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Pulse splitter for 2-photon glutamate uncaging and ex vivo electrophysiology

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We use this protocol and it's working

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Abstract

This protocol describes the setup and application of 2-photon glutamate uncaging (2PGU) for ex vivo electrophysiology (patch clamp) experiments, in particular with the use of an 8x pulse splitter for enhanced imaging and uncaging performance while minimizing photodamage.

Introduction

1 Summary

This protocol describes the setup of an 8x pulse splitter, intended for enhanced 2-photon imaging and glutamate uncaging (2PGU) performance with reduced photodamage during ex vivo electrophysiology (patch clamp) experiments.

1.1 Materials for the 8x Pulse Splitter

The following is a list of parts used for the 8x pulse splitter (described in **Step 2**), including all essentials mentioned in this document, as well as some accessories. Many of them are interchangeable with similar products; the list is based on the actual parts used in the pulse splitter in **Fig. 1a**, and should be regarded as a suggestion. Basic materials such as screws, optical posts, mounting bases, and others are omitted from the list; they are readily available from different vendors and can also be easily adapted per specific needs. The abbreviations (BS, PBS, HWP1, HWP2, T) refer to the optical elements shown in **Fig. 1 & 2**. Catalog numbers are for Thorlabs unless otherwise mentioned.

** Important:* for all optical elements, make sure that they are of correct optical profile suitable for the specific experimental needs, such as the wavelength of the laser to be used. All of the following parts are intended for 2PGU with 720 nm excitation; other applications (e.g. GCaMP imaging in vivo) may require the use of different parts for optimal efficiency (e.g. PBS123 instead of PBS122).

- Non-polarizing 50:50 (R:T) beamsplitter plate (BS, for the 4x splitter): BSW11
- Non-polarizing 50:50 (R:T) beamsplitter cube (BS, for the additional 2x split): BS005
- Polarizing beamsplitter cube (PBS): PBS122
- Fixed mount for beamsplitter plate: Polaris-B1G
- Platform mount for polarizing/nonpolarizing beamsplitter cube: BSH05
- Silver mirrors: PF10-03-P01
- Kinematic mirror mounts: Polaris-K1 or KS1
- Posts for Polaris mirror mounts: PLS-P1
- Clamping arm: Polaris-CA1
- Achromatic half-wave plate (HWP2): AHWP05M-980
- Rotation mount for HWP2: RSP1
- Zero-order half-wave plate (HWP1): Newport 05RP02-46
- Rotation mount for HWP1: Newport MT-RS
- Telescope (T; beam contractor, beam expander): GBE03-B
- Kinematic mount for telescope: KS2
- Iris diaphragms: ID20

- Right-angle adjustable clamp: RA90
- Nexus breadboard: B1212F
- Optical adhesive: Norland NOA81

1.2 (Extra) Materials for 2PGU Application for ex vivo Electrophysiology

The following are materials used for 2PGU application for ex vivo electrophysiology (patch clamp) experiments. Similar to the list of parts for the pulse splitter, they are not exhaustive and could be adapted according to the experimenter's needs, preferences, and the availability of materials; they should therefore be regarded as a suggestion.

- Patch clamp rig with 2-photon excitation microscopy (2PEF) capability
- Pressure delivery system (Parker Picospritzer III)
- Microforge to shape puffer pipettes (Narishige MF2)
- MNI-caged-L-glutamate (Tocris 1490)

8x Pulse Splitter Setup

2 Summary

The purpose and function of the pulse splitter is to split each laser pulse into temporally separated subpulses of smaller intensity, such that pulse repetition rate is increased n -fold from the original output according to the number of subpulses. Increasing the pulse repetition rate has the benefit of enhancing desired photoexcitation at a reduced cost of photodamage, compared to alternatives such as increasing the pulse intensity instead. For a detailed description, refer to the paper by Ji et al. (2008).

The 8x pulse splitter in this protocol is conceptually similar to Fig. S3c of Ji et al. (2008), with some adaptations. It can also be interpreted as a 4x splitter further expanded by 2x, resulting in an 8x split. Note that the pulse splitter in this protocol is different from what is described in the main text of the referenced paper, in that the former uses an arrangement of beam splitters and mirrors (**Fig. 1a**) instead of two interfaced media with different refractory indices as in the latter. The benefit of this alternative is that it is easier to construct at least for lower split numbers, at the potential expense of subpulse frequency, temporal precision of separation, throughput, and ease of alignment; however, these factors were not found to be critical for the experimental needs associated with 2PGU for ex vivo electrophysiology.

