MHz femtosecond laser. There is no obvious bleaching in both 40 MHz laser (blue) and 80 MHz laser (orange) two-photon imaging.

In our simplified design, all the electronic modules and components required for this

laser pulse frequency divider are commercially available and can be easily assembled in a laboratory setting. This laser pulse frequency divider can be seamlessly integrated into existing two-photon microscopy (2PM) setups as a modular unit. The simple addition of this laser repetition frequency divider leads to a remarkable increase of around 100% in image intensity in two-photon imaging, while maintaining a given average excitation power. This improved image intensity is not only beneficial for enhancing image quality in general multi-photon imaging but also crucial for cut-edge applications such as deep tissue imaging, fast volumetric imaging and voltage imaging[6], which always suffer from weak signal. The divider provides two channels 40 MHz femtosecond laser pulses, which can be shared by two independent imaging systems (Fig. 1a). These two laser pulses have a time delay of 12.5 ns, making them suitable for temporal multiplexing application[7,8]. The Pockels cell used in this setup achieves over 90% efficiency in both reflect and transmit direction. By employing two stages of such dividers in series, it becomes possible to divide an 80 MHz laser into 4 channels 20 MHz laser pulses with 12.5 ns temporal delays between them, therefore making it more suitable for spatiotemporal multiplexing application[8].

References

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Methods

Electronics

The electronic circuitry driving resonant Pockels cell includes a frequency divider, a RF amplifier and an impedance transform. An inductive coil L1 was connected to Pockels cell in parallel for a 40 MHz resonant frequency.

For femtosecond laser without 80 MHz synchronization output, an additional photodetector module was needed for synchronization signal. A commercial laser pulse detector (Thorlabs APD430A/M) can be used. Here we also presented a simple integrated laser pulse detector circuitry (Extended Data Fig. 1). A photodiodes S13773 chip was used to detect the laser pulse and amplified by a commercial 60 dB RF amplifier. All electronic components can be integrated onto the RF amplifier board. An adjustable 6-12 V DC supply was used to adjust the output attitude to matching the frequency divider input. A 5% reflector or a simple 0.17 coverslip was used to split a small laser energy onto the photodiodes.

A HMC432 frequency divider chip can also be simply integrated onto the RF amplifier board (see Extended Data Fig. 1). Or alternatively, a commercial HMC432 frequency divider module can be used to divide 80 MHz synchronization signal into 40 MHz signal. The output of the divider is 40 MHz square wave signal with <1.0 Vpp (Fig. 1 and Extended Data Fig. 4). This weak synchronizing signal was amplified by a RF power amplifier.

A commercial 40 MHz or 1-200 MHz broadband RF power amplifier was used to amplify the 40 MHz synchronization signal up to near 8 W. An adjustable 3V- 24V DC supply was used to adjust the output power of the RF power amplifier (Extended Data Fig. 4). A 50Ω RF resistance can be connected to the output of amplifier for power estimation. The output was around 30 Vpp with a 50Ω load under 8 W power. Use a low power for test at the beginning for device safety (Extended Data Fig. 4a and b). Notably, not power-on a RF power amplifier when the output is open.

The inductive coil L1 was wound with 1 mm enameled copper wire and with 8 turns and 8mm outer diameter for a Qioptiq LM 0202 P Pockels cell. It was shunt on the connector of Pockels cell for a 40 MHz resonant frequency. An RF impedance transformer was used to feed the RF power from RF amplifier to Pockels cell resonant circuitry. A 1:10 RF transformer can be simply wound with Teflon coated wires through the hole of magnetic ferrite core. Two copper tubes inside the holes of ferrite cores were used as the input side (1 turn). A piece of 1 mm diameter Teflon covered wire was wound through the copper tubes for 10 turns as the output side (Extended Data Fig. 2). The size the transformer is around 28x30x14mm (Extended Data Fig. 2 and 3). This transformer B1 was then mounted inside a copper box and connected with inductive coil L1 (Extended Data Fig. 2 and 3). The resonant status of the Pockels cell can be monitored with a 1 turn coil close to the RF transformer. Simply shortcutting the oscilloscope input probe with ground alligator clip can

be used as a 1 turn inductive coil. The tightness of coil L1 can be adjusted for a better resonant.

