Innovation in Neural Interface Technology

OPERATING INSTRUCTIONS FOR THE ELECTROPHYSIOLOGY MODULE USING THE IntanRHX SOFTWARE

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Last updated 31 October 2023

1 Purpose of this Document and Disclaimer

The purpose of this document is to provide operating instructions for BMSEED's Electrophysiology Module to guide the user through sample preparation, equipment setup, impedance measurements, and recording and stimulation of electrophysiological activity. The module can be used with standard microelectrode arrays (MEAs) made on glass substrates and with stretchable microelectrode arrays (sMEAs) made on soft, elastomeric silicone substrates. BMSEED is using electronic components from Intan Technologies in the electrophysiology module. Further details about the electronics and operating that are beyond the scope of this manual can be found online at https://intantech.com/files/Intan_RHX_user_guide.pdf.

2 User Requirement, Safety, Liability and Warranty

2.1 User Requirements

Only trained and qualified users should be allowed to work with the electrophysiology module. BMSEED LLC does not guarantee for the accuracy of the measured data. A qualified user familiar with the operation of the electrophysiology module must assess the quality and accuracy of the obtained data using standard laboratory protocols.

Qualified users are those that:
- are familiar with the laboratory and work safety requirements to operate the module,
- have the professional knowledge and skills to use the module, and
- have read this document prior to using the module.

2.2 Safety

Always follow OSHA rules when working with the product.
Always follow the laboratory and other safety regulations issued by the state, municipality, and organization where the product is used.

For best results, always follow good laboratory practices.
Do not use the product or its software for medical applications or on humans.

2.3 Liability and Warranty

The Sales Agreement between BMSEED LLC and the customer will be provided upon delivery of the system.
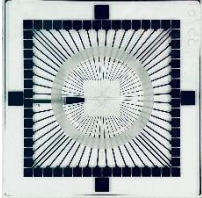
BMSEED's liability and warranty in the event of damage to the product or injury of personnel is excluded when the product is (i) operated outside its operating regime, (ii) incorrectly operated by the user, or (iii) tampered with in any way. Examples of such liability and warranty exclusions are, but not limited to,
- incorrect and inappropriate use of the product,
- operating the product when safety and protective features are defective,
- improper operation of the product,
- unauthorized or inappropriate alteration or repair of the product, and
- water or other physical damages that occur after the product was delivered.

3   Materials and Equipment

Make sure that the shipping box provided by BMSEED contains the items listed in Table 1 below.

Table 1: List of components for the electrophysiology module.

Component	Image
Stimulation/Recording Controller with power cable and USB cable	
1-4 headstages	
1-4 SPI cables	
Interface board	
sMEAs or glass MEAs (depending if and how many you ordered)	stretchable MEA (sMEA) standard glass MEA
EMI reduction enclosure* (Faraday Cage)	

Optional

If you ordered the expansion set to 120 channels, the shipping box should contain a total of four headstages and four SPI cables.

4   IntanRHX Software Download

1) Before setting up the hardware, download the IntanRHX software at
   https://intantech.com/downloads.html?tabSelect=Software

2) Scroll down until you see the "RHX software" bullet.
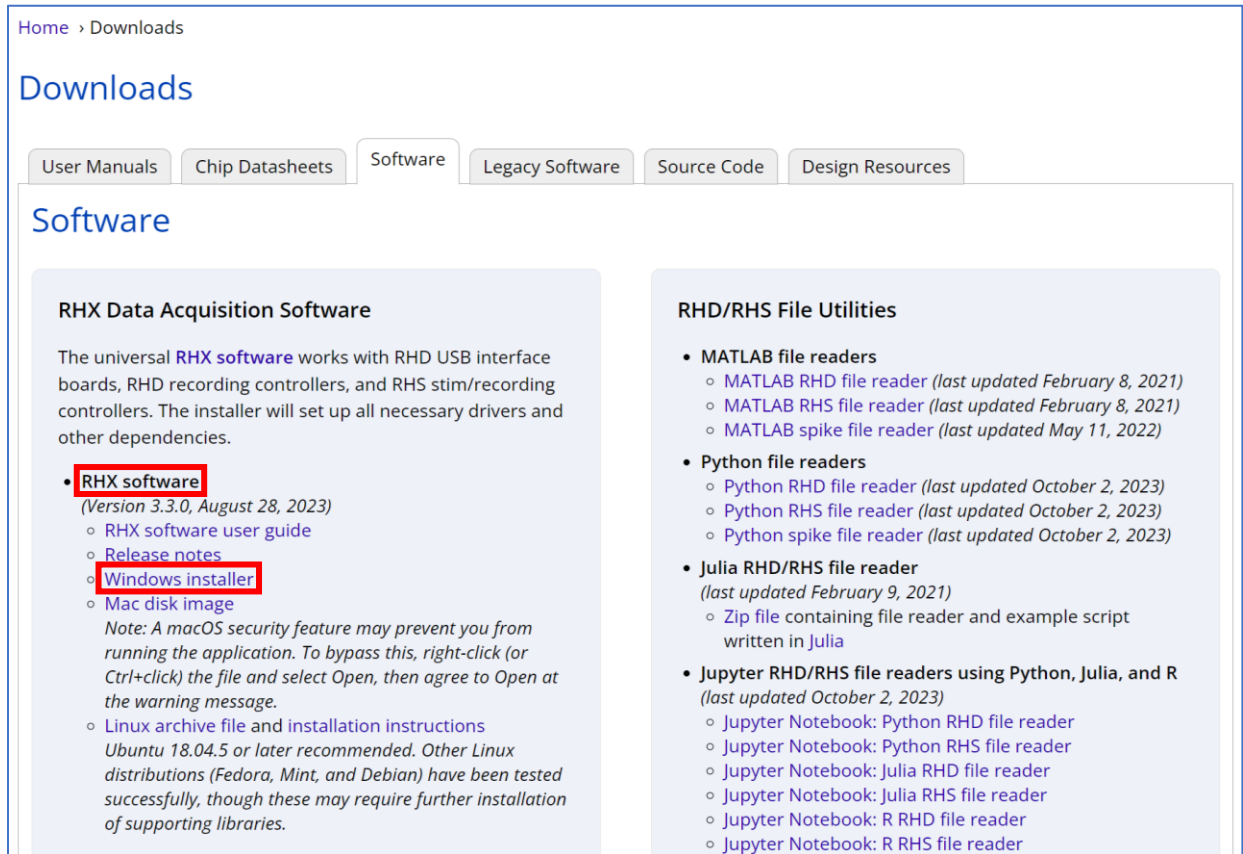
Fig. 1: Intan website screenshot (taken on 10/17/23).

3) Click on "Windows installer" or "Mac disk image" depending on your operating system.
   Double-click the installer file and follow the prompts on the Intan RHX Setup installer.
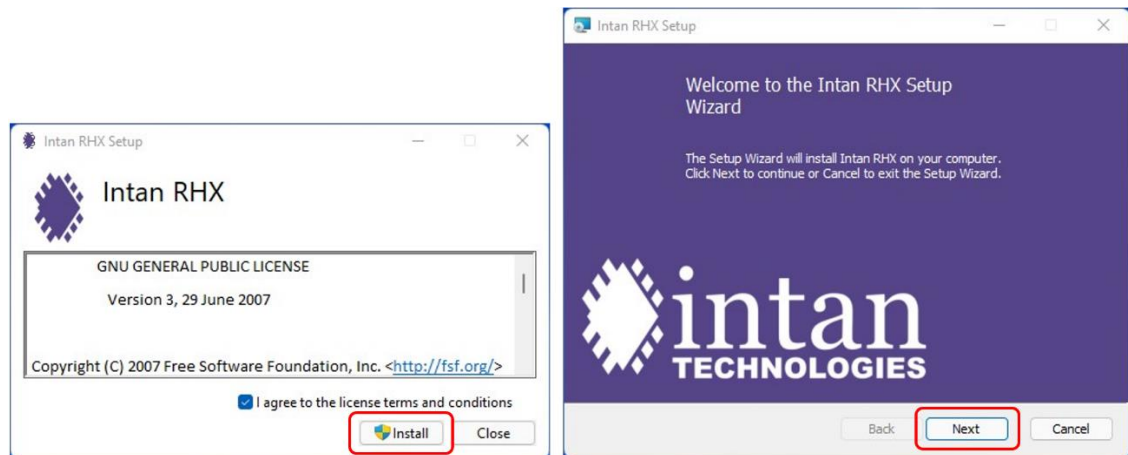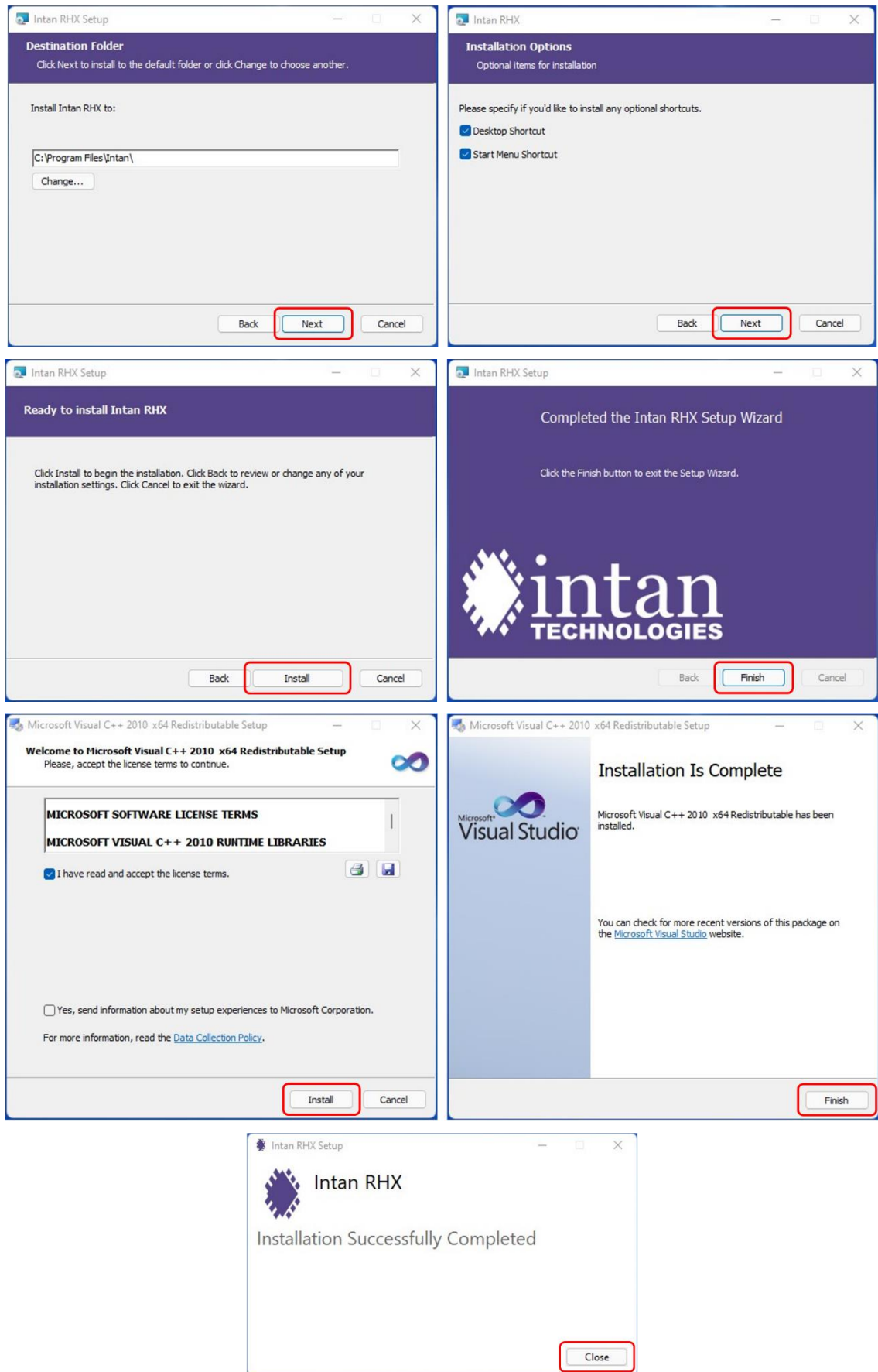
Fig. 2: Intan RHX Setup prompt windows (for Windows Installer).

4) You can now run the software or test the software in demo mode.

5 Sample preparation

5.1 Scope

This document provides a procedure to culture hippocampal tissue slices on BMSEED's stretchable microelectrode arrays (sMEAs) and stretchwells (no electrodes). The procedure is intended as a starting point for users and likely needs to be adjusted to accommodate the specific conditions and tools available in your lab. We recommend using stretchwells for the optimization of your culturing protocol because they are much cheaper than sMEAs, and a protocol optimized for stretchwells will work for sMEAs as well because the form factor and the substrate are the same.

DISCLAIMER: BMSEED does not guarantee that the information contained in this document is correct, nor does BMSEED provide a warranty of any kind for sMEAs or stretchwells. BMSEED recommends consulting the relevant literature before conducting experiments.

5.2 General recommendations

Both, standard microelectrode arrays (MEAs) on glass substrates as well as stretchable microelectrode arrays (sMEAs) need to be plasma treated prior to use. This is particularly true for sMEAs because the PDMS is hydrophobic. Plasma oxidation turns the PDMS surface from hydrophobic to hydrophilic.

Without plasma treatment, the recording sites of the microelectrodes are usually covered with air pockets after filling the well with the electrolyte solution (**Fig. 3a**). Even with plasma treatment of sMEAs, these air bubbles sometimes happen. These bubbles need to be removed (**Fig. 3c**) before electrophysiological measurements because they prevent the electrolyte solution from contacting the recording sites of the electrodes.

Additionally, to improve the wetting of the surface of the sMEA, SodiumDodecylSulfate (SDS) can be added to the PBS for a concentration of 40mM.

<div align="center">(a)   (b)   (c)</div>
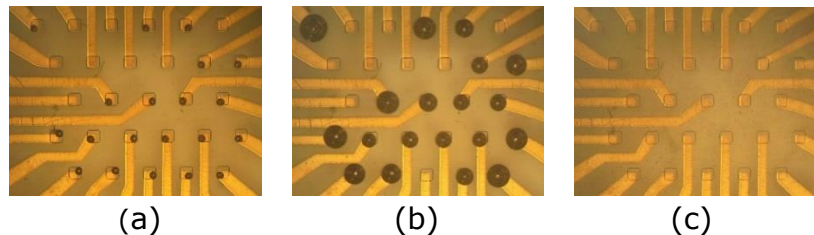
Fig. 3: Recording sites of a gen4e sMEA **(a)** with air bubbles on most contact holes after adding PBS, **(b)** air bubbles coalescing after pulling vacuum for 10min, and **(c)** after pulling vacuum for an additional 10min or blowing away the large bubbles using a pipette.

Instructions:

1) Place the sMEA or MEA in a plasma tool and expose it for 30-60s at level 1 (or low) to air plasma.

2) Add PBS or culture medium to the culture well (**Fig. 3a**).

3) Place sMEA in a desiccator and apply vacuum for 10 min. The bubbles tend to coalesce into one large or a few bubbles (**Fig. 3b**) which can be blown away with a pipette (**Fig. 3c**).

⚠️ Never touch the surface of the electrodes on MEAs or sMEAs to avoid damaging them.

5.3 Stretchable microelectrode array (sMEA)

BMSEED's sMEAs are made of a thin gold film that is sandwiched between two layers of the biocompatible silicone poly(dimethylsiloxane) (PDMS). The bottom PDMS layer is the substrate on which the gold film is deposited. The top PDMS layer is the encapsulation, which contains contact holes to the recording sites in the center of the sMEA and to the data acquisition system at the periphery. The PDMS bilayer with the gold film is compression bonded between two printed circuit boards (PCBs) with a circular hole in the center. The **gen4 sMEA** configurations have 28 recording electrodes and 2-4 reference electrodes (**Fig. 4a-c**), with square rec. sites that are $100{\times}100$ μm^2 and spaced 400 μm apart center-to-center. The **gen6 sMEA** configuration has 56 recording electrodes and 4 reference electrodes (**Fig. 4d**), with round recording sites that are 70 μm in diameter spaced 370 μm apart center-to-center.

(a)   (b)   (c)   (d)
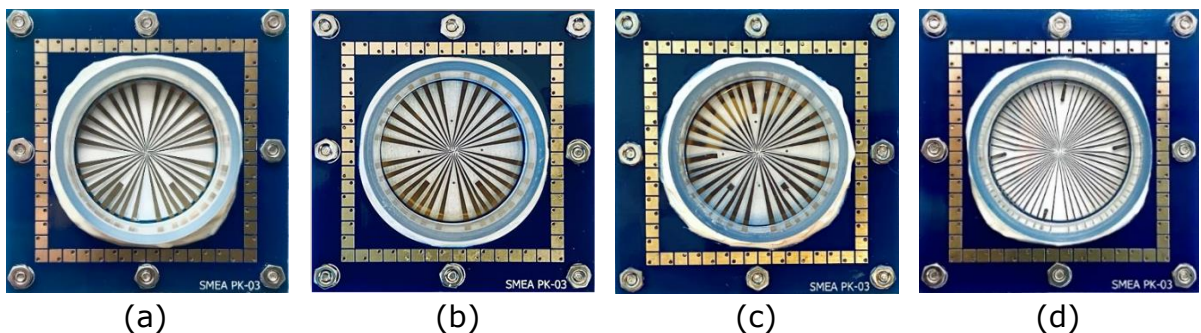
Fig. 4: Images of a **(a)** gen4a sMEA, **(b)** gen4d sMEA, **(c)** gen4e sMEA, and **(d)** gen6 sMEA.

In most cases, sMEAs will be used to stretch cells for neurotrauma or tissue engineering applications. sMEAs might also be used to take advantage of the softness of the PDMS compared to rigid MEAs on glass substrates. To stretch cells, they need to adhere well to the underlying PDMS layer with the electrodes. Below we provide a protocol for seeding organotypic hippocampal tissue slices and cardiomyocytes on an sMEA. Other cell or tissue types might require slight variations of the cell seeding protocol provided here.

sMEAs can be re-used 5-15 times depending on the cells and tissue cultures as well as the sterilization and cleaning procedure used.

5.3.1 Sample protocol for seeding organotypic hippocampal tissue slices

Materials Needed:
- Autoclaved, sterile water
- Sterile poly-L-lysine (320 µg/mL)/natural mouse laminin (80 µg/mL) solution
- Neurobasal-A media (with B-27 supplement, 1mM glutamine, 5 mg/mL D-glucose)
- 100 × 20 mm Petri dishes (1 per sMEA)
- Air or Oxygen plasma source
- UV light source
- Incubator with rocker

sMEA Pre-Sterilization (2 days):
1) Soak sMEAs in sterile, autoclaved water for 2 days.
2) Aspirate the excess water and dry the sMEAs in a vacuum oven, incubator or on a benchtop overnight at 37°C.

sMEA Sterilization (2 days):

1) To maintain sterility at all times, all sterilization procedures are performed in a biosafety cabinet.
2) The sMEAs (in Petri dishes) are sterilized by exposing them to UV light for 1-2 hours on each side (if the petri dish is plastic, remove the cover since UV light does not penetrate well).
3) The sMEAs are treated with low vacuum plasma for 0.5-3 minutes each (the exact time depends on the power of the plasma source) to turn the PDMS surface hydrophilic; this is needed for the coating (see below) in to be effective; plasma tools from Harrick, Plasma Etch, or Plasma Technology are suitable for this application.
4) Add sterile water to the well of the sMEA immediately after plasma treatment for 2-3 hours.
    - The silicone substrate of the sMEAs (polydimethylsiloxane, PDMS) turns hydrophobic again over time, and it is therefore important to minimize the time between plasma treatment and water soak.
    - This step is very important, do **NOT** skip it!

⚠️ Sterilization in an autoclave is possible, but not recommended because the heat softens the glue that holds bonds the culture well to the printed circuit boards, which can result in leakage.

⚠️ Do NOT use alcohol to sterilize sMEAs because the alcohol gets partially absorbed into the PDMS substrate and is difficult to remove, thus affecting the health of the cells.

sMEA Preparation (2 Days):

1) Immediately after gas plasma treatment, coat each sMEA with sterile poly-L-lysine (320 µg/mL)/natural mouse laminin (80 µg/mL) solution (diluted in autoclaved water, 0.5 mL per SMEA).
2) Incubate the sMEAs flat overnight at 37°C.
3) Aspirate the coating solution, rinse with sterile, autoclaved water.
4) Add Neurobasal-A media (with B-27 supplement, 1mM glutamine, 5 mg/mL D-glucose) (1.0 mL per SMEA).
5) Incubate overnight at 37°C on rocker.

Tissue Preparation (on Day 5):

1) Prepare slices using your standard protocol (for more details see: Morrison et al, Journal of Neuroscience Methods, 2016, 150:192– 201).
2) Aspirate neurobasal media from three sMEAs.
3) Place one slice in each sMEA and put back in incubator; make sure that the slice rests on the recording sites of the electrodes.
4) Allow to incubate for 5 minutes to allow slice to stick to the membrane.
5) Add sterile water to the underside of the PDMS membrane of the sMEA to keep the osmolarity of the medium inside the well constant; the added water permeates through the PDMS and replenishes the water lost from the media by evaporation in the incubator, thus minimizing the effect on osmolarity.
6) Add neurobasal-A media (start with 0.5 mL but adjust as needed) and place on rocker in incubator. Make sure that the slice alternates between being submerged in media and exposed to air.

7) Replace about 1/3 of the media in the sMEA with fresh neurobasal-A media every day.

8) After about 5 days-in-vitro (DIV) in the incubator, the slices will adhere to the silicone substrate of the sMEA and are ready for stretching.

5.3.2 Sample protocol for seeding cardiomyocytes

Materials Needed:
- Sterile Deionized Water (DI water)
- Petri dishes
- Gelatin solution in DI water (0.1% wt/v)
- cardiac culture medium
- UV light source
- Air or Oxygen plasma source

sMEA Washing and Hydration (2 days):
1) Wash sMEAs with DI water 3 times under aseptic condition (inside biosafety cabinet hood).
2) Fill sMEAs with DI water and keep them for 2 days in closed petri dishes.

sMEA Sterilization (2-3 hr):
1) In petri dishes, the sMEAs are sterilized under UV light under aseptic condition (inside biosafety cabinet hood) for 1-2 hours on each side. Make sure to remove the petri dish's lid.
2) Treat sMEAs with gas plasma for 1 minute each (e.g., Harrick, Plasma Etch, or Plasma Technology).

sMEA Preparation (2-3 hr):
1) Coat each sMEA with sterile gelatin solution (0.1% wt/v, diluted in sDI water, 1 mL per sMEA) immediately after gas plasma treatment.
2) Incubate the sMEAs for at least 2 hr or maximum overnight 37°C. Place DI water containers (small petri dishes) inside the sMEA container to avoid dehydration of gelatin solution overnight.
3) Aspirate the coating solution gently from the edge of the sMEA well, rinse with DI water once gently.

Cardiomyocyte Seeding:
1) Plate the desired number of cardiomyocytes (here: 10^6 cells were plated) on the sMEAs immediately after aspirating and washing the gelatin coating.
2) Fill the sMEA well with 2 mL of cardiac culture medium and incubate at 37°C overnight.
3) Exchange the media every other day.

5.3.3 Removal of tissue slices or dissociated cells from sMEA

After use, the tissue slices or cells need to be removed from the sMEA in order to reuse it. It is recommended to use solution based enzymatic digestion methods whenever possible to digest or detach the tissue/cells from the PDMS membrane of the sMEA. Any physical force that is applied to remove the tissue/cells will reduce the number of times the sMEA can be reused. While BMSEED does not guarantee a certain number of uses of an sMEA, users should expect to be able to reuse sMEAs >10 times when the cells are fully digested (typically for dissociated cells), and 5-10 times for tissue slices.

5.3.4 Sample protocol for storing an sMEA with tissues on them

1) If recording from an sMEA, try to always keep the cap (or any type of cover) on the sMEA whenever the sMEA is outside the hood to minimize the chance of infection.
2) Replace the recording solution (e.g., artificial CSF) with culture media after an experiment.
3) Return the sMEA to the incubator.

5.3.5 Sample protocol for storing an sMEA in between different tissue slices

1) Once all experiments on a specific tissue have been completed, add trypsin for 30 minutes to remove the cells and keep the sMEA in the incubator.

2) Store the sMEA in autoclaved water.

3) When you are ready to reuse the sMEA again for culturing, remove the sMEA from water and let it dry.

4) Sterilize the sMEA:
   i) Treat the sMEA with ethylene oxide.
   ii) Plasma clean the sMEA.
   iii) Add autoclaved water to the well of the sMEA immediately after plasma treatment for 2 hours.

5) Prepare the sMEA:
   i) Add the coating solution (e.g., PLL, laminin) and leave overnight.
   ii) Add the culture media (e.g., neurobasal media) and leave overnight.

6) Place the new tissue slice or dissociated cells following your protocol.

5.4 Standard glass microelectrode array (MEA)

BMSEED's MEAs consist of TiN electrodes and contact pads on glass substrates. The leads are made of Ti, and the insulation is silicon nitride (SiN). The 60 electrodes (1 reference electrode) are arranged in an 8×8 grid with interelectrode spacing (center-to-center) of 200 μm and recording site diameters of 30 μm. Each of these electrodes can be used for recording and stimulation. The impedance of the electrodes is in most cases 200±100 kΩ at 1kHz, and the peak-to-peak recording noise is typically 10μV. The glass MEAs are fabricated at the NMI in Reutlingen, Germany (www.nmi.de). The glass MEAs can be reused up to 30 times, depending on cell type, the coating material, and the cleaning procedure.

⚠️ It is recommended to avoid exposing MEAs to basic solutions (pH>7) to avoid damaging the TiN electrodes. Rinse the MEA thoroughly with distilled water after cleaning.

⚠️ Do not use too high stimulating currents to avoid damaging the electrode.

5.4.1 Seeding tissue slices or cell cultures on the MEA

Glass MEAs have been in use for over two decades and many seeding protocols have been reported in the literature for different tissue/cell types. Here is a brief overview.

Materials Needed:
- Autoclaved Water

- 100 × 20 mm Petri dishes
- Autoclave
- Air or Oxygen plasma source (e.g., Harrick, Plasma Etch, or Plasma Technology)

MEA Pre-Sterilization:
1) soak MEAs in sterile, autoclaved water for 2 days
2) aspirate the excess water and dry the MEAs

MEA Sterilization:
The MEAs are sterilized by either one of the following methods:
- exposure to UV light for 1-2 hours on each side
- rinsing in 70% alcohol solution (no soaking for extended periods of time)
- vapor autoclavation (125°C for 15 min)
- dry heat sterilization (125°C for 15 min)

⚠️ It is recommended to store the MEAs in between use with DI water in the culture well. The surface will remain hydrophilic for longer than dry storage.

MEA Preparation:
1) The MEAs are gas plasma treated for 30 seconds.
2) Coating of the MEA to promote tissue/cell attachment to the surface with:
    - sterile poly-L-lysine and laminin solution
    - poly-D-lysine or Polyornithin in combination with laminin
    - Nitrocellulose solution
    - Fibronectin solution
    - Collagen solution
3) Incubate the MEAs flat overnight.
4) Aspirate the coating solution, rinse with sterile, autoclaved water.
5) If using laminin: incubate with laminin solution over night; then aspirate solution.
6) Seed the tissue/cells.

6   Equipment Setup

This section describes the experimental setup of BMSEED's electrophysiology module, i.e., what do you need to do before your cells are ready for recording/stimulation on the MEA or sMEA.

1) Place the Interface Board on the custom holder of the MEASSuRE system (**Fig. 5a**).
   <u>Note</u>: If you do not use MEASSuRE, place the Interface Board in a Faraday cage (**Fig. 5b**) or wrap with Aluminum foil (**Fig. 5c**).
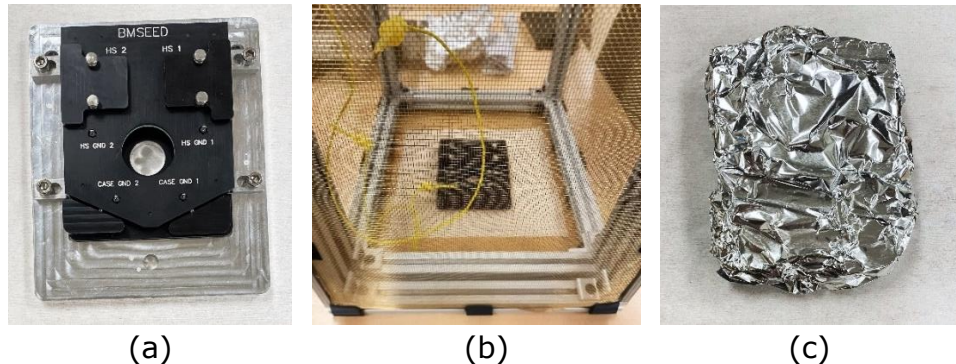

(a)   (b)   (c)

Fig. 5: Interface Board **(a)** on custom platform for MEASSuRE, **(b)** in a Faraday cage, or **(c)** wrapped with Aluminum foil.

2) Unscrew the protective shield of each headstage from the top of the Interface Board (two screws for each cover; **Fig. 6**).

(a)   (b)
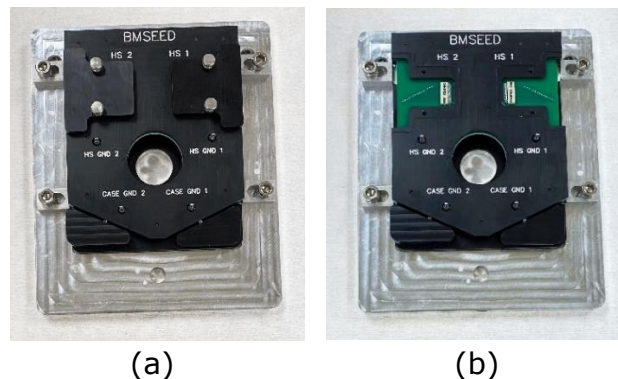
Fig. 6: Headstage covers **(a)** screwed into the Board and **(b)** unscrewed and removed from the Board.

3) Connect one of the red SPI cables to each headstage (**Fig. 7a**); you can connect either end of the SPI cable to the headstage.

4) Connect each of the two headstages to an Omnetics connector on the Interface Board (**Fig. 7b,c**).

(a)   (b)   (c)

Fig. 7: (a) One end of the SPI cable connected to one headstage, and **(b)** the headstage connected to the Omnetics connector in the Interface board; **(c)** both headstages and SPI cables connected to the Interface Board, labeled "HS1" and "HS2".

5) Screw the protective shield of each headstage back into the EIB (**Fig. 6a**), which will protect the headstages from mechanical damage or spillage of culture medium.
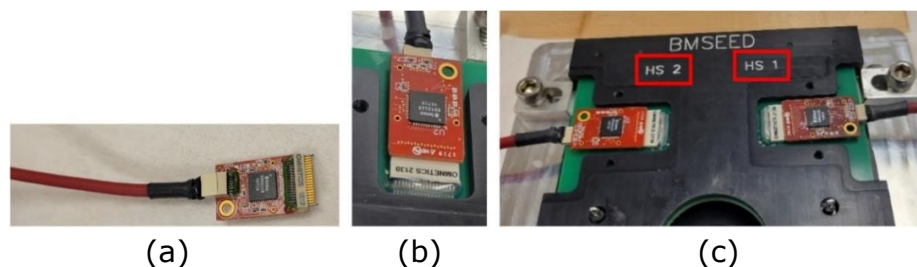
6) Connect the other end of the SPI cables (coming from the headstages on the Interface Board) to Port A and Port B on the controller (**Fig. 8**), or Ports C and D.

> It is important to connect the ports on the controller with the SPI cable from the CORRECT headstage (HS; **Fig. 7c**).
> Port **A** or **C**: connect the SPI cable from the headstage for **HS 1**
> Port **B** or **D**: connect the SPI cable from the headstage for **HS 2**

Fig. 8: Recording/stimulation controller with SPI cables connected to Port A and Port B.

7) Plug in the power cord on the Intan Stimulation/Recording Controller into the power outlet.

8) Connect the computer and the controller with the provided USB cable; the USB cable plugs into the slot on back panel of the controller.

9) Turn on the power in the back of the controller; a red LED should light up on the right front side of the front panel (**Fig. 8**, right), and all 4 ports should have a green and yellow light lit up (**Fig. 8**, left).

10) If you use a function generator, connect the "Output" BNC of the generator to the "Analog In 1" BNC contact on the front panel of the controller (**Fig. 9**).

Fig. 9: "Output" BNC of the function generator (left) connected to the "Analog In 1" BNC contact in the Intan controller (right).

11) Skip to step **15)** below if you use the Electrophysiology Module as a Stand-Alone data acquisition system, and you do not use the MEASSuRE platform.

12) To shield EMI from affecting the electrophysiological measurements, do the following:

   (i) Ground the imaging platform to the MEASSuRE frame to keep them both at the same potential (red cable in **Fig. 10** below). If the frame is anodized, connect to a non-anodized screw on the frame.
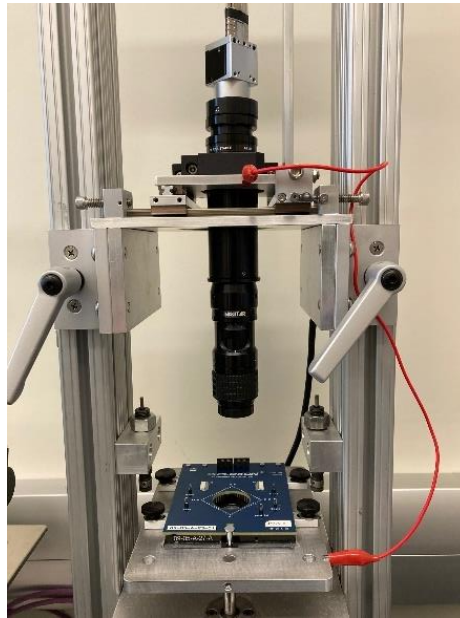
Fig. 10: Connecting the imaging stage to the MEASSuRE frame.

(ii) Connect the "chassis ground" on the rear panel of the Intan controller to the MEASSuRE frame (yellow cable in **Fig. 11** below). If the frame is anodized, connect to a non-anodized screw on the frame.

(iii) Connect the "I/O ground" on the rear panel of the Intan controller to one of the ground pins of the interface board (black cable in **Fig. 11** below).

Fig. 11: Connections of the I/O and chassis ground ports on the back of the Intan.

(iv) Ensure there is a ferrite choke on the USB cable connecting the Intan controller to the computer (red arrow in the left image in **Fig. 12** below).

(v) Place the EMI shielding cover (right image in **Fig. 12** below) with a sliding opening over the sample around the entire interface board.

Fig. 12: Ferrite choke on USB cable and EMI shielding cover for the interface board.

13) **Steps 13)(i)-(iii)** are **optional** and may help with the signal recording process.

   (i) Before each use, open the Interface Board and clean the bottom of the gold pins with ethanol using a Texwipe or similar wipe (**Fig. 13a**).

   (ii) Before each use, take the sMEA and clean the gold PCB pads of the sMEA with ethanol using a Texwipe or similar wipe (**Fig. 13b**).

   (iii) Before each use, use a pencil eraser as a gentle abrasive on the sMEA PCB pads after the ethanol has dried (**Fig. 13c**).
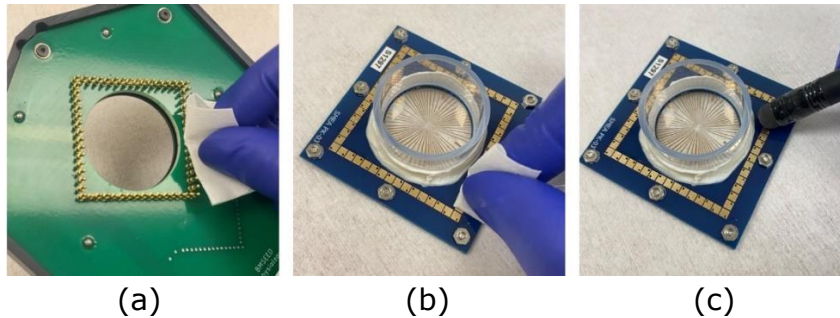

| (a) | (b) | (c) |

Fig. 13: Before each use, **(a)** the Interface Board pins are cleaned with ethanol, **(b)** sMEA PCB pads cleaned with ethanol and **(c)** gently abrased with a pencil eraser.

14) Open the Interface Board (**Fig. 14a**) and place the sMEA or MEA in the recess on the board (**Fig. 14**); it is important that the arrays are placed in the correct orientation so that the electrode label on the software matches the electrode label on the array.

   Note: For better contact with the EIB pogo pins, the sMEA or MEA can be "boosted" with a support PCB underneath (~0.5mm thick; **Fig. 14b**).
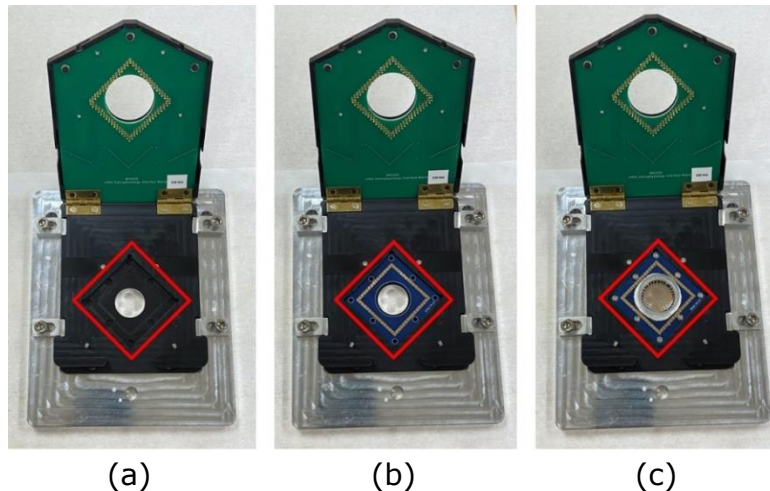

| (a) | (b) | (c) |

Fig. 14: Interface Board **(a)** opened with **(b)** support PCB placed in recess of board and **(c)** sMEA placed on top of support PCB in recess of board.

Make sure to **place the sMEA or MEA in the correct orientation** into the board:

   For **sMEAs**: Place sMEAs such that the edge with the white label "SMEA" is located along the edge with the engraved arrow. (**Fig. 15d,e**). In **gen4** sMEAs, the reference electrodes will contact pins on the board that are located on this side (**Fig. 15a**). In **gen6** sMEAs, there will be one reference electrode on each side, with the shortest located on this side (**Fig. 15b**).

   For **MEAs**: Place the MEA such that the edge with the large reference electrode

(Electrode #57) faces along the edge to the left of the engraved arrow (**Fig. 15c,f**).
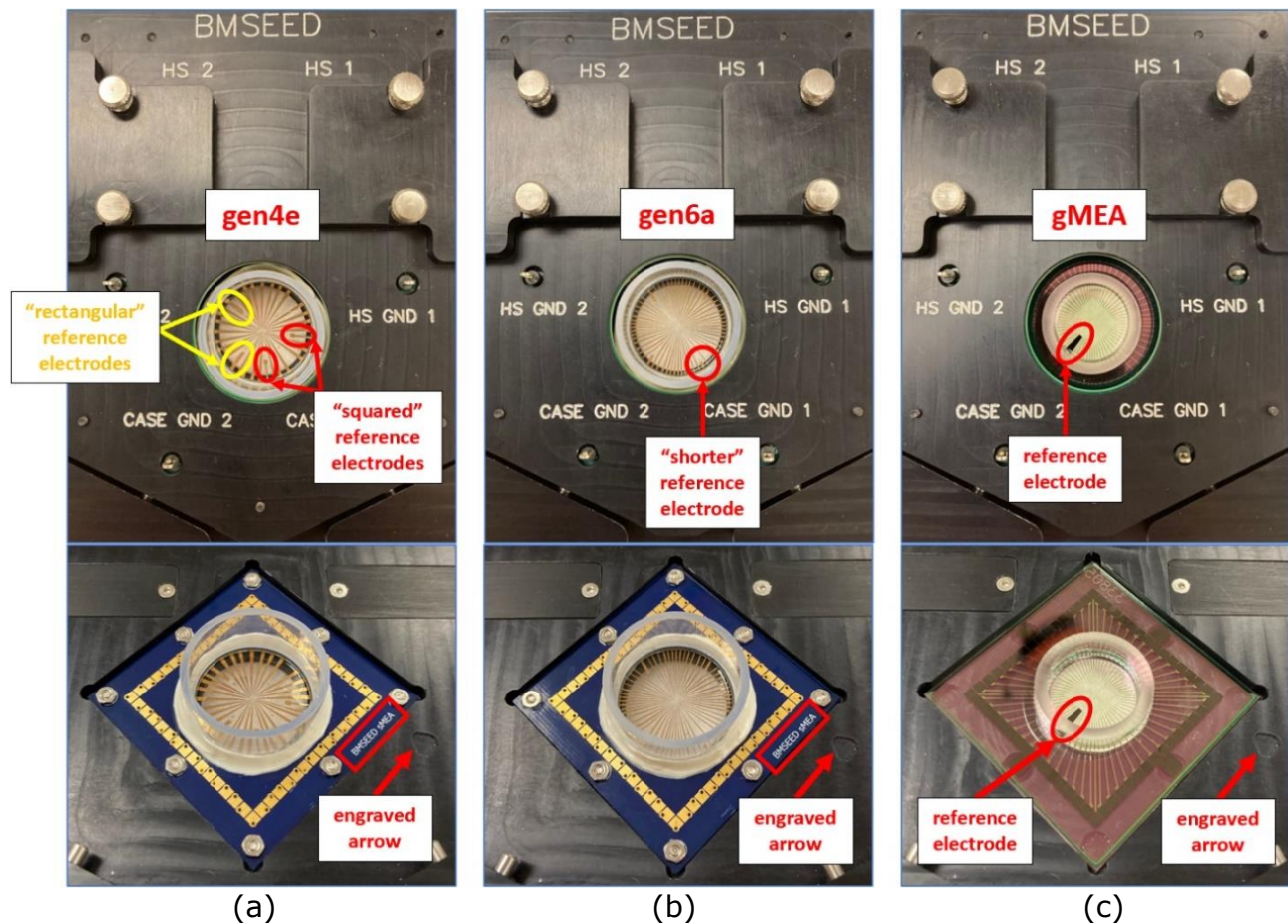

(a) (b) (c)

Fig. 15: The **(a) gen4e** sMEA, **(b) gen6a** sMEA, and **(c) glass MEA** loaded correctly for recording/stimulation. <u>Note</u>: **gen4d** is placed like gen4e, just without the two extra "rectangular" reference electrodes highlighted yellow.

