Protocols Overview

Protocol S1 – Shopping and Machining list of MINI2P system

Protocol S1 includes all the components' key features such as the supplier, the product name, the model (or item reference) and its approximate price in Euro. Products whose price is marked as Self-made* are manufactured in a workshop which is not considered here.
Documents of the drawings (2D and 3D), available on folder Hardware, can be accessed to obtain more specifications of the components and a link to the supplier and/or directly to shop the item.

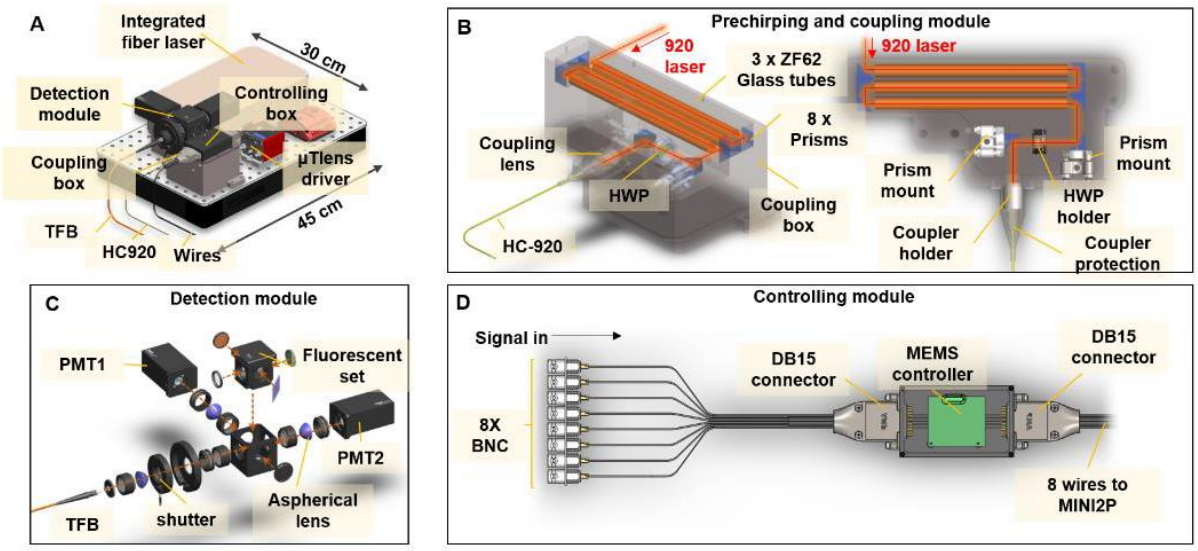
Protocol S2- MINI2P System building protocol

Protocol S2 includes all the procedures with detailed steps to assemble the three main modules of the MINI2P platform: I) Core Optics Assembly; II) Scope-mounting Assembly; III) Mobile Cart Assembly. Each Protocol starts with a short-list of main reagents and tools needed, and an overview schematic of the module, followed by a table with the main products. Finally, orderly steps are described to explain how to build the modules and in parallel, schematics are shown to better illustrate them.

The MINI2P system is divided in three modules, as illustrated in Fig. S15:

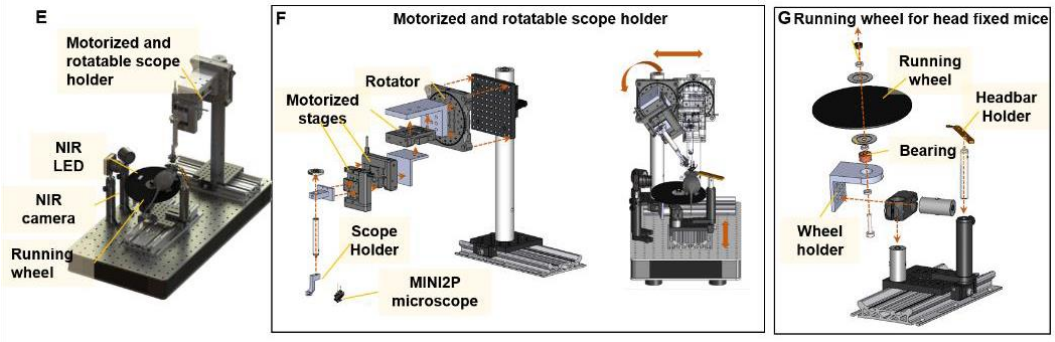
I. Core optics assembly Fig. S7 (A-D)

Core optics assembly (A) is the module where the short pulse laser light travels from the integrated fiber laser source, through a prechirping and coupling module (C), to the hollow-core photonic-crystal fiber (HC-920), and where the fluorescence from the tapered fiber bundle (TFB) is coupled to the detection module (B). The MEMS scanner and the µTlens are controlled by the controlling module (D).
A complementary tutorial video can also be found on the link.