2.1 Design

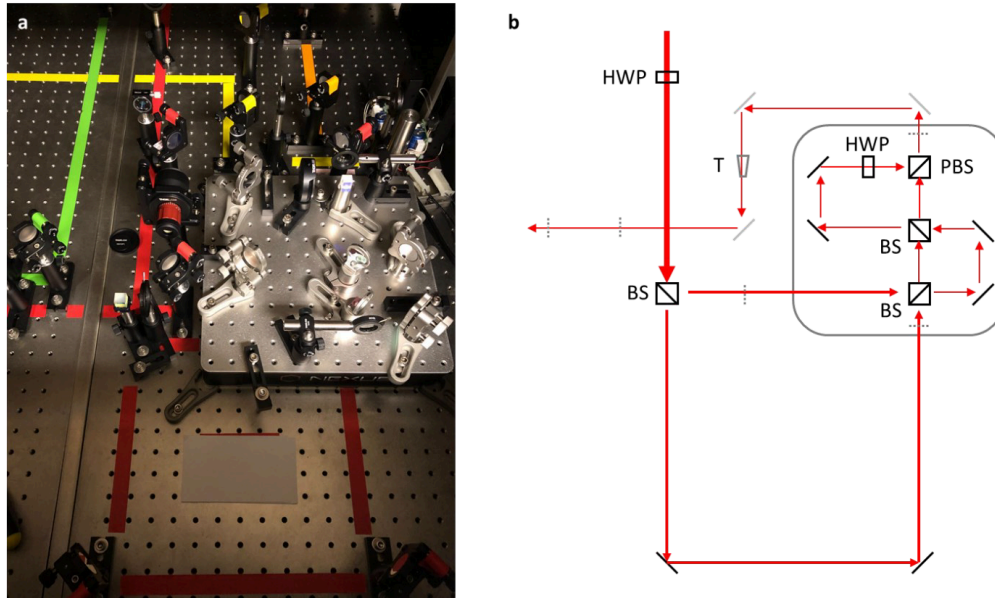


Figure 1. (a) 8x pulse splitter. (b) Schematic diagram of the 8x pulse splitter. HWP, half-wave plate; BS, nonpolarizing beam splitter; PBS, polarizing beam splitter; T, telescope. Unlabeled angled lines represent mirrors. Unlabeled dotted lines represent iris diaphragms.

Fig. 1a shows the setup for the 8x pulse splitter. Only the path labeled in red and the elements along that path are relevant to the pulse splitter, which are depicted in the simplified diagram in **Fig. 1b**. Laser beam approaches from the top of the picture past an EOM (not shown), travels through the splitter, and is directed to the scope towards the left side of the picture (not shown). The path is bounced around the splitter only because of the space available; there is no particular reason that it needs to be set up in that manner (aside from path length and alignment considerations, which will be described below).

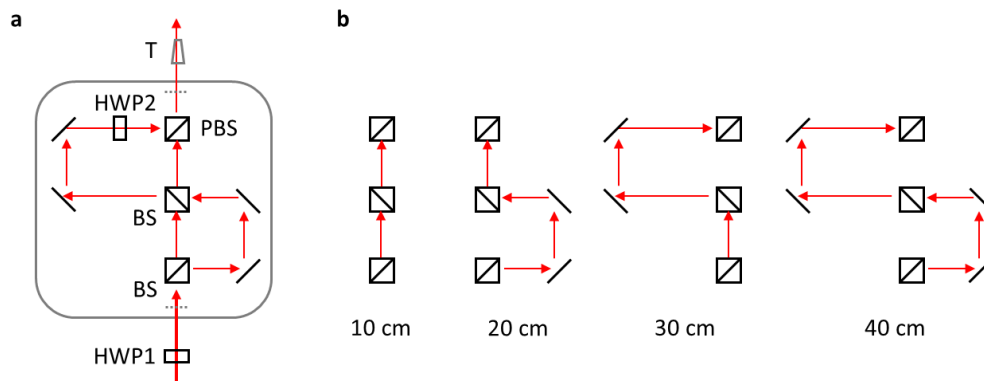


Figure 2. (a) Schematic diagram of the 4x splitter. (b) Optical paths of the 4x splitter.