15) Short one of the "HS GND" pins to one of the "CASE GND" pins.

16) Place a platinum reference electrode in the well of the sMEA or MEA, and connect the reference electrode to any of the four ground pins on the board (**Fig. 16**).
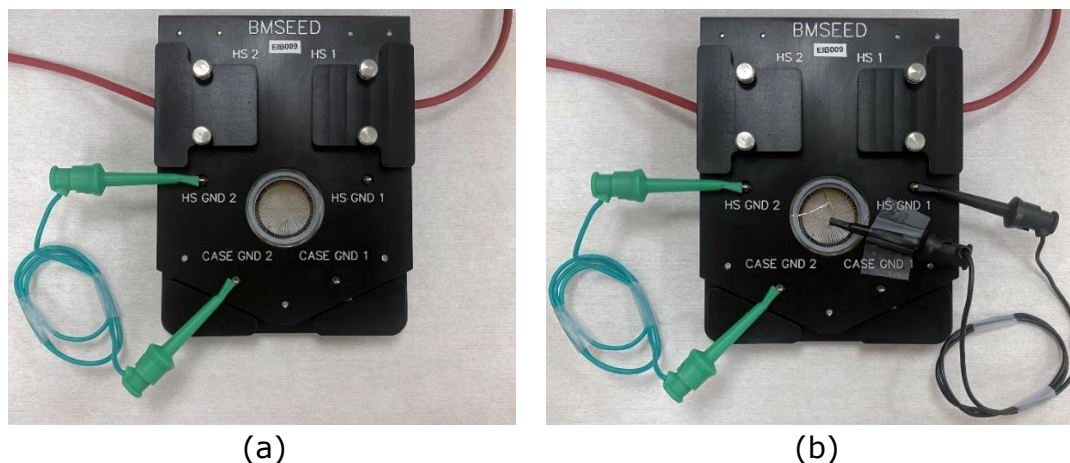

(a) (b)

Fig. 16: a) One of the "HS GND" pin shorted to one of the "CASE GND" pins and **b)** Platinum electrode placed inside of the culture well of the sMEA or MEA and connected to one of the ground pins on the Interface Board.

7   Software Setup

1)   Start the "IntanRHX" software by double-clicking the icon:

2)   When the software starts, USB ports are scanned and a list of connected Intan controllers is displayed. Select "RHS 128ch Stim/Recording Controller" and click "Open".
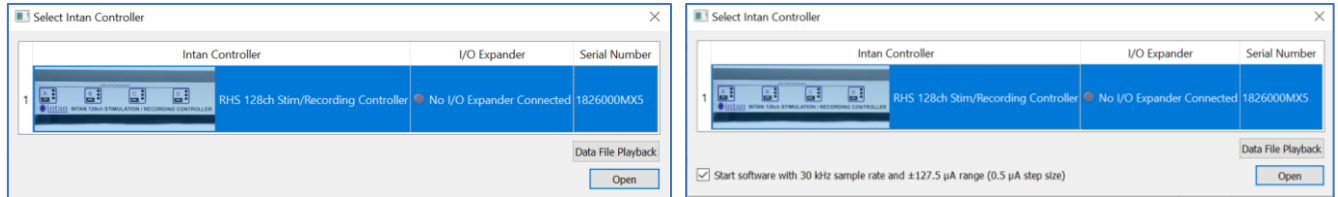
(a)   (b)

Fig. 17: Pop-up window asking user to select Intan controller with **(a)** unsaved stimulation settings or **(b)** stored stimulation settings from a previous run.

If no Intan controller is connected to the computer, the following window will popup allowing the user to select between one of three demos or the "Data File Playback" mode.
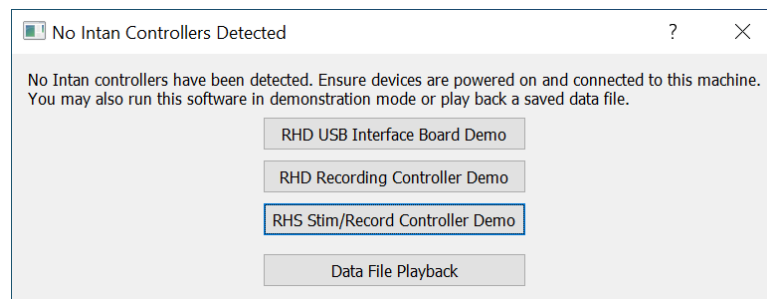

Fig. 18: Pop-up window if no controller is connected.

When using the stim/recording controller (either one connected to the computer or in demo mode), the user can set the sampling rate current stimulation range and step size in the "Select Sample Rate" window (**Fig. 19**). To access this window, do the following in the "Select Intan Controller" window or "No Intan Controllers Detected" window:

- If a connected controller is available but no sample rate and stimulation range from a previous run has been stored, click the "Open" button (**Fig. 17a**).
- If a connected controller is available and a sample rate and stimulation range has been stored from a previous run, uncheck the box "Start software with # kHz sample rate and ±# µA range (# µA step size)" and then click "Open" (**Fig. 17b**).
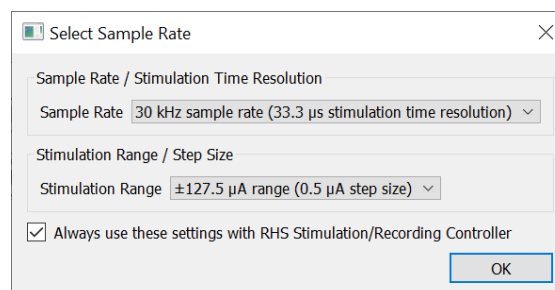- If no connected controller, select "RHS Stim/Record Controller Demo" (**Fig. 18**).

Fig. 19: Pop-up window to select the sample rate and stimulation range.

Once these two parameters have been specified, click "OK."

3) The software will load, and you will see the main IntanRHX software window (**Fig**. **20**).

Fig. 20: IntanRHX software loading banner.

4) Feature overview of Intan software (from Intan manual)

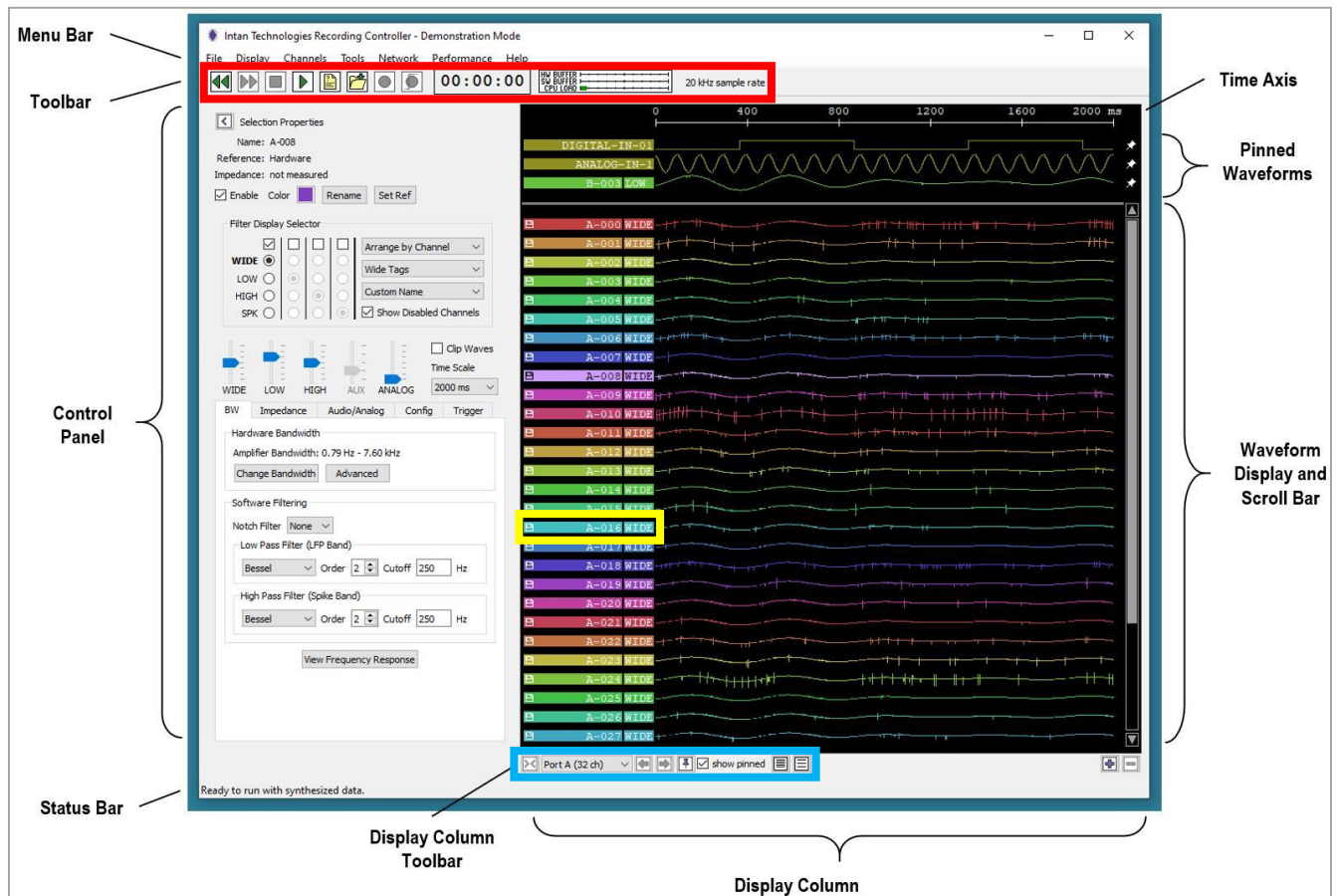The main features of the RHX window are shown below.

Fig. 21: IntanRHX software main window.

We explain below the main features of the different sections of the GUI. More details about the use of these features are discussed in the relevant sections further down in the manual.

Main toolbar (red box):
The contents of the Toolbar are shown below for normal data acquisition mode (**Fig. 22**). The **Run** and **Stop** buttons start and stop data acquisition. After some data has been acquired and the system has been stopped, the **Rewind** and **Fast Forward** buttons can be used to review the last 30 seconds of data on the display, which are stored in RAM. The **Record** and **Triggered Recording** buttons start data acquisition and save the results to disk, but these buttons are disabled until the **Select Filename** button is used to select

a path and base filename for the saved data files. The **Choose File Format** button is used to choose between various data formats.

The **Record Time** indicator starts counting in hh:mm:ss format when recording begins. It also shows the negative time position when rewinding. The **Load Indicator** shows three horizonal bar graphs which provide information on how well the computer is able to keep up with reading and processing the data from the Intan controller. HW Buffer indicates the capacity of the FIFO in the Intan controller that feeds data to the USB bus; if the CPU cannot read the USB bus fast enough, this buffer will fill up. SW Buffer indicates the capacity of the main software FIFO used to store data read from the USB port while it is being processed. CPU Load indicates the capacity of the processor to perform all its tasks while maintaining real-time operation. Ideally these should all be short green bars, or completely invisible. Long yellow or red bars indicate that the CPU and/or USB bus cannot keep up with the data from the Intan controller. The **Sample Rate Indicator** shows the sample rate that was chosen when the software was started. This can only be changed by restarting the software.
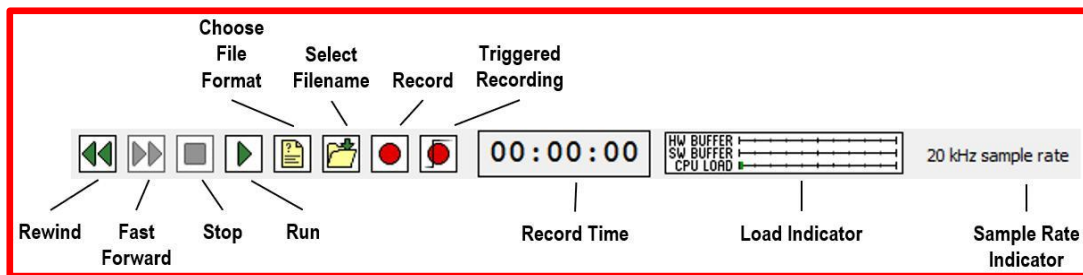

Fig. 22: IntanRHX software toolbar.

In Data File Playback mode, the Toolbar looks a bit different (see **Fig. 23** below). There are additional buttons to **Jump to Start**, **Jump Back 10 Seconds**, **Jump Back 1 Second**, and **Jump to Position** (to a specified time in the saved data). For technical implementation reasons there is no smooth rewind in Playback mode, but there is a fast forward option. The **Record** and **Triggered Recording** options are still present to allow users to re-record data in a different file format. The Sample Rate Indicator shows the sample rate that was used to acquire the data in the file.
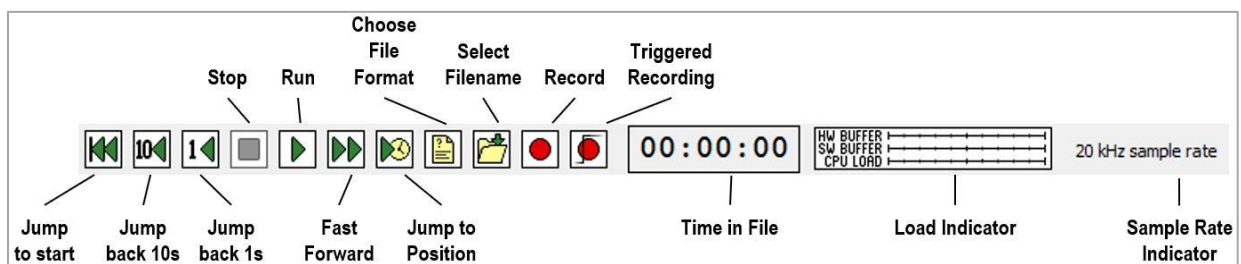

Fig. 23: IntanRHX software toolbar when in "Data File Playback Mode."

Display Column Toolbar (blue box):
The Display Column Toolbar is shown below. The Hide Column button allows you to temporarily hide a display column. (You must have more than one column on the screen to hide a column. At least one column must always be showing.) The Port Selector is a pulldown combo box that allows you to select which port to view in the Waveform Display. The Move Column Left and Move Column Right buttons allow you to rearrange multiple columns on the screen. The Add Pinned Waveform button brings up a dialog that allows

you to select any signal from any port to add to the Pinned Waveforms at the top of the Display Column. The Show Pinned check box allows you to temporarily hide all pinned waveforms. Other buttons allow you to select Less Spacing or More Spacing between waveforms in the display (you may click these multiple times to adjust the vertical spacing), and to Add or Delete Columns.
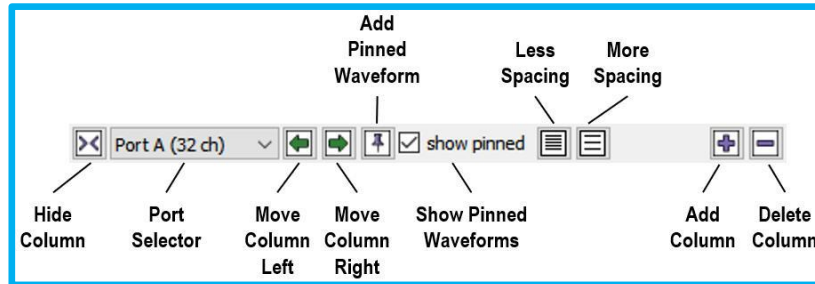

Fig. 24: Display Column toolbar.

Waveform tags (yellow box):

All waveforms displayed on the screen have a label or "tag" on the left side of the Display Column. An example of a waveform tag is shown below. By default, the channel name is displayed (A-006 in this example; channel 6 from the Port A headstage). You can change what is displayed here in the Display menu or a combo box in the Control Panel. For example, you can show the last measured electrode impedance instead of the channel name.
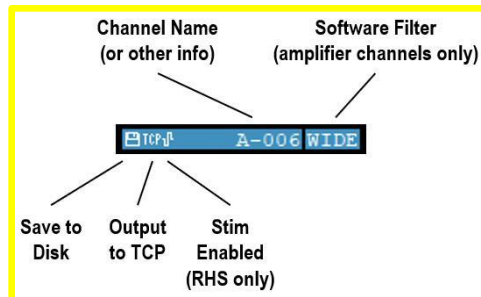

Fig. 25: Waveform tag on the left side of the Display Column.

There are three icons which may appear on the left side of the waveform tag (see diagram above): The Save to Disk icon appears if this channel will be saved to disk during recording. The Output to TCP icon appears if this channel is selected for TCP Output (see Remote TCP Control section for more information). The Stim Enabled icon appears if stimulation has been enabled on this channel (RHS systems only). If you wish to allocate more screen area for the waveforms themselves, you can visit the Control Panel where the default Wide Tags option can be changed to Narrow Tags or Hide Tags.

5) Basic setup of Intan software

After you started in the Intan software, make sure that the ports with the SPI cables on the controller have the third (red) LED lit as well (**Fig. 26**).

Fig. 26: Third (red) LED light above the yellow and green lights is lit after opening Intan software.

Initially, only one waveform column with channels of one port will be displayed (**Fig. 27**).
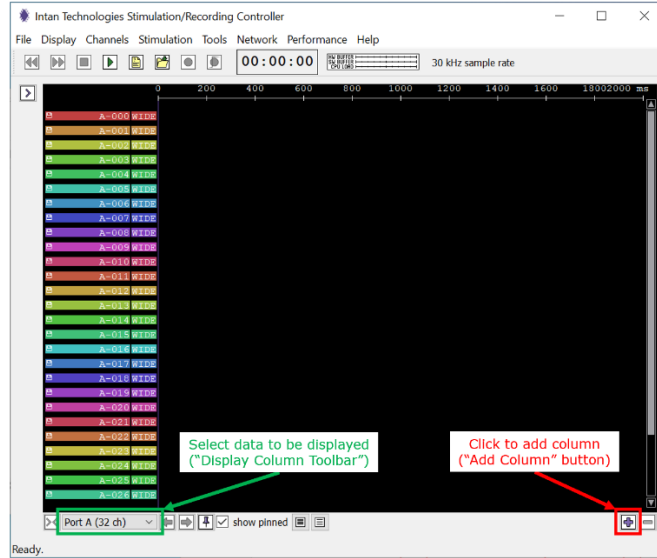

Fig. 27: Graphical User Interface (GUI) for the stimulation/recording controller.

By clicking on the "Add Column" (+) on the bottom right corner, a second column of data will be added (**Fig. 28**). You can select which data will be displayed in each column by using the dropdown menu at the bottom of the displayed column (Display Column Toolbar). These data can be waveforms from Ports A through D (if connected to an Intan headstage), analog input ports, analog output ports, digital input ports, and digital output ports. Even if only one port is being displayed on the GUI, data from all enabled channels and ports are saved for impedance measurements as well as recording or stimulation experiments. More than two columns can be displayed by clicking the "Add Column" (+) tap again.

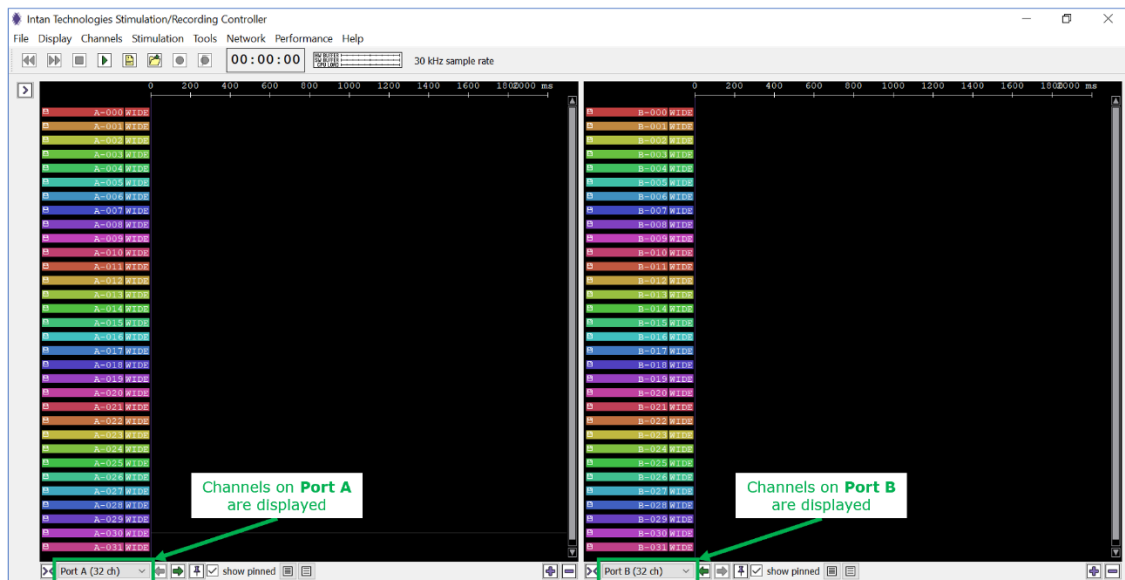**Fig. 28:** Graphical User Interface (GUI) after adding a second data column.

6) The port selector on the display column toolbar in the GUI will only list ports connected to an Intan headstage via an SPI cable. Connected ports will have all three LEDs lit (red, yellow, and green; Port A and Port B in the image below). Unused ports will only have two LEDs lit (yellow, and green; Port C and Port D in the image below).

Fig. 29: Recording/Stimulation Controller connected to a function generator.

7) To expand the GUI to display additional control parameters, click on the arrow on the top left ( ) of the window to display the control panel ("Show Control Panel" button) which allows the user to change the parameters of the recordings. You can collapse the GUI in the same way by clicking on the same arrow (pointing in the opposite direction) in the expanded GUI (**Fig. 30**).
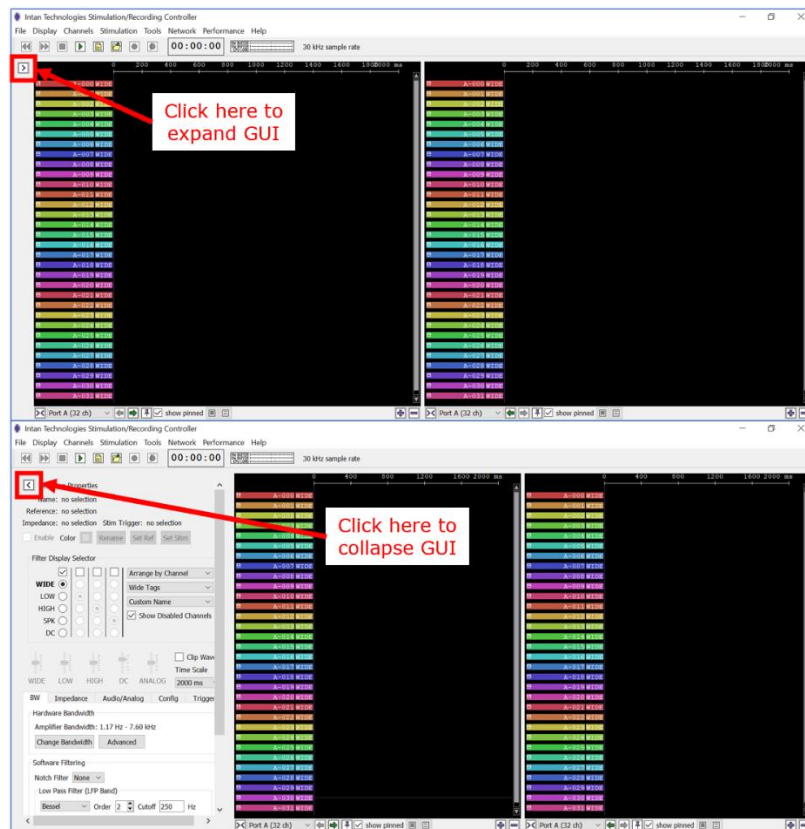

Fig. 30: GUI displaying the control panel.

8) The last step before starting your experiments is to load the Intan Setup File (ISF), which links the proper electrode number on the sMEA or MEA with the pins on the Interface Board and the contacts of the SPI cable.

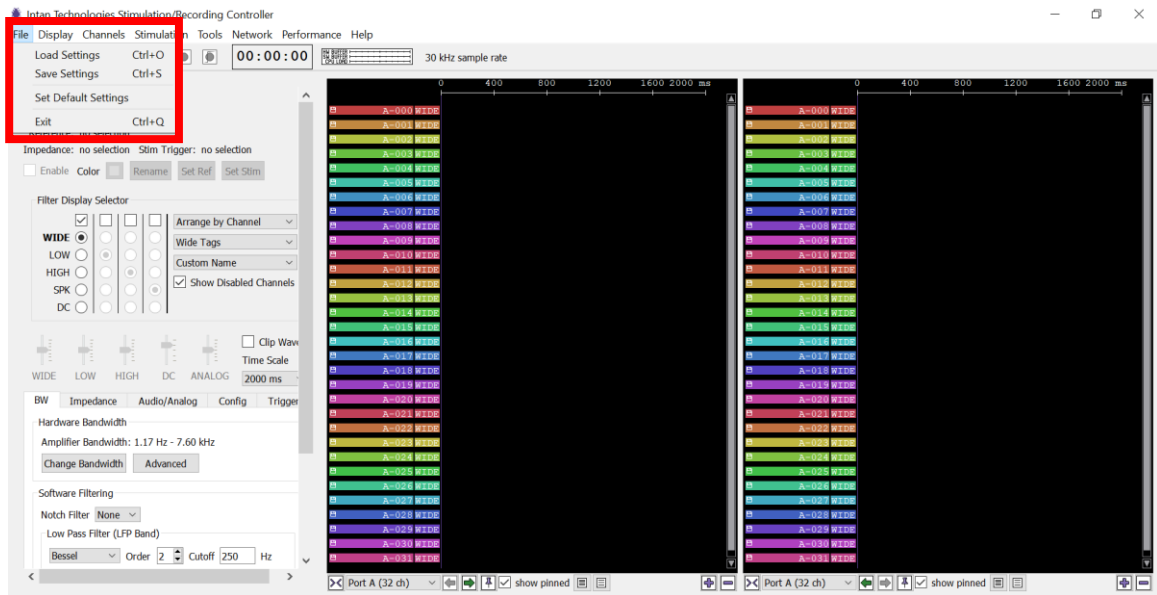9) Select "File" and "Load Settings" (**Fig. 31**) and select the file location.

Fig. 31: "File" tab and "Load Settings" button location in the GUI.

10) Go to the folder that contains the ISF files and select the appropriate file for the sample that is being used. The ISF (provided by BMSEED) depends on:
   - the type of sample: sMEA or glass MEA
   - the type of sMEA: gen4a-d, gen4e, gen6
   - ports of the Intan Stimulation/Recording Controller to which the sample is connected to
   - the orientation of the sample in the interface board (standard or 90° rotated).

Note: Ensure that you have the latest Intan software version (no older than **version 3.2.0**) for the ISF files to work properly. If you have an older software version, uninstall and reinstall the Intan program (see Section 4).

Table 2: Mapping files for the different types of sMEAs and glass MEAs.

sMEA gen4	sMEA gen4e	sMEA gen6	glass MEA	Mapping File Name
✓				• Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4_Ports-AB_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4_Ports-AB_90deg_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4_Ports-CD_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4_Ports-CD_90deg_20220426.xml
	✓			• Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4e_Ports-AB_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4e_Ports-AB_90deg_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4e_Ports-CD_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen4e_Ports-CD_90deg_20220426.xml
		✓		• Chs_sMEA-IB_RHS2000_RHX_sMEA-gen6_Ports-AB_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen6_Ports-AB_90deg_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen6_Ports-CD_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_sMEA-gen6_Ports-CD_90deg_20220426.xml
			✓	• Chs_sMEA-IB_RHS2000_RHX_glassMEA_Ports-AB_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_glassMEA_Ports-AB_90deg_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_glassMEA_Ports-CD_Sdrd_20220426.xml • Chs_sMEA-IB_RHS2000_RHX_glassMEA_Ports-CD_90deg_20220426.xml

*Note: IntanRHX settings files as of this user manual's last update.

It takes 3-4 seconds for the mapping file to load. Wait until the "Run" button at the top left of the GUI is not grayed out anymore (**Fig. 32**).
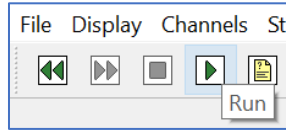

Fig. 32: "Run" button colored green (i.e. not grayed out).

For example, loading the Intan settings file for a gen4 sMEA in the standard orientation (in the EIB) and connected to Ports A and B will update the IntanRHX main window as shown below.
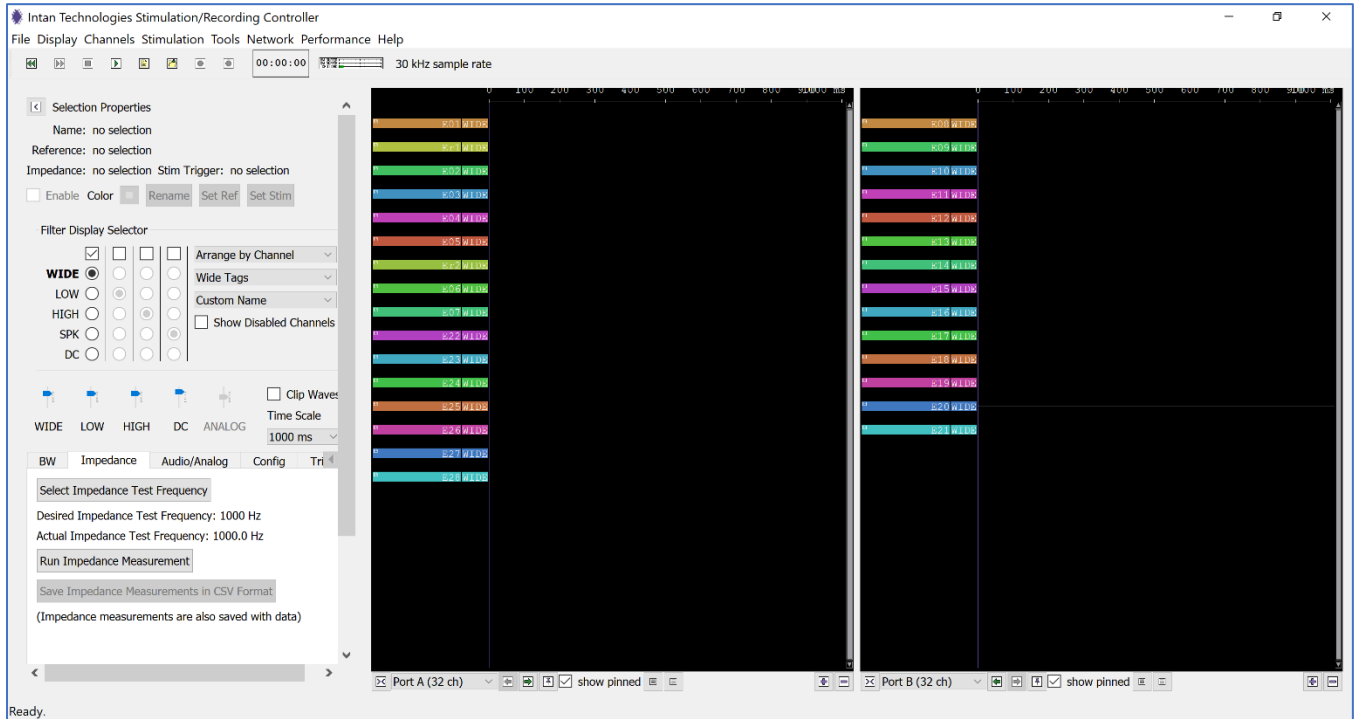

Fig. 33: GUI displaying Ports A and B channels with a gen4 sMEA mapping file.

If you see the warning message below (**Fig. 34**), save the mapping file under a different name and reload this new file. The warning message will disappear.
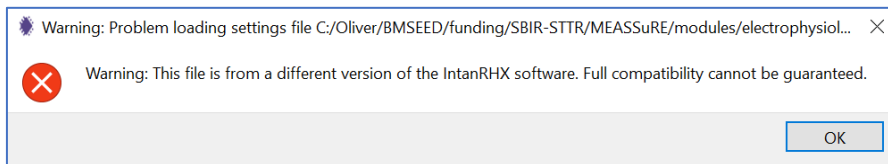

Fig. 34: Warning message if the chosen mapping file is incompatible.

11)   <u>Tip</u>: Press F12 to display a list of keyboard shortcuts.

Keyboard Shortcuts   ?   ✕

Waveform Plot

/ or ? Key: Toggle roll/sweep mode

< or , Key: Zoom in on time scale

> or . Key: Zoom out on time scale

+ or = Key: Zoom in on voltage scale

- or _ Key: Zoom out on voltage scale

Ctrl+P: Pin selected channels

Ctrl+U: Unpin selected channels

Mouse Wheel: Scroll through channels

Ctrl+Mouse Wheel: Adjust vertical spacing of channels

Cursor Keys: Step through channels

Page Up/Down Keys: Scroll through channels

Home Key: Scroll to first channel

End Key: Scroll to last channel

Spacebar: Enable/disable channels

Ctrl+G: Group selected channels

Ctrl+Shift+G: Ungroup selected channels

Ctrl+1: Display custom channel name

Ctrl+2: Display native channel name

Ctrl+3: Display impedance magnitude

Ctrl+4: Display impedance phase

Ctrl+5: Display reference

Ctrl+T: Cycle through waveform display labels

Ctrl+Z: Undo

Ctrl+Y: Redo

Spike Scope Plot

< or , Key: Zoom in on time scale

> or . Key: Zoom out on time scale

+ or = Key: Zoom in on voltage scale

- or _ Key: Zoom out on voltage scale

Mouse Wheel: Zoom through voltage scale

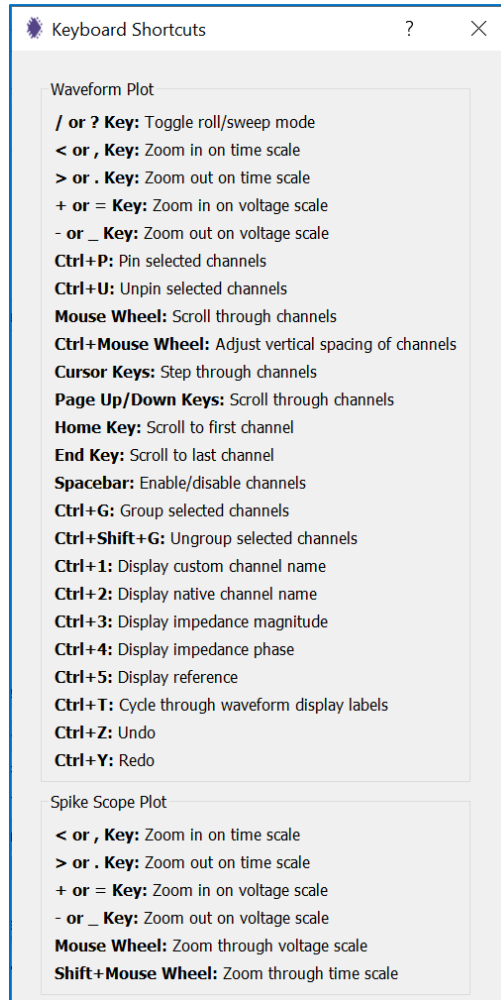**Shift+Mouse Wheel:** Zoom through time scale

Fig. 35: List of keyboard shortcuts.

8 Impedance measurement

Impedance testing is often used to determine the source of a poor recording, which could be caused by high electrode impedance, improper shielding/grounding or other issues. Impedance measurement of sMEAs and MEAs is typically done with PBS or the cell culture medium as an electrolyte.

⚠️ **IMPORTANT**: Data acquisition must be stopped to access this control. Otherwise, the software might freeze, and you might need to relaunch the software.

The GUI with the relevant tabs for impedance measurement highlighted in green is shown below. The data acquisition must be stopped for impedance measurements.
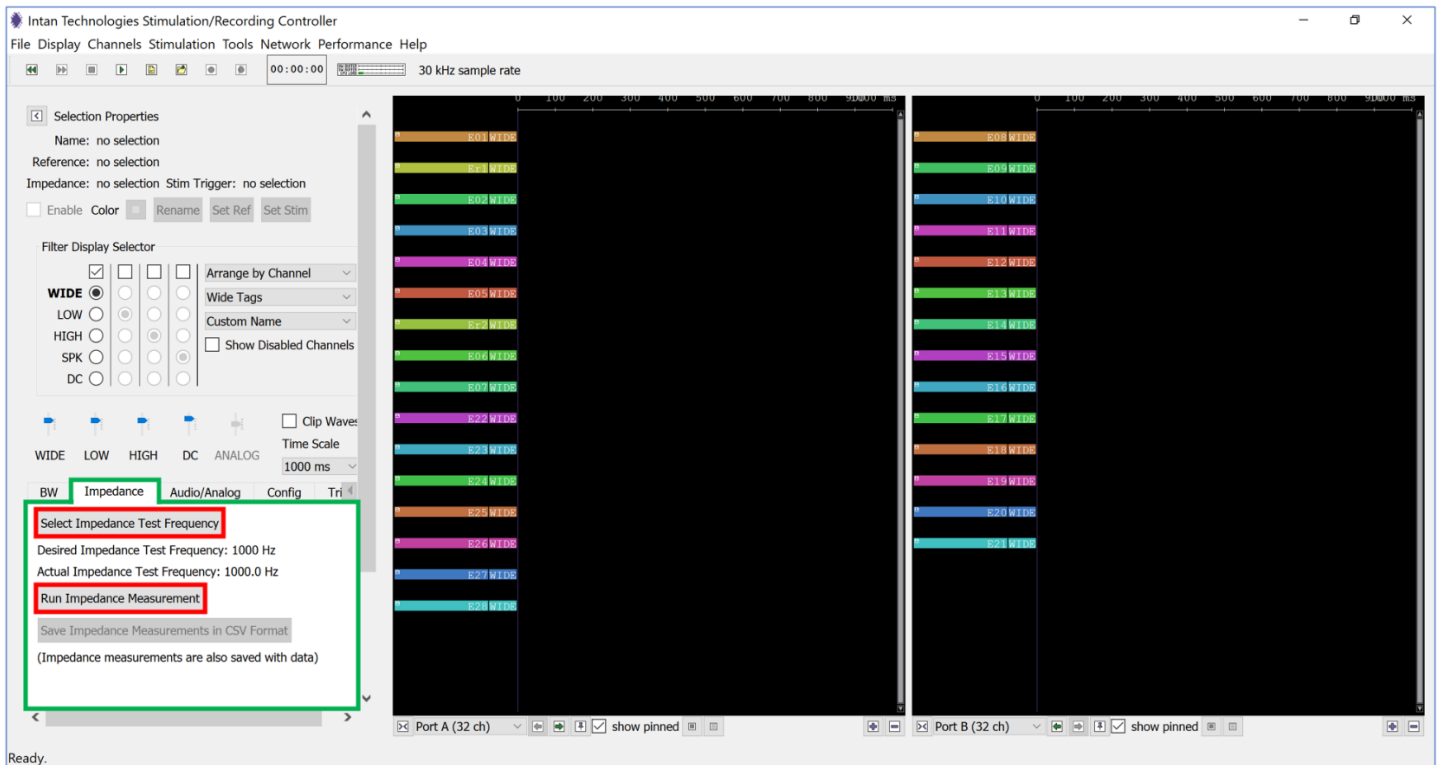

Fig. 36: GUI with impedance measurement features highlighted in green.

1) Click on the "Impedance" tab in the GUI (**Fig. 36**, highlighted green).

