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Modified Frame-projected Independent Fiber Photometry (FIP) System_Triggering system

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Kenta M. Hagihara[1]

[1]Allen Institute for Neural Dynamics

Allen Institute for Neural...

Kenta M. Hagihara
Allen Institute for Neural Dynamics

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Protocol status: Working
We use this protocol and it's working

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Keywords: fiber photometry, calcium imaging, neuromodulator imaging, CMOS-based photometry

Abstract

Frame-projected independent fiber photometry (FIP) is a method to measure fluorescent sensor signals through optical fibers implanted in living animals, using a camera to record video of the fiber faces. To precisely control timings of excitation and camera frame acquisition for FIP, and of other external stimulation apparatus (e.g. LEDs for photo-stimulation), we use a Teensy 4.1 microcontroller, which generates voltage pulses without relying on continuous communication with the operating system of the experimental computer for timing. This protocol is for the 3 excitation LED + 2 collection CMOS cameras design.
Triggering-controlling software (an Arduino sketch) and data aquisition software (a Bonsai workflow) can be found in an associated github repository.

Guidelines

Teensy 4.1 only provides ~3.3V. If the device you want to trigger requires 5V (or more), the voltage needs to be up-regulated.

Materials

Equipment	
Teensy 4.1	NAME
PJRC	BRAND
DEV-16771	SKU
https://www.pjrc.com/store/teensy41.html	LINK

Equipment	
Terminal Block Breakout Board Module for Teensy 4.1, Screw Mount Version	NAME
Breakout board	TYPE
OONO	BRAND
D-1395P	SKU

Software
Arduino software
Teensy extension
AIND FIP DAQ Control github repo

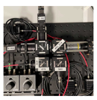
Cables and connectors
USB2.0 cable (USB-A to Micro USB)
Jumper (or bare) wires
BNC cables
BNC sockets (Amazon link for an example product)
BNC connector (Amazon link for an example product)
FLIR camera GPIO cable (6-pin Hirose)

Miscellaneous
Solder (we used: Lead free wire from Amerway)
Soldering tools (we used: Weller WX2)
3D-printer (we used: Stratasys Dimension 1200es and IC3D Virago 700, with ABS and PETG filament, respectively)
A computer that can run Arduino/Teensy software. We used Windows 10 PCs but Max/Linux would work too.

Before start

Please see the accompanying protocol for the main FIP hardware design:

Protocol



NAME

Modified Frame-projected Independent Fiber Photometry (FIP) System_Hardware

CREATED BY

Kenta M. Hagihara

PREVIEW

Preparing an enclosure

1 As the Teensy 4.1 is a bare board, it should securely covered in an enclosure. The enclosure should be able to securely house Teensy 4.1, at least 5 BNC sockets, and optionally a small breadboard. Plastic 3D-printing (CAD design can be found here: 0294-100-01_X01.step 246KB 0294-100-02_X01.step 3MB) would be sufficient and could be any materials and designs.

Setting up an experimental PC and Teensy 4.1 device

25m

2 Install Arduino app on your experimental PC from the following link: **Arduino software**.

10m

3 Add Teensy extension to Arduino app by following **the official instructions**.

10m

4 Download .ino sketch from the **github repo**.

5 Connect Teensy 4.1 device to the experimental PC using a USB cable, set proper COM port in the Arduino app, and upload the .ino sketch to the device. How to find and set up the corresponding COM port can be found here: https://support.arduino.cc/hc/en-us/articles/4406856349970-Select-board-and-port-in-Arduino-IDE.

5m

Connecting the Teensy 4.1 device to hardware to be controlled

50m

6 Place the Teensy 4.1 device on a breakout board (or a generic electric breadboard).

5m

7 Attach the breakout board and BNC(female) sockets to the enclosure.

10m

8 Solder wires between the breakout board and the BNC sockets according to this diagram for 3 excitation LEDs and 2 CMOS sensors configuration.

