P4 - System operate instruction

Part1: Software needed to be installed

Table1: Overview of Software needed in MINI2P Platform (updated version Jan2022)

Software	**version**	**company**	**Source**	**License (Y/N)**
MATLAB	2019b or newer	Mathworks		Y
LabVIEW	2021 or newer	National Instruments		Y
PI Software Suite	PIMikroMove 1.0.7.2	Physik Instrumente	CD	N
ScanImage	Basic 2021 or newer	Vidrio		Y
TOPAS	FemtoFiber Ultra 920	Toptica	USB flash drive	N
PulseLink	APE pulseLink	A-P-E	.exe	N
PMT2100	latest	Thorlabs	.exe	N
ThorCam	latest	Thorlabs	.exe	N
Fiji	Imagej	Imagej	.exe	N
Pylon	Pylon 6 or newer mp4 package	Pylon	.exe	N
MINI2P Distortion Cleaner	DistortionCleaner	Kavli NTNU	.exe	N
MINI2P SI device	Distortion app (plugin app)	Kavli NTNU		N

Guidelines

Step1. Start by installing MATLAB and LabVIEW that are needed to run the MINI2P control software ScanImage, widely used in laser scanning microscopies.

MATLAB: Install 2019b or a newer version. This requires a MATLAB license.

LabVIEW: Install 2020 SP1 including the packages below. NI-DAQmx

- NI-VISA
- PXI Platform Service
- FlexRIO for Modular I/O
- FlexRIO for Integrated I/O
- NI-IMAQ
- NI-IMAQdx

Please deselect all additional items.

Note! For more information on the NI-IMAQ and NI-IMAQdx versions, please check the supported versions with the operating system provided by National Instruments in the link:

https://www.ni.com/en-no/support/documentation/compatibility/17/vision-acquisition-software--vas--ni-imaq-ni-imaqdx-ni-imaqi-o-a.html

Step2. Download and install the imaging control software, ScanImage Basic-2021 or newer, available in your Vidrio account after purchase (see link in Table1). Install also RDI drivers and possibly update vDAQ Firmware. Follow more instructions provided in https://vidriotechnologies.com/drivers

Step3. Install the software that runs controller of the DC motors, an executable file **PISoftwareSuite** provided in a CD by Physik Instrumente.

Step4. Connect the controller (item 91). For this, run the controller PIMikroMove program. Then connect to the correct controller by choosing C-884 in the USB tab. Click connect.

Step5. The next step is to reference the three DC motors (item 82). For that, identify the three linear stages M-112.2DG (item 82) as motors 1 to 3 (physically confirm they connected with that attribution in the back panel of the controller). Click **reference** and wait for the motors to move all the way, away from the motor's cable. Finally click **done**, and when closing, save the settings to avoid having to reference the DC motors again.

Caution! Before referencing the motors, confirm there is no object on the way that can crash against. Otherwise, click **stop** while referencing.

Step6. Installation and connection of laser control software, FemtoFiber Ultra 920 (item 29 & 93)

The laser can be connected to the workstation via USB. To install the USB drivers, follow point 5.2 of **FemtoFiber Ultra 920 manual**. To install the laser control software, TOPAS FemtoFiber Ultra, follow point 5.3 of the same manual. Finally, to connect for the first time, follow the manual's point 5.4.

Step7. Install the autocorrelator software that measures the pulse width, PulseLink Check by running the executable file provided by APE (See link on Table 1)

Step8. Installation of the light detectors software, Photomultiplier Tubes (PMTs), PMT2100. Read **Chapter 6** of **PMT2100 Series Photomultiplier Tubes User Guide.** For installation of the detector software PMT2100, follow steps 6.1 to 6.2, possibly 6.3.

Step9. Start-up the detector software PMT2100 and for both detectors, PMT1 and PMT2, type the respective serial numbers that are printed on the side of the detector, and click OK.

Step10. Install the software (ThorCam, see link in Table 1) of the camera used to monitor the miniscope while moving its motorized scope holder (item 79). Read the user manual, more specifically chapter 3 and follow its installation guidelines.

Step11. Copy the folder SI settings provided in GitHub MINI2P/toolbox to your local drive so you can fetch it later when running ScanImage via MATLAB (see step 14).