2) Click on the "Select Impedance Test Frequency" button (**Fig. 36**, highlighted red). This is typically set to 1 kHz.
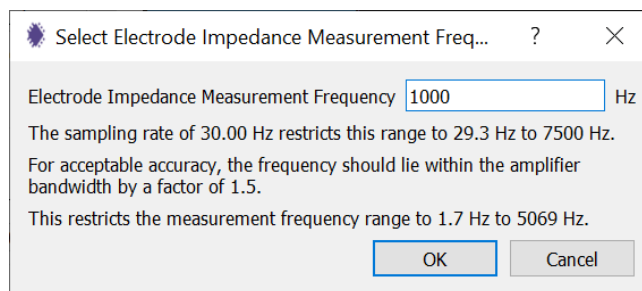

Fig. 37: Setting the frequency for measuring impedance.

3) Click the "Run Impedance Measurement" button (**Fig. 36**, highlighted red), and the impedance measurement will start. The measurement takes 4-10 seconds, and the measured impedance values will be displayed on the channel tags.
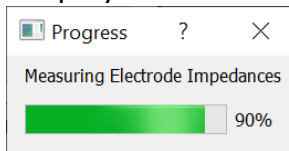

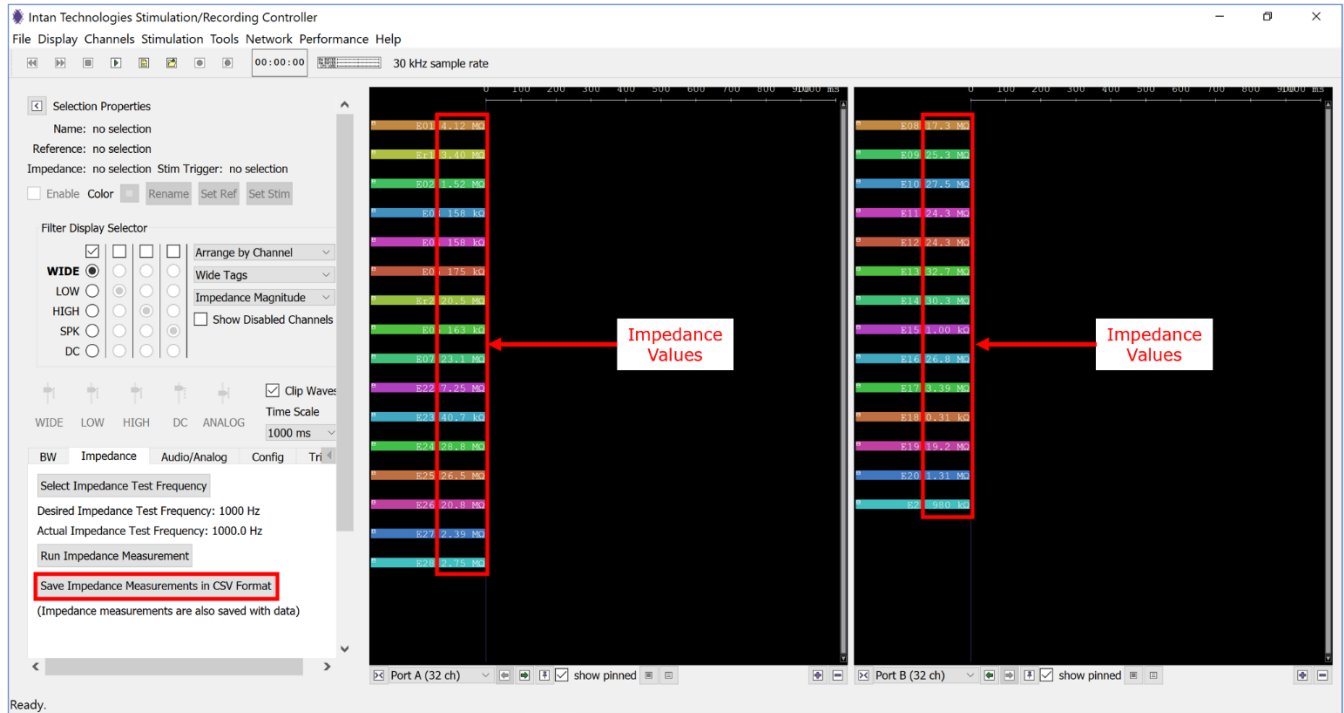**Fig. 38:** Progress bar as impedance measurements are taken.

Fig. 39: GUI displaying impedance measurements.

4) If you want to save the data, click on the "Save Impedance Measurement in CSV format" button in the "Impedance" tab (**Fig. 39**, highlighted red). Note that this option is only available after impedance measurements have been taken.

9   Manual Recording of Electrophysiology Activity (without Stimulation)

This section describes the procedure for manual recording of electrophysiological activity (see further down for information on stimulation).

The GUI with the relevant tabs for file recording format and filename (red box in **Fig. 40**) is shown below.
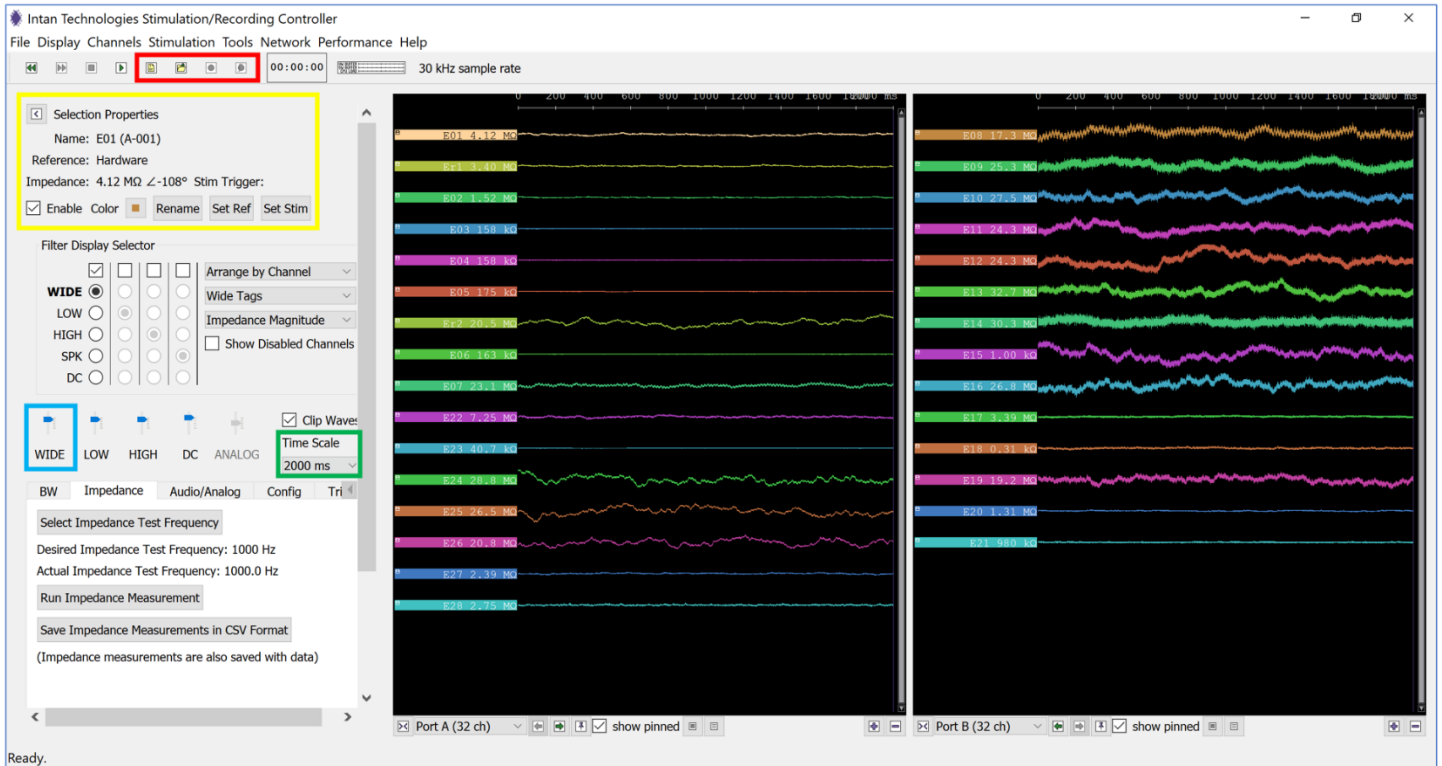

Fig. 40: GUI with options for file recording format and filename, channel renaming, and time/voltage scaling.

9.1 Configuring the GUI and Manual Recording

1) Make sure you loaded the correct mapping file.

2) Change the time and voltage scale

   a. The "time scale" (x-axis of display section in the GUI) is selected from the drop-down menu (green box in **Fig. 40**).

   b. The "voltage scale" (y-axis of display section in the GUI) is adjusted using the level indicators of the wide analog bands of the channel (blue box in **Fig. 40**).

   c. Alternatively, the voltage and time scales can be adjusted by selecting the "Display" tab on the top left of the GUI (**Fig. 41a**). Click on "Zoom In Time Scale" to reduce the time scale or simply click on "<". Equivalently, zoom out in time scale or zoom in/out in voltage scale by using the corresponding commands.

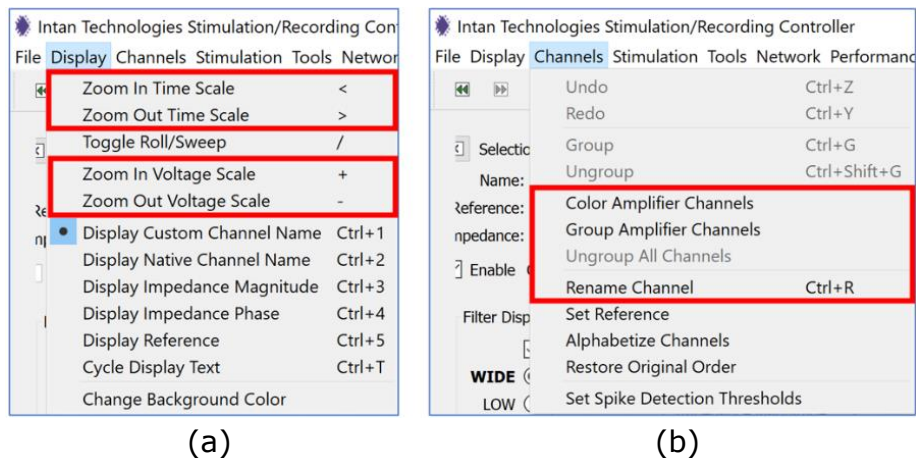(a)   (b)

Fig. 41: (a) "Display" and **(b)** "Channels" tabs on GUI.

3) Reorder, rename, recolor, group
   Using the commands in the "Channels" tab (**Fig. 41b**), you can rename, set the color, and group individual channels. An alternative way to rename and set the color is to select the channel you want to rename/recolor, and use corresponding tabs in the "Selection Properties" section of the GUI (yellow box in **Fig. 40**).

4) Reorder waveforms
   You can reorder the waveforms on the display by clicking and dragging a tag up or down the Waveform Display. You can select multiple waveforms by holding down Shift or Ctrl while clicking on the tags.

5) Disabling channels
   You can hide unused channels by first disabling them. A channel can be disabled by first selecting it with the left mouse button followed by either:

   a. Pushing the space bar
      Note: Pressing the space bar toggles between enabling or disabling the channel. If you have disabled a channel and want to re-enable it, check the "Show Disabled Channels" box (red box in **Fig. 42**). Select the channel you want to re-enable by clicking on it and hit the space bar again.

   b. Un-checking the "Enable" box in the "Selection Properties" section of the GUI (red arrow in **Fig. 42**).
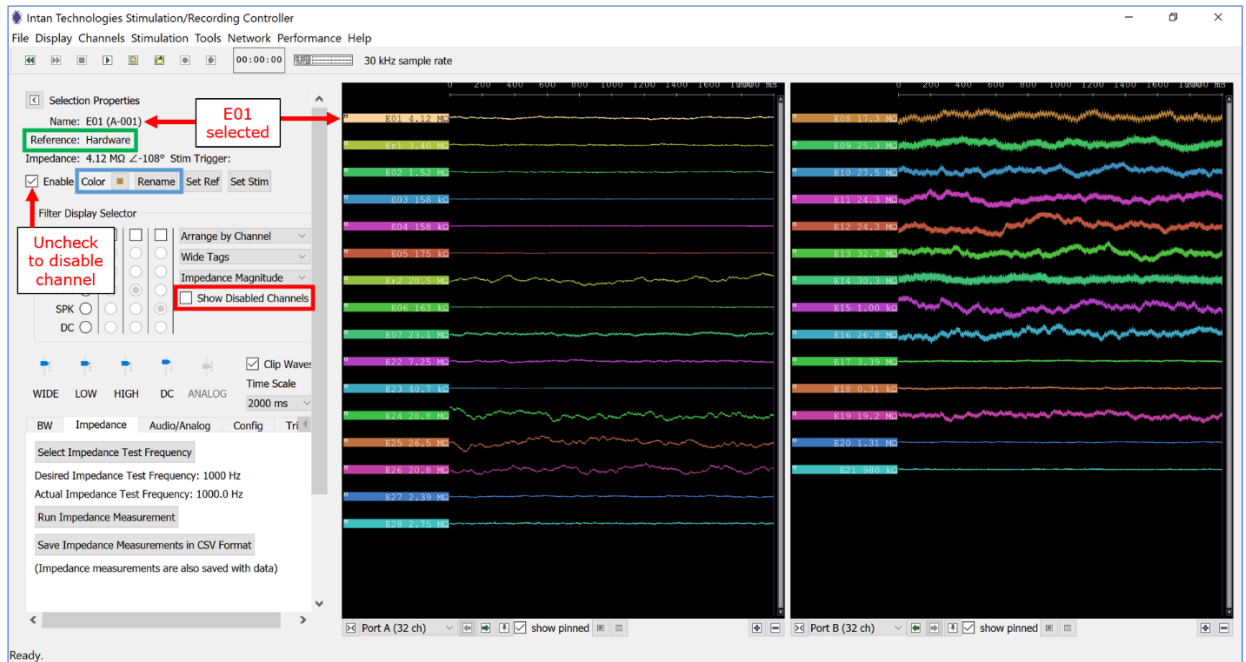
Fig. 42: GUI showing how to disable channels.

Disabled channels are **not** plotted and are **not** saved to disk. Users are encouraged to disable all unwanted channels to save disk space when recording data.

6) Reference potential
   Each channel on an RHS2000-series chip amplifies the electrode signals with respect to a reference potential. There are three options for this reference potential:

   • Hardware Reference:
     This setting uses the REF input on the headstage as reference, which is connected to the "Ground" contacts on the BMSEED Interface Boards. This hardware reference is typically a Ag/AgCl or Pt wire electrode in the culture medium. The Hardware Reference is the default reference electrode if no other option is selected. The hardware reference is often connected to a local ground near the recording sites to reduce pick-up of common-mode signals, such as AC line noise and movement artifacts. This is the **default method** we recommend using (green box in **Fig. 42**).

   • Software Reference: All Enabled Channels on Port
     The average of enabled channels is used as reference potential. There are two choices:
     a) Average of all enabled channels on all ports
     b) Average of all enabled channels on a one specific port

   • Software Reference: Custom Channel List
     Use single channel or average of multiple channels as reference.

   While we recommend using a hardware reference, in some cases it is desirable to use a software reference. With a software reference, the IntanRHX software performs a digital subtraction using a single channel or an average of multiple channels as reference potential.

The reference potential can be specified for individual electrodes, i.e., different electrodes can have different reference potential providing high flexibility. To check which reference is used for a specific channel, click on the channel in the display column. The name of the channel, the selected reference electrode and its impedance is shown on the top left of the GUI under "Selection Properties" (yellow box in **Fig. 40**). For instance, in the screenshots below, channel A-001 is using channel A-002 as reference (red underlines), and channel A-004 is using the hardware reference (blue underlines).
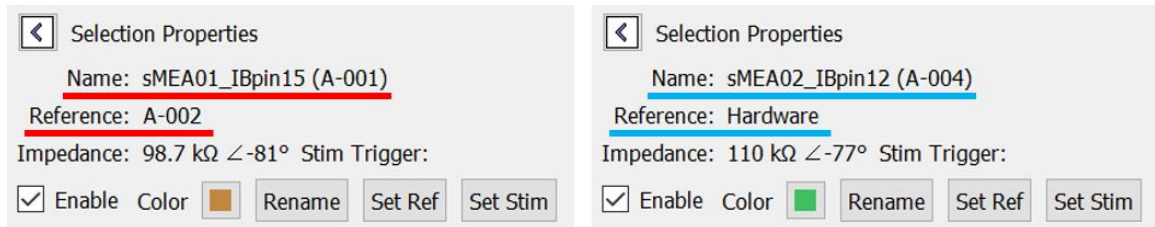

Fig. 43: Channels showing two different types of references.

To set the reference for a specific electrode, select the channel in the waveform display (left mouse button click), which automatically creates a check mark in the "Enable" tap under "Selection Properties". Click the "Set Ref" button under "Selection Properties", and the "Select Reference" window will pop up (**Fig. 44**). You can set the reference potential for multiple electrodes by holding the "CTRL" key on your keyboard and left-clicking on all channels that you want to set the same reference for in the waveform display section of the GUI. In addition, holding the "SHIFT" key on your keyboard and clicking on two channels in the waveform display section of the GUI will select all channels in between the two clicked ones.
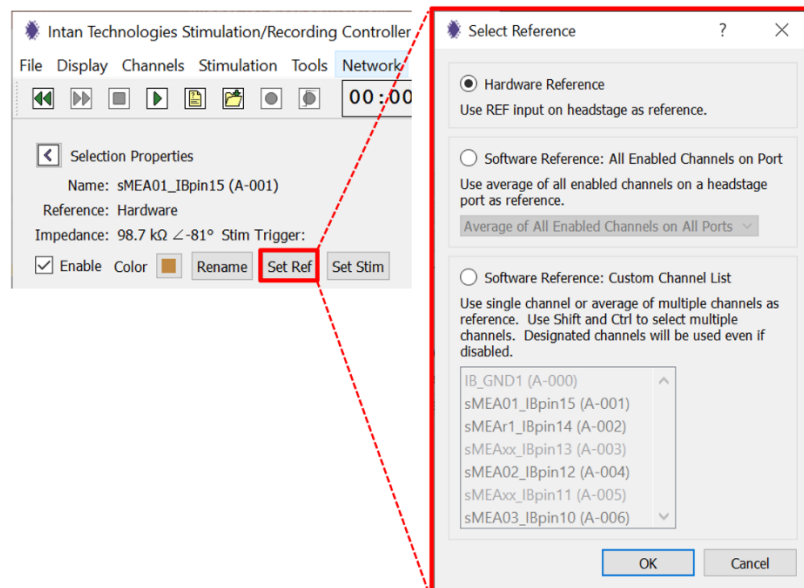

Fig. 44: Setting the reference potential.

Make sure to select one or multiple amplifier channels with no localized electrophysiological activity to serve as reference. The data on this digital reference will be subtracted from all other amplifier channels in real-time.

Note: If using a software reference, knowing the reference channels will allow the user to reconstruct the original signal prior to digital re-referencing.

⚠️ If the background noise on all channels is approximately Gaussian, this digital reference subtraction will increase the background noise by 41%.

7) Filter Display Selector

The Filter Display Selector in the Control Panel (red box in **Fig. 45**Fig. 40) allows you to show different versions of the amplifier waveforms and to customize their arrangement. Each amplifier channel can be shown as a wideband signal (WIDE, unfiltered), a low-pass filtered signal (LOW), a high-pass filtered signal (HIGH), a spike raster (SPK), and a low-gain DC amplifier signal (DC). The default setting when the software starts is a checked box over the leftmost column, and WIDE waveform is selected. You can change the selection from WIDE to LOW, HIGH, or SPK while data acquisition is running, and the waveforms in the display will change to these various filtered versions of each amplifier channel. Bear in mind that this selection only affects the displayed waveforms, NOT the waveforms that are being saved (see below for more information). Notice also that the right side of the waveform tags change to reflect the filter currently displayed.

You can check up to three additional square boxes at the top of the Filter Display Selector (red box in **Fig. 45**) to display additional waveforms for each amplifier channel: (i) a low pass filtered waveform, (ii) a high pass filtered waveform, (iii) a spike filter, and (iv) a DC waveform. Additionally, you can select the order for them to be displayed by clicking on the round boxes in the "Filter Display Selector" section. The red box in **Fig. 45** shows an example where four additional waveforms are selected to be displayed in sequential order.
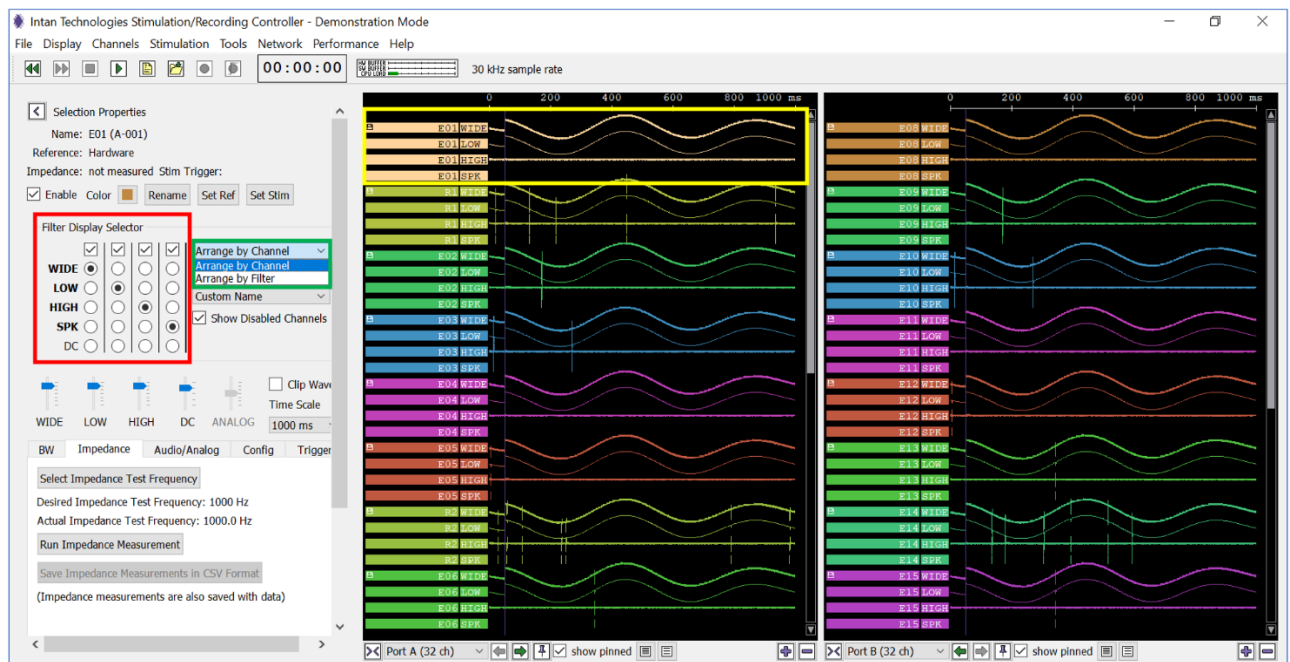

Fig. 45: GUI with additional waveforms selected in Filter Display Selector (red box).

You can arrange the waveforms to be displayed by Channel or the type of Filter using the pull down the Arrange by Channel combo menu (green box in **Fig. 45**). Arranging

the waveforms by channel displays all selected waveforms of a given electrode together, as shown in **Fig. 45** (yellow box). Arranging the waveforms by filter shows the waveforms of all electrodes with the first selected filter first, followed by the other selected filters.

If you hover the mouse pointer over a waveform, a vertical scale bar will appear, showing the voltage amplitude of the signal (e.g., 200μV in the yellow box in **Fig. 46**). You can change the voltage amplitude scale by clicking on the "+" sign on your keyboard to increase and the "-" sign to decrease the scale. Alternatively, you can use the slider scale in the middle of the control panel (red box in **Fig. 46**). Checking the box labeled "Clip Waves" (green box in **Fig. 46**) will not allow adjacent waveforms to overlap on the screen.
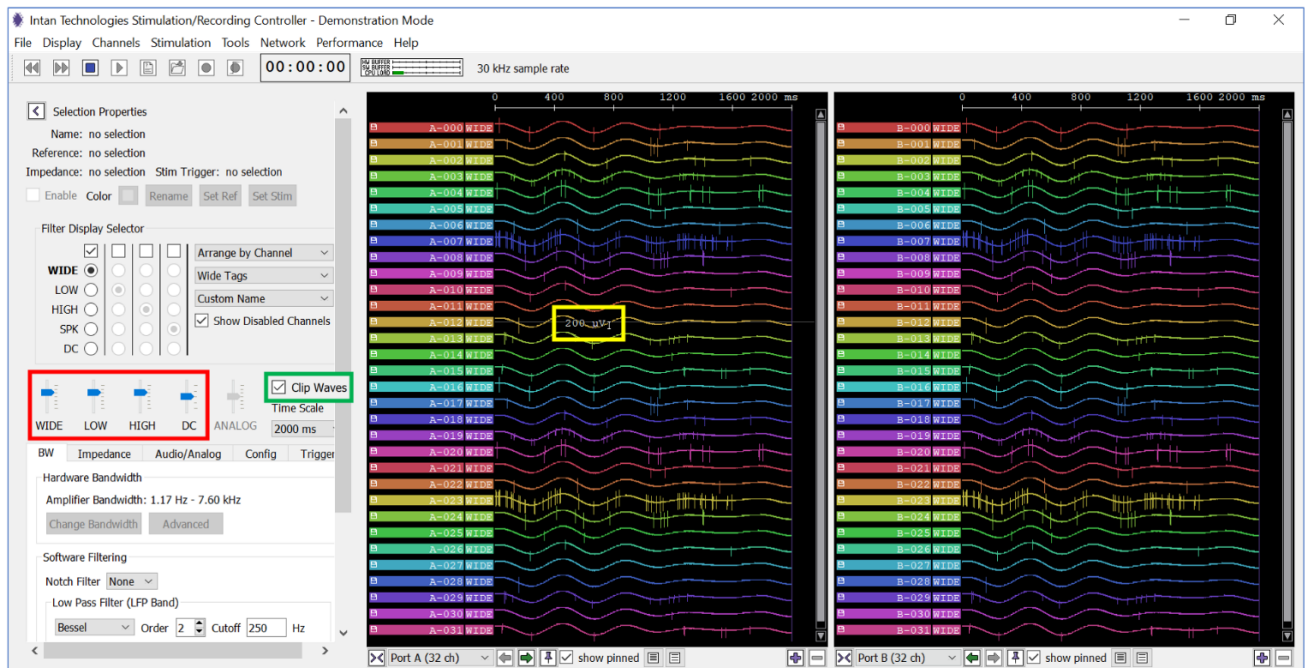

Fig. 46: Changing the scale of the voltage (y-) axis in the Waveform Display.

8) <u>Low- and High-Pass filtered waveforms</u>
See **Section 9.4.1** Bandwidth for details.

9) <u>Spikes</u>
The user may also specify a larger Artifact Threshold that can be used to reject large artifacts (e.g., due to movement or electrical stimulation). The "Set Spike Detection Thresholds" option in the "Channels" menu (**Fig. 41**) allows users to automatically set the thresholds for all enabled channels to an absolute level or to a multiple of RMS level.

10) <u>A short note regarding DC amplified signals</u>
Each channel in an RHS headstage contains two types of amplifiers: a high-gain AC-coupled amplifier used for monitoring weak electrophysiological signals, and a low-gain DC-coupled amplifier that may be used to monitor larger voltage excursions induced by stimulation. The DC amplifiers have a very low resolution and cannot be used to observe any known electrophysiological effects. They are only intended to provide a gross indication of electrode voltage.

Note: The Voltage Scale selector changes to a new set of ranges (from ±1V to ±10V) when DC amplifiers are selected.

It is important to note that displaying additional waveforms does not necessarily mean that the additional waveforms are also saved. If you selected the "Traditional Intan File Format", only the "Wide" (unfiltered) waveform is displayed. This is shown in **Fig. 47**.
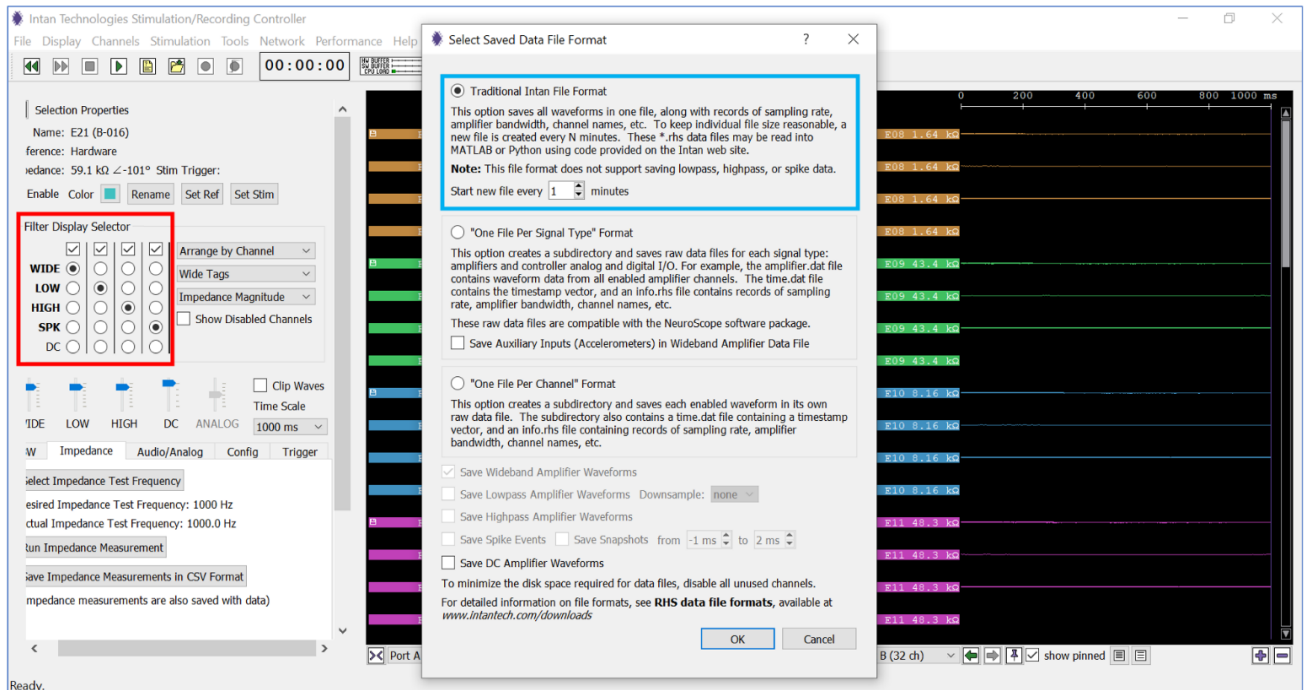

Fig. 47: GUI with additional waveforms selected in Filter Display Selector (red box), but Traditional Intan File Format is selected, saving only the "Wide" waveform (blue box).

To ensure that the additional waveforms are saved, select the "One File per Channel" Format and ensure that all desired waveforms are checked in the square box. **Fig. 48** shows that the 4 additional waveforms that are selected in the Filter Display Selector (red box) are also selected in the Select Saved Data File Format tab (blue box).
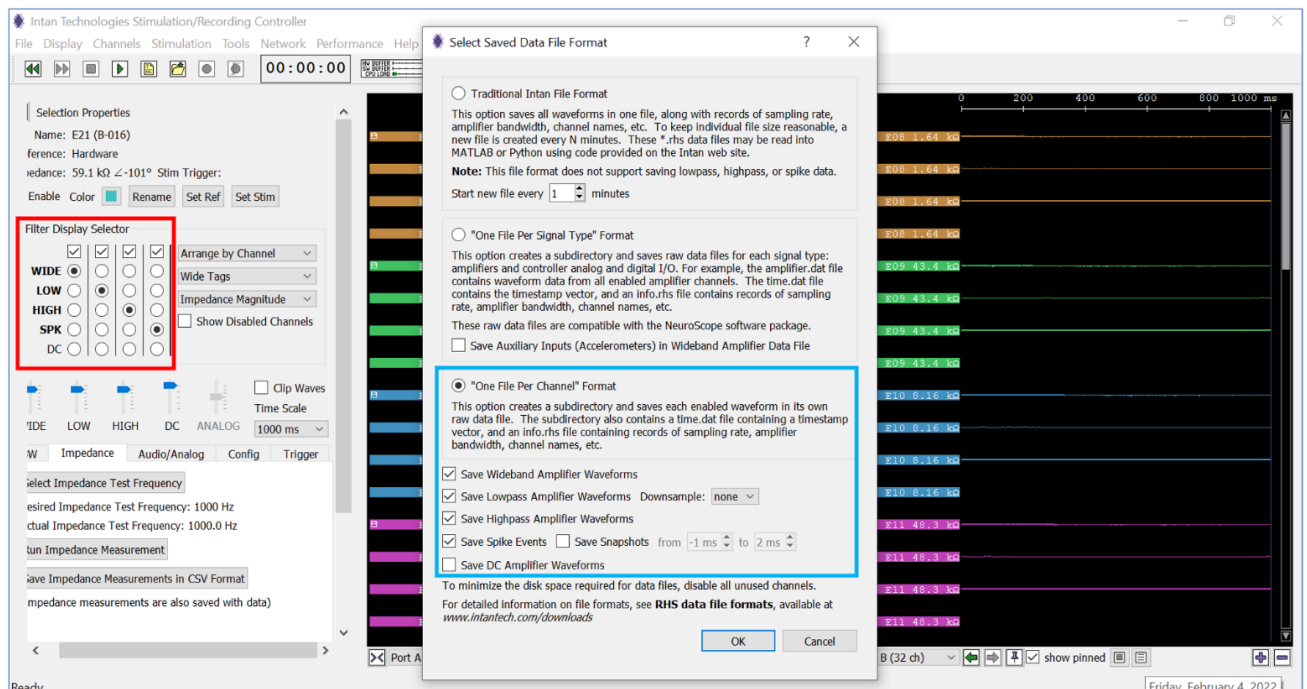
Fig. 48: GUI with additional waveforms selected in Filter Display Selector (red box), and also in the Select Saved Data File Format tab (blue box).

Click "OK" and the additional waveforms will be saved to the drive, indicated by a Floppy Disk icon next to the displayed waveform (yellow boxes in **Fig. 49**).

Fig. 49: GUI with Floppy Disk icon next to the additional waveforms selected.

11) Pinned Waveforms

Pinned Waveforms can be added at the top of the Waveform Display for any "global" signals you wish to view alongside the signals from each port. Pinned Waveforms have two important properties: (1) they stay fixed at the top of the display regardless of the scroll bar position; and (2) they may be signals from other ports. For example, if Port A is displayed on a particular Display Column, channels from Port B/Digital Input/Analog Input can also be displayed in this column (blue box in **Fig. 50**).

To pin a channel, click on the pin in the toolbar of the display column (red box in **Fig. 50**). You can select to pin a signal from any headstage or the controller I/O (green box in **Fig. 50**). For pinning headstage signals, you can use any signal from any headstage that is connected to the controller or any of the processed/filtered signals (low pass, high pass, spike, DC). For pinning controller I/O signals, you can use any of the digital or analog in/out signals.
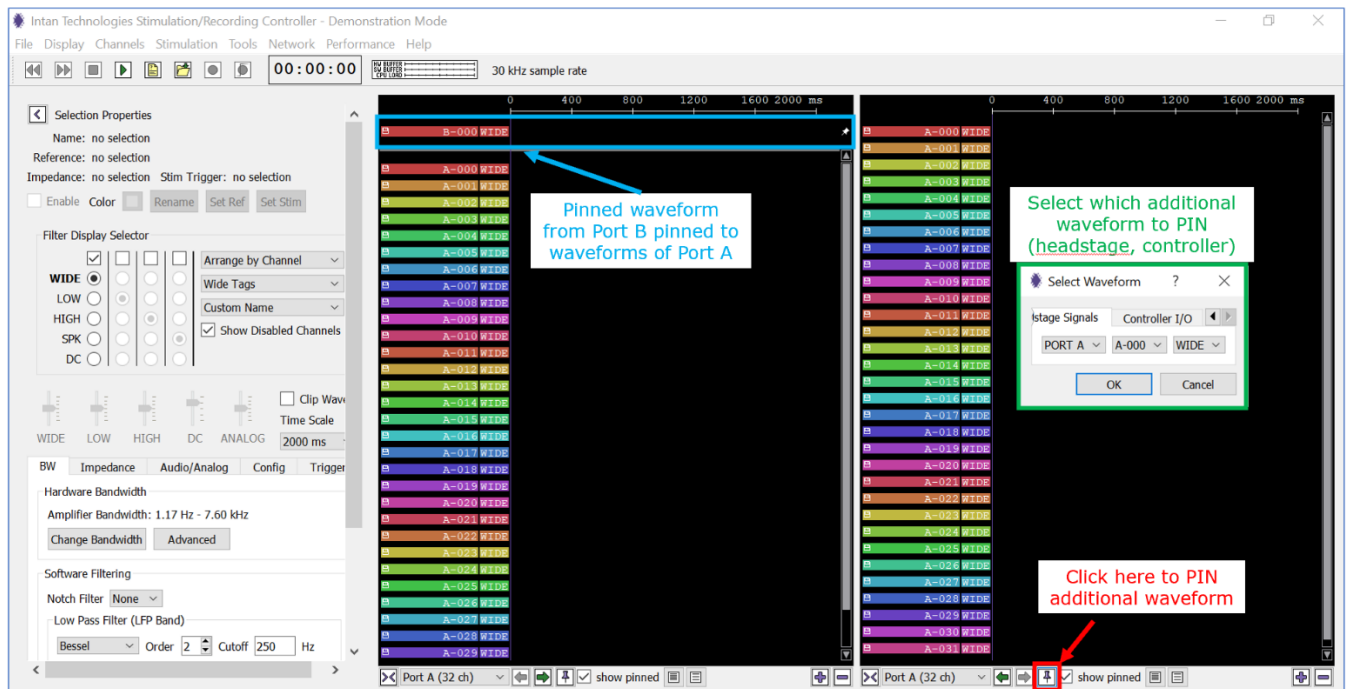
Fig. 50: Pinning waveforms at the top of the Display Column.

If the pinned signal does not show up in the GUI, check the "Show Disabled Channels" box, select the pinned channel, and hit the space bar.

After a waveform has been pinned, it can be unpinned by clicking on the angled pin icon to the right of the waveform: You can also pin and unpin waveforms by pressing Ctrl+P and Ctrl+U.

9.2 Manual Recording

1) To visualize the signals, click "Run" (blue box in **Fig. 51**) to start displaying the recorded voltages from each channel. The data are **not** saved, and the run mode is intended for viewing the recorded signals only. In the GUI shown below, the Intan channels not connected to an sMEA electrode have been disabled (disabled channels are shown in gray color). You can remove the disabled channels from the waveform display by un-selecting the "Show Disabled Channels" tab (green box in **Fig. 51**). Also, note that the "Record" and "Trigger" functions (red box in **Fig. 51**) are disabled. The file format and filename need to be specified first for these functions to be enabled (see below).
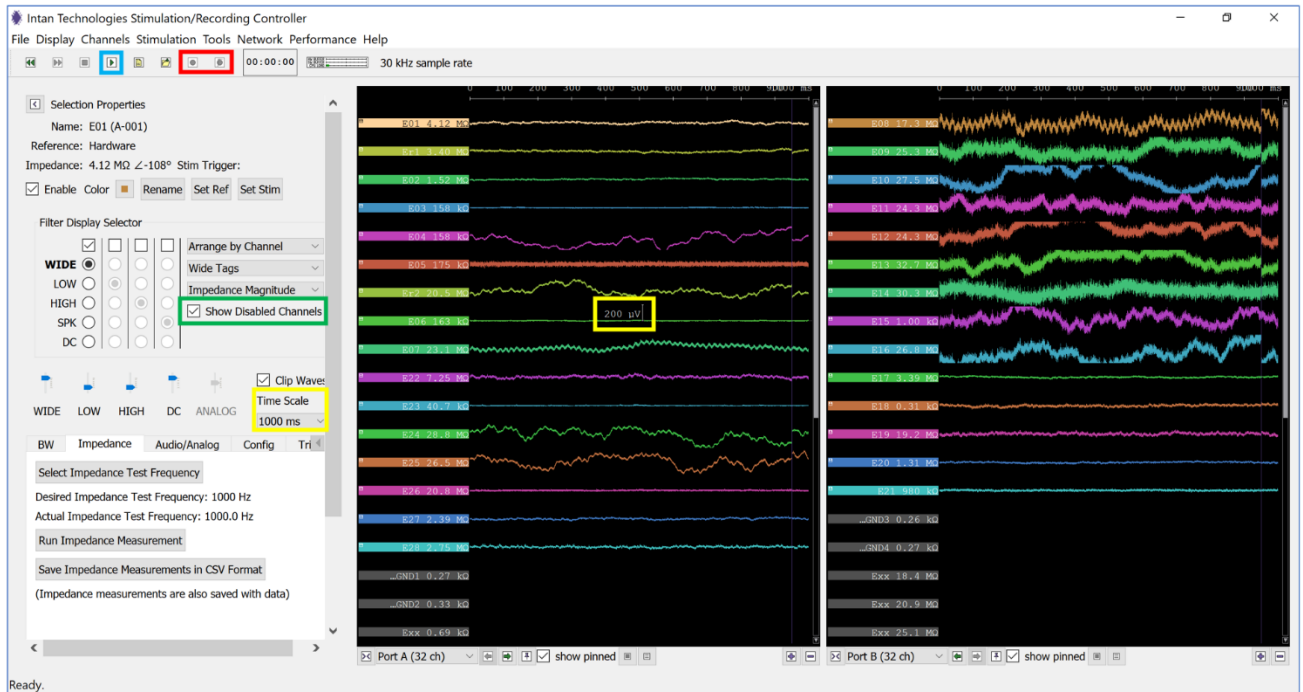
Fig. 51: GUI in "Run" mode with multiple channels disabled (grayed out).