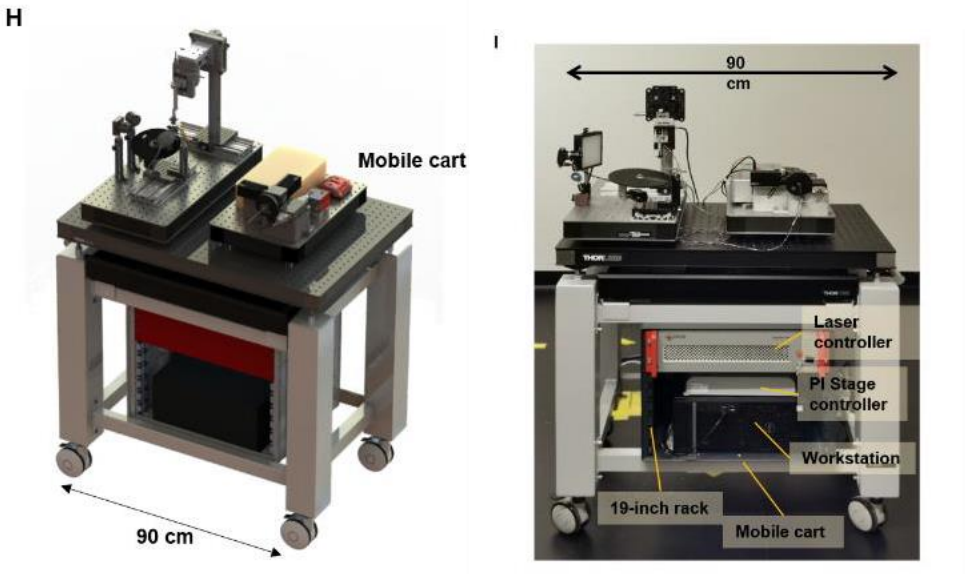
II. Scope-mounting assembly Fig. S7 (E-G)

Scope-mounting assembly (overview in E) incorporates both motorized and rotatable scope holder (F), head-fixed running wheel (G) and finally, illumination (NIR LED) and recording (NIR camera) components.



III. Mobile cart assembly Fig. S7 (H,I)

This module comprises a mobile cart (H) with a rack where all functional components are integrated (I) (workstation, laser controller, motors driver, and modules I and II, as shown in Fig. 1 below).



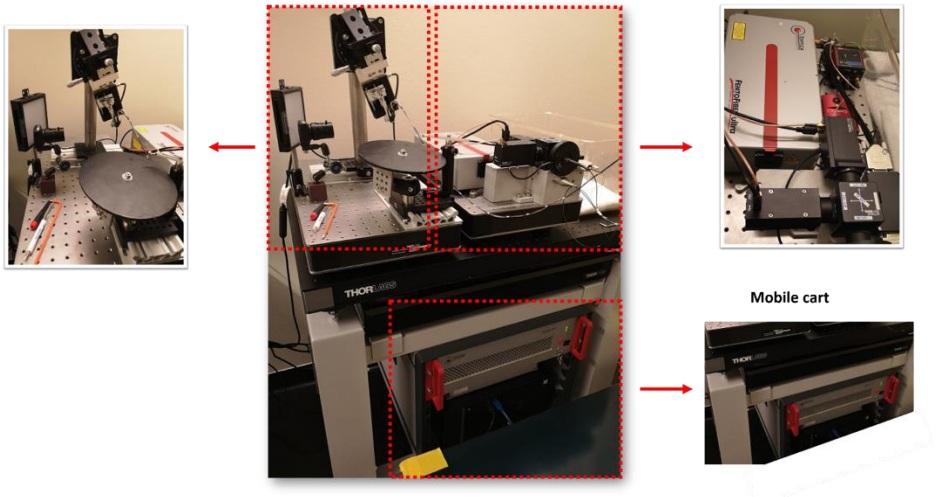


Figure 1 Overview of Mobile Cart Module with the two other integrated modules: Core Optics and Scope-mounting assemblies.

Protocol S3 – MINI2P assembly protocol

The most important part of the system is a miniaturized two-photon microscope (MINI2P). Short 920nm wavelength pulses are collimated at the entrance of Mini2P, before the laser light passes through a stack of tunable lenses (together termed the µTlens) which facilitate z-scanning. Next, the light hits a microelectromechanical System (MEMS) scanner that sweeps the (x,y) plane followed by the scan lens which translates the optical deflection angle of light reflected from the MEMS to a positional change. The light is reflected by a dichroic mirror, and focused onto the sample or object plane after passing through an objective. Fluorescent light emitted by the object passes through the mirror, and is focused into an image plane, being collected by a tapered fiber bundle (TFB) to the Detection Module of Core Optics. How to assemble these MINI2P optical components is described in this section.