Step12. To finish the installation part, also install the remaining software easily by downloading and running the .exe files (See links in Table 1)

- Fiji for reading and processing data in format of .tiff
- Pylon, the software that communicates with the Basler camera (item XX) for animal tracking. Install also the .mp4 package to save the videos in that format.
- MINI2P Distortion Cleaner that runs in MATLAB to find Transformation Matrices and calculate FOV (see more in Protocol P5 – Standard Testing Protocol)

Part2: Instructions to start-up ScanImage

More information and details can be found in:
https://docs.scanimage.org/Configuration/index.html

Step13. Run the MATLAB version installed. To the MATLAB directory, click SET PATH to add the necessary packages. Firstly, the ScanImage folder which can typically be found in the computer hardrive, as C:\Program Files\Vidrio, and MATLAB toolbox folder.

Step14. Start the imaging control software, ScanImage.
Type 'scanimage' on MATLAB command window. A ScanImage dialogue window will pop-up. Upload your license or login and select it.
Then, also select a Machine Data File (MDF) and User Configuration file. Each file is provided in Github: MINI2P toolbox/Software/SI settings. Click "Start ScanImage" (See Fig.1-top).

Step15. Wait for SI to load. In the meantime, minimize or move MATLAB/SI window to be able to visualize a display that enable the DC motors connector. Click on the USB tab, select C-884 +SerialNumber > Click OK (See Fig.1-bottom).

Step16. If loaded correctly, the display should look like Fig2.

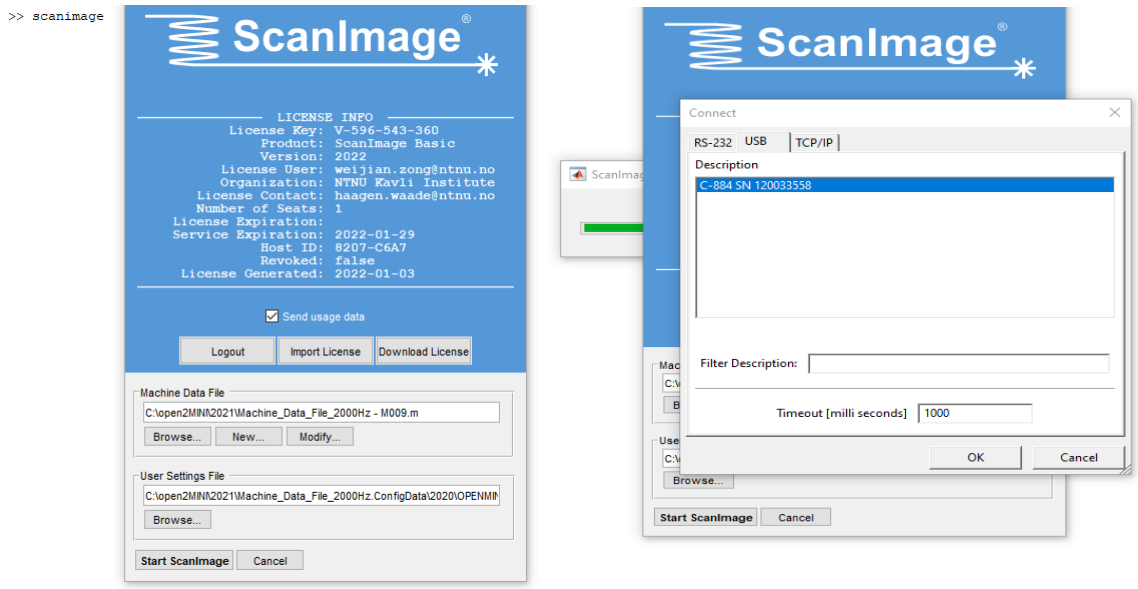

Figure 1: ScanImage starting dialogue windows with MATLAB command window on the background.

<u>Test Laser and Calibration of Laser Look-Up-Table (LUT)</u>

To change laser voltage in the following steps, click View> vDAQ Testpanel. Modify the voltage of the Analog Output channel that is connected to the laser by introducing its voltage, and clicking Set.

Step1. Run APE Pulse Link when the laser is aligned to the Autocorrelator (item q). Compare the Pulse Width @ 5V to the technical report provided by Toptica. Also measure the output power with power meter which has a suitable range (> 1.2W)

Step2. Update the laser LUT given in AOM.beamlut file. That Table has a set of datapoints [laser power/ P_max@2V, voltage], with values ranging from 0 to 1.0 and voltage from 0 to 2.0, in steps of 0.2, as:
[0, 0;
0.015, 0.2;
[0.054, 0.4;
…
[1.0, 2.0].