Note: At the top of the waveform display, the time axis is displayed. To view the voltage scale, move the cursor over one of the channel's waveform and the voltage scale will be displayed. For example, in the screenshot above, a time scale of 1000ms and a voltage scale of 200μV have been set (yellow boxes in **Fig. 51**).

Under the "Display" menu, the user can select what is displayed on the channel's tag to the left of each waveform. For instance, the custom channel name or the impedance magnitude can be displayed (red and yellow boxes in **Fig. 52**, respectively).
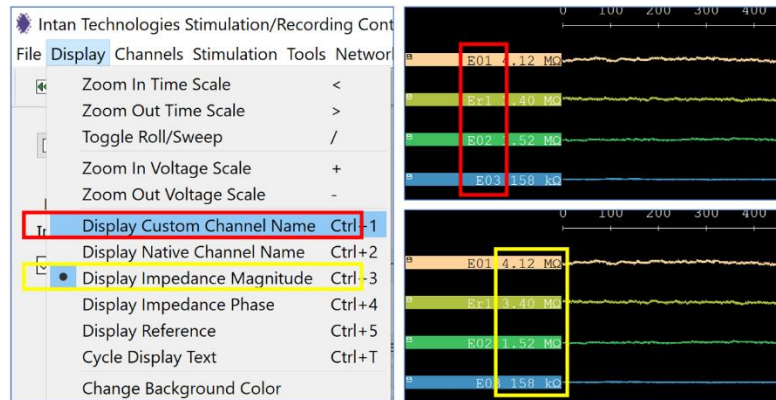
Fig. 52: A few channels displaying custom channel name and impedance magnitudes.

2) Before **recording data you want to save**, click the "Choose File Format" icon (; green box in **Fig. 54**). The following window will appear.
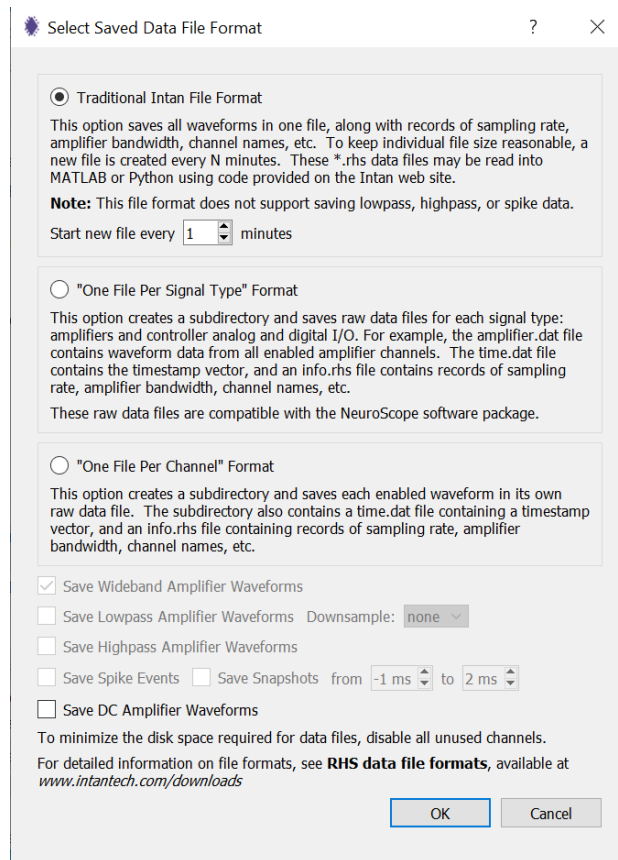
Fig. 53: Settings for the saved data file format.

3) Choose the format of the saved files, which will have the suffix "*.rhs". The "Traditional Intan File Format" is the standard format in which the user can specify the time interval at which new data files are created. If "1 minute" is set as in the screenshot above, a new data file will automatically be created every minute with a unique time stamp. If "2 minute" is chosen, a new data file will be created every 2 minutes, etc.

4) Click the "Select Filename" icon (; yellow box in **Fig. 54**) to specify the folder where the data file will be saved to and its filename. After setting the file name, click "Save."
The "Record" icon (; red box) and "Trigger" icon (; blue box) buttons will no longer be grayed out and can be selected by the user.
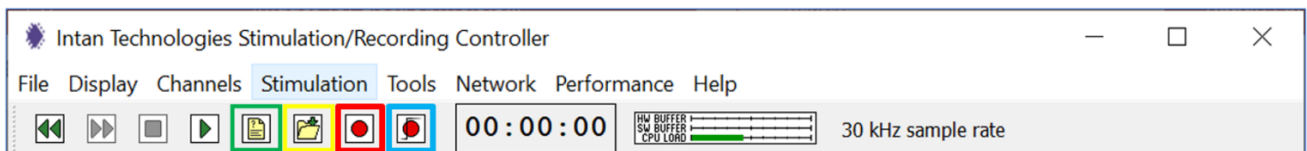

Fig. 54: GUI top toolbar after selecting the file format and setting the base filename.

5) Select "Record" () to save all the recorded data to a file. Press the "Stop" button () to end the recording.

Note: Pressing the "Run" () button instead of the "Record" button will NOT save the recording!

When you click the "Record" button, a folder with the specified filename is created. In this folder, a new file with the chosen filename and a time stamp (*year-month-day_hour-minute-second*) will be added every 1 minute (or whatever interval you chose for the Data File Format window in step 7 above). Each file has the following format: "basefilename_yymmdd_hhmmss.rhs". See below for a sample filename "test4".

Name	Date modified	Type
settings	7/29/2021 3:17 PM	XML File
test4_210729_151700.rhs	7/29/2021 3:18 PM	RHS File
test4_210729_151800.rhs	7/29/2021 3:18 PM	RHS File

Plexon board
software
test4_210729_151700
images

Fig. 55: Example Intan recording filename.

9.3 Triggered Recording

Section 11 of this manual will describe the use of triggers for electrical stimulation. Recording of data can also be triggered by an external signal. In this mode, the recordings are only saved as data after a user-specified trigger signal has been detected. For "Triggered Record", set up the software as shown above. In addition, you need to connect an external analog or digital signal which provides the trigger. In the example in **Fig. 56**, we use the pulse wave from a signal generator, which is connected to the "ANALOG IN" port on the controller.
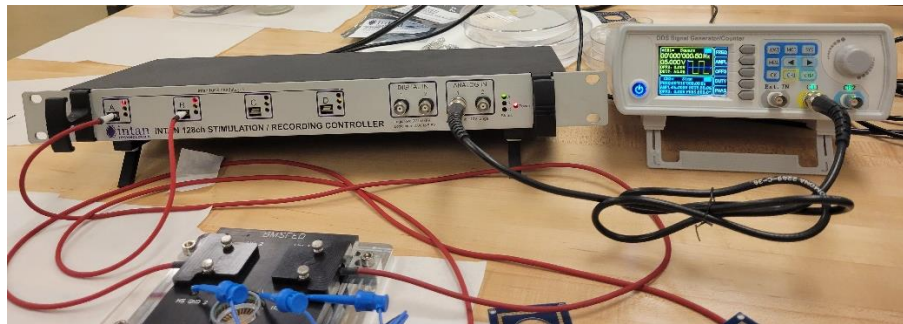

Fig. 56: Settings for the saved data file format.

To make sure that the trigger signal is detected, pin the trigger signal to all used ports. **Fig. 57** shows a 5V, 0.5Hz signal from the signal generator displayed in the pinned channel for Port A and Port B (red boxes).

⚠ If you use a voltage trigger, the supplied total voltage (peak-to-peak) needs to be at least 3.4V.

Avoid using square wave signals as triggers as there appears to be a glitch in the software. We recommend to use a pulsed trigger with a 4V amplitude, 0V offset, and a 10% duty cycle.
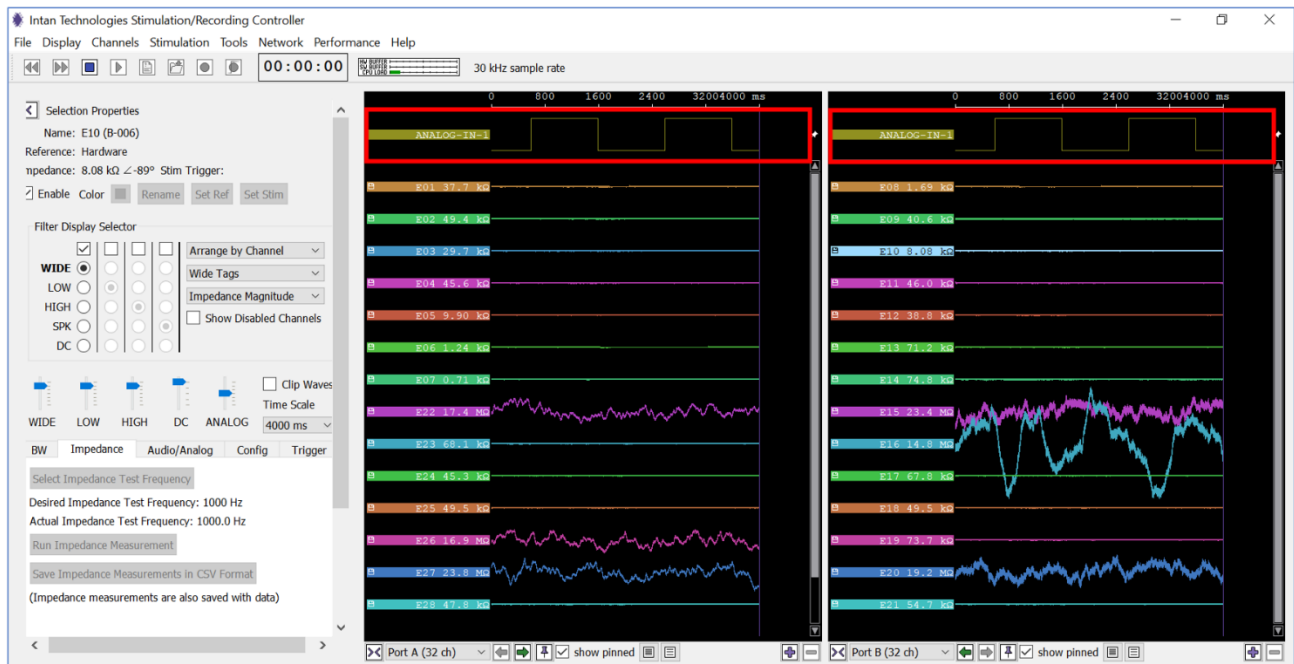
Fig. 57: Settings for the saved data file format.

When you click on "Triggered Record" (⬤), the "Episodic Triggered Recording Control" window will pop up (**Fig. 58**).
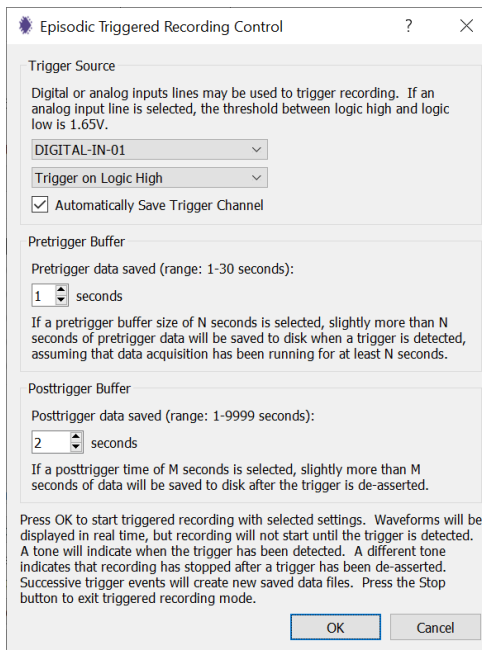

Fig. 58: Settings for the episodic triggered recordings.

Enter the input channel that will provide the trigger and select whether the trigger occurs when transitioning to a logic high or a logic low. You can also specify how many seconds are saved pre- and post-trigger detection. Once these parameters have been set, click "OK" and the software will start running, displaying waveforms in real-time. Keep in mind that data will not be saved until the trigger signal is detected.

Press the "Stop" button (⬛) when the triggered recording has completed.

9.4 Additional Parameters

Additional useful functions can be found in the lower left of the control panel (red box in **Fig. 59**).
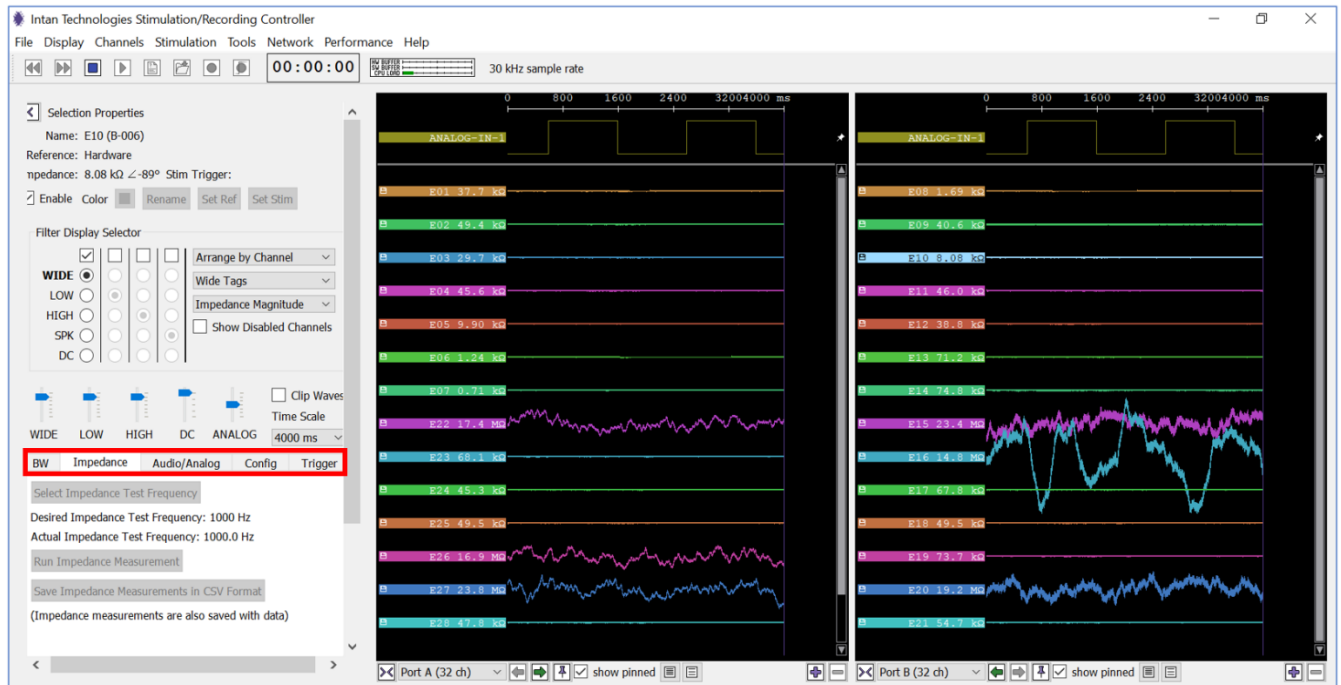

Fig. 59: Additional functions in the control panel.

9.4.1 Bandwidth tab

The Bandwidth "BW" tab, highlighted in red in the screenshot below, allows the user to set hardware bandwidth and software filtering parameters. Data acquisition must be stopped to access these controls.
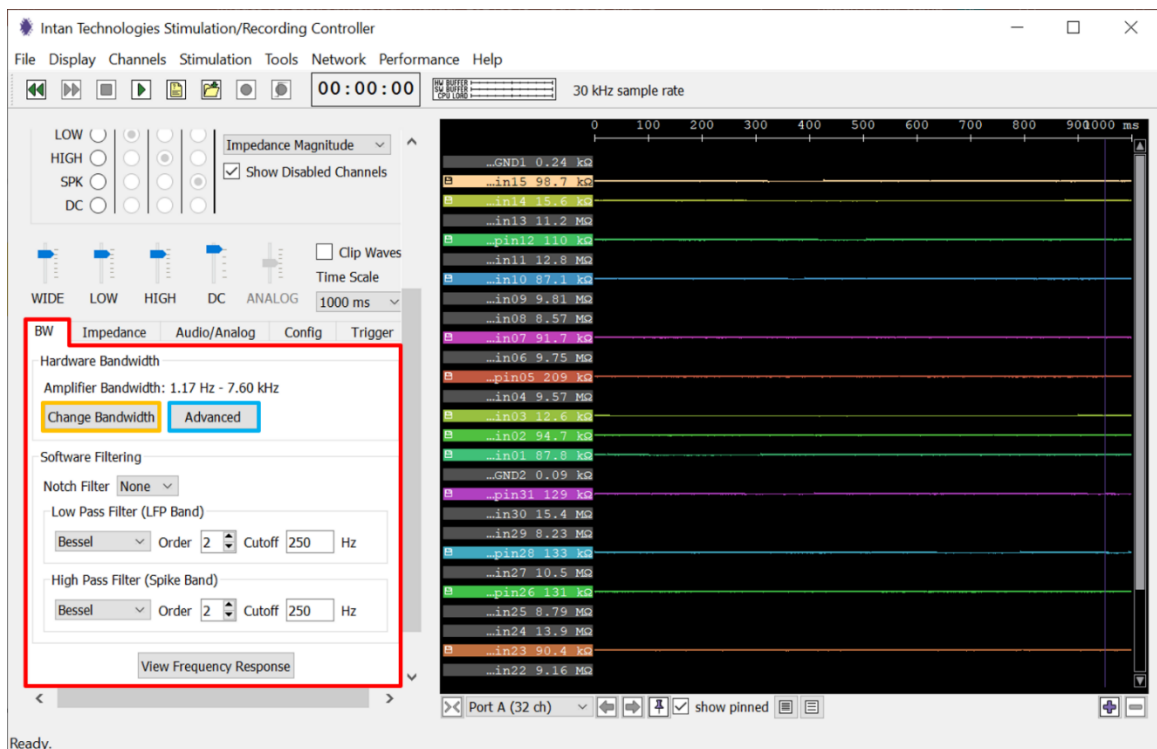

Fig. 60: Bandwidth tab in the control panel.

The bandwidth tab consists of two sub-sections: Hardware Bandwidth and Software Filtering.

9.4.2 Hardware Bandwidth

This section allows the user to set the bandwidth of the waveforms that are displayed in the Display Columns. Under "Change Bandwidth" (orange box in **Fig. 61**) the amplifier low frequency and high frequency bandwidths can be set. Intan recommends that most users set the amplifier bandwidths using this menu. The Low Frequency Bandwidth ranges from 0.1 Hz to 500 Hz and the High Frequency Bandwidth ranges from 100Hz to 20kHz. In the example below, the Low Frequency Bandwidth is set to 1Hz and the High Frequency Bandwidth is set to 7,500Hz, which means that the waveforms with frequencies between 1Hz and 7,500 Hz are recorded.

However, under "Advanced" (blue box in **Fig. 61**) the user can set amplifier analog cutoff frequencies, as well as the on-chip DSP offset removal filter cutoff frequency, directly (blue box in **Fig. 61**). It is recommended to set the "DSP Cutoff Frequency" to the desired low-frequency cutoff, and to set "low frequency bandwidth" to between 2× and 10× lower than this frequency. If both are set to the same or similar values, the actual cutoff frequency will be higher than either frequency due to the combined effect of the two filters.
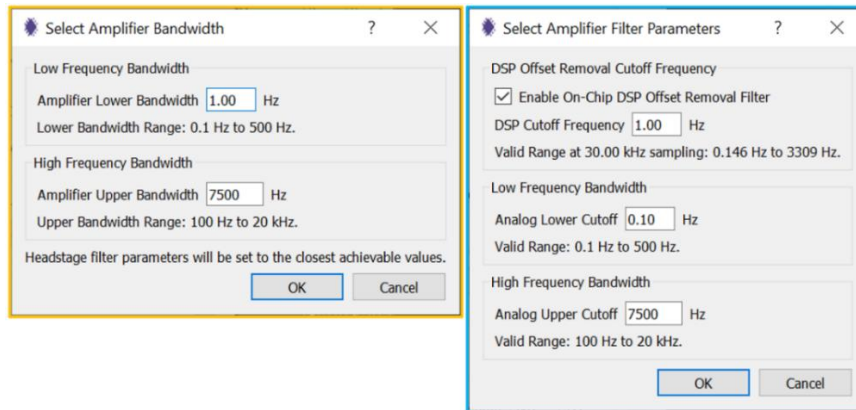

Fig. 61: Setting amplifier bandwidth and filter parameters.

Note: The Amplifier Upper Bandwidth is recommended to be at the most half of the sample rate. That is, for a sample rate of 30kHz, the upper Bandwidth should be set to 15kHz or less. Otherwise, a red warning saying "Nyquist frequency violation" will pop up (see **Fig. 62**). If a Nyquist frequency violation occurs, information about the signal will be lost.
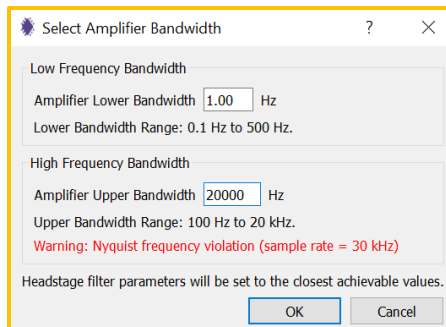

Fig. 62: Warning message when amplifier bandwidth is set to greater than half the sample rate.

9.4.3 Software Filtering

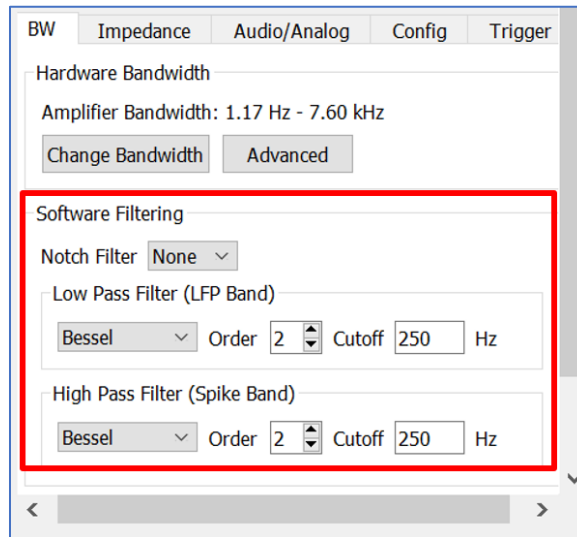Under the "BW" tab, software filtering can also be enabled.

Fig. 63: Bandwidth tab with optional software filtering highlighted in red.

- Notch filter: An optional 50Hz or 60Hz software notch filter can be enabled to help remove mains interference. A notch filter is a type of band-stop filter, which is a filter that attenuates frequencies within a specific range while passing all other frequencies unaltered. Important: unlike previous versions of the software, note that the notch filtered data is saved to disk if it is enabled.

  It is **recommended to disable the notch filter for stimulation experiments** because it can introduce low frequency post-stimulation artifacts. The notch filter will increase the CPU load for the data transfer.

- Low-pass filter (LFP Band): These parameters allow low pass filtering for the LFP ("LOW") band of the channel waveform. Only frequencies <u>below</u> the cut-off can pass the filter (250Hz in **Fig. 63**).

- High-pass filter (Spike Band): These parameters allow high pass filtering for the "SPIKE" band of the waveform. Only frequencies <u>above</u> the cut-off can pass the filter (250Hz in **Fig. 61**). This filter is very useful for observing neural activity immediately following a stimulating pulse because it reduces the stimulation artifact recovery time while allowing action potentials to pass. We recommend setting the frequency to 200-800 Hz (250Hz in **Fig. 63**).

Fig. 64 shows how clicking the "View Frequency Response" button brings up a graph that shows the various frequency bands graphically.
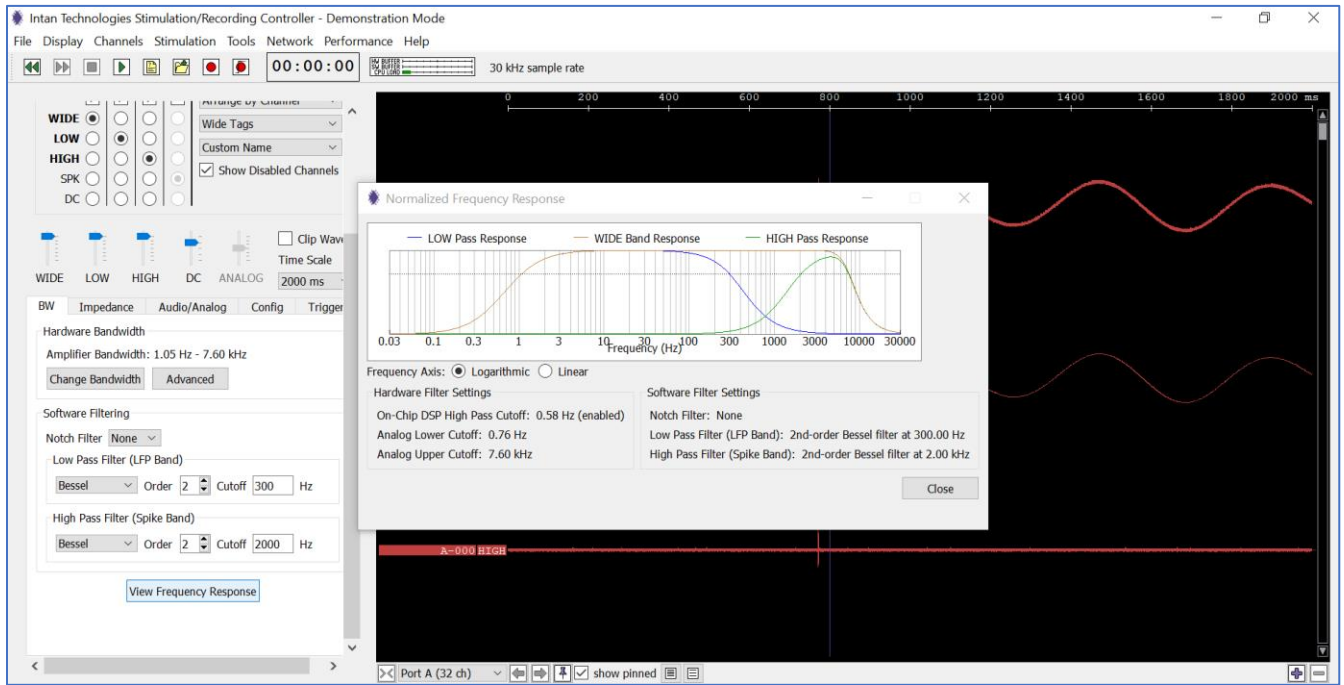
Fig. 64: Visualization of the bandwidth selection.

The low pass filtered, high pass filtered, and wideband waveforms can be displayed together in the Display Column by selecting the corresponding waveforms in the Filter Display Selector (green box in **Fig. 65**). The Display Column in **Fig. 65** shows the wideband waveform with frequencies of 1Hz to 7.5kHz, the low pass filtered waveforms for frequencies from 1Hz to 250Hz, and the high pass filtered waveforms for frequencies from 250Hz to 15kHz (yellow box in **Fig. 65**). The low pass filtered waveforms are typically used to examine low frequency potentials whereas the high pass filtered waveforms are used to examine action potentials.
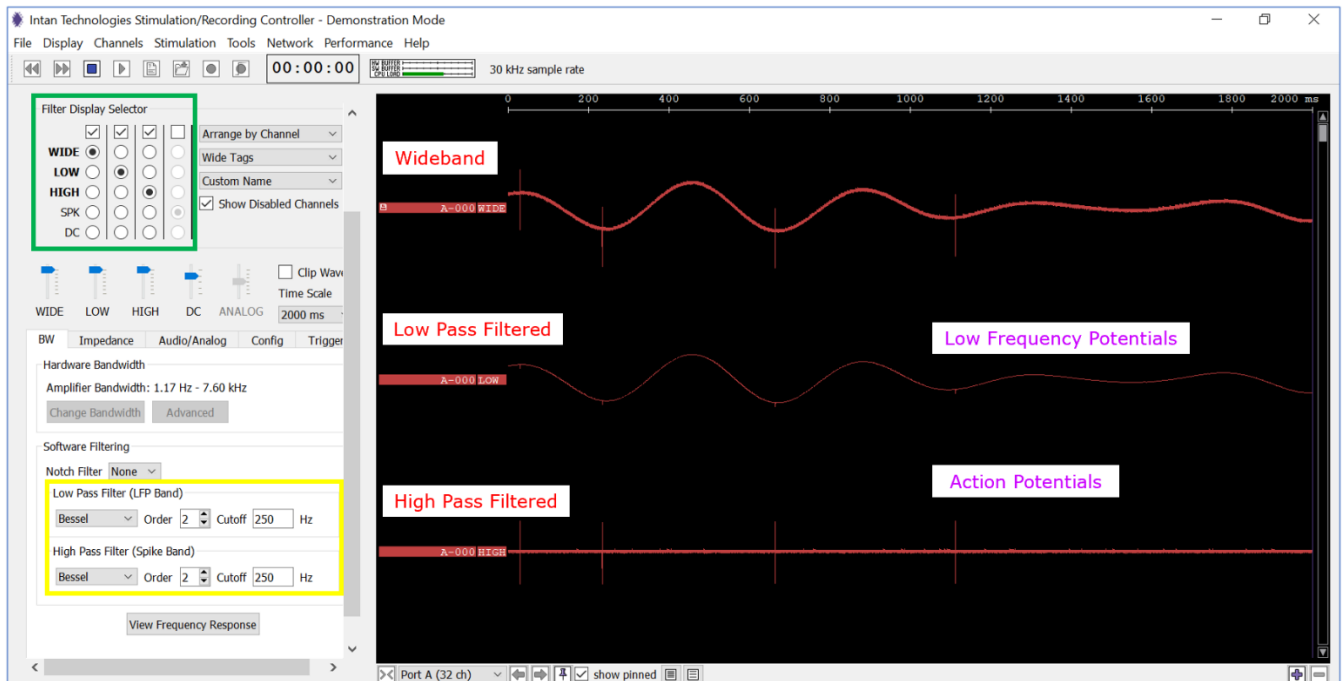

Fig. 65: Wideband, low pass filtered, and high pass filtered waveforms.

9.4.4 Audio/Analog tab

This tab controls the routing of select amplifier channels to the computer audio. It has four sections:

i) <u>PC Audio</u> (red box in **Fig. 66**)
This section controls the volume and background noise (Noise Slicer) of the audio signal. The level set by the noise slicer is the minimum signal level (prior to amplification) that will cause an audible sound.

ii) <u>Hardware Analog Out/Audio</u> (blue box in **Fig. 66**)
This section controls the amplification (gain) of the selected signal to be sent by the Analog-Out ports. A slider at the top allows users to select the total Electrode to ANALOG OUT gain (ranging from 1.6 mV/μV to 204.8 mV/μV). It is important to remember that the analog outputs limit at ±10.24V; large gain settings coupled with large signals from recording electrodes may lead to signal saturation.

iii) <u>Analog Out</u> (green box in **Fig. 66**)
This section controls the selection of the signal that is routed to the Analog-Out ports.

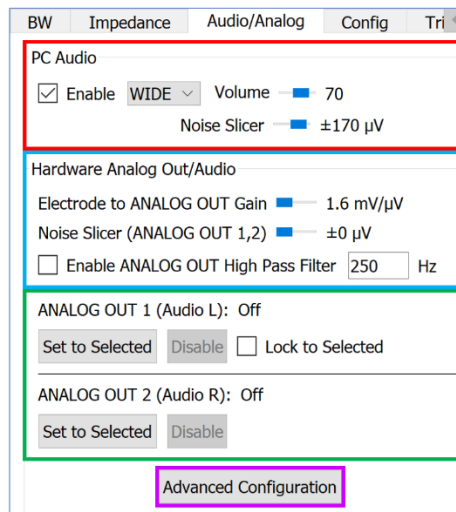iv) <u>Advanced Configuration</u> (purple box in **Fig. 66**)
See below.

Fig. 66: Audio/Analog tab showing Analog-In-1, Analog-Out-1, two stimulating and two recording channels.

9.4.5 Config tab

This tab allows the user to rescan all ports on the Intan controller and check for connected SPI cables. The software recognizes all ports with an SPI cable (third light on port turns red) upon startup. However, when SPI cables are disconnected from one port and reconnected to another, the Rescan Port function (red box in **Fig. 67**) allows to user to proceed without having to restart the software entirely.

In addition, the "Notes" box (blue box in **Fig. 67**) includes three single-line text boxes in which users may add informative text that will be saved in the header of any recorded data file. This may be used to annotate various experimental parameters. The "Live Notes" box (yellow box in **Fig. 67**) contains a text box and "Add Live Note" button. During a recording session, the user may type notes into the text box and click "Add Live Note"

at any time. A notes.txt file will be created in the recording subdirectory and timestamped Live Notes will be appended to this text file. This can be used to note important events during the course of an experiment. Each line in the notes.txt file lists the timestamp index (i.e., sample number), the timestamp in hh:mm:ss format, and the text note.
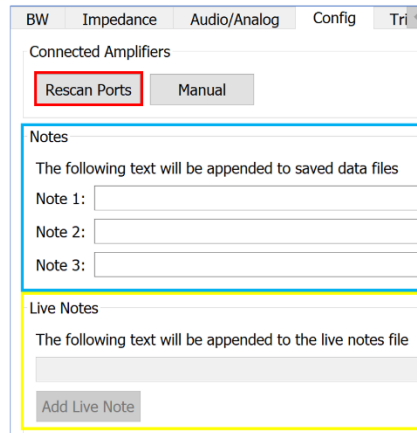

Fig. 67: Config. tab showing connected amplifiers, notes, and live notes.

9.4.6 Trigger tab

The Trigger tab allows the user to display the recorded data only for a set period of time before and after the trigger was enabled, i.e., not continuously.

Note: This function does not affect if the data are saved or not. You need to use the "triggered recording" button to save the data to disk.

The Trigger tab in the Control panel has 4 functions.

i)   Check the "Enable" box if you want to use this function (green box in **Fig. 68**). If the box is not checked, the waveforms are displayed continuously.

ii) The "Trigger Source" allows the user to select the input that triggers the display of data. In **Fig. 68**, the "Analog In 1" input from a signal generator is used as trigger (red box).

iii) The "Trigger polarity allows the user to choose between the recording being displayed for a "Rising Edge" or "Falling Edge" signal. In **Fig. 68**, the display of recorded data starts with a falling edge, i.e., when the trigger signal goes from a high value to a low value (pinned channel in blue boxes in **Fig. 68**).

iv) The "Trigger Position" tab allows the user to position the trigger on 6 different positions within the chosen timescale. The trigger can be placed on 1/10, ¼, ½, ¾, or 9/10 of the timescale (yellow box in **Fig. 68**). In the example in **Fig. 68**, the timescale is 4000ms. The Trigger Position tab allows the user to place the trigger at 1/10 (400ms), ¼ (1000ms), ½ (2000ms), ¾ (3000ms) or 9/10 (3600ms).
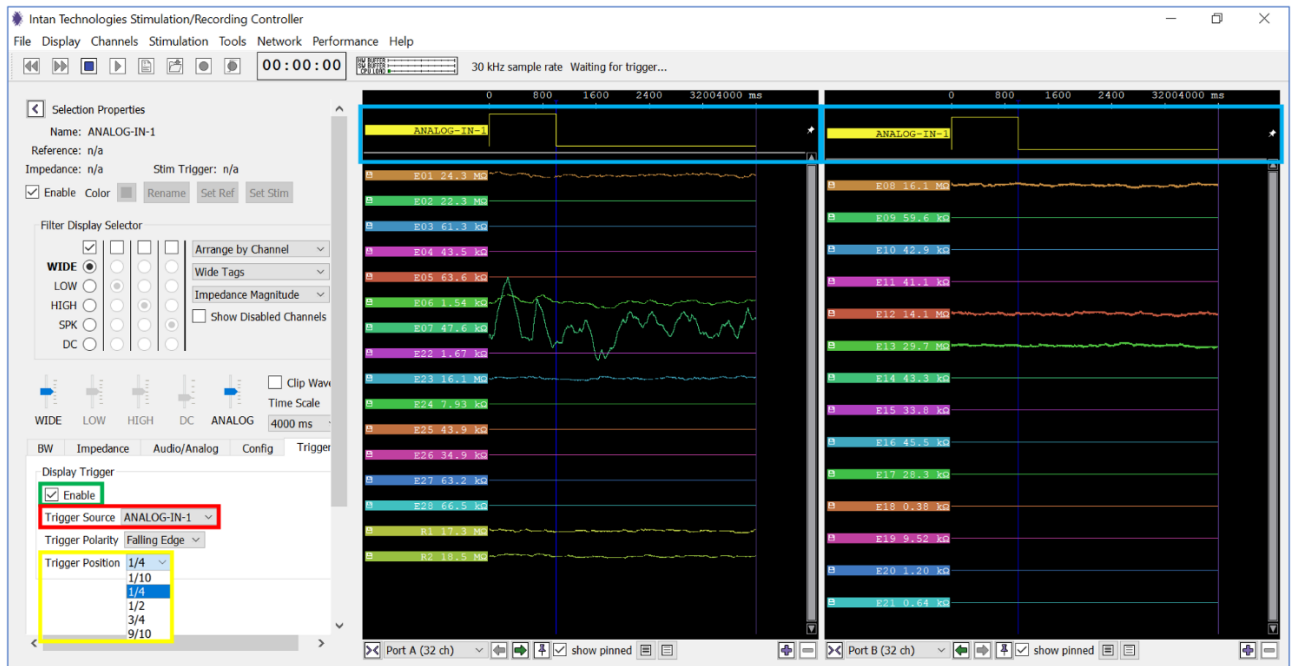
Fig. 68: Display waveforms after before and after a trigger.

10 Stimulation

The focus of this section is to demonstrate the stimulation using an analog input.

⚠️ If you are in "Run" or "Record" mode, click "Stop" before setting the stimulation parameters. Otherwise, the software will crash.

10.1 Setting Stimulation Parameters and Running a Stimulation Run

1) Select the channel you want to use for stimulation with a left mouse click in the waveform tag.

2) Click "Set Stim" under Selection Properties and the "Stimulation Parameters" window will pop up (red box in **Fig. 69**).
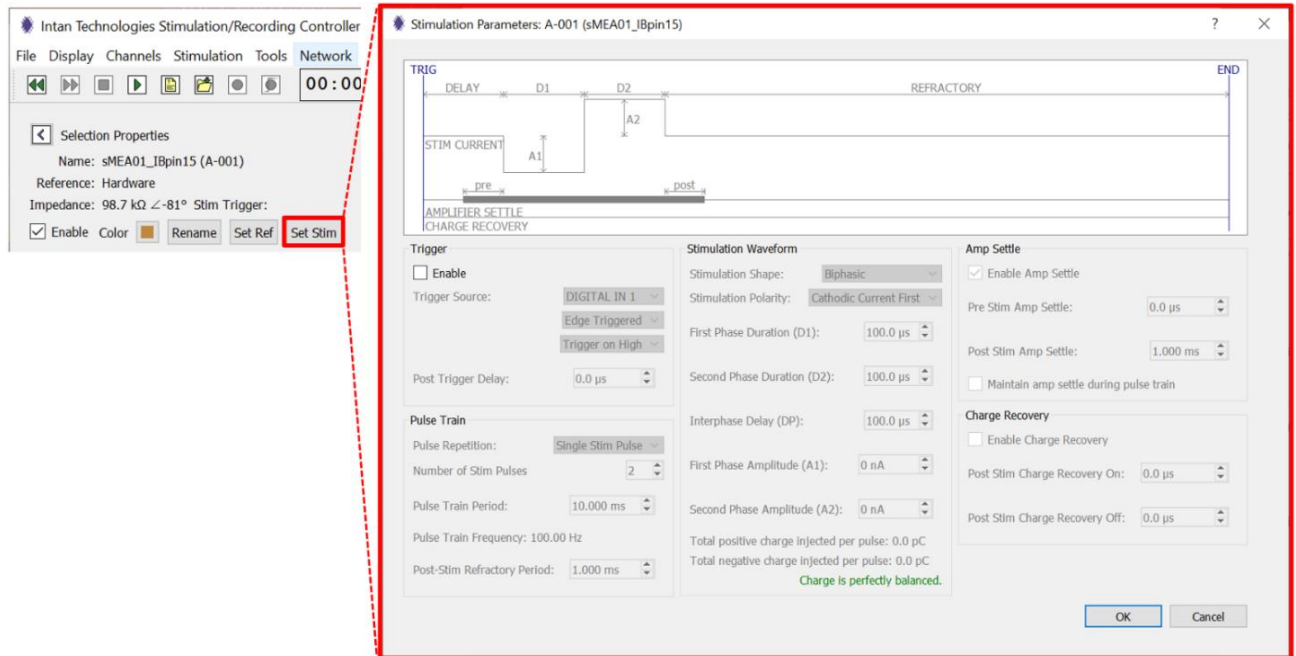

Fig. 69: "Stimulation Parameters" window.