Setup the resonant Pockels cell behind the 80 MHz femtosecond laser. Carefully adjust the angle (normally only yaw angle) of the resonant Pockels cell. Another photometer module was used to monitor the output laser pulses. Half of the pulses at intervals can be found modulated by the Pockels cell (Extended Data Fig. 4a and b). Adjust the phase difference by change the length of the cables to get the right phase of driving signal and maximize the modulation of laser pulses (Fig. 1b and Extended Data Fig. 4b). Increasing the RF power can get a fully modulation of the interval pulses. When the angle of the resonant Pockels cell is right and the RF power is enough (less than 10 W), the 80 MHz femtosecond laser will be split into two 40 MHz femtosecond lasers with near equal power (A and B in Fig. 1a).

The capacitance value of Pockels cell will change with temperature. A constant temperature heating module was need to warm the Pockels cells to 31-32℃ for a fast starting and stable working. We used a commercial temperature control module to drive a 40℃, 10 W PTC (positive temperature coefficient) piece. PTC heater provide additional safety to avoid overheating. The L1 coil has quite large current inside during resonance when using a Conoptics Pockels cell. A small fine is need for L1 cooling.

Animal and 2PM calcium imaging

Two-photon calcium imaging was performed in one adult rhesus macaques. Experimental protocols were approved by the Peking University Animal Care and Use Committee. Detailed procedures of macaque 2PM imaging has been described elsewhere[10]. Briefly, two surgeries were performed for a craniotomy, AAV vectors injection (AAV1.hSynap.GCaMP5g.WPRE.SV40, Penn Vector Core) and imaging window implantation.

Two-photon imaging was performed using an Ultima IV (In Vivo) 2PM (Bruker Nano, FMBU, formerly Prairie Technologies) with an Ti: Sapphire laser (InSight X3 or InSight DeepSee, Spectra Physics) at 1000 nm wavelength. An 600mmX600mm field of view (FOV) was imaged with a 16X objective (0.8-N.A., Nikon). Two-photon images were acquired with fast-resonant scan at 30 frames per second (fps) and stored at 15 fps (average every 2 frames) during rest period of monkey.

Data analysis

All data analyses were performed using customized Matlab software (The MathWorks, Natick, MA). The images from each session were first realigned to a template image (the average image of 1000 frames) using a normalized cross-correlation-based translation algorithm, to correct the X-Y offset of images caused by the motion between the objective

and the cortex[10]. The psnr function was used to calculate the SNR of single frame or averaged frame (Fig. 2). An averaged template image (1000 frames) was used as the reference image.

Data availability

The data that support the plots within this paper and other findings of this study are available from the corresponding authors upon reasonable request.

Code availability

The code that support the plots within this paper and other findings of this study are available from the corresponding authors upon reasonable request.

