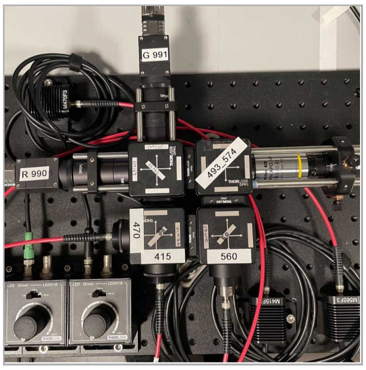

JUL 10, 2023

Modified Frame-projected Independent Fiber Photometry (FIP) System_Hardware

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We use this protocol and it's working

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ABSTRACT

This is a step-by-step protocol to build a modified FIP (Frame-projected Independent FP) system. FIP was first implemented and reported by Kim et al. (2016). Their protocol is available here.

We modified previous design so that it can be built mostly with Thorlabs products (still some products from other manufactures are required), which are off-the-shelf and easy to be purchased within a reasonable lead time. The system described in this protocol is designed to record signals from 1)GFP-based sensors, 2)mApple-based sensors, and 3) iso sbestic signals from up to 4 sites. When applied to measurements of other fluorescent protein-based sensors or spectrally shifted sensors, selection of optical filters and/or excitation light sources should be modified accordingly. Also, the selection
of fiber patch cable should be based on the number of simultaneously recorded locations, and diameter/NA of fiber implants.

We thank Takeo Katsuki Ph.D., (Thorlabs Japan) and Sho Yagishita M.D., Ph.D., (U.Tokyo, Japan) for sharing their galvano-mirror-based photometry design described in Iino et al. (2020). TK also kindly provided CAD model used in this protocol.

MATERIALS

Thorlabs

product#	Description	#
Excitation		
M415F3	LED415nm	1
M470F3	LED470nm	1
M565F3	LED565nm	1
LEDD1B	t-cube LED driver(1200mA)	3
KPS201	PowerUnit for T-cube	3
FB410-10	410 bandpass	1

product#	Description	#
FB470-10	470 bandpass	1
FB560-10	560 bandpass	1
M59L01	SMA-SMA 1000um 0.48NA fiber	3
KAD11F	collimator mount (kinematic)	2
AD15F	collimator mount	1
F220SM-532	collimator	2
F950SMA-A	HighNA collimator	1
General cage system (30mm cage)		
DFM1	kinematic filter cube	4
RS1P	post	4
CP33	cage plate	5
ER025	rod	16
ER2-P4	rod	2
ER4-P4	rod	1
SM1CP2	Externally SM1-Threaded End Cap	4
SM1L05	LensTube	4
SM1L03	LensTube	2
-	Breadboard with a proper size	1
Emission		
SM1A12	M25*0.75 Objective adaptor	1
AC254-080-A-ML	achromatic lens f=80	2
SM1A9	C-Mount/SMA adaptor	2
SM1AB2	C-Mount/SMA adaptor	2
SM1P1	Optic mount	2
Fine-tunable Fiber Mount		
CXYZ05	xyz fiber mount	1
SM05L03	lens tube	1

product#	Description	#
SM05FC	fiber adapter	1
SM05T2	capla for lens tube	1

Note, Thorlabs products can get obsolete routinely; replace them with corresponding newer products.

Semrock

product#	Description
FF493/574-Di01-25x36	493/574 nm BrightLine® dual-edge standard epi-fluorescence dichroic beamsplitter
FF01-520/35-25	520/35 nm BrightLine® single-band bandpass filter
FF01-630/69-25	630/69 nm BrightLine® single-band bandpass filter
FF562-Di03-25x36	562 nm edge BrightLine® single-edge standard epi-fluorescence dichroic

Nikon

product#	Description
MRD70170	CFI60 PLAN APOCHROMAT LAMBDA D 10X

Edmund Optics

product#	Description
#69-899	500nm, 25.2 x 35.6mm, Dichroic Longpass Filter
#69-898	450nm, 25.2 x 35.6mm, Dichroic Longpass Filter

Doric

Fiber patch cable should be selected based on application.We recommend "Low-Autofluorescence Bundle Branching Fiber-optic Patch Cord" from Doric for standard applications.