The channel name is shown in the window title bar on the top ("A-001 (sMEA01_IBpin15)" in the **Fig. 69** above). A schematic illustration of the stimulation sequence (not to scale) is shown in the upper part of the "Stimulation Parameter" section. When the stimulation parameters are chosen in the lower section of this window, the relevant arrows and labels will be highlighted in the schematic with the white background on top.

3) To access and adjust the stimulation parameters, the stimulation trigger must first be enabled by checking the "Enable" box (red box in **Fig. 70**).
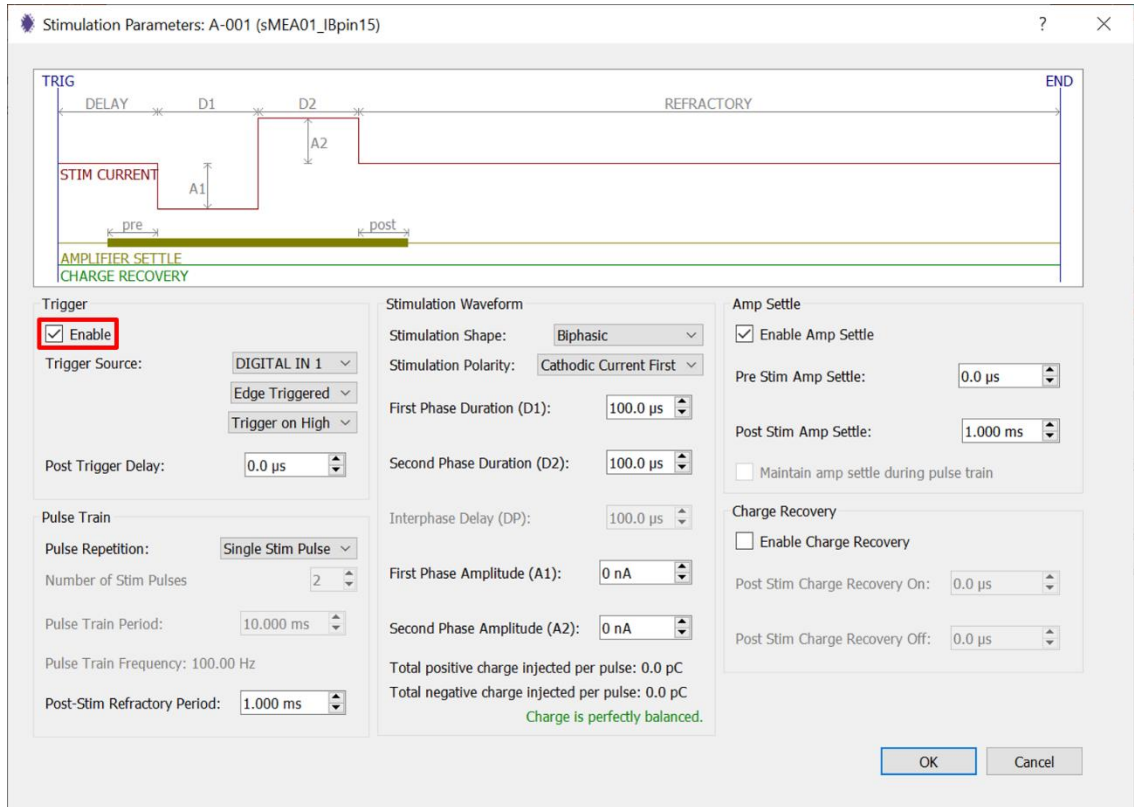

Fig. 70: Stimulation Parameters window with the "Enable" box checked.

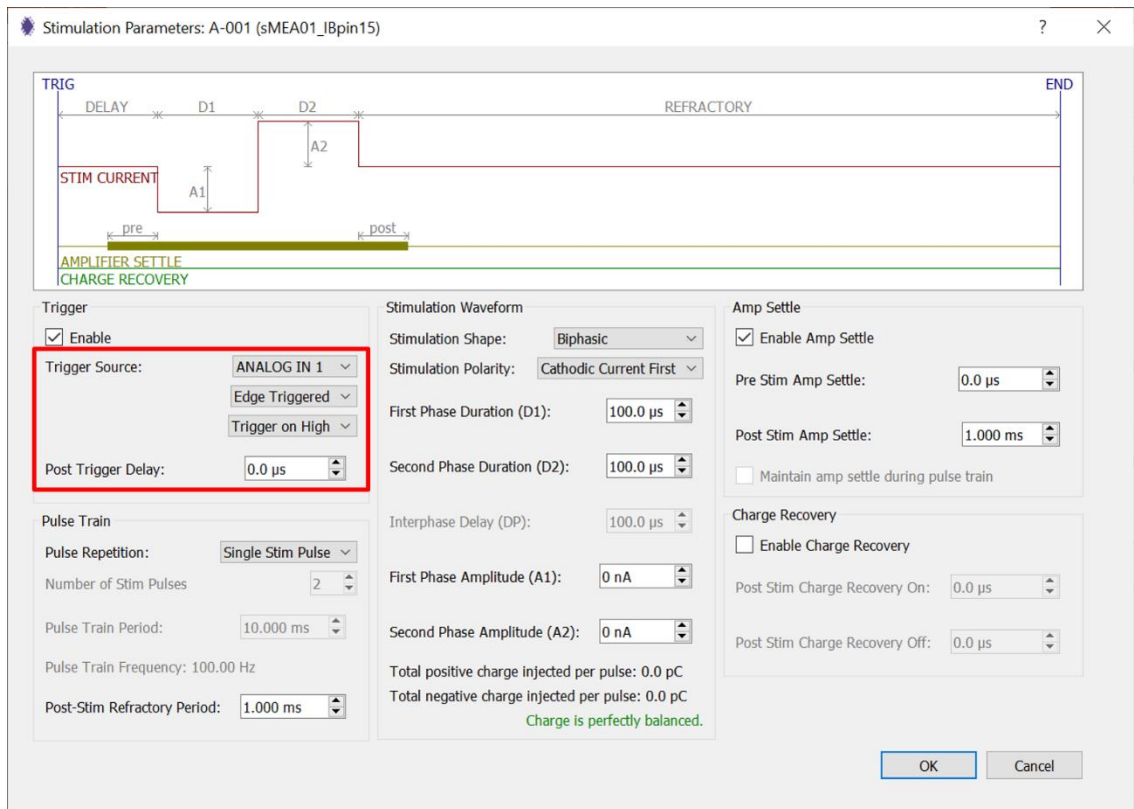4) The "Trigger" panel allows the user to set trigger parameters (red box in **Fig. 71**).

Fig. 71: Stimulation Parameter window with the trigger options highlighted.

- The **trigger source** can be either an analog or digital input, depending on the nature of your trigger. In the setup shown below, an analog signal from a function generator is used as a trigger, and "ANALOG IN 1" is selected for triggering. For triggering purposes, analog signals act like digital signals when the threshold of +1.65V is surpassed (i.e., signal is $\geq$ +1.65V). We recommend, whenever possible, using a signal generator and set the trigger source as "ANALOG IN 1."
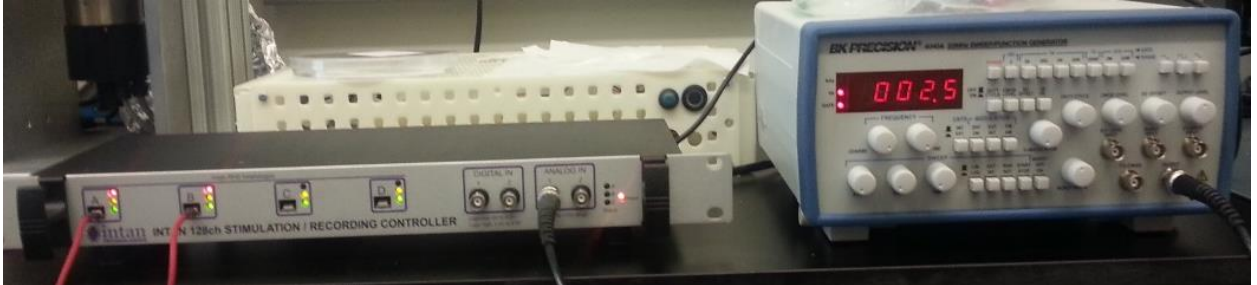

Fig. 72: Equipment setup with a function generator as the analog input trigger.

Alternatively, key presses on the host computer with keys "F1" through "F8" can be used as manual triggers as well.

Tests performed at BMSEED suggest that key presses generate artifacts with larger amplitudes and longer durations than analog signals when used as triggers. Thus, we recommend avoiding key presses as trigger sources when possible.

- The **trigger type** can be set to either "edge triggered" or "level triggered." Selecting "edge triggered" causes a stimulation sequence to execute once every time the trigger transitions from an inactive state to an active state. Selecting "level triggered" causes a stimulation sequence to execute and then repeat as long as a trigger source remains in the active state. We recommend selecting "edge triggered." Note that the stimulating electrodes are labeled with a symbol of a stylized stimulus pulse (red box in **Fig. 73**).
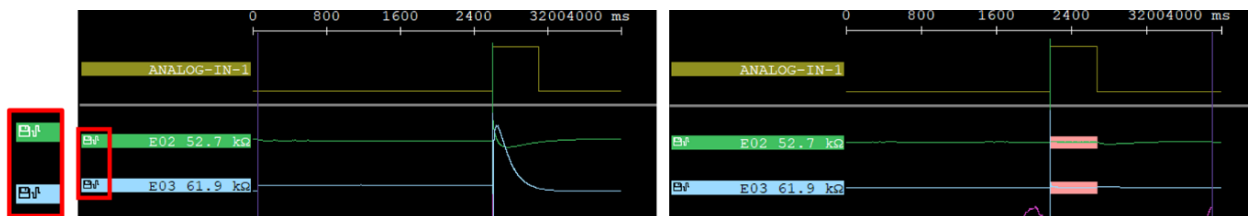

Fig. 73: Edge Triggered (left) and Level Triggered (right), both triggered on high.

- **Trigger activation** can be set to either "trigger on high" or "trigger on low." "High" or "low" refers to the logic level that will be considered as active.

  o When defining the trigger source as an analog input from a function generator, setting the "trigger on high" (**Fig. 74**, left) means that the stimulus pulse will be provided when the signal voltage transitions 1.65V from a lower voltage state (e.g., from 0V to +2V). Conversely, setting the "trigger on low" (**Fig. 74**, right) means that the stimulus pulse will be provided when the signal voltage transitions 1.65V from a higher voltage state (e.g., from +2V to 0V)

  o When defining the trigger source as key presses, a "high" logic level corresponds to a when the key is pressed; conversely, a "low" logic level corresponds to when a key is not being pressed.
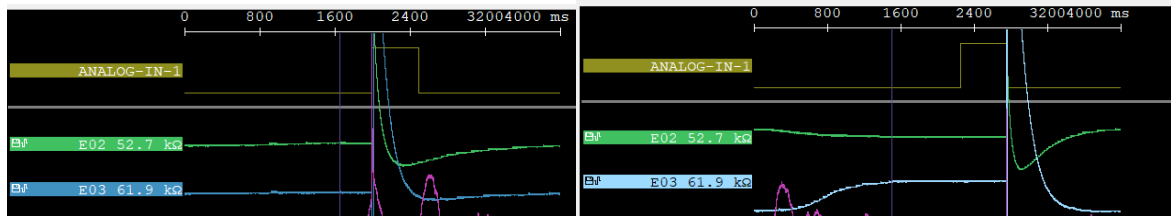
Fig. 74: Trigger on high (left) and low (right).

- o We <u>recommend</u> selecting "trigger on high."

- By default, the stimulation sequence is executed immediately following a trigger event. The **post trigger delay** allows to delay the execution of the stimulation sequence by up to 500ms.

5) The "<u>Pulse Train</u>" panel (**Fig. 75**) allows the user to set stimulus pulse parameters.
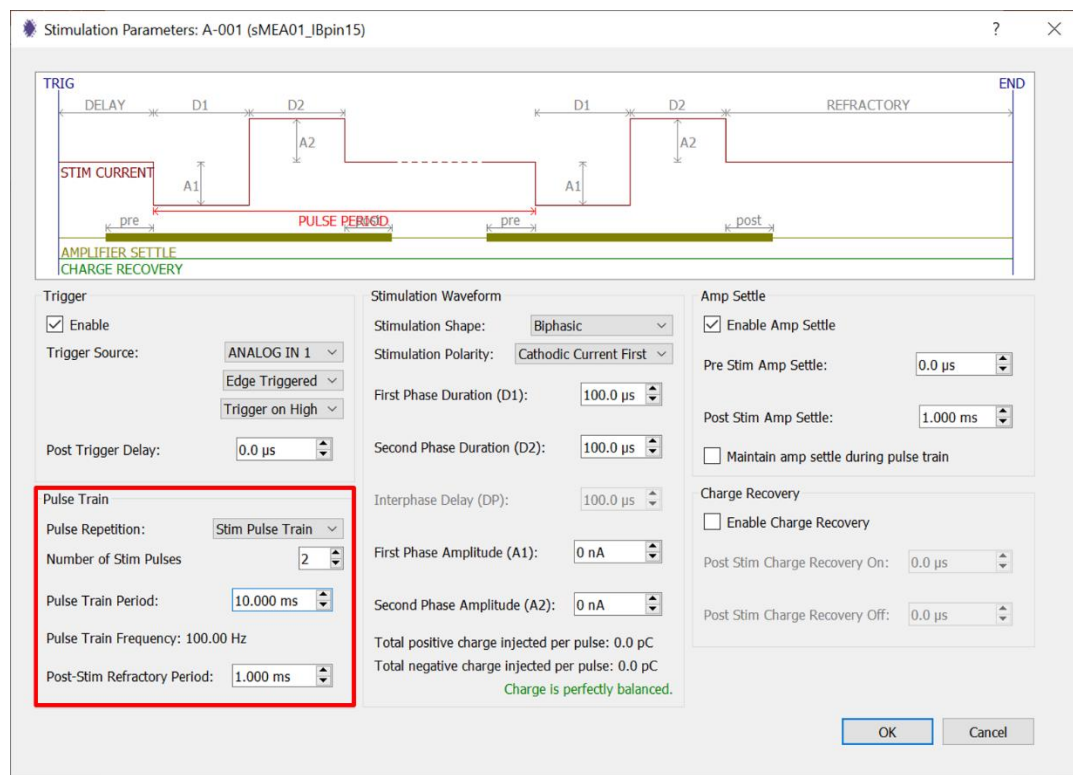

Fig. 75: Stimulation Parameter window with the pulse train panel highlighted.

- A stimulation sequence may consist of a single stimulation pulse or a train of multiple, identical pulses. The pulse repetition selector allows the user to choose between these two options. The **pulse repetition** can be set to either "single stim pulse" and "stim pulse train." Set to this menu to "stim pulse train" if a trigger needs to set off more than one pulse.

- The **number of stim pulses** can be set between 2 and 256 pulses. This option is only available when pulse repetition is set to "stim pulse train."

- The **pulse train period** is the time from the beginning of a pulse to the beginning of the subsequent pulse in the pulse train. This parameter can be set between 200.0μs and 999.99ms. This option is only available when pulse repetition is set to "stim pulse train." The corresponding frequency is displayed underneath the pulse

train period menu. This option is only available when pulse repetition is set to "stim pulse train."

- After a stimulation sequence has completed, additional trigger events will be ignored after during the **post stim refractory period**. This parameter can be used to configure pulse trains of indefinite duration by selecting single stimulation pulses along with level triggering. As long as the trigger source remains high, the single stimulation pulse will repeat at a rate set by the post-stimulation refractory period. This period may be up to one second in length. This option is available when pulse repetition is set to either "single stim pulse" or "stim pulse train."

- **Fig. 76** shows two examples for pulse train stimulation. The top panel (red box) shows a train of 4 pulses with the pulse train period of 100ms. The image shows 4 pulses that are triggered on high spaced at 100ms. The bottom panel (blue box) shows a train of 100 pulses with the pulse train period of 10ms. The image shows 100 pulses that are triggered on high spaced at 10ms.
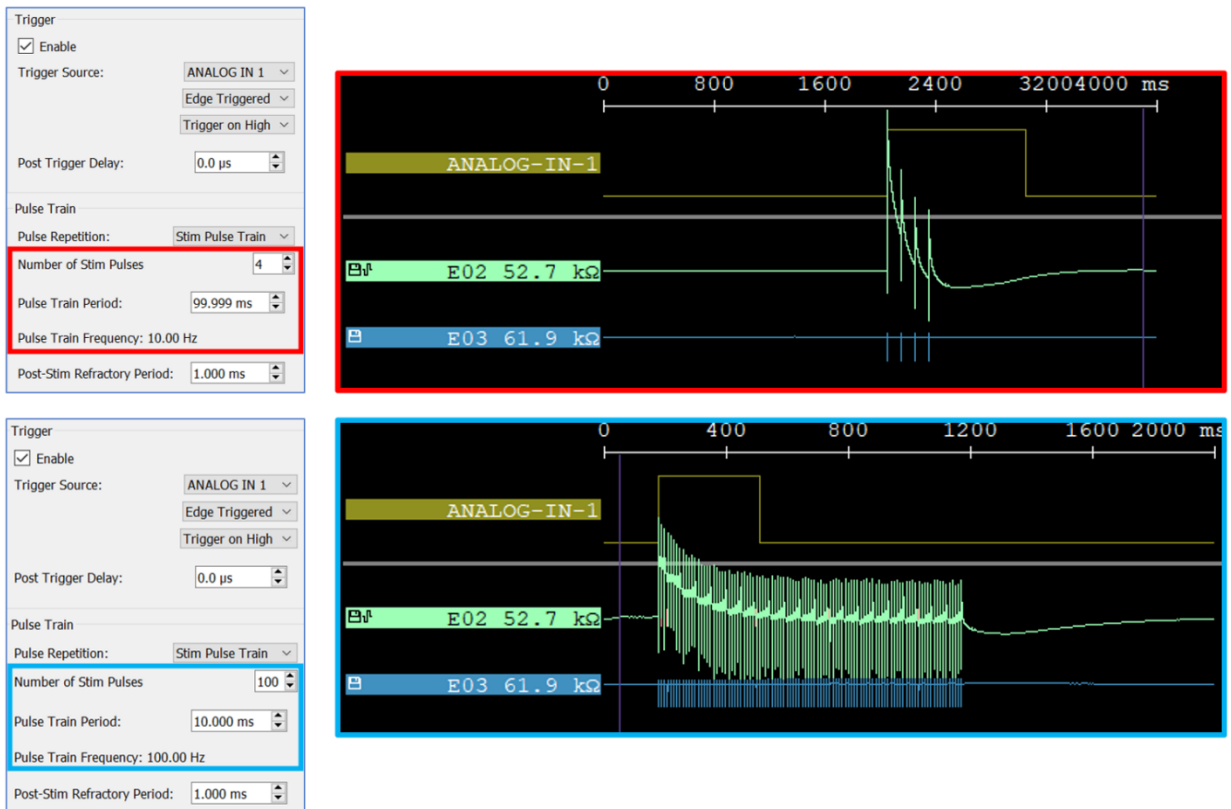

Fig. 76: Stimulation Parameter window with the pulse train panel highlighted.

6) The "Stimulation Waveform" panel (red box in **Fig. 77**) allows the user to specify each stimulus (single or pulse train) parameters, such as waveform shape, polarity, phase duration, and phase amplitude.
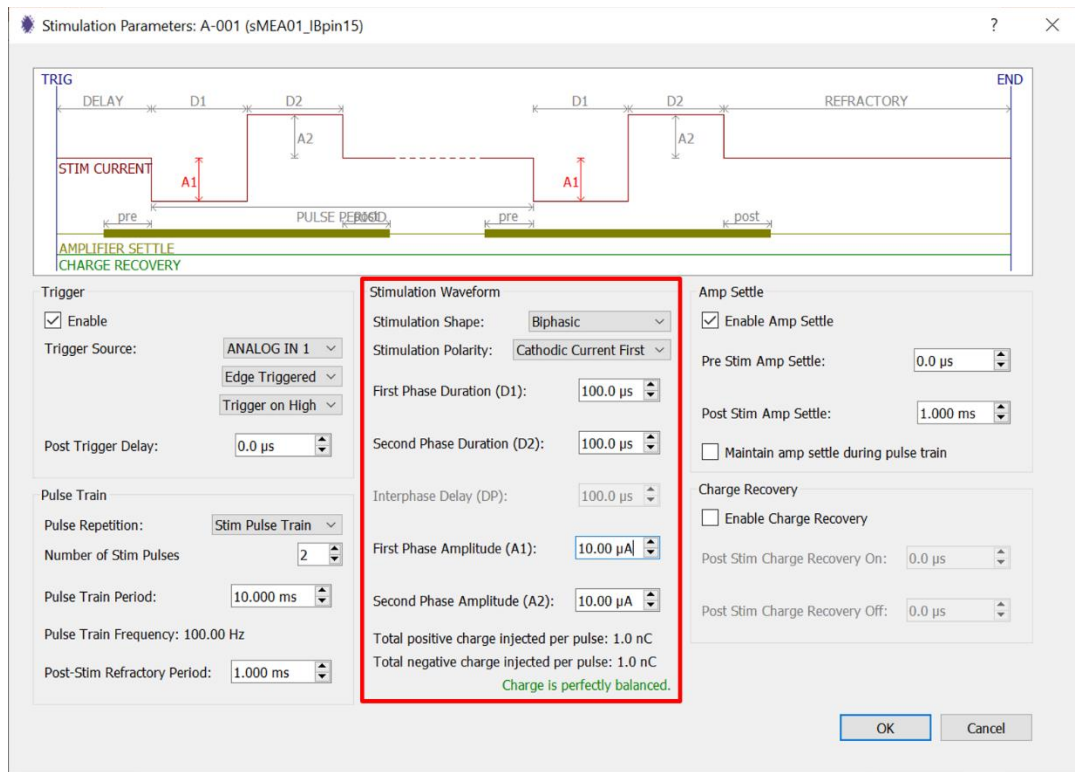
Fig. 77: Stimulation Parameter window with the stimulation waveform panel in red.

- Three **stimulation shapes** are supported: biphasic, biphasic with interphase delay, and triphasic.
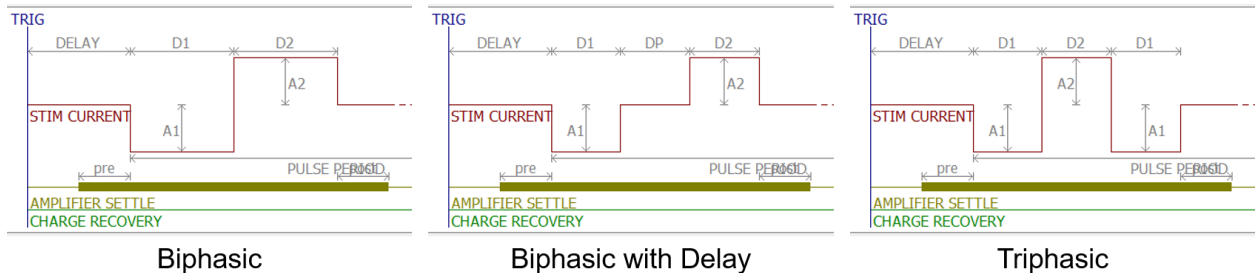

Biphasic   Biphasic with Delay   Triphasic

Fig. 78: Schematics of the three available stimulation waveforms.

- The **stimulation polarity** can be set to either "cathodic (negative) current first" or "anodic (positive) current first." The waveforms shown in the figure above show a cathodic current first polarity, which is the standard practice in most stimulation experiments.

- The **first phase duration (D1)** can be set between 0µs and 5ms.

- The **second phase duration (D2)** can be set between 0µs and 5ms.

- The **interphase delay (DP)** can be set between 0µs and 5ms. Note that this parameter is only available if the stimulation shape is set to "biphasic with delay."

- The **first phase amplitude (A1)** can be set between 0nA and 2.55mA.

- The **second phase amplitude (A2)** can be set between 0nA and 2.55mA.

⚠️ **IMPORTANT:** Note that the maximum stimulation current as well as the time and amplitude resolution are set when selecting the sampling rate when launching the IntanRHX software.

The product of current amplitude and duration for each stimulation phase equals the **charge injected** in this phase. To reduce electrochemical reactions at the electrode and to extend electrode life, it is generally recommended that the net charge injection during the entire pulse is zero, i.e., that the positive and negative charges injected during the cathodic and anodic phases are equal and cancel each other out. Displayed text at the bottom of the "Stimulation Waveform" window shows whether the charges are balanced (green) or unbalanced (red).

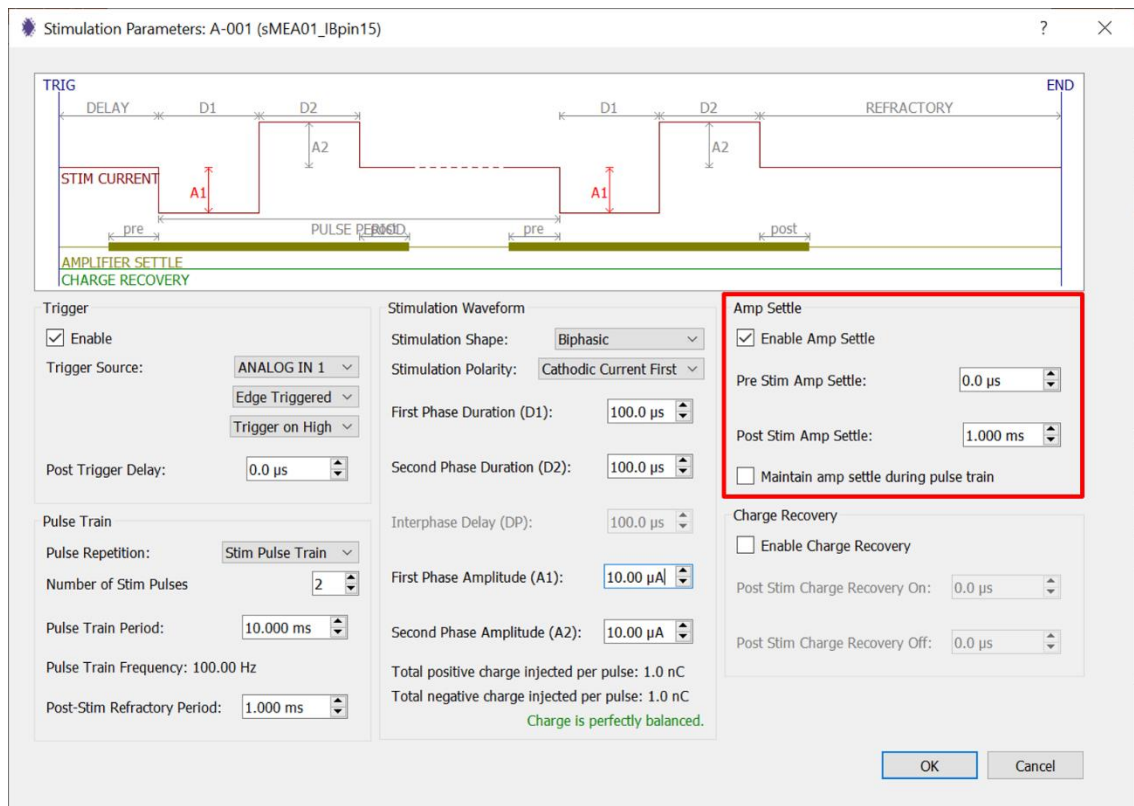7) The "Amp Settle" panel (**Fig. 79**) allows the user to set amplifier settle function parameters.

Fig. 79: Stimulation Parameter window with the "Amp Settle" functions highlighted.

- A stimulation pulse induces a large voltage transient in microelectrodes, which makes it difficult to observe weak electrophysiological signals immediately following a stimulation pulse. The "**enable amp settle**" function can help reduce the time it takes for the voltage produced during the stimulation pulse to return to baseline level after experiencing a large stimulation artifact. Checking the Enable Amp Settle box activates this functionality.

- The **pre stim amp settle** time is the time before the stimulation that the "amp settle" function should engage. This value is typically set to 0µs.

- The **post stim amp settle** time is the time the "amp settle" function should remain engaged after the stimulation pulse has ended. Experiments by Intan have shown

that a value of 1ms seems to work well. Since the optimal value could be different, the user is encouraged to experiment with it.

- The **maintain amp settle during pulse train** box can be checked to keep the "amp settle" function enabled during the execution of a pulse train stimulus.

8) There are two methods for settling the amplifier after a stimulation pulse, and either of these methods may be selected in the amplifier settle configuration window (see figure below), which is accessed by selecting "Amplifier Settle Settings" under the Stimulation menu. The window below on the right will pop up.
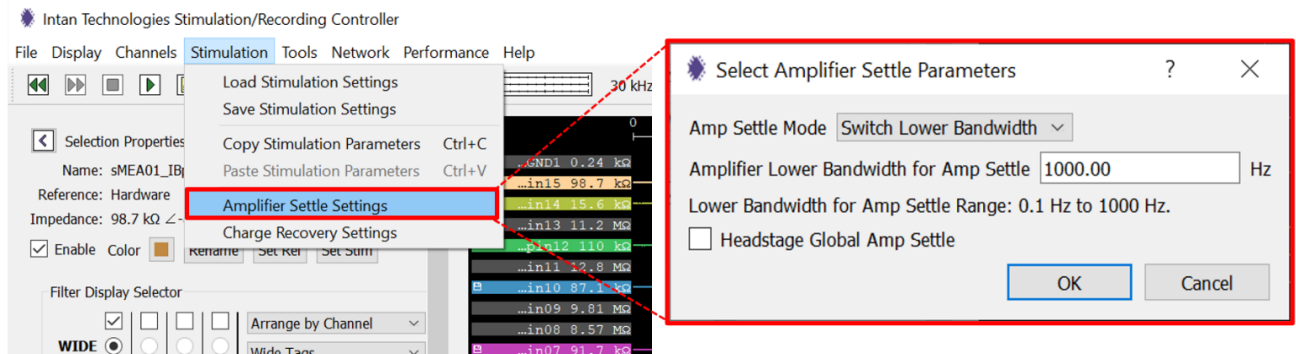

Fig. 80: Accessing the "Select Amplifier Settle Parameters" window.

- **Amp settle mode** can be set to either "switch lower bandwidth" and "traditional fast settle." "Switch Lower Bandwidth" is the default and <u>recommended</u> mode in which the lower bandwidth of the amplifier is changed to a new value during the "amp settle" period.
The "Traditional Fast Settle" mode was a technique used in previous Intan series of amplifier chips that does not seem to be as effective in recovering from stimulation artifacts as the "switch lower bandwidth" mode.

- The default and <u>recommended</u> **amplifier lower bandwidth for amp settle** value is 1000Hz, but values between 0.1Hz and 1000Hz could be used as well.

- The **headstage global amp settle** function activates the amplifier settle function on all channels of the headstage. If this box is checked, an "amp settle" event on any channel in a particular headstage will activate the amp settle functions in all channels on that headstage. The yellow shading in the waveform plots makes it clear when this is happening. The Omnetics connector used on Intan stim/record headstages has approximately 0.15pF of capacitance between adjacent pins which can lead to crosstalk between channels, especially when a volt-level stimulation pulse occurs while trying to observe microvolt-level electrophysiological signal on a nearby channel. This headstage global amp settle function can help reduce this crosstalk.

9) The "<u>Charge Recovery</u>" panel (**Fig. 81**)
This function allows the user to set charge recovery parameters. Positive or negative current sources in each RHS headstage are not perfectly matched due to random variations in transistors. This means that ideal charge balance cannot be obtained in practice and charges may build up over time on the electrode even if the charges injected during the cathodic and anodic stimulation phases are perfectly balanced. For acute experiments this may not have any practical consequence, but for long-term

chronic experiments, residual post-stimulation charge may have deleterious effects. To mitigate the effects of these residual charges, RHS headstages have the capability to perform charge recovery by forcing an electrode to ground, or to another fixed voltage between -1.225V and +1.215V. For any channel, charge recovery may be enabled by checking Enable Charge Recovery, and the onset and duration of charge recovery after the end of a stimulation pulse may be specified. The green bar in the stimulation waveform display illustrates (not to scale) the timing of charge recovery.
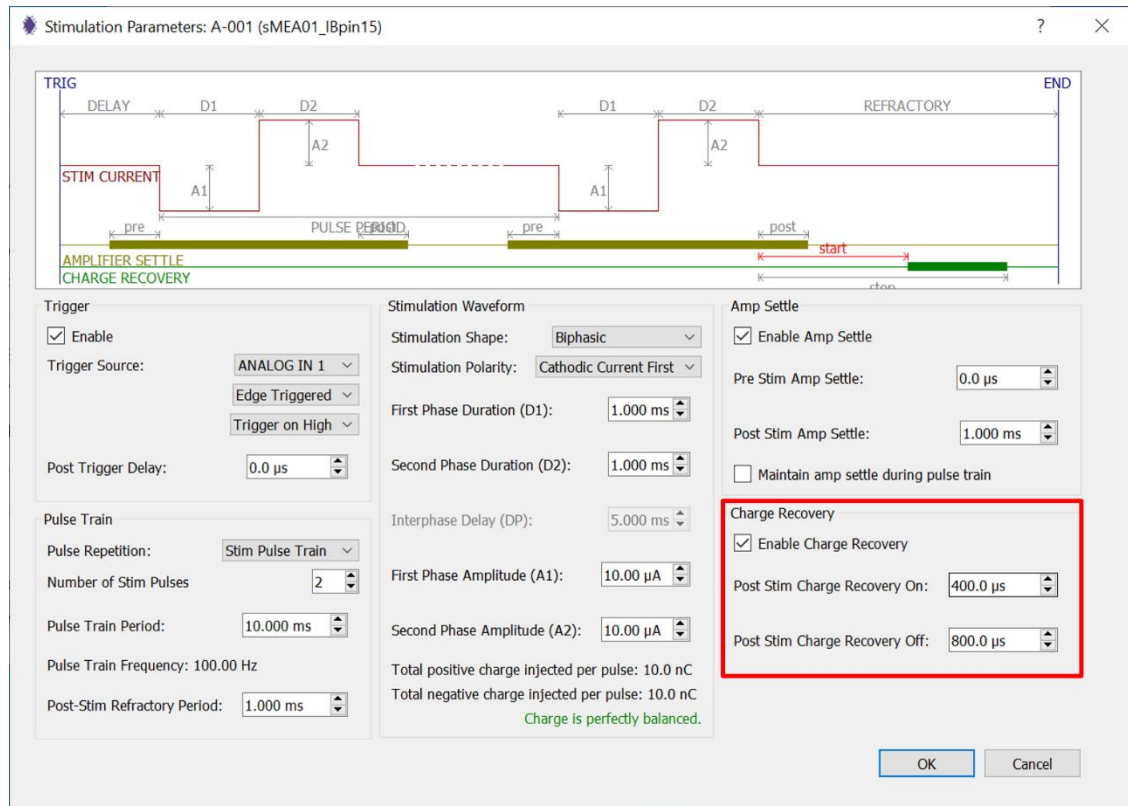

Fig. 81: Stimulation Parameter window with the "Charge Recovery" panel in red.

- The **enable charge recovery** box can be checked to enable the charge recovery function for a specific channel. By default, this box is unchecked or disabled.

- The **post stim charge recovery on** specifies the onset of the charge recovery function after the end of the stimulus pulse. This value cannot be greater than the post-stim refractory period.

- The **post stim charge recovery off** specifies the end of the charge recovery function after the end of the stimulus pulse. This value cannot be greater than the post-stim refractory period or lower than the post stim charge recovery on parameter.

IMPORTANT: The duration of charge recovery equals the difference in time between "Post Stim Charge Recovery On" and "Post Stim Charge Recovery Off".

10) There are two types of charge recovery that may be selected globally for all channels in all headstages. This selection is accessed in the charge recovery configuration window (**Fig. 82**) by selecting "Charge Recovery Settings" under the Stimulation menu.
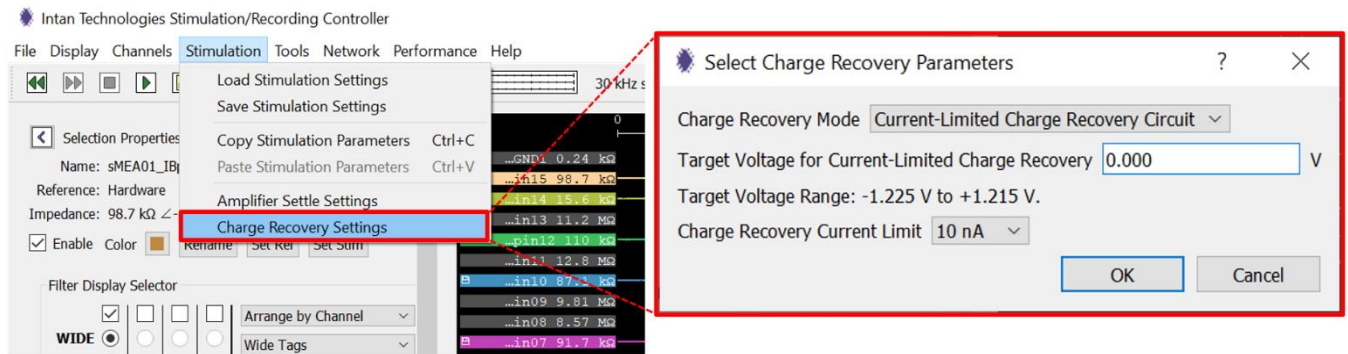
Fig. 82: Accessing the "Select Charge Recovery Parameters" window.

- The **charge recovery mode** can be set to either "current-limited charge recovery circuit" or "charge recovery switch."

- **current-limited charge recovery circuit**
  In this mode, the electrode potential is pulled towards a fixed voltage with a driver that has a limited current drive capability. The **target voltage for current-limited charge recovery** can be set to a range of -1.225V to +1.215V and the **charge recovery comment limit** can be set to values between 1 nA and 1 μA.

- **charge recovery switch**
  The "charge recovery switch" mode activates a transistor-based switch that connects the electrode to ground. This switch has an 'on' resistance on the order of 1kΩ.

⚠️ Charge Recovery events often induce artifacts during recording.

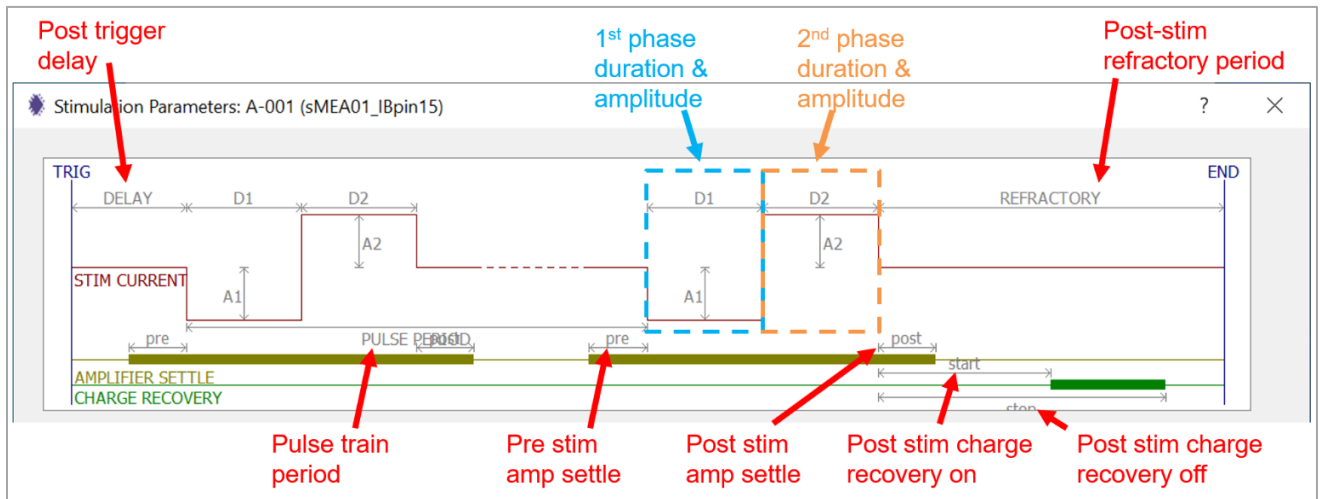11) Once all parameters have been set in the "Stimulation Parameters" window, click "OK."

Fig. 83: Schematic showing all stimulation parameters.

12) In the main IntanRHX window click the "Run" button (▶).

13) **Fig. 84** below shows a snapshot of the GUI running with channel "sMEA01_IBpin15 (A-001)" set as the stimulating channel. With the channel selected, the stim trigger is displayed as "AnalogIn01" under Selection Properties. In addition, the channel tag displays a pulse wave icon to label this channel as a stimulating channel.