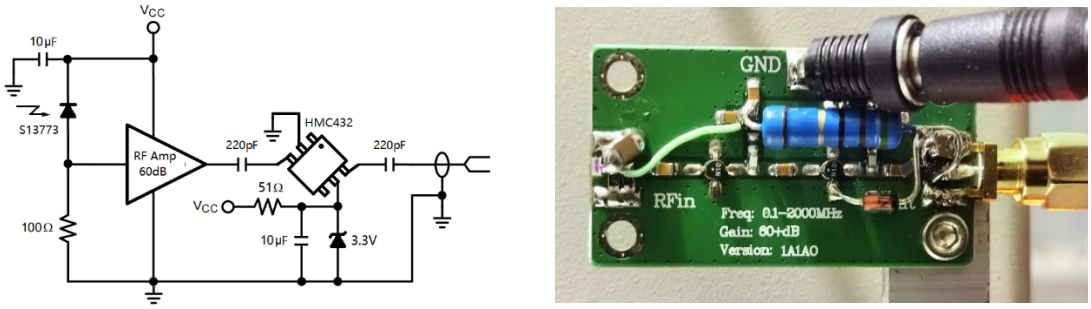
ACKNOWLEDGMENTS

We thank Jianwei Zong for help in manuscript revision, Yang Li and Hongfei Jiang for assistance in animal preparation. This work was supported by National Natural Science Foundation of China (grant no. 31730109 and U1909205) and funds from the Peking - Tsinghua Center for Life Sciences.

Additional information

Extended data

a **b**



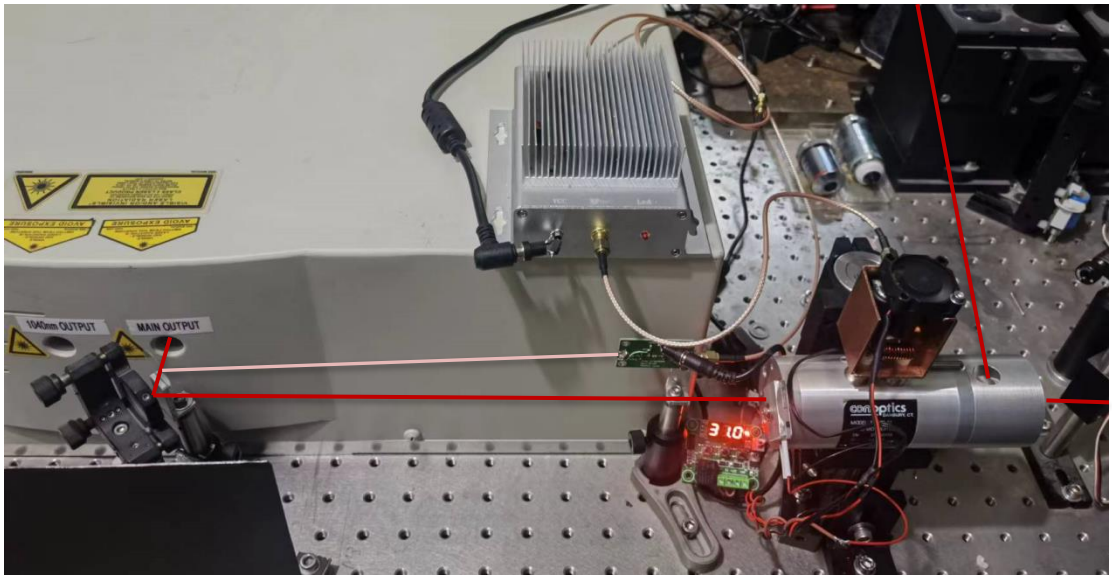
Extended Data Fig. 1| An integrated laser pulse detector and frequency divider module. **a**, The electronic circuitry of an integrated module for 80 MHz laser pulse detecting and frequency dividing. **B**, A demonstration of an integrated RF amplifier board with photodiodes and frequency divider.

a **b** **c**

Extended Data Fig. 2| Photos of impedance transformer B1 and resonant inductor L1. a, An 1:10 impedance transformer B1 with a SMA connecter. **b and c**, Impedance transformer B1 and resonant inductor L1 are assembled into a copper shielding box.

