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Multi-color fiber array imaging

In 1 collection

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ABSTRACT

We have developed a new micro-fiber array approach capable of chronically measuring and optogenetically manipulating local dynamics across over 100 targeted locations simultaneously in head-fixed and freely moving mice, enabling investigation of cell-type and neurotransmitter-specific signals over arbitrary 3-D volumes . This protocol includes the fiber array imaging setup and acquisition steps. Please contact us (mwhowe@bu.edu) if you are interested in using this technique.

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Imaging setup

1 Fiber bundle imaging for head-fixed experiments was performed with a custom microscope mounted on a 4' x 8' x 12" vibration isolation table (Newport). The setup is detailed in the substeps below.

1.1 Excitation light (470nm, 405nm, 415nm, and 570nm) for fluorescent sensors was provided by high-power Solis LEDs (Thor labs, No. SOLIS-470C, SOLIS-405C, SOLIS-415C, SOLIS-570C) which were combined using a series of two dichroic filters (Chroma No. ZT532rdc and 405rdc).

1.2 Light from the LEDs was filtered (Chroma No. ET405/10, ET555/25, ET473/24) and coupled into a liquid light guide (Newport No. 77632) with lenses (f = 60mm and 30mm, Thor labs No. LA1401-A and LA1805) and a collimating beam probe (Newport No. 76600). The liquid light guide was coupled into a filter cube on the microscope and excitation light was reflected into the back aperture of the microscope objective (10x, 0.3NA, Olympus No. UPLFLN10X2) by a dichroic beam-splitter (Chroma No. 59009bs).

1.3 Light power measured at the focal plane of the objective was set to 65-90 mW which produced ~1.6-2 mW/mm^2 power at the fiber tips.

1.4 Fluorescence from the fiber bundle was collected by the objective then passed through the dichroic beam-splitter used to direct the excitation light. A second dichroic (Chroma, No. 532rdc) reflected green and passed red fluorescence, and bandpass filters for red and green (Chroma, No. 570lp and 525/50m respectively) blocked residual excitation light and autofluorescence.

1.5 A tube lens in each path (Thor labs, No TTL165-A) focused emission light onto the CMOS sensors of the cameras to form an image of the fiber bundle (Hamamatsu, Orca Fusion BT Gen

III).

1.6 The microscope was attached to a micromanipulator (Newport No. 9067-XYZ-R) to allow fine manual focusing and mounted on a rotatable arm extending over the head-fixation setup to allow for coarse positioning of the objective over the mouse.

Imaging acquisition

2 Imaging data was acquired using HCImage Live (Hamamatsu). The parameters are detailed in the substeps below.

2.1 Single wavelength excitation and emission was performed with continuous, internally triggered imaging at 30Hz.

2.2 For dual-wavelength excitation and emission, two LEDs were triggered by 5V digital TTL pulses which alternated at either 11Hz (30ms exposure) or 18Hz (20ms exposure).

2.3 To synchronize each LED with the appropriate camera (e.g. 470nm LED excitation to green emission camera), the LED trigger pulses were sent in parallel (and decreased to 3.3V via a pulldown circuit) to the cameras to trigger exposure timing. The timing and duration of digital pulses were controlled by custom MATLAB software through a programmable digital acquisition card ("NIDAQ", National Instruments PCIe 6343). Voltage pulses were sent back from the cameras to the NIDAQ card after exposure of each frame to confirm proper camera triggering and to align imaging data with behavior data.