In the example shown in the screenshot below, the stimulation is triggered by a signal generator connected to the Analog Input 1 port with the following settings:

- Waveform: pulse wave
- Frequency: 2Hz
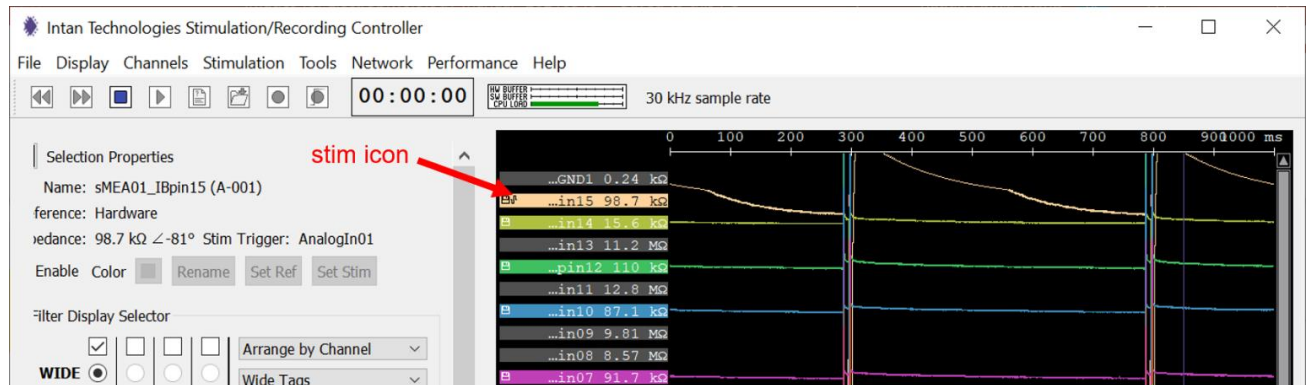- Amplitude: 2V
- Offset: 2V
- Duty cycle: 50%

Fig. 84: Stimulation run with "sMEA01_IBpin15 (A-001)" set as the stimulating electrode.

10.2 Copying, pasting, saving, and loading stimulation parameters

Once stimulation parameters have been set for one channel, they can be copied and pasted to other channels. Follow these steps:

1) Select the channel already configured with the stimulation parameters.

2) Click "Stimulation" on the top toolbar and select "Copy Stimulation Parameters."

3) Select the channel you want to paste the stimulation parameters.

4) Click "Stimulation" on the top toolbar and select "Paste Stimulation Parameters." Note that his option will only be available if stimulation parameters have been copied.

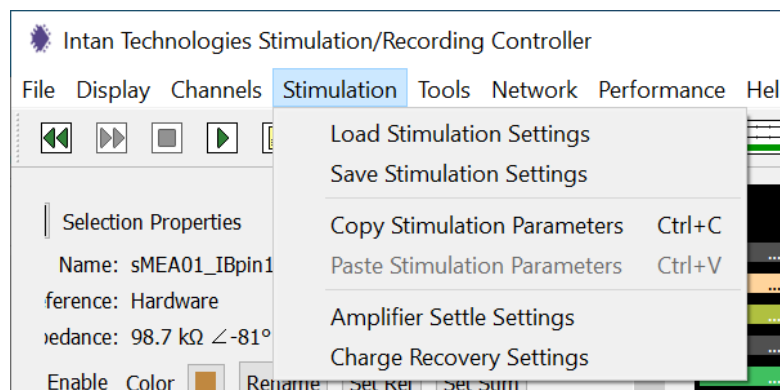5) Repeat steps 1 through 4 for additional channels you want to paste the stimulation parameters.

Fig. 85: Accessing the stimulation parameters and stimulation settings functions.

The stimulation settings for all channels can be saved in XML format by selecting "Save Stimulation Settings." To load the stimulation settings, click "Load Stimulation Settings", and select the file with the stimulation parameters that you want to use.

⚠️ Stimulation settings are saved separately from general settings and should be loaded after general settings.

10.3 Color coding of waveform plots

When a stimulation pulse occurs, background areas of the stimulating channel waveform are color coded to indicate the different parts of the stimulus:

- When a stimulation pulse occurs, the background of the waveform plot is highlighted in **light red** for the **duration of the current pulse**. Note that no graphical distinction is made between anodic and cathodic current.

- When the **amp settle is engaged**, the background is highlighted in **light yellow**.

- If the charge recovery function es enabled, the **duration of the charge recovery** will be highlighted in **light green**.
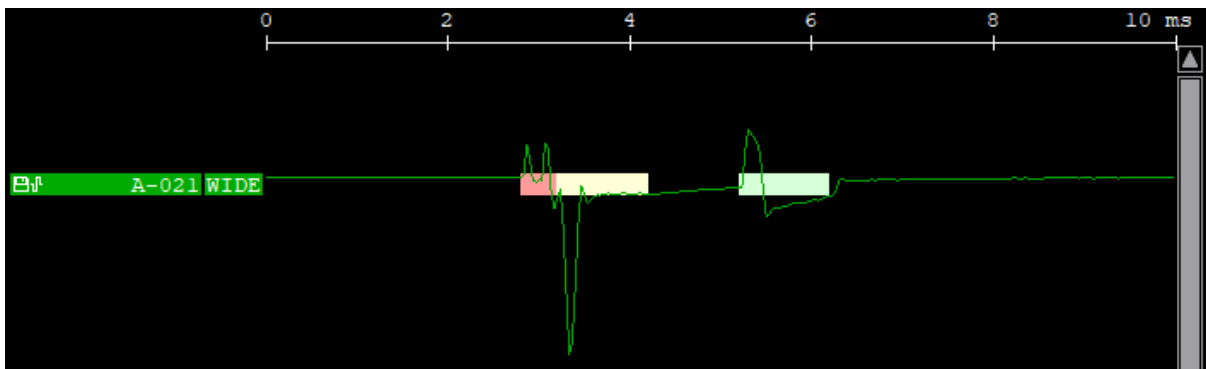

Fig. 86: Example of color coding of a stimulating channel waveform. Image extracted from Intan's "IntanRHX User Guide."

If the voltage during stimulation is close to the compliance limit of the power supply of ±9V, a **compliance limit warning** is shown by a **dark red highlight** on the waveform plot. This warning often indicates that the electrode impedance is too high to produce the requested current within the compliance voltage limit, which often indicates a damaged or broken electrode.
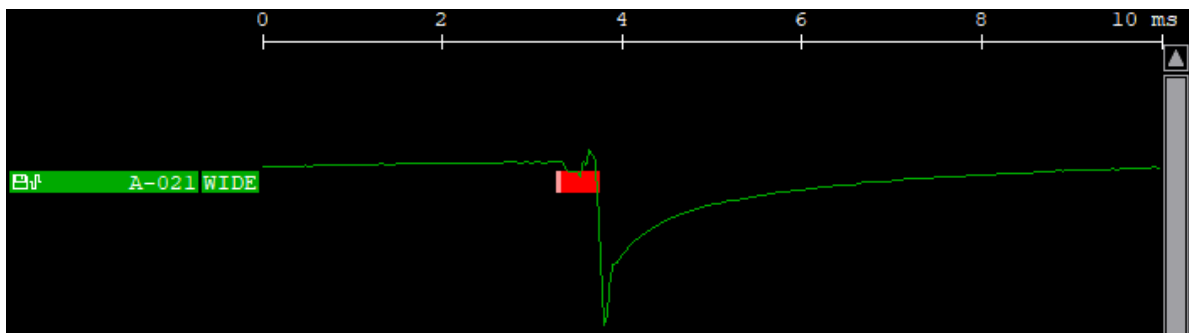

Fig. 87: Example of a stimulating channel exhibiting a compliance limit warning. Image extracted from Intan's "IntanRHX User Guide."

Reducing the stimulus current and/or duration, or using a lower impedance electrode are methods to circumvent the compliance limit issue.

10.4 Viewing other data streams

In the "Port Selector" drop-down menu in the "Display Column" toolbar, the user can select to display the analog input ports, analog output ports, digital input ports, and digital output ports.

For example, to display analog input 1: select "Analog In Ports" in the port selector, select channel "ANALAOG-IN-1", and enable this channel.
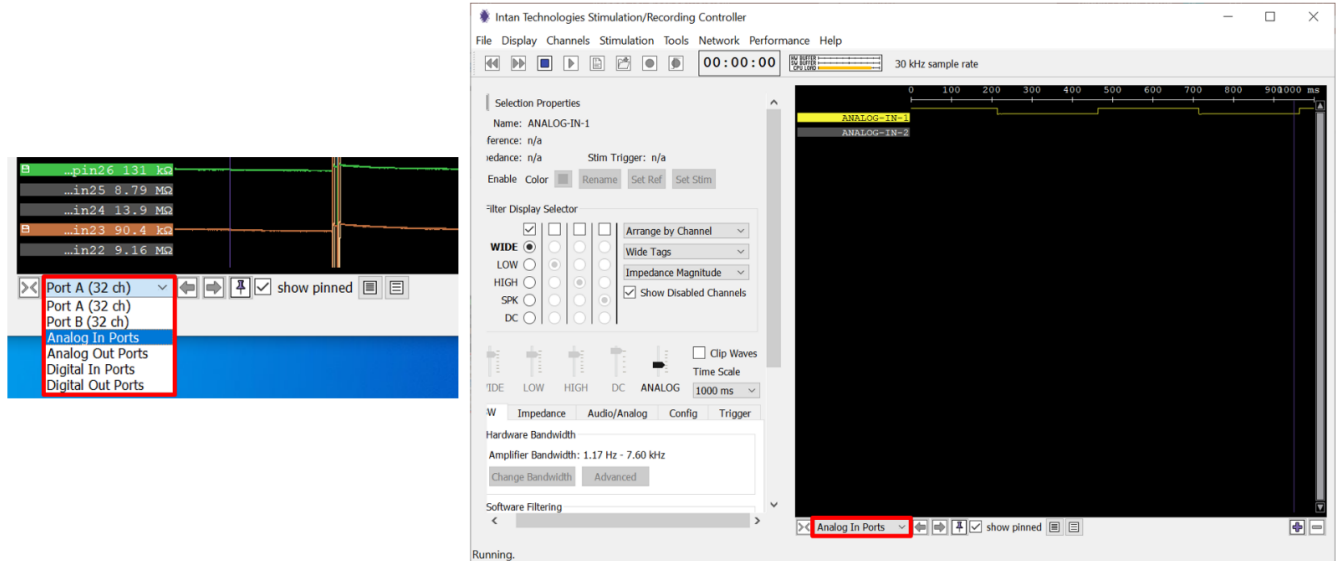

Fig. 88: The Port Selector menu and GUI displaying analog input 1 waveform from the signal generator.

11 Triggered Recording of Electrophysiology Activity with Stimulation

This section describes the procedure for automatically recording electrophysiological activity using the stimulation signals as a trigger. This feature is useful when only the signals that immediately follow the stimulation pulse are of interest (**Fig. 89**). Setting up triggered recording saves disk space. We are using the Audio/Analog tab in the Control Panel for this purpose.
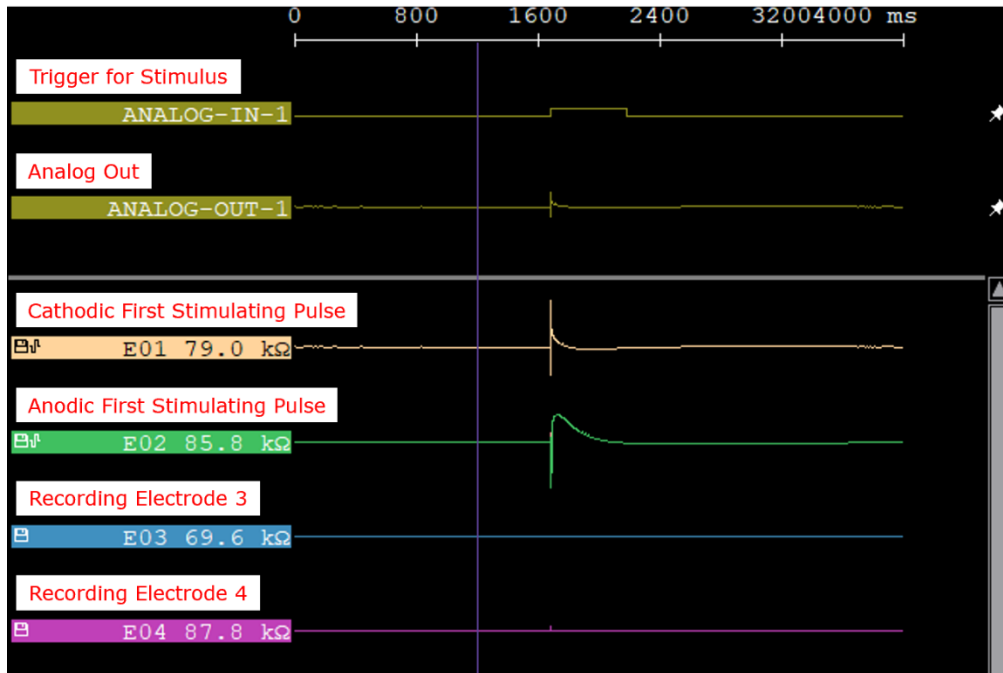

Fig. 89: Analog-In-1, Analog-Out-1, two stimulating and two recording channels.

1) As described above for triggered recording, connect the output of the signal generator to the Analog Input 1 of the Intan controller. This signal will be used to trigger the stimulus, which in turn will be used to trigger data recording. We show below how this is accomplished.

2) Set the stimulation parameters as described in **Section 10.1** steps 1) through 11).

3) Connect the Analog Output 1 port (back of the Intan controller) to the Digital In 1 port (front of the controller) using a BNC cable (**Fig. 90**). The headstages communicate with the controller using purely digital signals, so this allows the selected signal(s) to be reconstructed to analog signals.

Fig. 90: "Analog Out 1" connected to "Digital In 1" on Intan controller.

4) Set the analog output signal that will be used as the trigger for data recording after stimulation:

   i) Select the "cathodic current first" stimulating channel (left-click). Following the example used in this manual, channel "E01" was selected.

ii) Go to the "Audio/Analog" tab of the Control Panel (red box in **Fig. 91**).

iii) Under the "ANALOG OUT 1 (Audio L):" section, click "Set to Selected" (blue box in **Fig. 91**). The stimulating channel will now be displayed next to the "ANALOG OUT 1" label (blue underline in **Fig. 92**). The signal from the selected channel (A-001) will now be routed to the Analog-Out-1 port.

iv) Note that the generic name of the channel with be displayed (here: A-001), not the name of the channel if it was changed (here: E01).
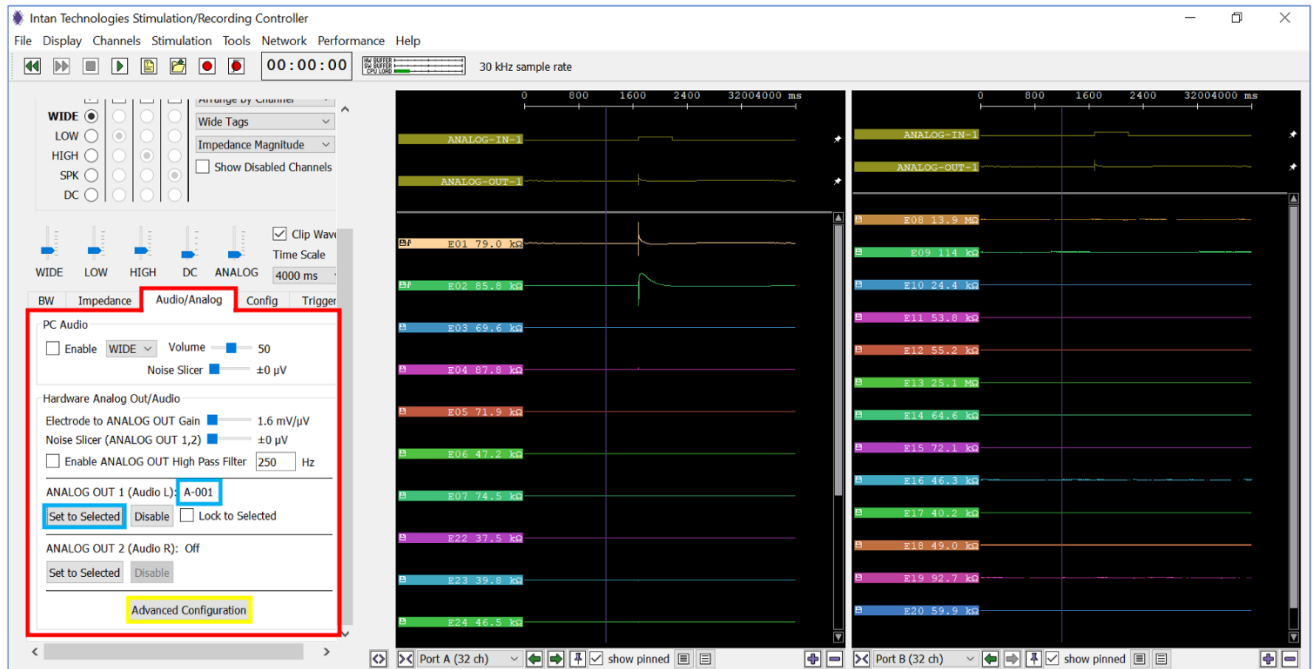
Fig. 91: Setting the analog output 1 signal to the stim channel to trigger data recording.

v) Click the Advanced Configuration button (yellow box in **Fig. 91**).

vi) In the "Analog Output Configuration" window, set the "DIGITAL OUT 1 Threshold" to 6000µV and check the "Enable" option to the right (red underlines in **Fig. 92**).
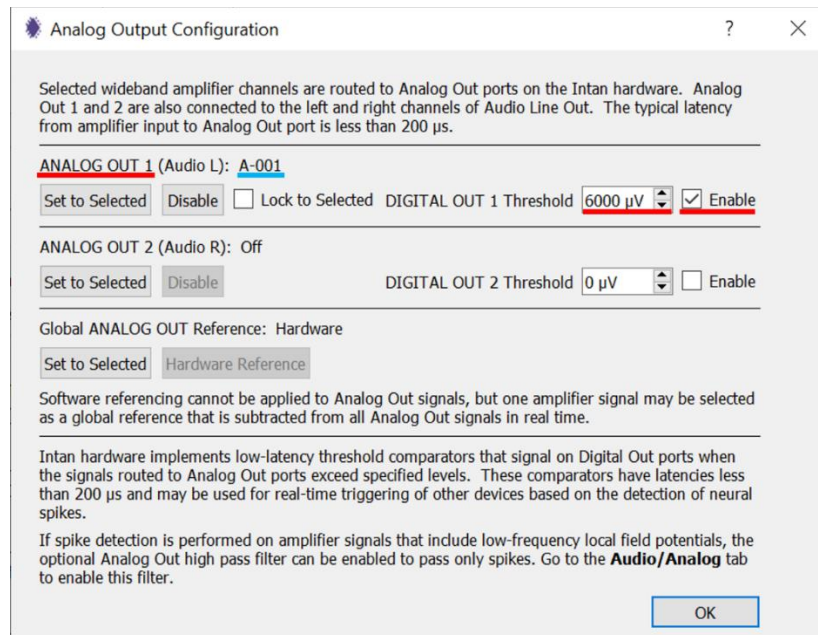
Fig. 92: Setting the parameter of the digital output 1 (linked to the stim channel).

vii) Click "OK."

5) <u>Set the trigger for data recording</u>:

i) In the IntanRHX main window, click the "Select Filename" button (⬚) and set the filename.

ii) Click the "Triggered Record" (⬚). Note that this option will only be available after a base filename has been specified.

iii) In the "Episodic Triggered Recording Control" window, set the following
- Trigger Source: DIGITAL-IN-01
- Set to "Trigger on Logic High"
- Pretrigger Buffer: 1 second (or any desired duration up to 30s)
- Posttrigger Buffer: 2 seconds (or any desired duration up to 999s)
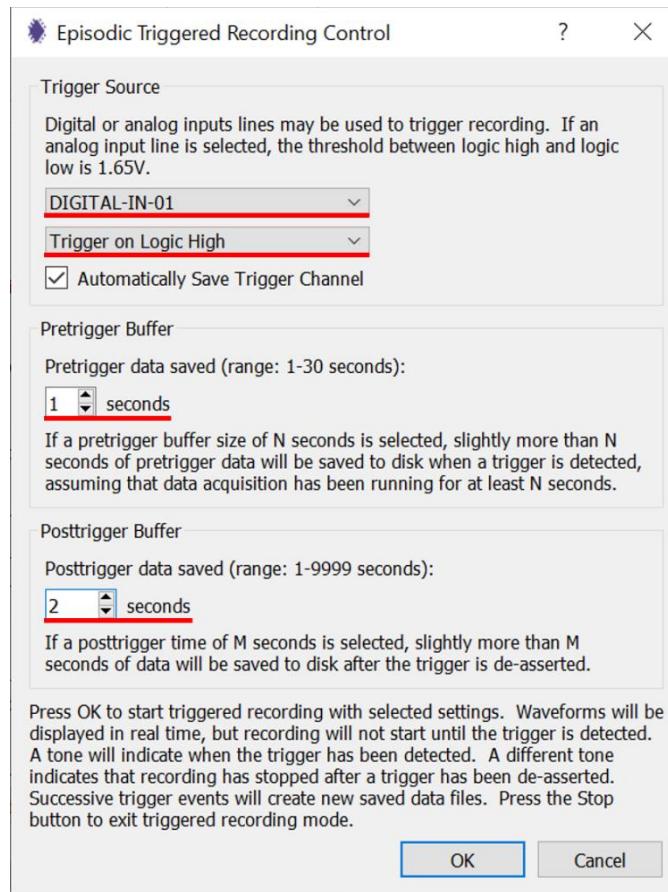
Fig. 93: Setting the triggered recordings.

iv) Click the "Ok" button.

The Episodic Triggered Recording Control window will close, and the Intan software will run. A single datafile will be recorded for each stimulus, which is determined by the frequency of the signal generator output signal. If "PC Audio" is enabled in the "Audio/Analog" tab, an audio cue will play once a recording starts, and a different audio cue will play once the recording is completed.

Note: The software and recordings will continue until the "Stop" button (◼) is pressed.

v) Click the "Stop" button (◼) to stop the recordings.

Note: The Intan rhs datafiles will be ready for use.

12 Remote TCP Control Using Matlab

The IntanRHX software includes a remote TCP control feature, which allows other software within the same computer or in another computer (as long they are both connected to the same network) to communicate with IntanRHX, to either send or receive data.

This functionality is particularly useful in experiments where changes to the stimulation parameters need to be updated within short windows of time as the user can now program a sequence of commands for the IntanRHX software to perform.

Various programs and languages (e.g., Matlab, C++, Python) can be used for remote TCP control as long as they allow TCP connections to be made. The following steps show how to run IntanRHX using the Matlab script "*IntanRHXStimRecord.m*" provided in Intan's website (click on "RHX TCP Control" at https://intantech.com/downloads.html?tabSelect=Software).

1) Launch the IntanRXH software with a stim/recording controller connected to the computer and display the control panel.

2) Load the Intan setting file by clicking "File" on the top toolbar, selecting "Load general settings," and selecting the appropriate mapping file based on the interface board, type of sMEA, and orientation of the sMEA in the interface board.

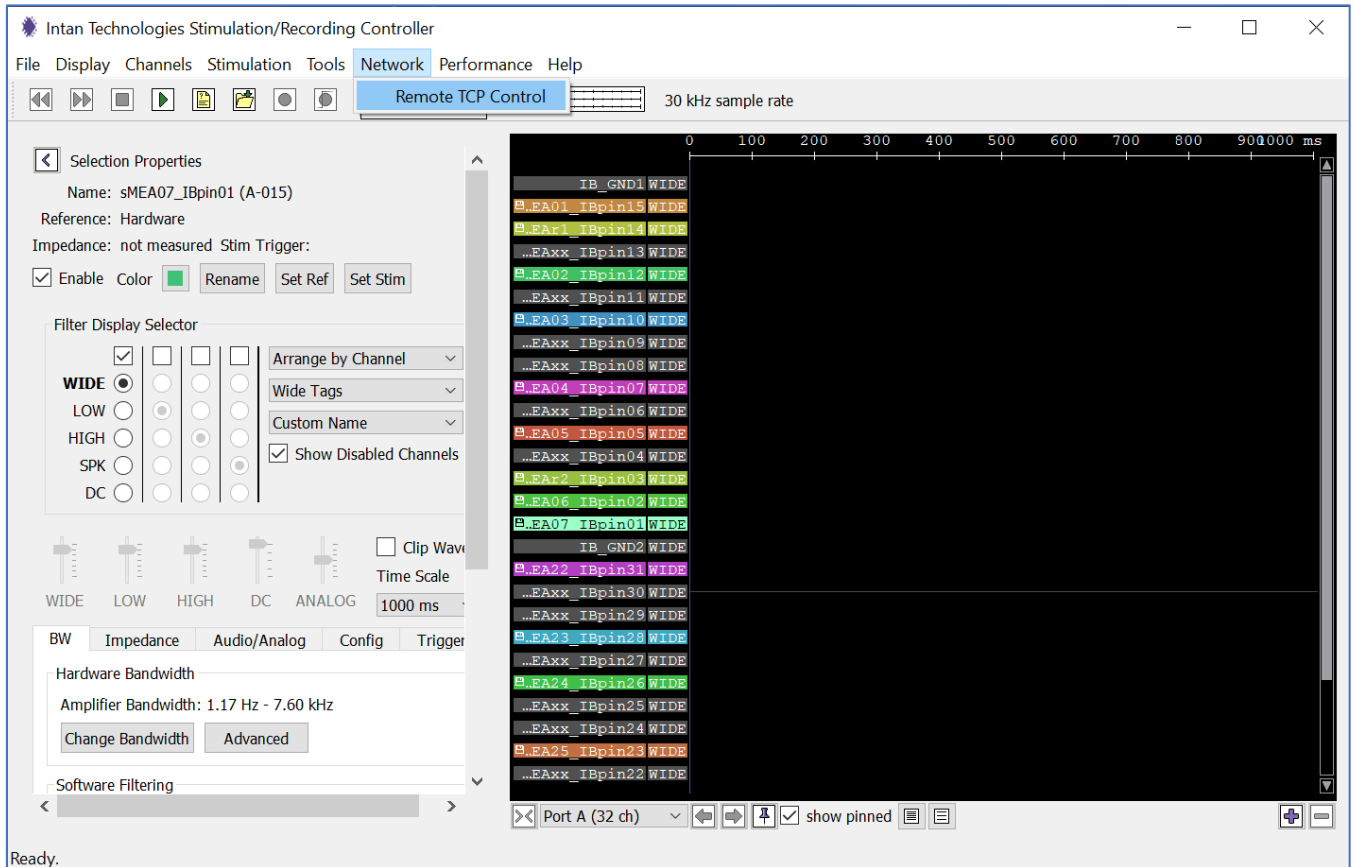3) Enable remote TCP control by clicking "Network" in the top toolbar and selecting "Remote TCP Control."

Fig. 94: Accessing the "Remote TCP Control" feature.

The "Remote TCP Control" window will pop up. The "Commands" and "Data Output" tabs will look as shown in the figure below.
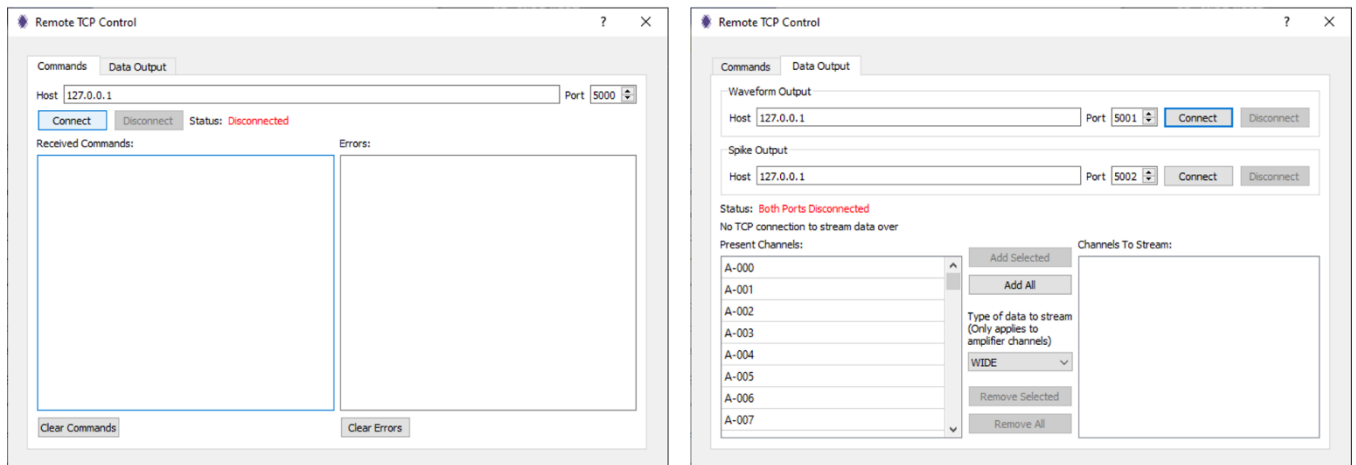

Fig. 95: The "Remote TCP Control" window and its two tabs.

4)   Click "Connect" for all three data ports, one port on the "Commands" tab on two ports on the "Data Output" tab. Status labels should now display "Pending."

The Commands port allows sending commands to the IntanRHX software for execution; this can be considered IntanRHX's input port. The Waveform Output and Spike Output ports allows receiving data from the IntanRHX software. The data can then be used in the client software (in this example, Matlab) to stream the data and perform any type of analysis on the data in real-time.
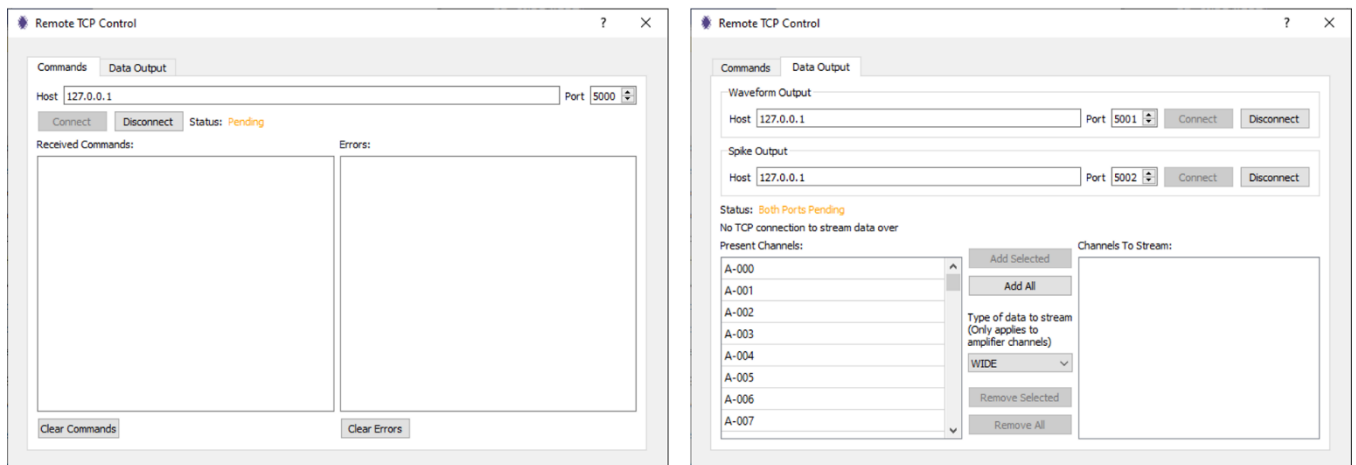

Fig. 96: After clicking connect on all three data ports.

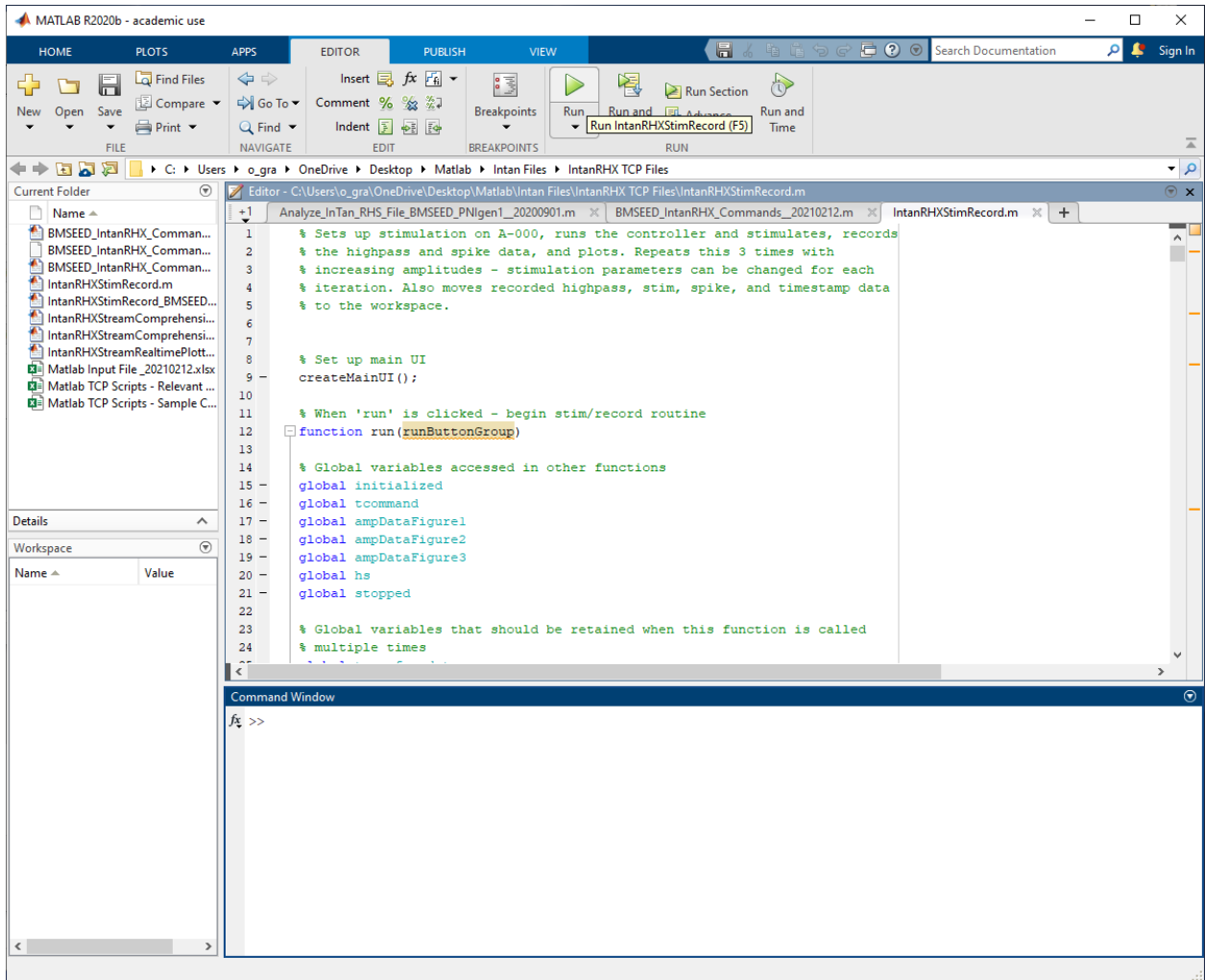5) Click "Run" in Matlab to run the script "IntanRHXStimRecord."

Fig. 97: Running the sample Matlab script "IntanRHXStimRecord" provided by Intan.

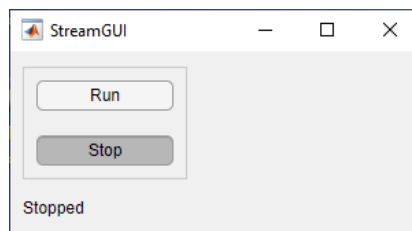6) In the Matlab pop up window "StreamGUI," click "Run."

Fig. 98: "StreamGUI" window generated by the Matlab script.

As the Matlab script is running, the IntanRHX software is also running. Disrupting either of these programs will result in a disruption of the other program.

7) Once completed, the IntanRHX software and Matlab will automatically stop, and results can now be examined.

In IntanRHX the "Remote TCP Control" window should have been updated displaying the commands that were sent to and executed by IntanRHX ("Commands" tab), as well as the data streams that were sent from IntanRHX to Matlab ("Data Output" tab).
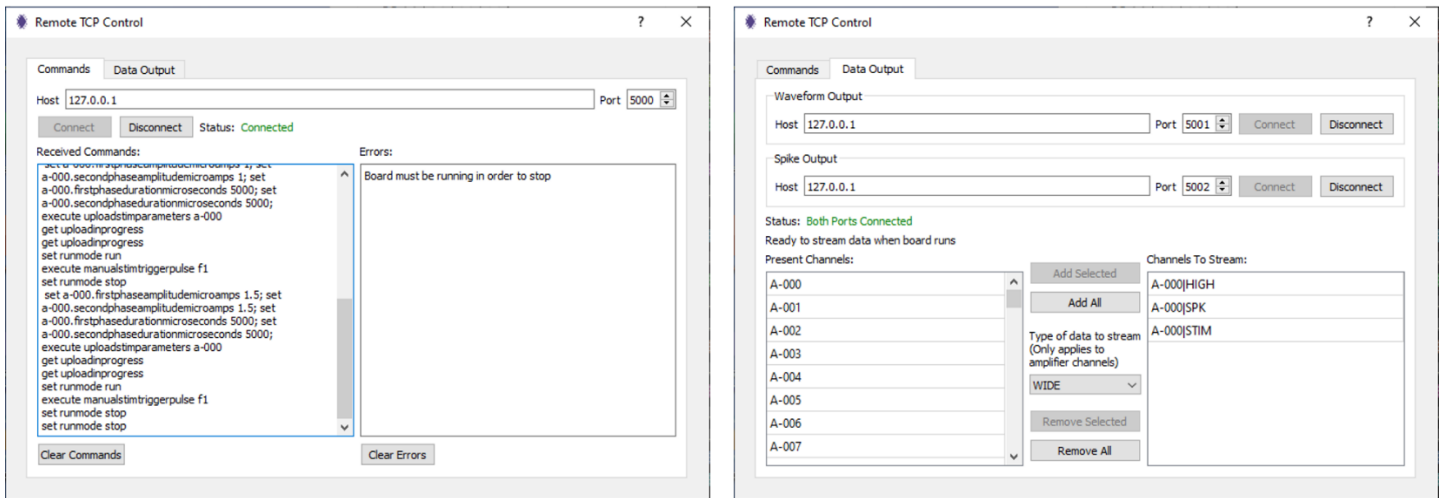

Fig. 99: The Remote TCP Control after Matlab script execution.

The GUI will display the most recent data it captured while running.
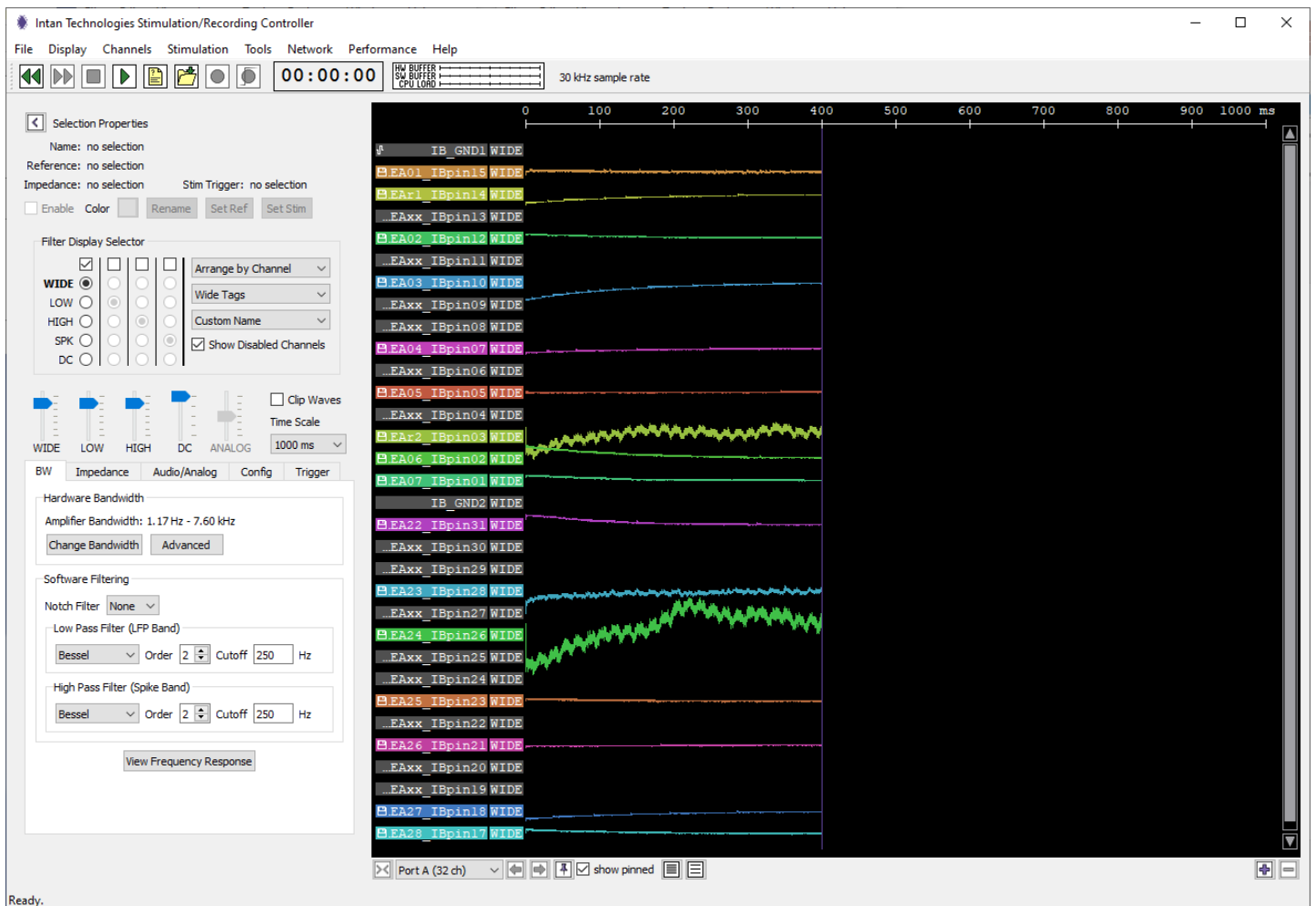
Fig. 100: GUI displaying the most recent data it captured while running.