30m

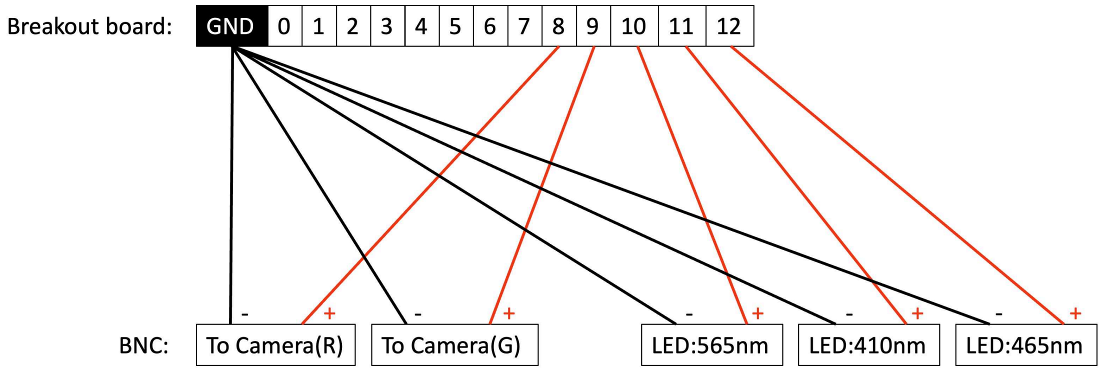
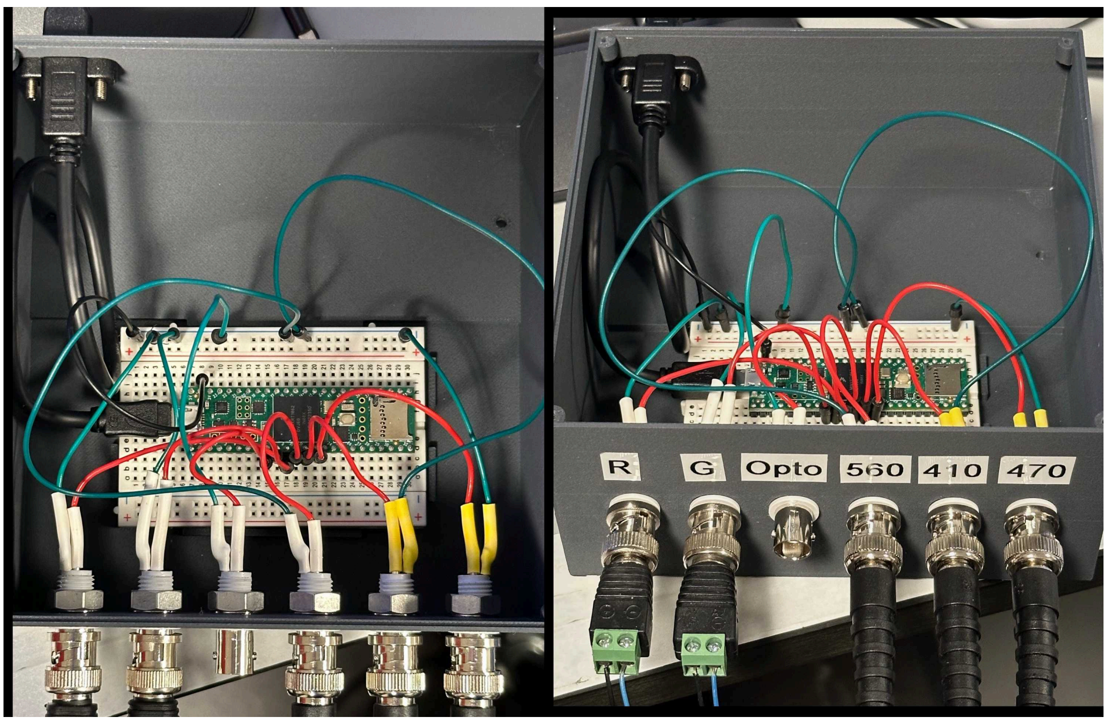


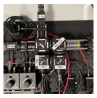
Fig1. Diagram for connections



Red wires (hot), green wires (ground)