FLIR-Teledyne

CMOS sensors should be selected based on required frame rate and sensitivity. We use "BFS-U3-20S4M-C", which is based on Sony IMX422 sensor, as default CMOSs.

SAFETY WARNINGS

Although resultant FIP setup will be a fairly optically closed system, in particular during building, experimenter(s) could be exposed to stray light from LEDs. In general, inappropriate use of any Fiber-Coupled LEDs may result in permanent eye damage. To prevent injury, use the LEDs in accordance with the International Standard “Photobiological Safety of Lamps &
Lamp Systems” IEC 62471. The LEDs used in this system fall under RG2 - Moderate Risk Group in accordance to the
standard IEC 62471:2006.

System Overview

1

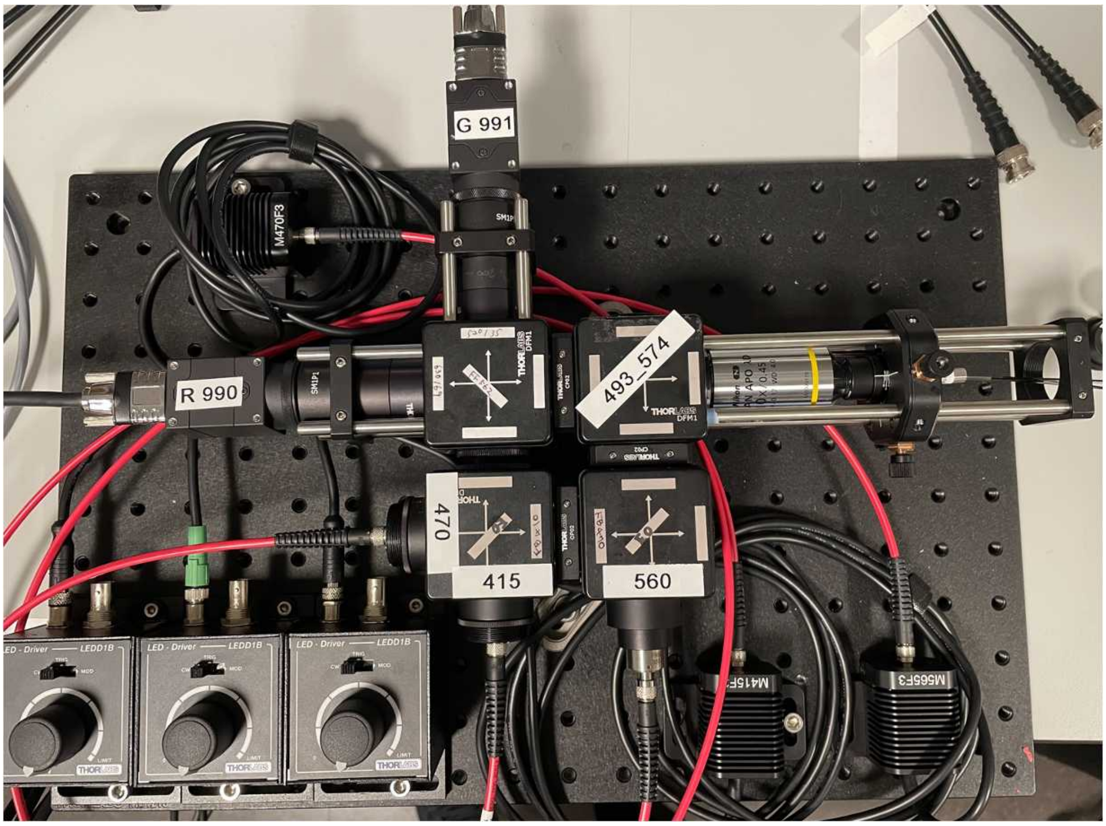

Fig. 1. System Overview

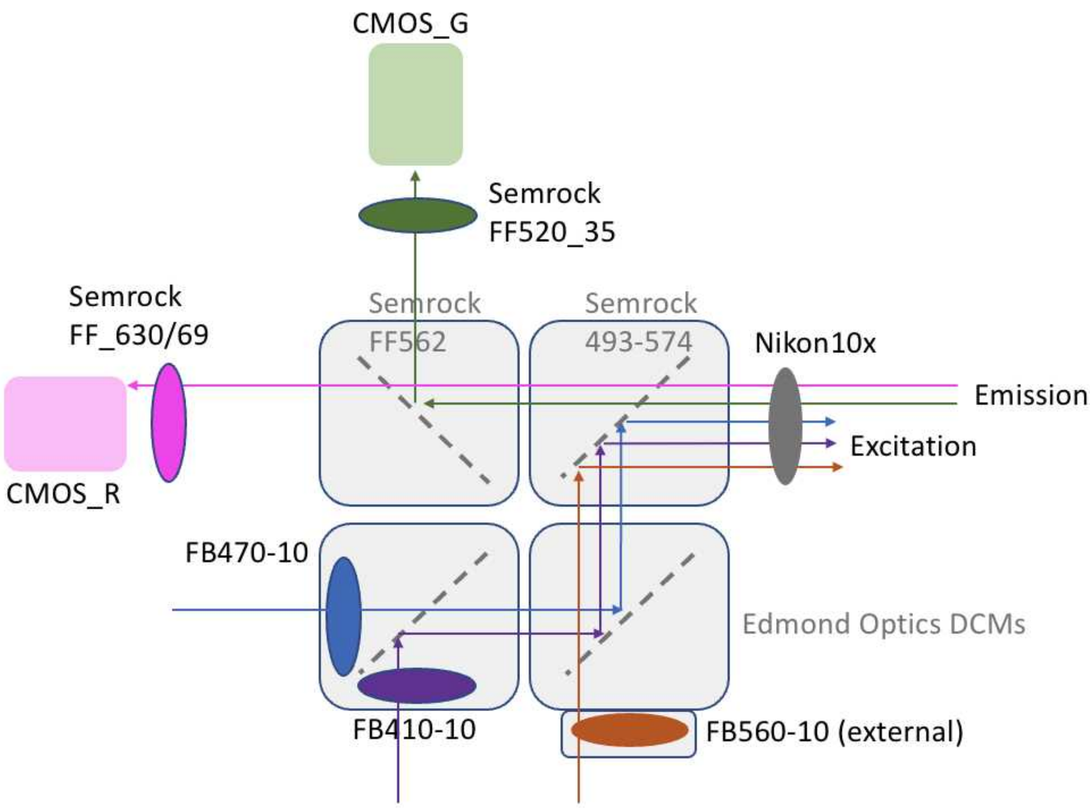

Fig. 2. Design diagram

See the **Materials** section for products used in the setup.