In Matlab three figures should have been created, each representing a different stimulation run, in which the stimulation amplitude was increased in each iteration (as specified in the Matlab script).

Fig. 101: Three figures created by Matlab as specified by the script.

The workspace will now contain a list of variables created during the Matlab script execution.

Fig. 102: Matlab workspace containing the variables from the run script.

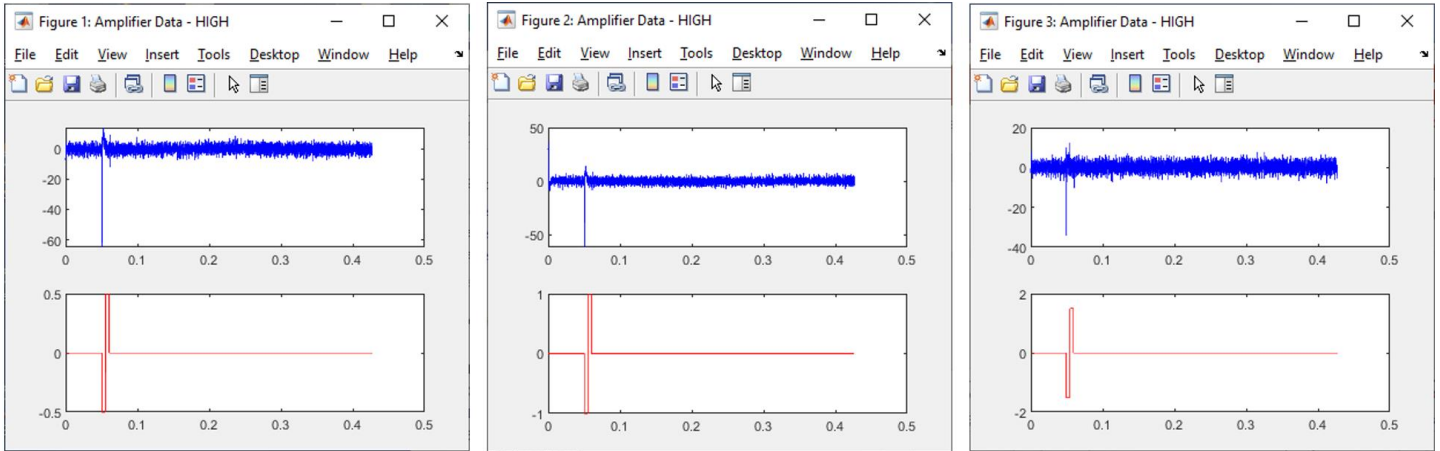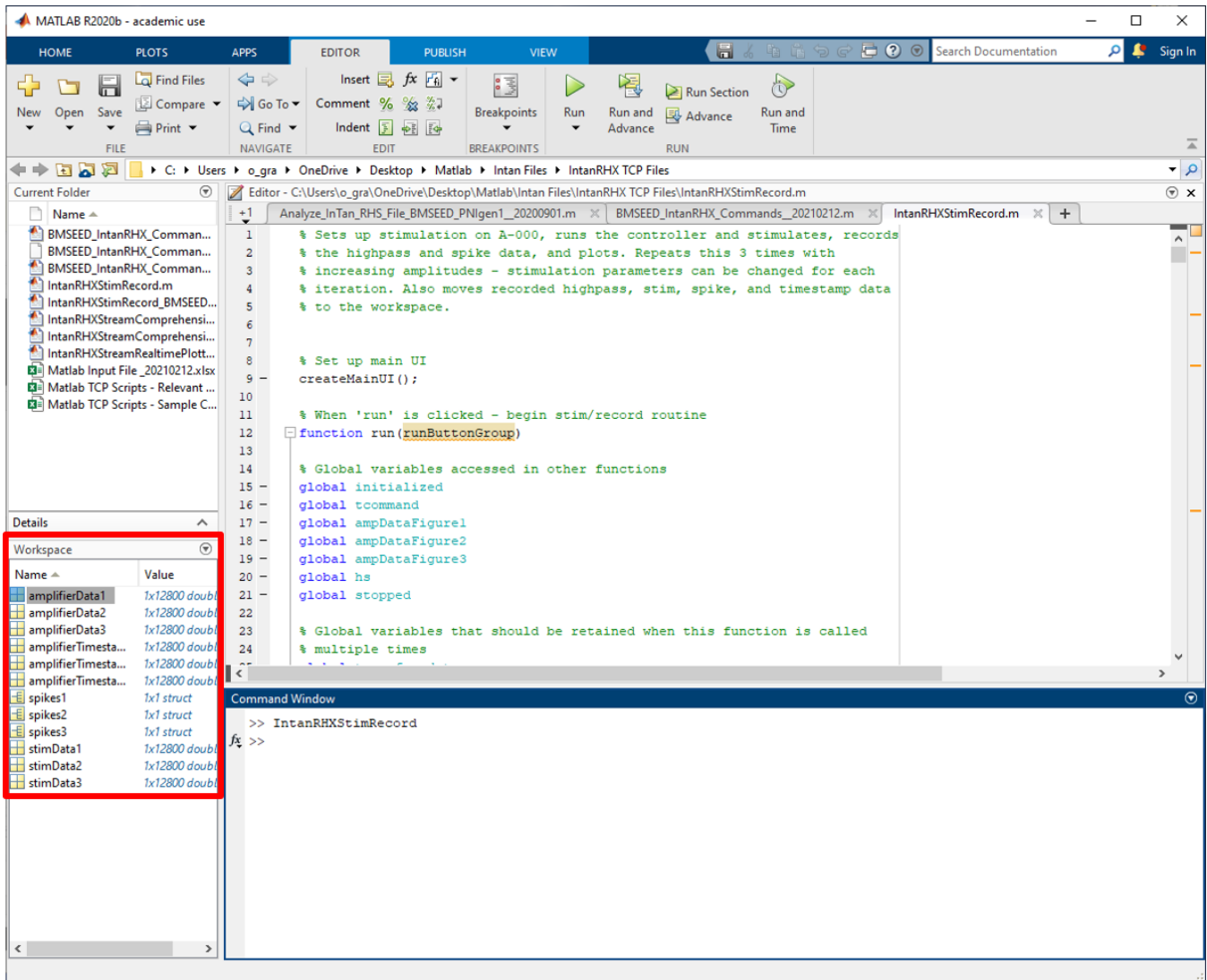# 13 Sample Matlab Script for TCP Control: SR Curve Experiment

The script "***SR_Curve_Experiment__Matlab_Script_for_IntanRHX _TCP__20210527.m***"
was developed by BMSEED to automate the experimental protocol to produce an SR curve.
Once run, the user is asked to input a file (i.e., an Excel workbook) that will contain important
information for accurate execution of the experiment.

- "Settings" sheet: This sheet lists setting parameters specific to this SR curve experiment.
    - type of sample ("sMEA gen4", "sMEA gen4e", "sMEA gen6", or "glass MEA")
    - sample orientation ("standard" or "90° turned")
    - ports connected ("A+B" or "C+D")
    - trigger source ("Analog input 1" or "F1 key press")
    - trigger type ("Edge triggered" or "Level triggered")
    - trigger on level ("Trigger on high" or "Trigger on low")
    - pulse repetition ("Single stim pulse" or "Stim pulse train")
    - sample's biphasic cathodic- and anodic-first channels
    - amp settle time ("Enabled" or "Disabled")
    - pre-stim amp settle time (must be between 0 and 500,000 μs)
    - post-stim amp settle time (must be between 0 and 500,000 μs)
    - time interval between stimuli (must be between 0 and 60 sec)
    - number of stimulation cycles (calculated from "Mapping" sheet)
    - the sample's biphasic cathodic and anodic-first channels with the Intan headstage
      numbering scheme (determined previous input and from "Mapping" sheet).
- "Stimulation" sheet: This sheet lists the stimulation cycles or runs and the amplitude (μA)
  and duration (μs) for each stimulus pulse.
- "Mapping" sheet: This sheet contains mapping information between the sample's channels
  and Intan headstage channels.

The Matlab script sets up IntanRHX parameters based on the input file and cycles through the
different stimulation runs while running the IntanRHX software in trigger mode (i.e., the
software runs and only after a stimulus trigger is detected, data is recorded for a specified
period of time). To generate an SR curve the stimulus amplitude is increased from 0 μA to
40 μA. For every stimulation run, after a stimulus is detected in Matlab, the IntanRHX software is
stopped to allow for data recording. After a short pause of a few second, IntanRHX is run again
in trigger mode. This is repeated until all stimulation runs listed in the Excel workbook have
been performed. Prior to and after all stimulation runs, impedance measurements are recorded.

The following steps show how to run the Matlab script for an SR curve experiment:

1) Launch the IntanRXH software with a stim/recording controller connected to the computer
   and display the Control Panel.

2) Load the Intan setting file by clicking "File" on the top toolbar, selecting "Load general
   settings," and selecting the appropriate mapping file based on the interface board, type of
   sMEA, and orientation of the sMEA in the interface board.

3) If using the Digital Input 1 signal to trigger data recording (i.e., the Analog Output 1 port
   is connected to the Digital Input 1 port via a BNC cable), select the stimulating channel in
   the Main Display you will set with the "cathodic current first" stimulation polarity.
   In the example below, channel "sMEA07_IBpin01 (A-015)" is selected.

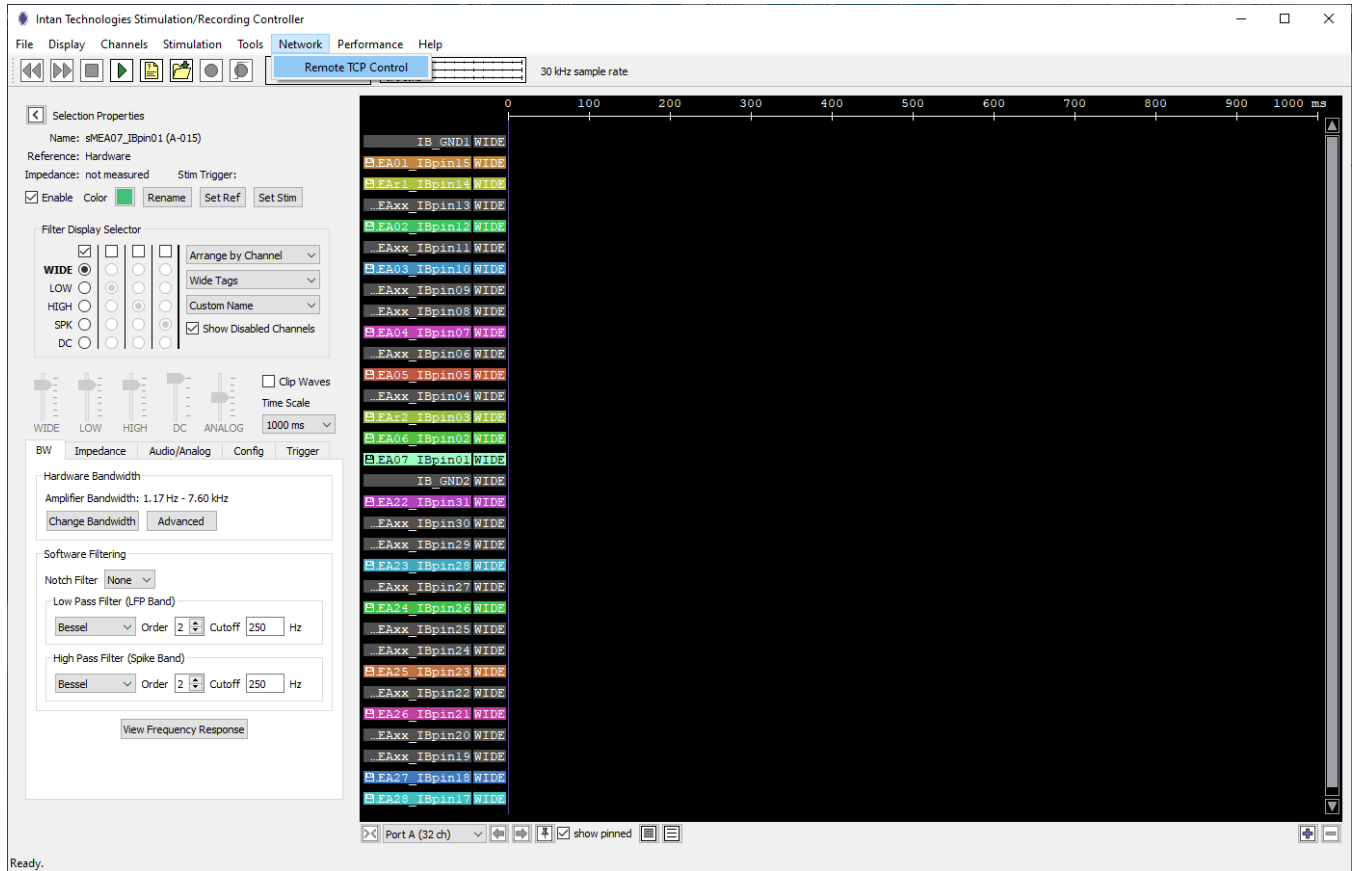4) Enable remote TCP control by clicking "Network" in the top toolbar and selecting "Remote TCP Control."

Fig. 103: Accessing the "Remote TCP Control" feature.

The "Remote TCP Control" window will pop up. The "Commands" and "Data Output" tabs will look as shown in the figure below.
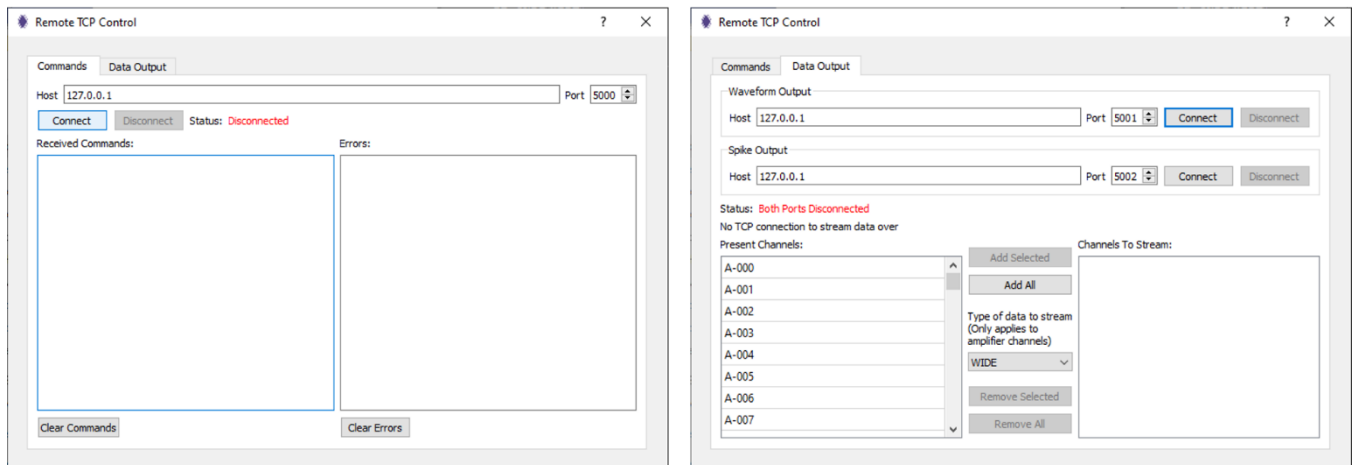

Fig. 104: The "Remote TCP Control" window and its two tabs.

5) In the Commands tab, click "Connect" to allow Remote TCP Control to an external program. The status label will now display "Pending."

Note: The Waveform Output and Spike Output ports in the Data Output tabs will be enabled by the Matlab script, so no need to click "Connect" for those.

The Commands port allows sending commands to the IntanRHX software for execution; this can be considered IntanRHX's input port. The Waveform Output and Spike Output ports allows receiving data from the IntanRHX software. The data can then be used in the client software (in this example, Matlab) to stream the data and perform any type of analysis on the data in real-time.
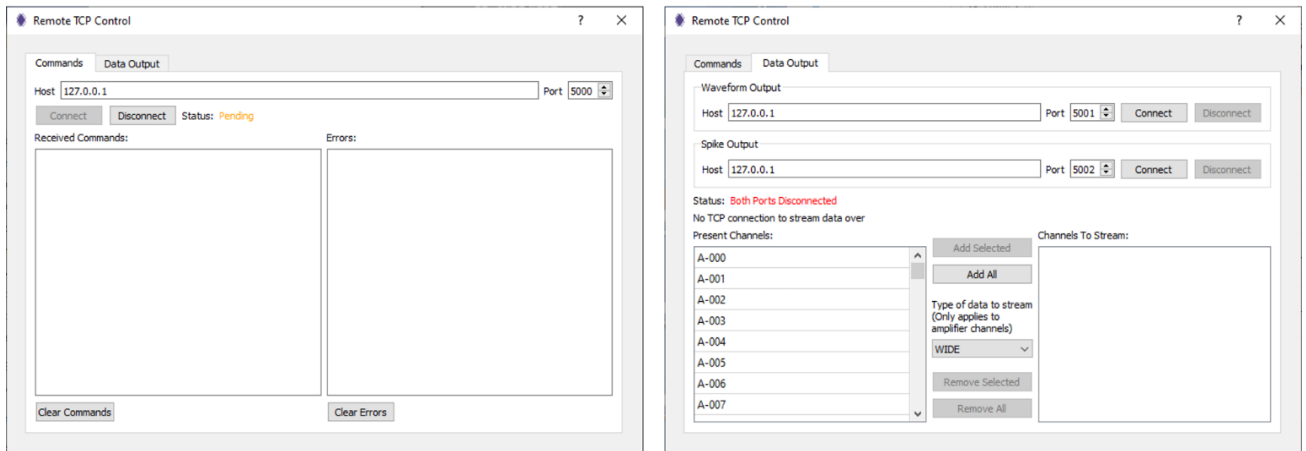

Fig. 105: After clicking Connect in the Commands tab only.
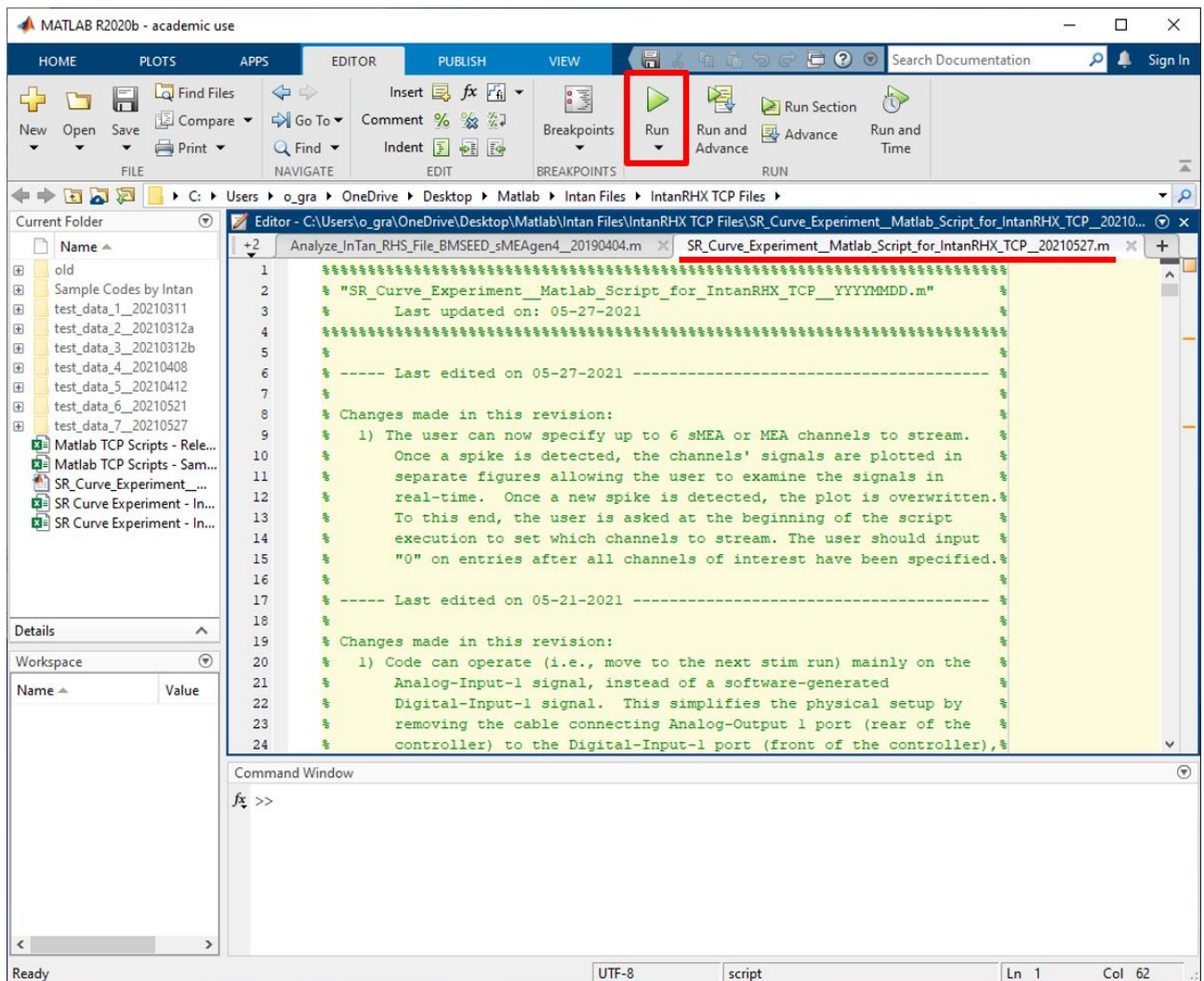
6)   Click "Run" in Matlab to run the script.

Fig. 106: Running the Matlab script to execute the SR curve experiment.

7) When prompted by Matlab, select the Matlab input file (in this example, "**_SR Curve Experiment – Input File for Matlab _20210412_**" Excel spreadsheet) that contains all relevant information regarding the SR curve experiment to be executed.
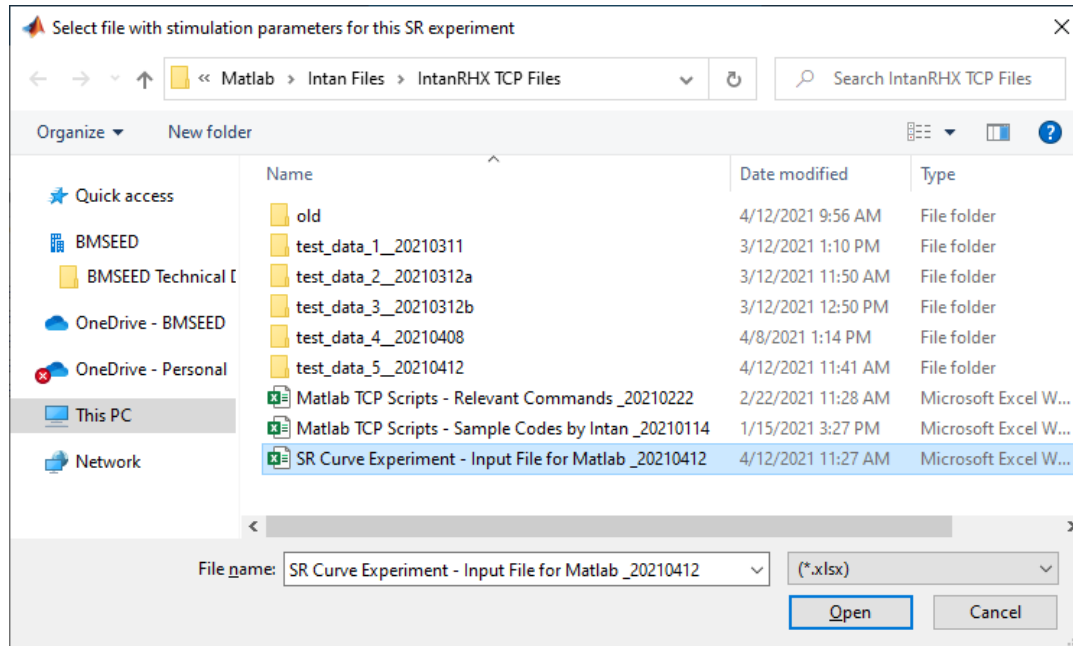

Fig. 107: Selecting the SR curve experiment Matlab input file.

8) When prompted by Matlab, select the folder (in this example, "_test_data_") where to save the impedance measurement and recording files.
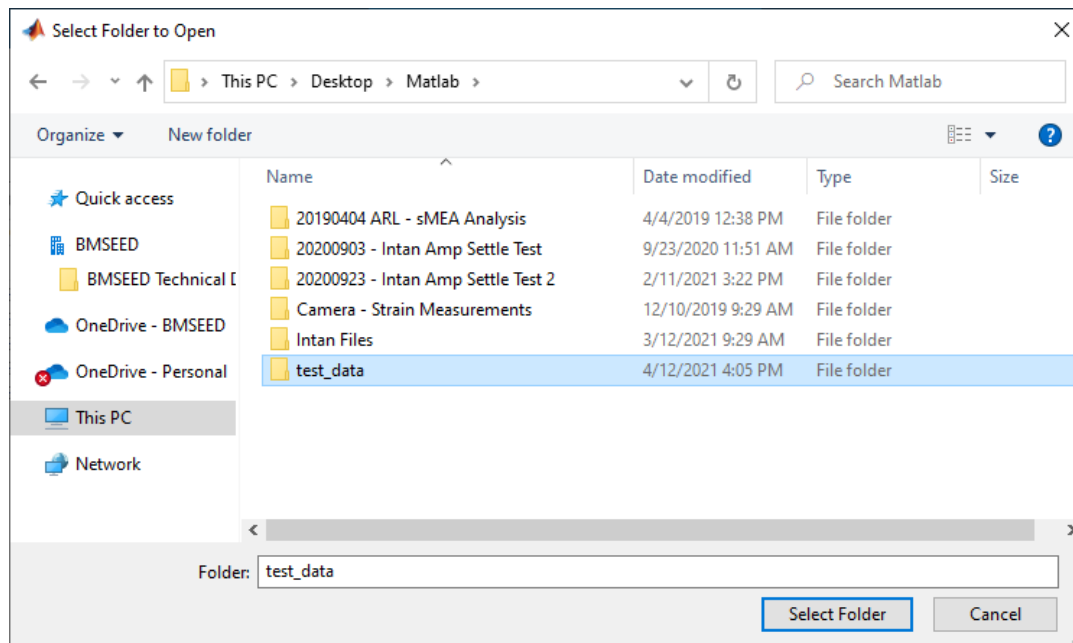
Fig. 108: Selecting the folder where Matlab output files will be saved in.

<u>IMPORTANT:</u> There should not be any spaces in the directory/path of the selected folder. Otherwise, output files will not be saved, and error messages will appear that will stop Matlab's script execution. For instance,
- "_C:/Users/UserName/Desktop/Matlab/test_data_" is **acceptable**.
- "_C:/Users/UserName/Desktop/Matlab/Intan Files_" is **not acceptable**.

- "*C:/Users/UserName/Desktop/Matlab/Intan Files/test_data*" is **not acceptable**.

9) When prompted by Matlab, specify the:

    a) base name for the IntanRHX impedance measurement files and ".rhs" recording files,

    b) type of trigger for data recording,

    c) sMEA or MEA channels to stream for real-time monitoring.

Note: By default, the analog input 1 signal is used as the trigger for data recording since the additional BNC cable between Analog Output 1 and Digital Input 1 is not needed.

Fig. 109: Specifying the base name for the output files.
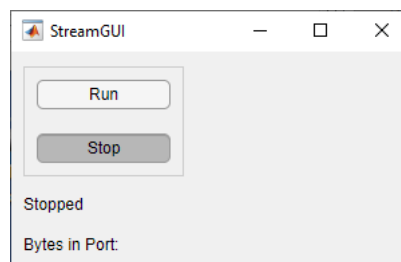
10) In the Matlab pop-up window "StreamGUI," click "Run."

Fig. 110: "StreamGUI" window generated by the Matlab script.

IMPORTANT: As the Matlab script is running, the IntanRHX software is also running. Disrupting either of these programs will result in a disruption on the other program.

11) The listed stimulation runs in the input file will be executed with varying phase amplitudes for both, the cathodic-first and anodic-first stimulating channels. Impedance measurements will be taken and saved before and after all stimulation runs.

Note: As the script is executed, Matlab will stream and display the selected trigger signal in real-time (**Fig. 112**). Spikes in the specified sample channels are only displayed when the stimulus trigger is detected (**Fig. 113**). This plot is overwritten for every trigger.
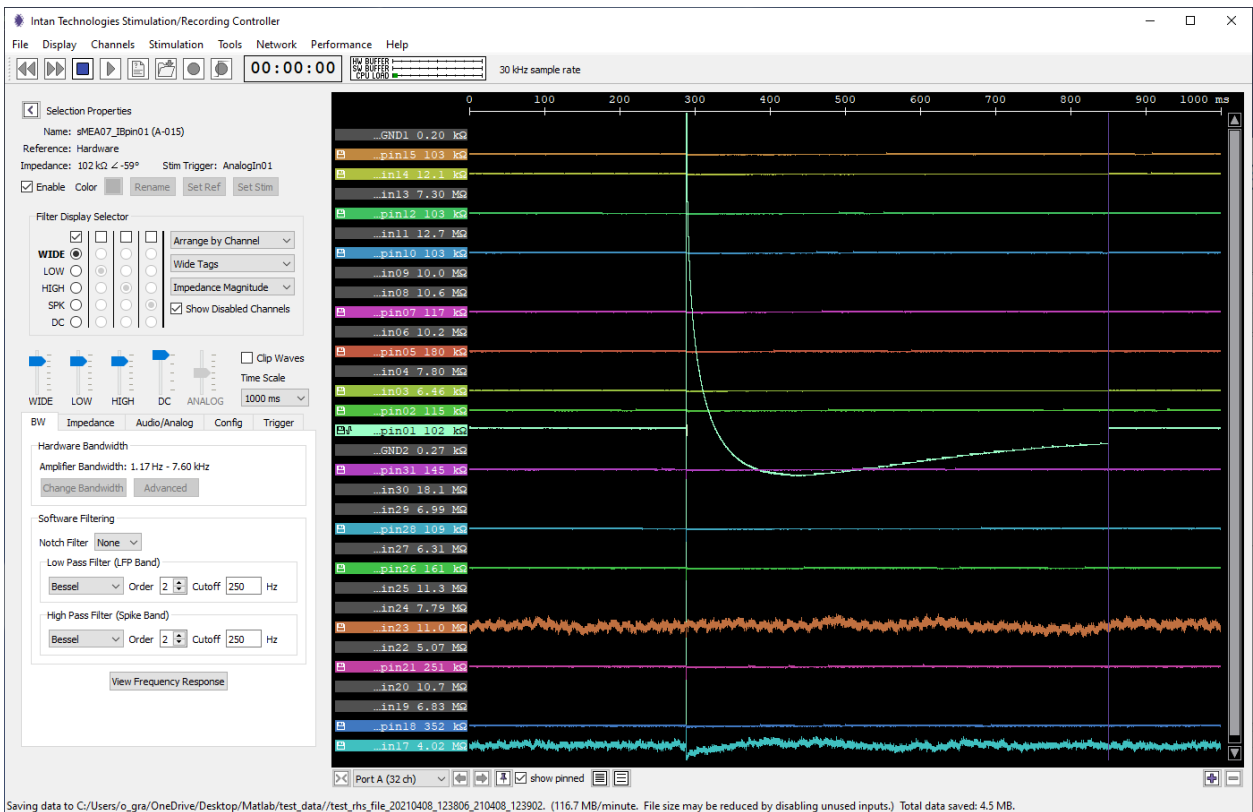
Fig. 111: Screenshot of the IntanRHX software running as the Matlab script is executed.
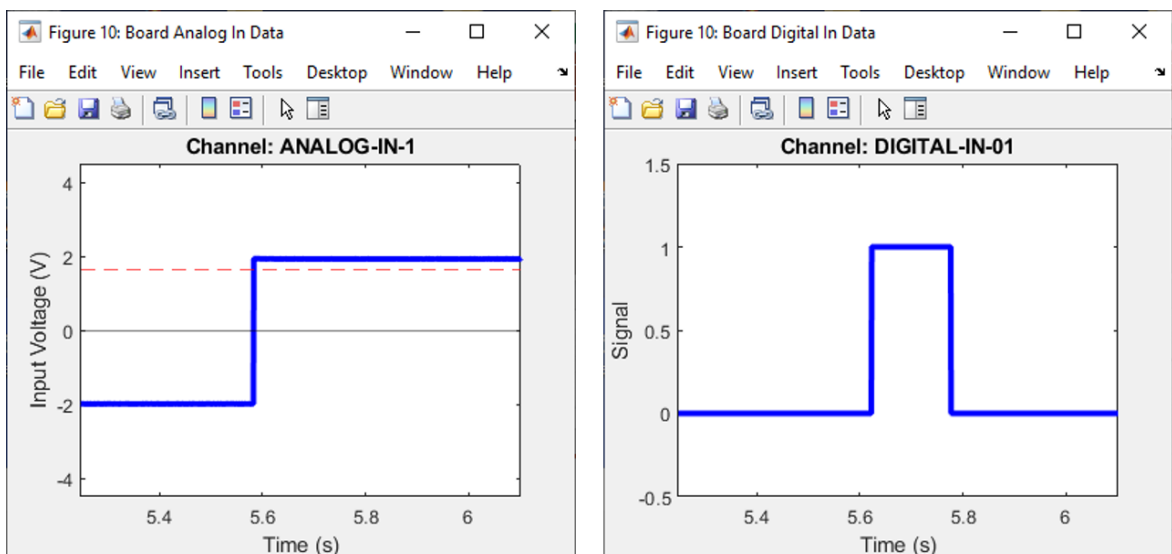
Fig. 112: Matlab figures showing examples when either an analog input or a digital input signal is used as the trigger for data recording.
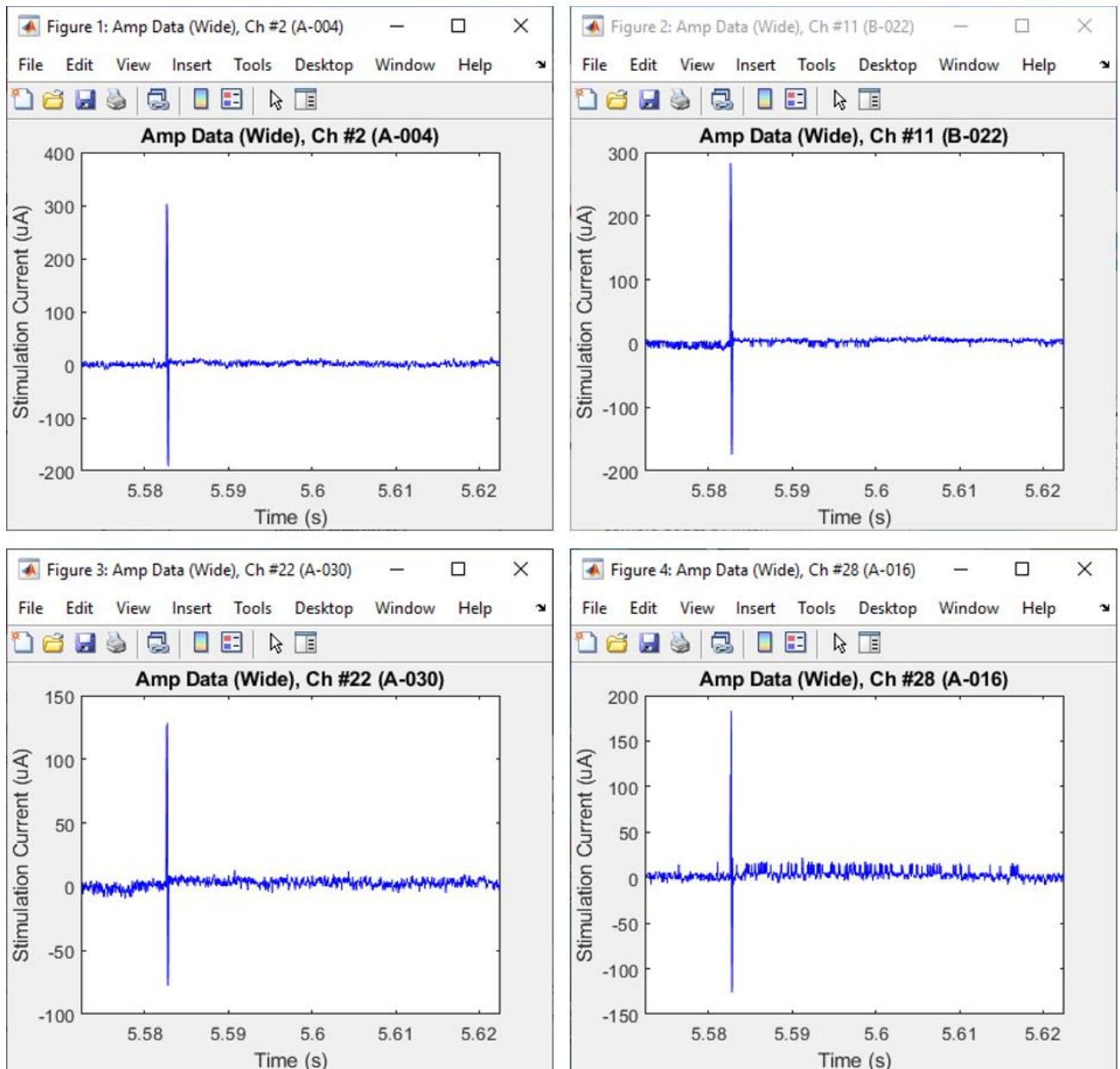
Fig. 113: Matlab figures showing examples of zoomed-in signals when the stimulation was triggered.

Fig. 114: Matlab Command Window displaying text as the script executes.

12) Once all stimulation cycles have been completed, the IntanRHX software and Matlab will automatically stop.

13) To be ready for the next Matlab script execution, click the "Disconnect" button in the Commands tab of the Remote TCP Control window of the IntanRHX software. The status should now change to "Disconnected" in red font (**Fig. 115**).

Note: The Waveform Output and Spike Output ports in the Data Output tabs are automatically disconnected by the Matlab script (**Fig. 116**), so no need to click "Disconnect" for those.
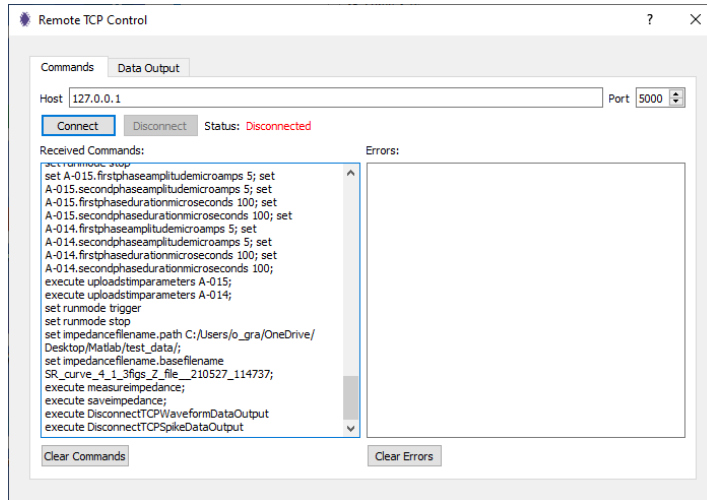
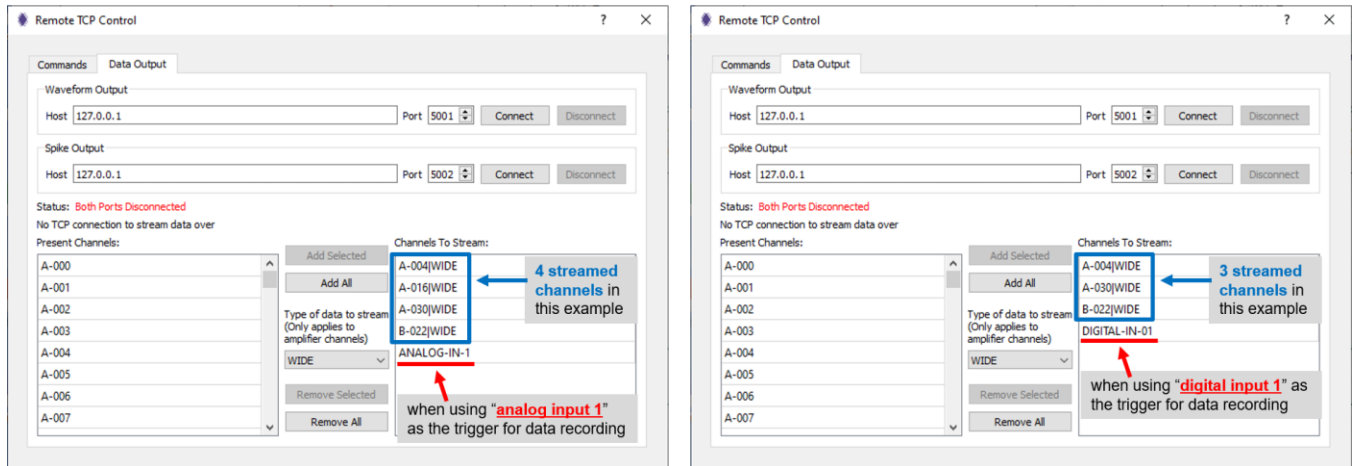**Fig. 115:** The Command tab of the Remote TCP Control window post disconnection.