9 Connect the device to hardware (cameras and LEDs) to be controlled using BNC cables. 10m

Protocol



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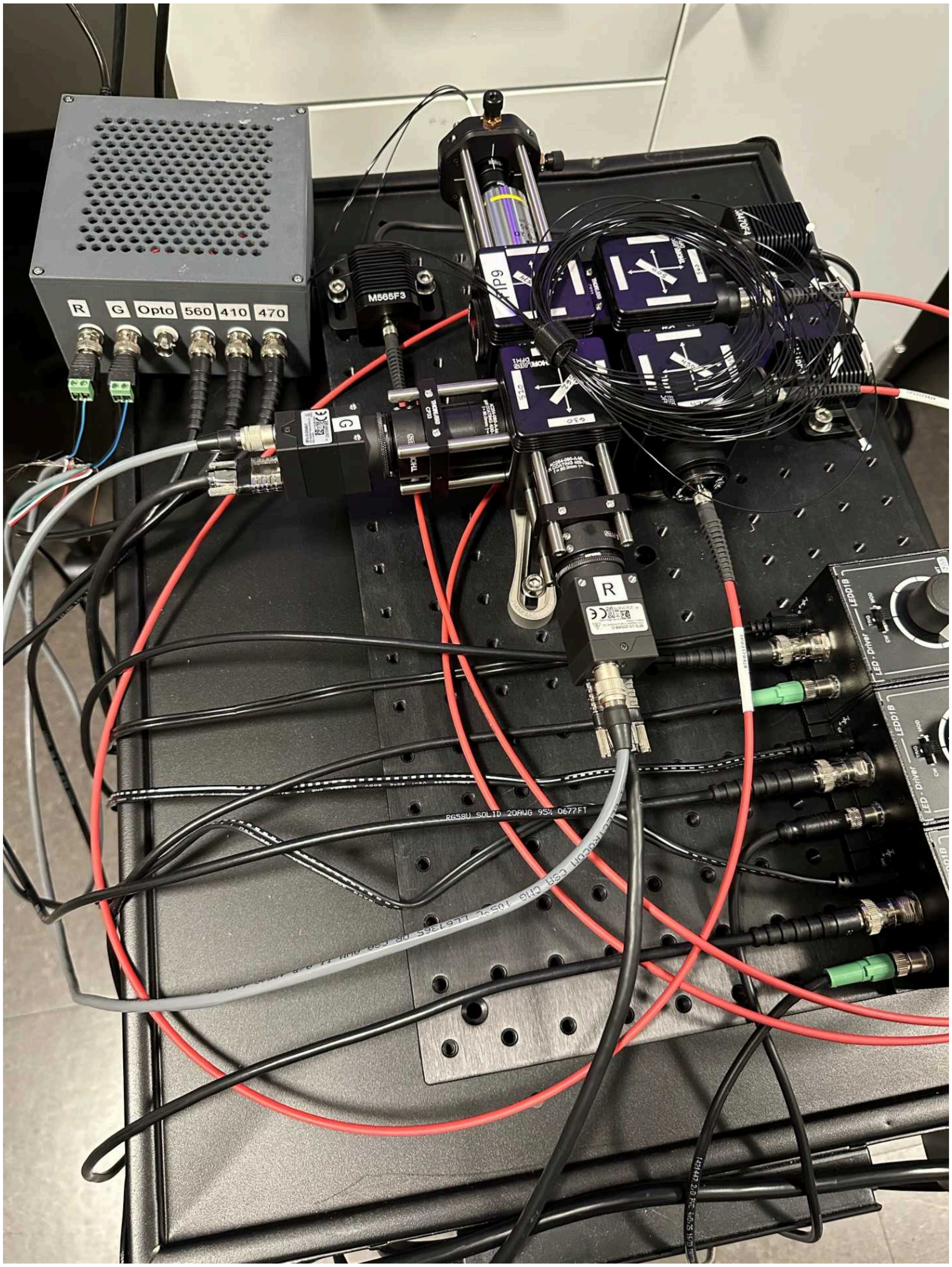
PREVIEW