Here, the design principles of the 4x splitter is introduced first, which will then be expanded to a $2 \times 4 = 8x$ split. The 4x splitter in **Fig. 2a** is identical to what is incorporated in our 8x splitter in **Fig. 1**; the upstream half-wave plate and the telescope past the splitter have been moved in the diagram to equivalent locations along the path for visual simplicity.

As the laser beam enters the splitter, it is split evenly (50:50, transmitted vs. reflected) at each beam splitter (BS; Thorlabs BSW11), resulting in a total of four paths with different lengths (**Fig. 2b**). The asymmetry in the placement of mirrors is intentional, with the purpose of producing an identical distance difference between each path (in this case ~ 10 cm) such that it will result in subpulses of temporally uniform separation. In reality, it may not be feasible to have these differences to be precisely identical due to the alignment process to be followed, but reasonably small differences are tolerable for the experimental needs of 2PGU. Note also that this spatial distance directly corresponds to subpulse frequency, as the split is achieved by having each subpulse delayed from the immediately preceding one by the increased travel distance; for example, our splitter is expected to have a subpulse separation of > 334 ps (assuming 10 cm difference, and longer in practice because $v < c$). In other words, higher subpulse frequency could be reached by having smaller distance difference between paths; however, there will be practical limitations due to the placement of optical elements.

The pulse splitter is intended for linearly polarized light. In the setup in **Fig. 1 & 2**, output from the source laser is in horizontal linear polarization, and remains unchanged before arriving at the splitter. Under some circumstances, the plane of polarization might have been altered to accommodate other needs, such as dividing the source laser into two separate microscopes. In any case, the polarization angle of the beam entering the pulse splitter is to be adjusted using the first half-wave plate (HWP1; Newport 05RP02-46) to achieve maximal throughput efficiency for the two of the four paths corresponding to the transmitted component through the polarizing

beam splitter (PBS; Thorlabs PBS122), and then again with the second half-wave plate (HWP2; Thorlabs AHP05M-980) for the remaining two paths reflected at the same PBS, after which all of these four paths will be combined. The difference in axis angles of HWP1 and HWP2 will consequently be close to 45 degrees relative to each other once the adjustments are complete. When correctly configured, it can also be expected to result in a relatively even split, assuming that the BS are evenly splitting and that power loss at each contact is negligible; at the time this document was written, the 8x splitter in **Fig. 1** produces a split of (1 : 0.90 : 0.87 : 1.11 : 0.99 : 1.04 : 1.00 : 1.01) from the shortest to the longest path, with 72.9% throughput under maximal attenuation at 720 nm.

The telescope (T; Thorlabs GBE03-B), or a beam contractor, is simply a beam expander placed in the reverse direction. The sole purpose of the telescope is to reduce beam dispersion as it travels from the splitter to the microscope. While technically not an essential component of the splitter, we found it to be practically necessary considering the distance traveled, along with other requirements. The aim is to have the beam arrive at the back aperture of the objective just large enough to fill it, so as to minimize power loss. The telescope can also be helpful during alignment, similar to how iris diaphragms can be used (described below). The platform at the base of most of the elements constituting the 4x splitter (represented as a square with round edges; Thorlabs B1212F) is likewise not essential, but will be extremely useful if the splitter ever needs to be relocated.