The "power" here varies between 0 and 1 because its value is divided normalized by the maximum measured power at 2V. For example, 159mW @2V, then the last datapoint of the table is [1.0, 2.0]. Nevertheless, measure the power after the miniscope's objective with the power meter's sensor (item g) and repeat this for all datapoints.

Step3. Set up the PMT2100 offset (see point 6.5 of Manual).

Part3: Start up Imaging & Recording

Step4. Mount the miniscope by screwing a M2.5 onto the Scope Holder (item 75)

Step5. Open the control software to the camera ThorCam software and click > Capture (camera software to monitor/visualize) purpose

Step6. Start the laser control software TOPAS, and click Connect. Turn on Laser and wait until Power stabilizes at ~ 1200mW (a couple of minutes)

Step7. Open PMT2100 software, check if Serial Numbers match the PMTs in the OPEN2MINI system, then click ok.

Caution! Never start the PMT (meaning click play, increase gain) when the lights of the laboratory are on.

Step8. Repeat step 11 to start ScanImage.

Note! It typically shows the previous MDF and User settings used, so if this is not the first time, then confirm this is correct and click Start.

Note! A window overview should appear as in Fig. 3 of Overview Documents (dependent on the user configuration selected)

Step9. Approach the miniscope to the target focus point yet guarantee the objective does not crash against anything on the way. While moving the scope holder down with Z-Motor, use the software opened in Step11 to better visualize the proximity to the object.

Step10. Click Focus on the Main Controls to visualize the Channel display of the FOV.

Step11. Move z until the image is in focus and optimize the (x,y) position.

Step12. On Main Controls, click Grab to record a FOV image. Before that, do not forget to check Save (see Fig.2)

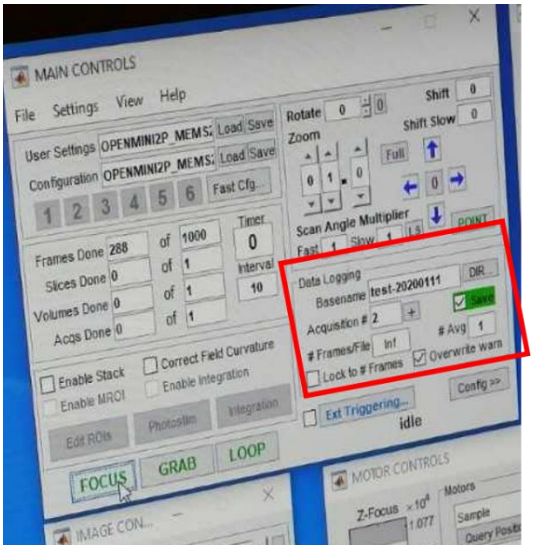

Figure2: Datalogging on Main Controls of Scan Image.

Step13. After finishing recording, run Fiji and drag the .tiff to check just-saved data

Preliminary tests

There are two main phases to go through before acquiring calcium imaging data, so that the MINI2P system is ready for final usage.

First, a quick check of FOV. One can optimize collimator position (and TFB/GFB) to maximize uniformity of the light, mostly in the center and edges. Secondly, laser calibration as shown in Step2. It is also advised to optimize the MEMS fast and slow axis using MDF files, but do not go above the maximum allowed voltage.

Now the user is ready to initiate the Standard Performance tests provided in the next protocol.

Quick Check to see if the miniscope is working

Step1. Prepare a Fluorescent sample: Cover a Lens Cleaning Tissue (item j) with Fluorescent Marker, painting uniformly thin rows. Place it beneath the miniscope.

Step2. Repeat Steps 19-24 until an image like in Fig.3 is visualized

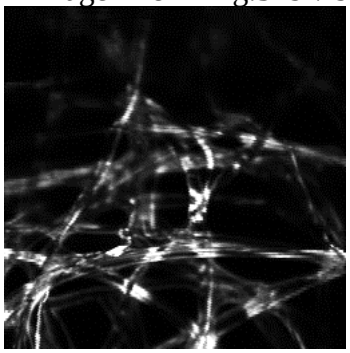

Figure3: Image of Fluorescent sample to efficiently test a miniscope before, for instance, in -vivo imaging.