a

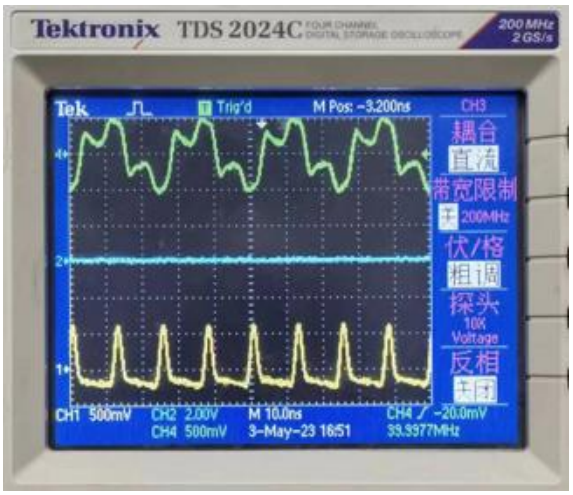
b



Extended Data Fig. 3| Demo 40 MHz femtosecond laser repetition frequency dividers. a, RF transformer unit connected with Qioptiq LM 0202 P. **b**, Photo of a laser repetition frequency divider with a Conoptics Model 350-80-02 Pockels cell. A 0.17 mm cover glass was used to reflect near 5% power from an InSight DeepSee femtosecond laser onto a photodiode

for an 80 MHz synchronization signal. The pink line indicates 5% laser power to photodiode reflected by a 0.17 mm cover glass. The dark red lines indicate the main laser power paths.

a



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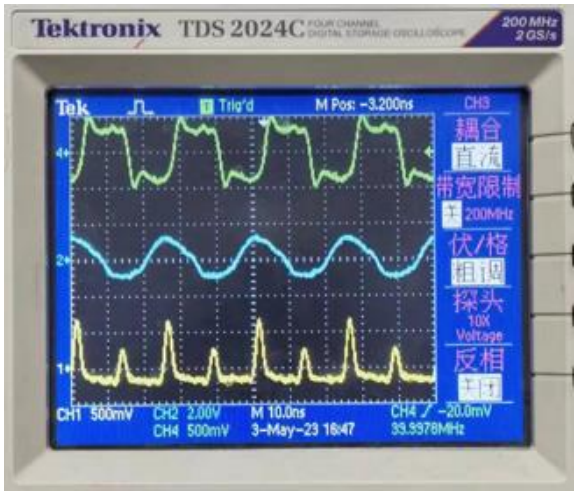

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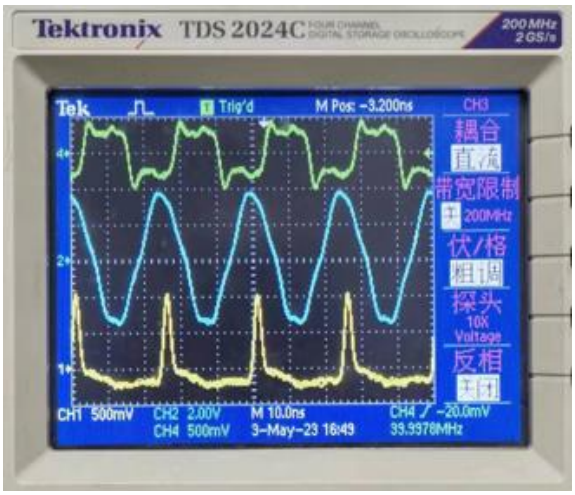

Extended Data Fig. 4| Electronic signals of a demonstration femtosecond repetition

frequency divider under different power supply voltages on the RF power amplifier. The green track at CH4 represents the 40 MHz synchronization signal, the blue track at CH2 represents the inductive signal from the resonant circuitry, and the yellow track at CH1 represents the output laser pulse signal detected by another photodetector. **a**, When the power supply voltage of the RF power amplifier is set very low (0-5 V DC), there is no pulse modulation observed. The output laser pulses maintain an 80 MHz repetition rate as there is no 40 MHz resonant voltage on the Pockels cell. **b**, As the power supply voltage is increased to 10 V, the interval between the laser pulses is partially reduced by the Pockels cell. **c**, When the power supply voltage is further increased to 18 V, the interval between the laser pulses is completely blocked by the Pockels cell (reflected to B direction as shown in Fig. 1a), resulting in the outputs a 40 MHz femtosecond laser pulse train.