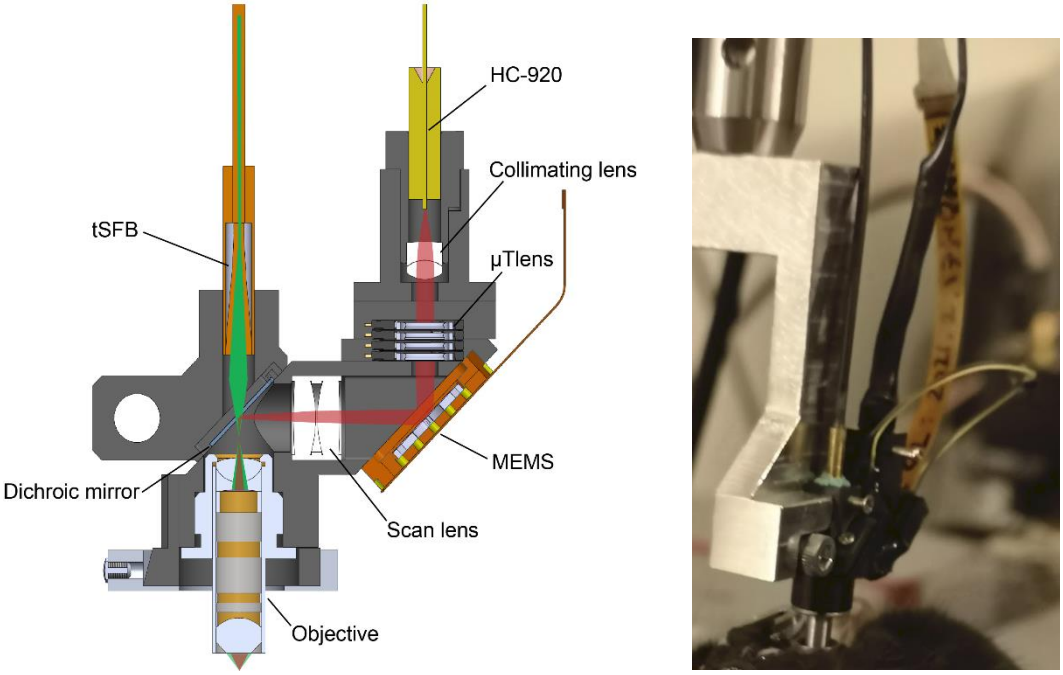


*Figure 2: Schematic of visible parts of **MINI2P** taken from Fig.2A(i) (left) and a photograph of the miniscope attached to the Scope Mounting module (right).*

Protocol S3 will be provided soon, but an assembly tutorial video can be found on the link: https://youtu.be/I0aYfi8GrIc

Title: **MINI2P Assembly Tutorial**
Description: A step-by-step video guide showing how we build MINI2P and important aspects to keep in mind

Protocol S4 – MINI2P system operation manual

This section includes software installation guidance, as well as a description on how to start and/or prepare for imaging. The first part, (i), includes a list of software and packages (with links and/or other procedures) to install. The second part, (ii) comprises instructions to start-up ScanImage, and other software necessary to visualize the miniscope approaching the target (using the camera application), as well as two-photon excitation source (laser PC-GUI) and recording (detector software). Finally, part (iii) comprises the steps to perform imaging and animal tracking, and some more advanced definitions with multiple scanning and stacking capabilities of ScanImage.

Protocol S5 will be provided soon.

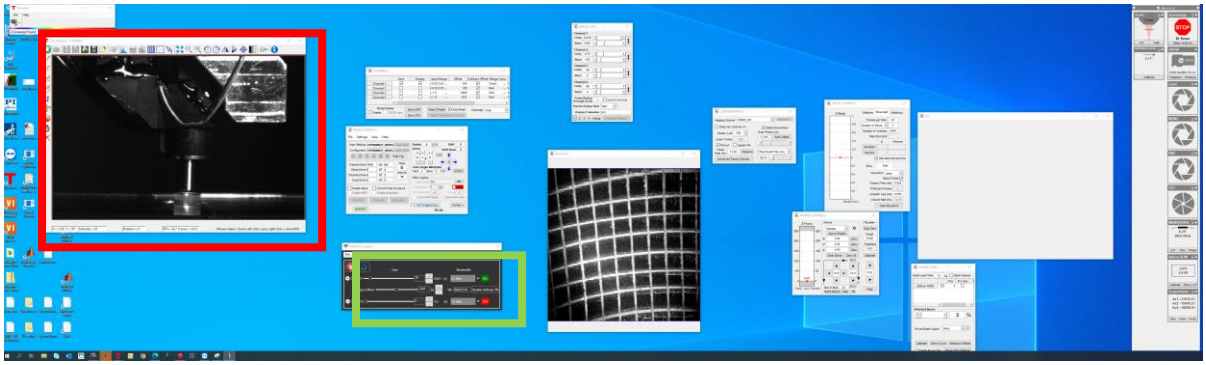


Figure 3 Overview desktop of MINI2P Calcium Imaging setup: ScanImage main controls at the right, Thorlabs software to operate the PMTs (green frame) and the monitoring Zelux Camera (red frame) at the top left to visualize the miniscope approaching a sample.

Protocol S5 – Performance Tests & Standard Testing Protocol

This section illustrates how to characterize and calibrate both the MINI2P device and system. Firstly the user can follow a standard testing protocol to estimate the Field-of-View (FOV), to generate a Transformation Matrix that corrects for distortion, and finally to estimate the resolution. Moreover, a method to calibrate the µTLens is also shown.
Secondly, there is an example of a system report (in the form of Table) with all the important parameters regarding both the miniscope and MINI2P system. The purpose here is to quantify the minimum acceptable thresholds to guaranty a high control platform.

Protocol S5 will be provided soon.