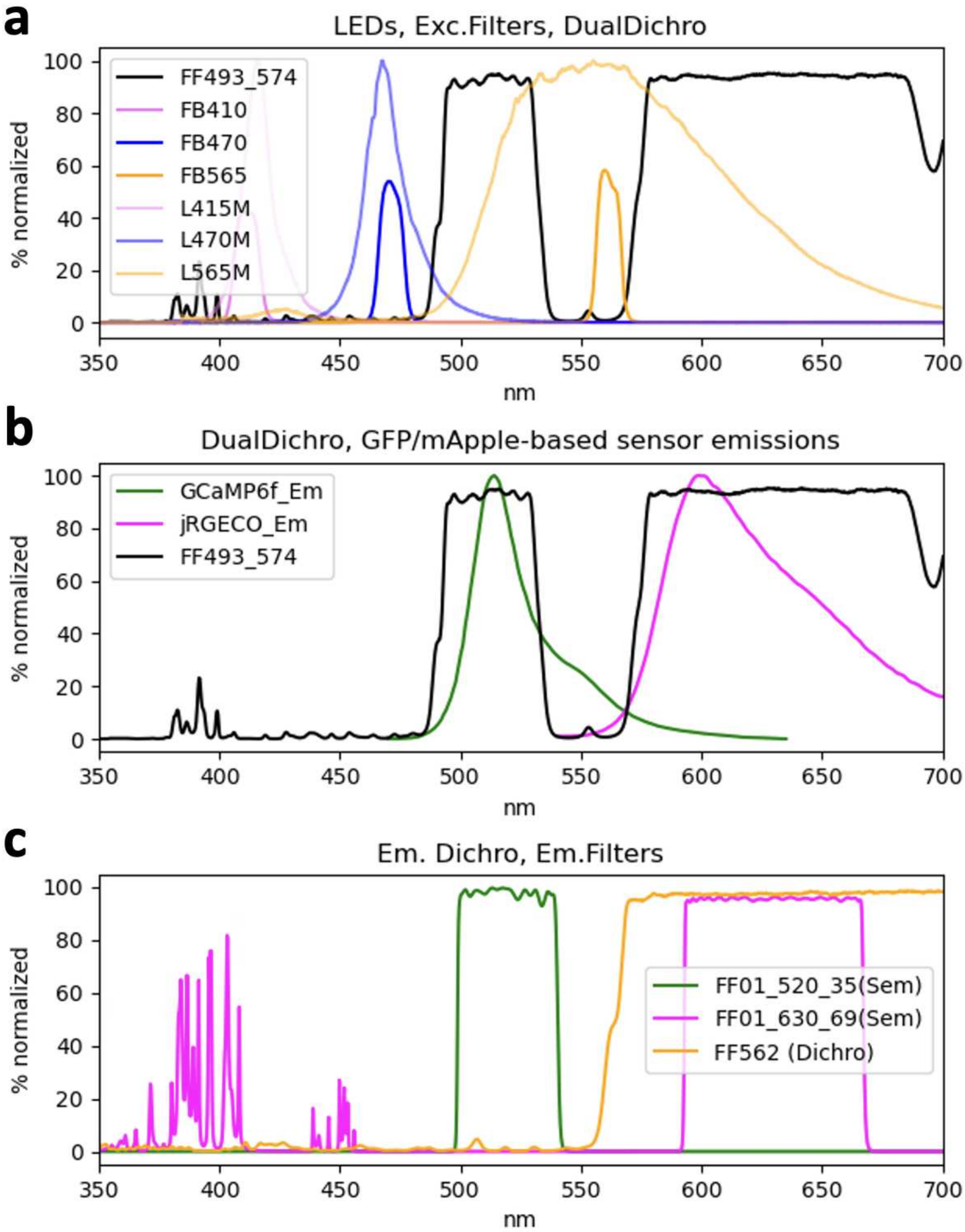

Fig. 3. Spectral features of LEDs, optical filters, and genetic sensor emission signals.
a, Normalized intensity of excitation LEDs (L415M, L470M, L565M), and transmission of excitation band-pass filters (FB410, FB470, FB560) and the dual dichroic filter (FF493_574).
b, Transmission of the dual dichroic filter (duplicated from **a**) separating emission spectrum from excitation spectrum (**Fig. 1**), and normalized intensity of GCaMP6f and jRGECO1a. Note, this design would be suited for most cpGFP- and cpmApple-based sensors.
c, Transmission of the dichroic filter separating green and red emission signals (FF562), and emission band-pass filters (FF01_520_35 for green and FF01_630_69 for red)

Assemblying optical parts

2h

2 Assemblying the cubes 30m

(This step-by-step part will have corresponding CAD models later.)

3 Adding optical filters, collimators, and the objective lens 30m

4 Assemblysing the fiber positioning module 15m

5 Assemblysing the CMOS modules 30m

6 Connecting excitation light sources with fibers 15m

Alignment

30m

7 Adjusting the fiber location so that fiber end makes a crisp image on CMOSs

8 Adjusting angle of collimators so that excitation light coming from fiber patch cables would get roughly uniform. 30m