1. System requirements

Spiral size, revolution and durations of the photostimulation protocol should be defined in PrarieView (version 5.4.64.40, Bruker Corporation). The access to the raw image data stream depends on PrairieLink (Bruker Corporation). The phase masks are uploaded to the spatial light modulator (OverDrive Plus SLM, Meadowlark Optics) using the Blink\_SDK dll (Meadowlark Optics). Analogue voltage outputs are generated using NI-DAQmx (version 15.5 National Instruments, ). ManagedCUDA and BitMirecle.LibTiff are used when motion correction is enabled (a NVIDIA GPU is required; tested on GeForce GTX 750 Ti). Software platforms used in the package include: VB.net (.NET Framework 4.5), Visual Studio 2013 (64 bits) and Matlab (2016a). Software has been tested on windows 7 desktop.

1. Installation guide

* Install Visual studio 2013,
* Install Measurement Studio (version 15) for VS2013
* Install NI-DAQmx (tested with version 15.5)
* Install CUDA toolkit 8.0
* Make sure Prairie View and Framework 4.5 are installed
* Connect an analog voltage output (tested with USB-6212, National Instruments) to the spiral trigger input of the Prairie system
* Download folder ‘RTAOI - dev’. Change the device ID and TCP/IP address as instructed in the .sln file and rebuild the solution

The whole installation procedure takes less than 1.5 hours on a ‘normal’ desktop computer.

1. Demo

Instructions:

* Start Prairie View
* In MarkPoints, import ‘gpl\_demo.gpl’; load series ‘mp\_demo.xml’; click ‘Run Mark Points’
* Run CL\_Blink.exe; Insert number of phase masks (15) then insert ‘Y’ to start waiting for RTAOI commands
* Start RTAOI
* Open ‘FOV\_with\_targets\_demo.bmp’ and select the marked ROI(s) in the FOV window (in the demo select click from 1 to 4)
* Configure experiment protocols in the RTAOI interface (e.g. change the threshold etc); check the ‘Below’ boxes to specify ROIs as trigger cells; click ‘lock threshold’; click begin experiment; click ‘Display off’ to speed up online processing (optional)
* Start data streaming in Prairie View

Expected output:

Photostimulation will be delivered to the ROIs that have intensity value below the thresholds (%)

Expected run time for demo:

A maximum 2000 photostimulation can be delivered; run time depends on online recorded intensity within the ROIs; the user can terminate the process at anytime by stopping data streaming and restart by clicking ‘New recording’

1. Instructions for use

* For basic use please refer to the instructions to run demo
* Detailed experimental protocols can be configured in separate panels
* Enable motion correction by loading a reference image (.tiff file) before recording and check ‘Use GPU’ and ‘Get shifts’
* If operating in ‘Playback’ mode, select the saved out .txt file (described below) and click ‘Playback’ button. Then all preset protocols will be ignored and the specified photostimulation patterns will be delivered at specified frames
* Click ‘Begin experiment’ – ‘New recording’ – ‘Start recording’ before starting data recording in Prairie View to ensure protocol not affected by previous recordings
* Save out photostimulation frame and pattern indices into a .txt file (optional)