All BNC ports connected to corresponding devices of the main hardware

Software parameter setting

30m

10 Based on acquisition frame rate and the CMOS cameras to be used, set the acquisition parameters in the .ino sketch.

30m

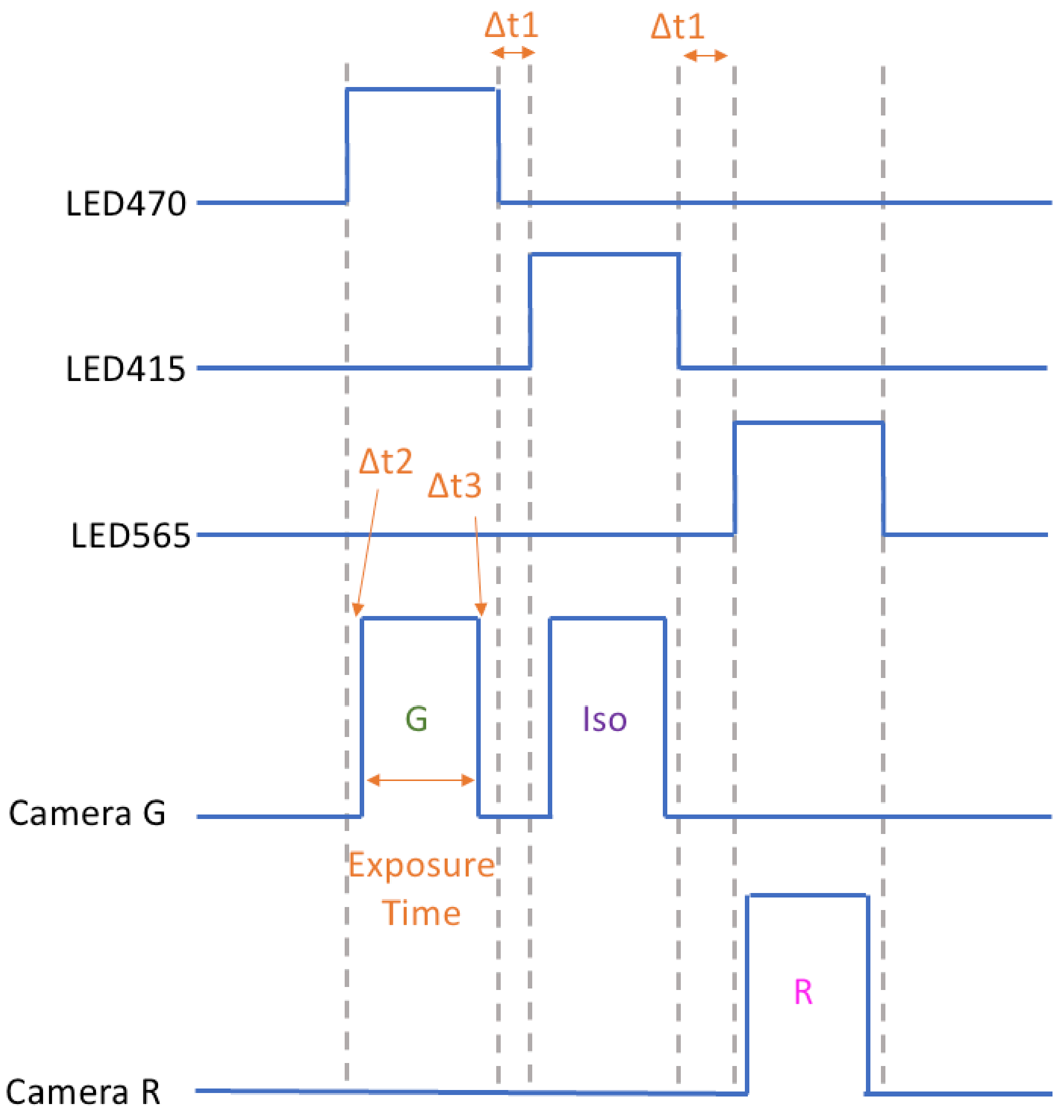


Fig2. Scheme of temporal multiplexing of excitation and data acquisition

When acquisition is 60Hz (20Hz for each channel) with sufficiently fast CMOS sensors (e.g. FLIR, BFS-U3-20S4M-C), an example parameter set could be:
ExpT = 15650 µs
Δt1 = 666 µs
Δt2 = 300 µs
Δt3 = 50 µs
ExpT+ Δt1+Δt2+Δt3 = 16666 µs (= 1/60 s)

For better signal, Δt1/Δt2/Δt3 should be minimized to make the exposure time longer. If they are too short, however, the camera will not be able to unload the data (when Δt1 is too short) or LED instability at the onsets (when Δt2 is too short) would lead to periodic noise generation. Based on excitation light sources and CMOS models, optimize these parameters empirically.

Protocol references

Kim, C., Yang, S., Pichamoorthy, N. *et al.* Simultaneous fast measurement of circuit dynamics at multiple sites across the mammalian brain. *Nat Methods* **13**, 325–328 (2016). https://doi.org/10.1038/nmeth.3770

TeensyDuino .ino sketch can be found at **AIND github repo**.

CAD design can be found here: 0294-100-01_X01.step 246KB 0294-100-02_X01.step 3MB

Acknowledgements

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