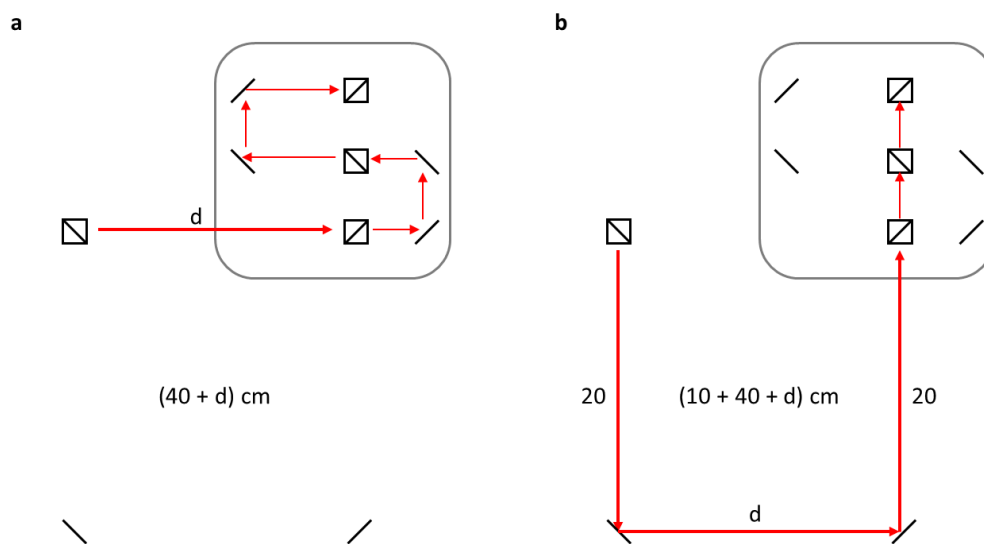


Figure 3. (a) Longest path of the 4x splitter, combined with the shorter path of the additional 2x split. (b) Shortest path of the 4x splitter, combined with the longer path of the additional 2x split. The difference between these two paths is intended to be identical to the original distance difference between paths of the 4x splitter illustrated in **Fig. 2b**, such that all 8 paths will have an approximately even distance difference.

Once the 4x splitter is complete, it can be readily expanded to an 8x splitter (**Fig. 1b**) by introducing an additional beam splitter (BS; Thorlabs BS005) between the first HWP and the first BS of the original 4x splitter. In this configuration, The initial beam entering the 4x splitter will now have been split at the newly incorporated BS before arriving at the next BS (along orthogonal paths) which was part of the 4x splitter. It is therefore crucial to use a nonpolarizing and evenly splitting BS, otherwise both the throughput and the evenness will be severely compromised. The two additional mirrors that are introduced alongside serve two important purposes: 1) They should be positioned in such way that the resulting 8 paths will retain approximately even differences in distance (**Fig. 3**); otherwise, the subpulses may overlap or be too separated in time, degrading the effectiveness of the splitter. 2) Having a pair of mirrors will be helpful and nearly crucial for alignment, which will be described in more detail in the section to follow.

2.2 Alignment

Spatial alignment of the splitter, or of the paths, can be done by adjusting the positions and angles of the optical elements. The basic idea of aligning the splitter is identical to that of directing any laser beam; a pair of reflective surfaces can be used to control both the angle and the translation of the beam, and in some cases, relocating those surfaces can be helpful to produce larger shifts whenever needed. Some tedious iterations will be required, but generally speaking for the splitter, the BS and the PBS can be placed first at roughly reasonable positions and angles, then the mirrors can be placed, again first at roughly reasonable places, after which fine adjustments can be made using the kinematic mount. Each of these elements may have to be repositioned if deemed necessary.

Alignment of two (or more) beams can be checked visually by cutting off the beam at arbitrary locations. During this process, it is crucial to look at at least two different locations along the intended path, preferably as far apart as possible from each other, to ensure that the beams are in fact aligned (i.e. arriving at the same point while also being collinear), instead of only being convergent onto the point at which the beams were cut off with different incident angles. Having a set of iris diaphragms will be almost crucial for the alignment process, in order to reduce beam diameter such that alignment quality can be examined by eye.

One approach that can be used for aligning the 4x splitter is to first align a selected pair of the four paths while obstructing the other two, then to repeat the process with different combinations of paths until alignment is complete for all paths. For example, blocking the reflected component at the first BS in **Fig. 2a** will block out the second and fourth paths in **Fig. 2b**, and alignment of the remaining paths will also no longer

involve the two mirrors to the right. Likewise, blocking the point between the second BS and the PBS in **Fig. 2a** will block out the first and second paths in **Fig. 2b**, but in this case the alignment of the remaining paths will involve all four mirrors, two of which will also affect the second path that had been blocked out. It will be easier to figure out this process through trial and error than from following a written description; keeping in mind the elements involved in each path and the hierarchy between them will be helpful in employing a systematic approach for alignment.

Alignment of the 8x splitter can be done by first aligning the 4x splitter, then finishing the alignment by adjusting the two mirrors that were introduced for the additional 2x split at the upstream. If correctly done, there should be no need to re-adjust the 4x splitter.

Alignment must be done with the same laser wavelength as intended for actual application, as it will be compromised at different wavelengths (due to different speed in non-vacuum medium). Splitter alignment should be examined regularly, as it can deteriorate over time from environmental factors such as thermal expansion of optical and mechanical components caused by temperature fluctuation. The angle of HWP axes, mentioned in the previous section, can and should be adjusted after all alignment is complete, while the power is measured to assess splitter efficiency and evenness; adjusting the HWP axes should not affect alignment.

Protocol references

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