Fig. 116: The Data Output tab of the Remote TCP Control window after Matlab script execution.

14) The Matlab "StreamGUI" window (which should now be set to "Stop") can be closed.
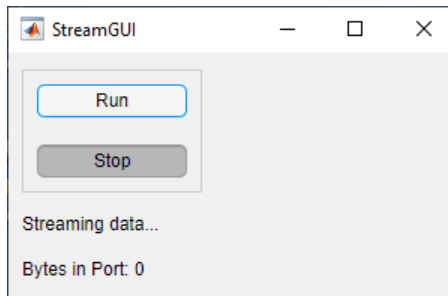
Fig. 117: The "StreamGUI" window after script execution.

15) Recordings can now be examined.

As shown in **Fig. 115** above, the "Remote TCP Control" window in IntanRHX should have been updated displaying the commands that were sent to and executed by IntanRHX ("Commands" tab). In the "Data Output" tab, the data streams that were sent from IntanRHX to Matlab are displayed as shown in **Fig. 116** above. Two examples are shown in this figure. In the example on the left side of the figure, four channels were streamed for real-time monitoring, and the trigger was set to the analog input 1 signal. In the example on the right side of the figure, three channels were streamed, and the trigger was set to digital input 1 signal.

The IntanRHX GUI will display the most recent data it captured while running.
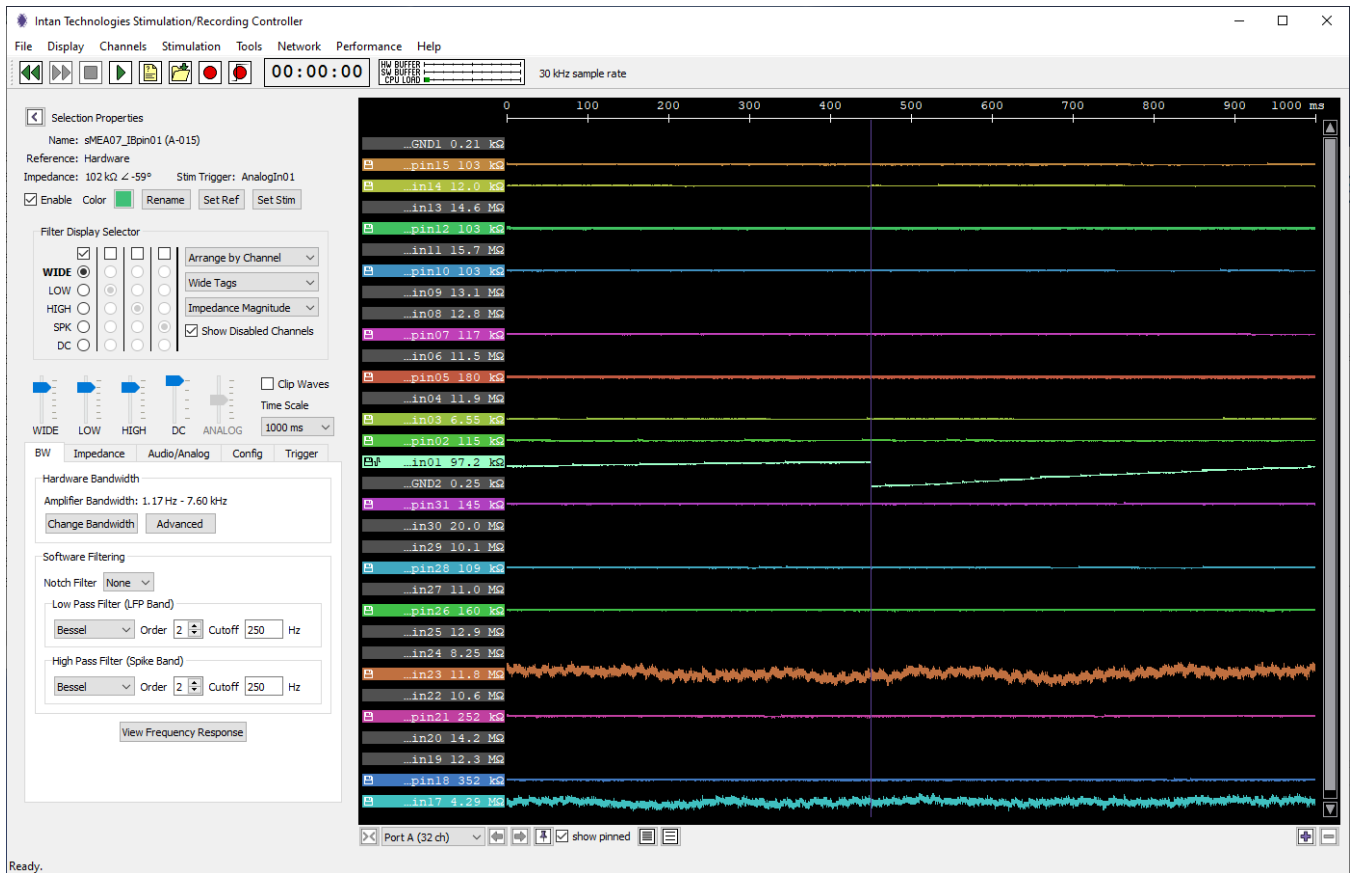
Fig. 118: GUI displaying the most recent data it captured while running.

In Matlab the different figures generated throughout script execution should remain: one figure for the trigger for the data recording (such as **Fig. 112**) and one figure for each sample channel specified at the beginning of script execution (such as **Fig. 113**).

Also, the Command Window in Matlab will display text from the last stimulation cycle executed.

Fig. 119: Command Window after script execution.

The specified save folder (in this example, "*test_data*") should now contain two impedance measurement files in ".csv" format and one recording data file in ".rhs" format for every stimulation run listed in the "Stimulation" sheet in the Matlab input file as shown in **Fig. 120**.
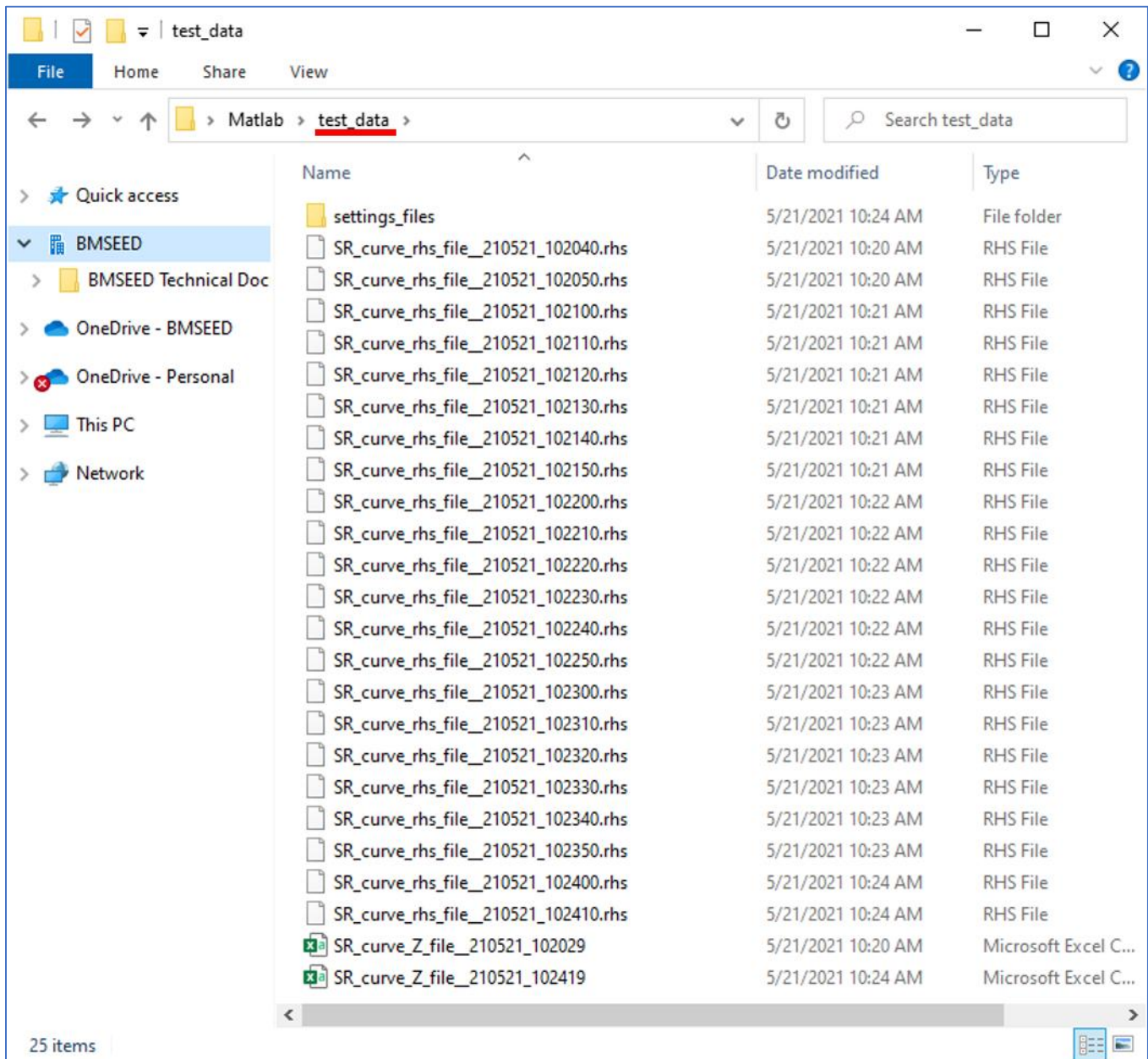
Fig. 120: Save folder with all output files after the Matlab script execution.

In addition to these impedance measurement and data recordings files, there should be a subfolder named "settings_files" which contains one settings ".xml" file for every stimulation run (as shown in **Fig. 121**). Each settings file contains the stimulation parameters for all channels, including those channels with stimulation disabled.
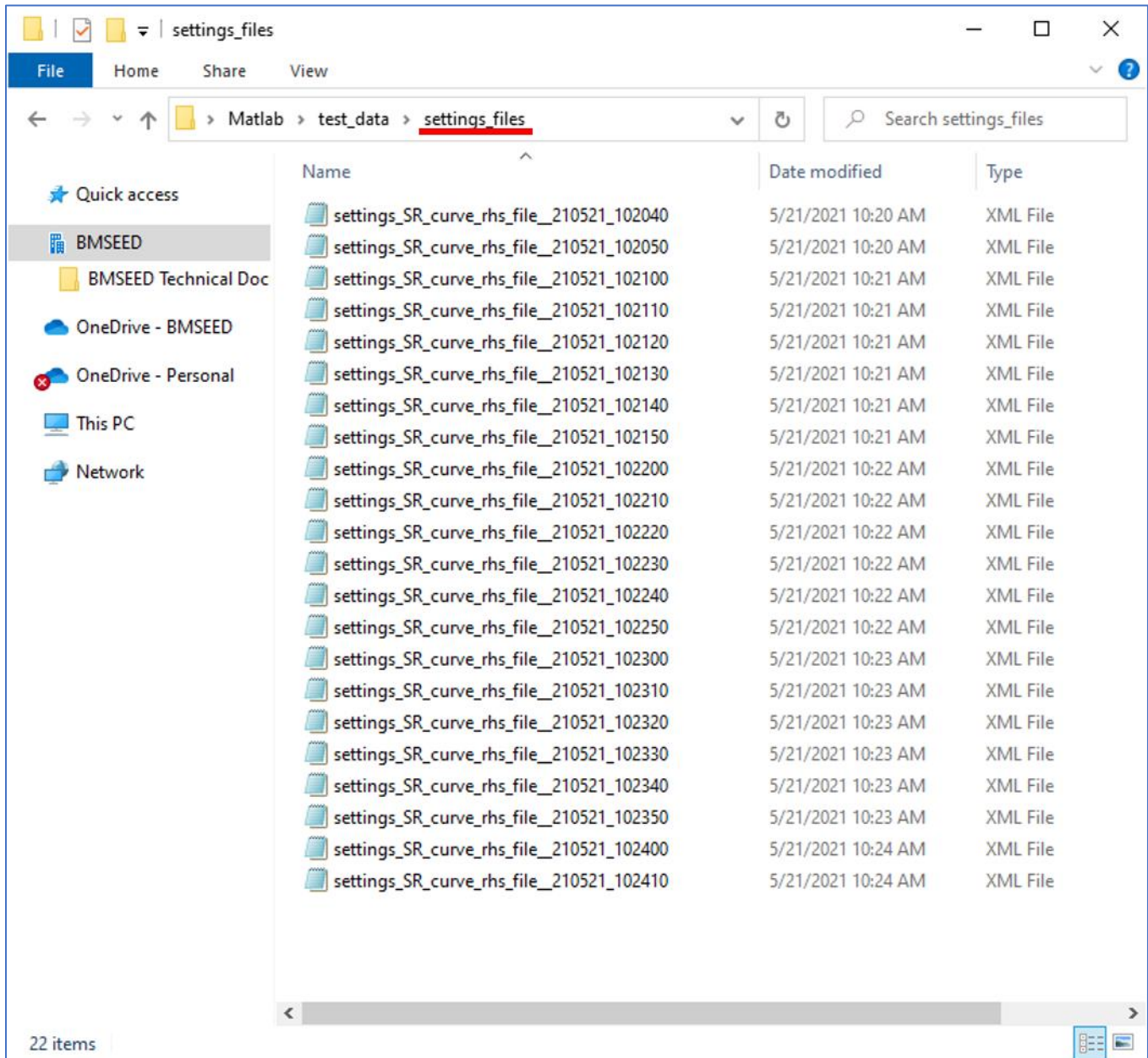
Fig. 121: "Settings_file" subfolder created to store stimulation setting files for all stim runs.

14 Loading Intan Data Files (.rhs) and Data Conversion

The data from the electrophysiology module are saved as ".rhs" file types. These files can be directly uploaded to the data analysis software NeuroExplorer (**http://www.neuroexplorer.com/**).

To convert the .rhs files to a .txt file, follow the instructions below.

⚠️ *You will need either Matlab or Octave (open source) to run the provided m-files.*

1) Load Matlab or Octave.

2) Open the m-file with the main code to examine the Intan rhs data file.
   If using Matlab, open the Matlab version of the code:
   - "*Examine_Intan_RHS_File_with_Matlab__20230322_BMSEED.m*"
   If using Octave, open the Octave version of the code:
   - "*Examine_Intan_RHS_File_with_Matlab__20230322_BMSEED.m*"

3) In the Editor section of the software, the code will be displayed.
   Note: The overall layout might vary depending on the user's setup/configuration.
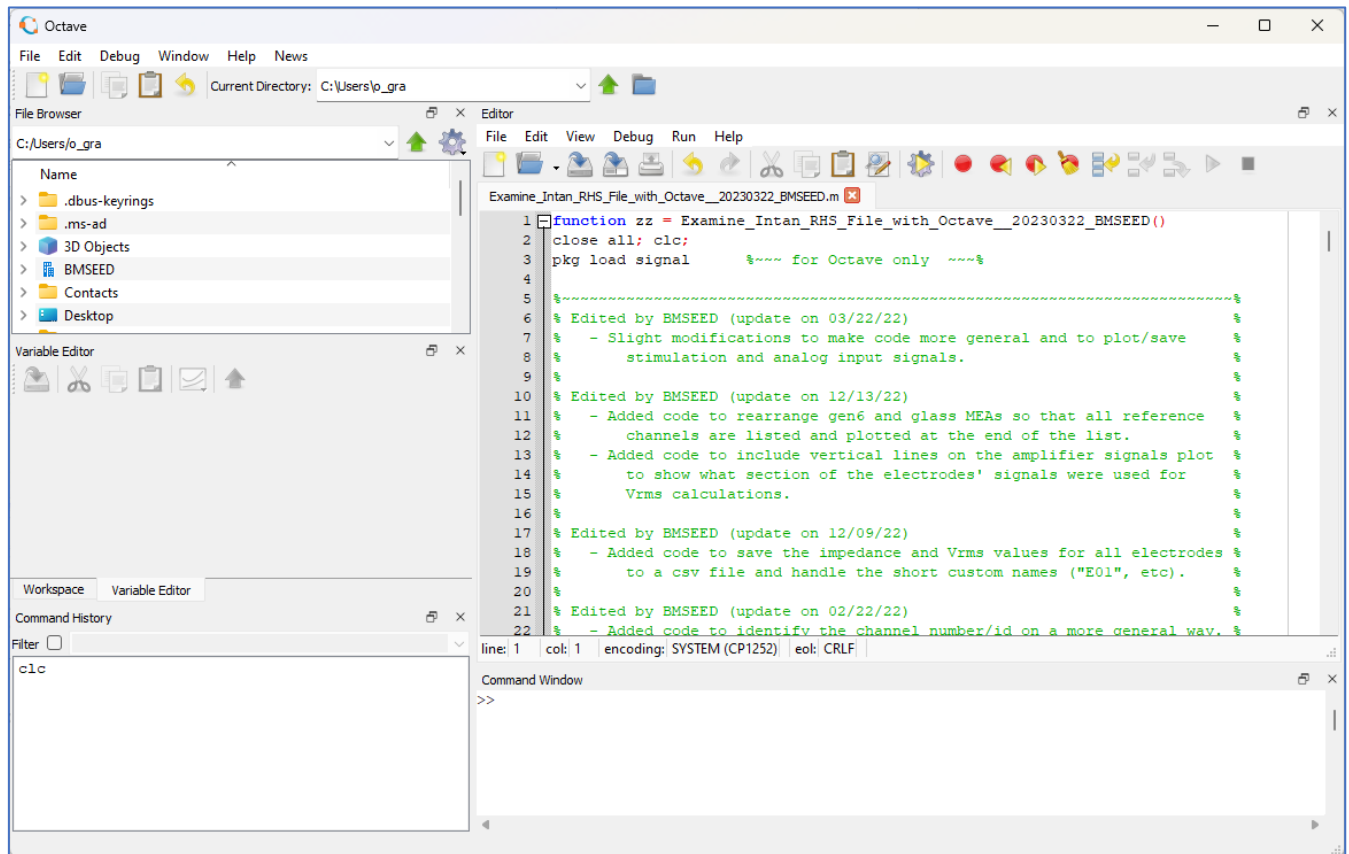
Fig. 122: Screenshot of Octave window with the provided code in the Editor.

4) Ensure that the "*read_Intan_RHS2000_file__BMSEED.m*" file is located within the same directory as the main code listed in step 2) above.

5) Left-click inside the Editor section and click the "Run" icon (i.e., "play" symbol).

6) If a pop-up window opens asking to change directory, click the "Change Directory" button.
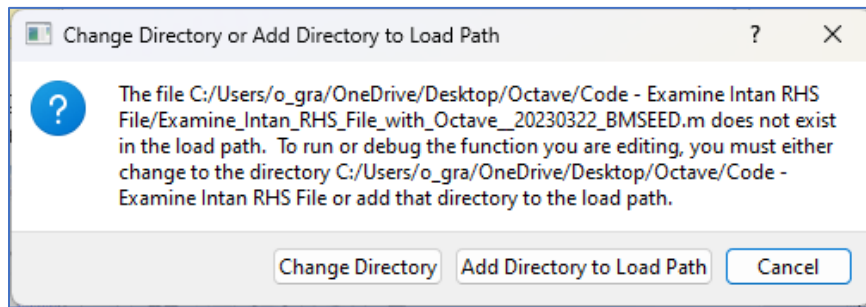
Fig. 123: Screenshot of the "Change Directory" pop-up window.

7) In the pop-up window locate and select the Intan rhs file that you would like to examine and click "Open."
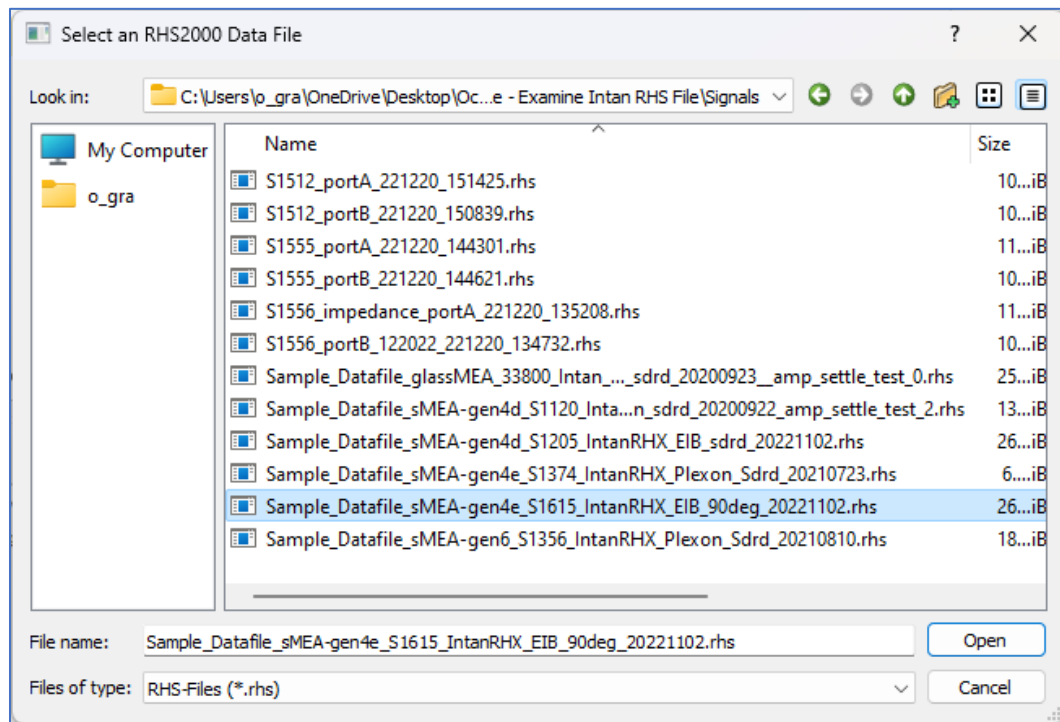
Fig. 124: Pop-up window asking for the Intan RHS data file.

8) A "User Input" window will open asking the user to provide some information. Fill-out the various fields and click "Ok".
   The five fields to be filled out by the user:

   - type of MEA being used, which should be set to either
     - "sMEA_gen4"   → if using a gen4a, gen4c, or gen4d sMEA
     - "sMEA_gen4e"   → if using a gen4e or gen4f sMEA
     - "sMEA_gen6"
     - "glass_MEA"
   - lower limit of the y-axis for all electrode signals (in μV) when plotting them
   - upper limit of the y-axis for all electrode signals (in μV) when plotting them
   - start time for the Vrms calculations (in sec)
   - end time for the Vrms calculations (in sec)

Fig. 125: User Input window with the default values.

9) A few figures and a second "User Input" window will pop-up.

Figures 1-2: show the voltage vs. time plot for all electrodes. If a gen4 sMEA is being examined, only one figure will appear showing all electrodes. If a gen6 sMEA or glass MEA is being examined two figures will appear since these have 60 electrodes. By default, the whole recording will be displayed. The dotted red lines show the section of the signal where Vrms was calculated. Each electrode plot is titled with the measured impedance and calculated Vrms.
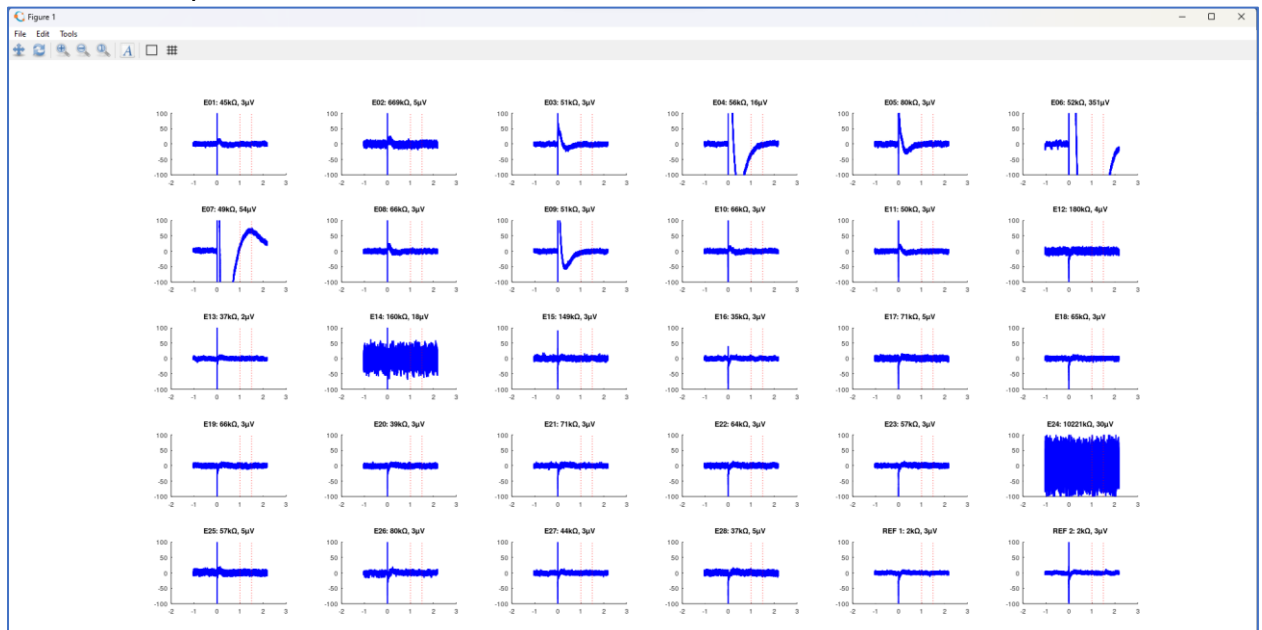
Fig. 126: Sample of the electrode signals (28 recording electrodes plus 2 reference electrodes of a gen4d sMEA).

<u>Figure 3-4</u>: show the stimulating current vs. time plot for all electrodes. Only the stimulating electrodes will show any activity (i.e., not a flat line).
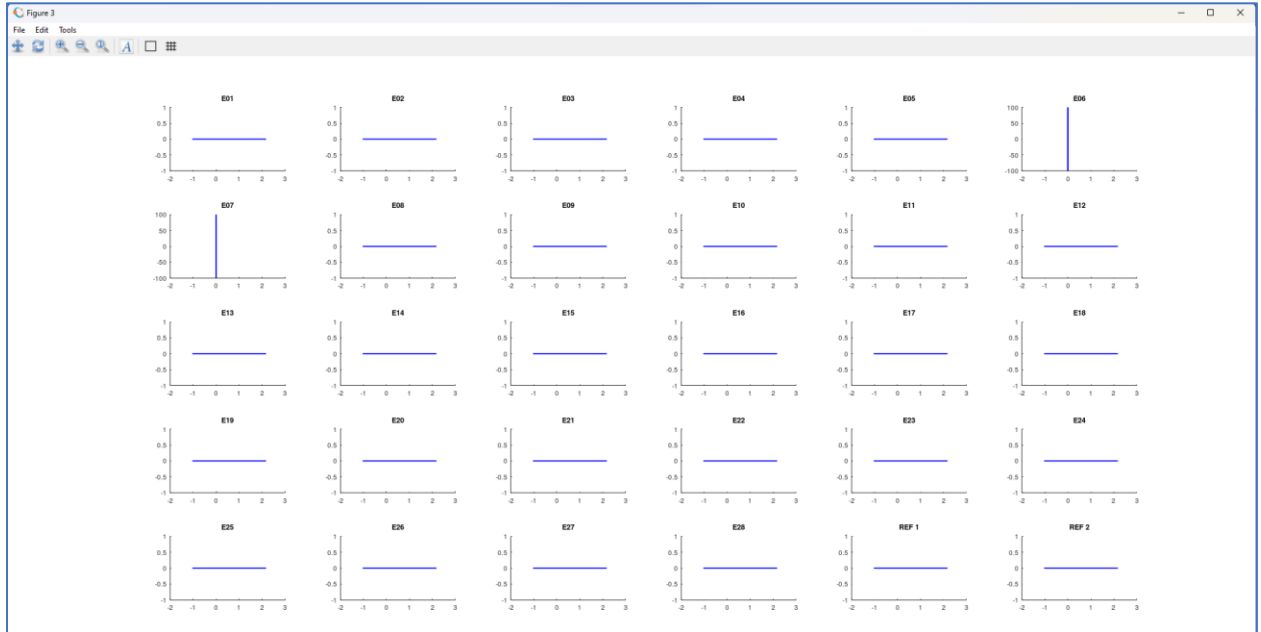
Fig. 127: Sample of the stimulation data. In this example, electrodes E06 and E07 were set as the stimulating electrodes.

<u>Figure 5</u>: shows the analog input signal from the function generator which served as the trigger for the stimulation pulse. This figure will not appear if no analog input signal was recorded.
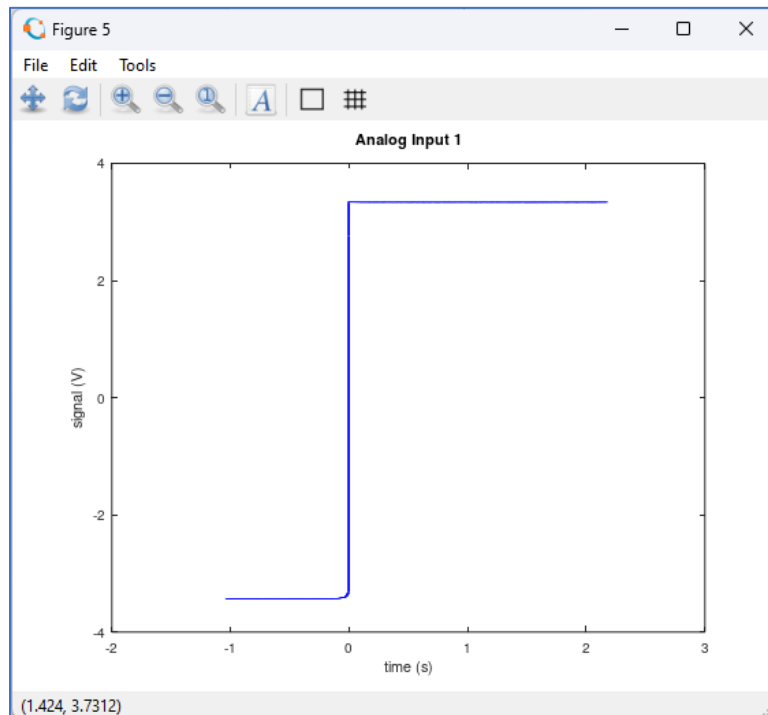
Fig. 128: Sample of the analog input data from a signal generator.

<u>Output File Name window</u>: This window will be used to separate the rhs file into multiple smaller text files. Only the IntanRHX software can open rhs datafiles as it is Intan Technology's proprietary file type. Exporting the data into text files will allow the data to be opened by other software, including Excel.
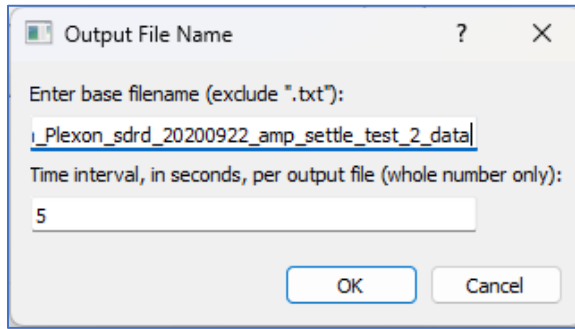
Fig. 129: Output File Name window.

A few remarks regarding this window:
- In the <u>first input field</u>, enter the base filename for the output data. By default, this is set to the filename of the Intan rhs file being examined.
- In the <u>second input field</u>, enter the time interval in seconds that you want to convert to text files.

  In the screenshot above, the data will be split into smaller (i.e., 5-second long) text files. The first file will have the suffix "_0to5sec" in its filename, the second file will have the suffix "_5to10sec", and so on, until the final section which will have the suffix "_#sectoEND."
- After completing these fields, click "OK."
- However, click "Cancel" if you do not want to export the data as text files.

10) If an output file name was created, it will appear in the same directory where the m-file is located. The newly created data text file can be opened with other software such as Excel and will contain columns with the following: time, electrodes' voltage, stimulating channels' current, and analog input (if any).
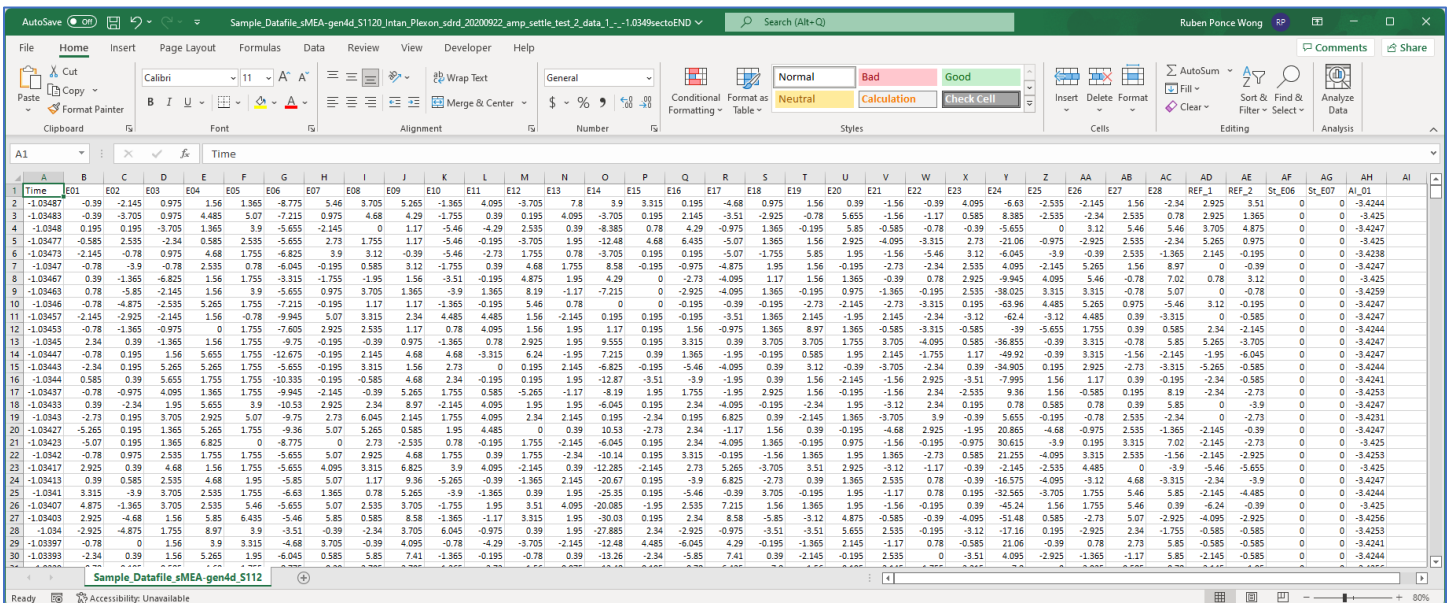
Fig. 130: Sample data file exported as a text file.

The provided m-file can be further customized. For instance, a few changes to the code include:

a. Updating the scale of the x- and y-axis of the figures plotting the electrode signals:
In lines 365 to 367 of the provided m-files, the x- and y-limits of the plots can be modified for all electrode signals. By default, the y-limits are set to the values entered in the User Input pop-up window and the x-limits are not specified, resulting in all the data being plotted. Specific values can be set by specifying these limits with set numbers (e.g., commented out for the x-limits in line 367 below) or with variables such as the Vrms calculations markers (e.g., commented out for the x-limits in line 366 below).

```
Examine_Intan_RHS_File_with_Octave__20230322_BMSEED.m ✕
349
350   % Plotting the amplfier data
351 ⊟ for j = 1:fig_num
352       %figure(j+0);
353       figure(j+0, 'position',[1,62,1918,940]);
354
355 ⊟     for k = 1:fig_plots
356           subplot(fig_rows,6,k); hold on;
357           %plot(time(start_sec_indx:step:end_sec_indx), data(start_sec_indx:step:end_sec_
358           plot(time(1:step:end), data(1:step:end,30*(j-1)+k), ...
359               'Color', 'b', 'LineWidth', 1);
360           plot(time(start_sec_indx)*ones(1,2), [y_limits(1) y_limits(2)], 'r:');
361           plot(  time(end_sec_indx)*ones(1,2), [y_limits(1) y_limits(2)], 'r:');
362           title([id_label{30*(j-1)+k} ': ' ...
363               num2str(round(Z_data(30*(j-1)+k)/1000)) 'k\Omega, ' ...
364               num2str(round(Vrms(30*(j-1)+k))) '\muV'])
365           ylim([y_limits(1) y_limits(2)]);
366           %xlim([start_sec_mark end_sec_mark]);
367       %xlim([33 45]);
368     end
369 ⊟
```
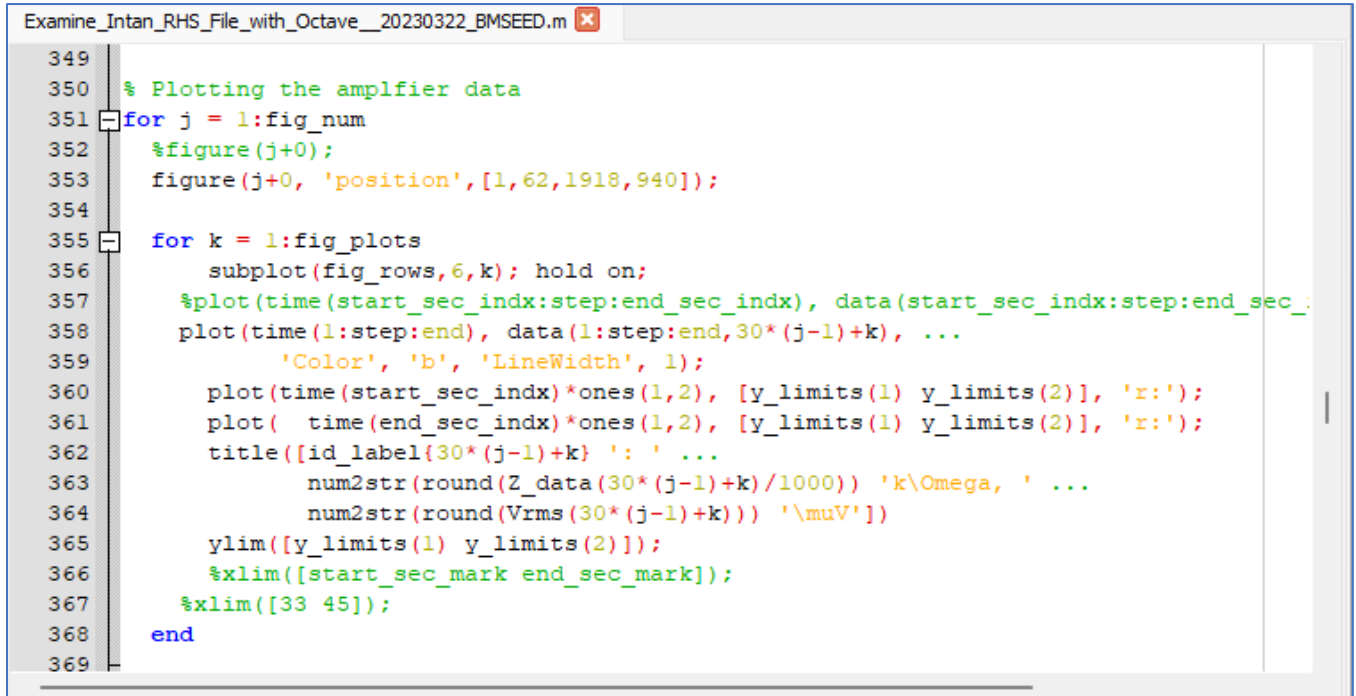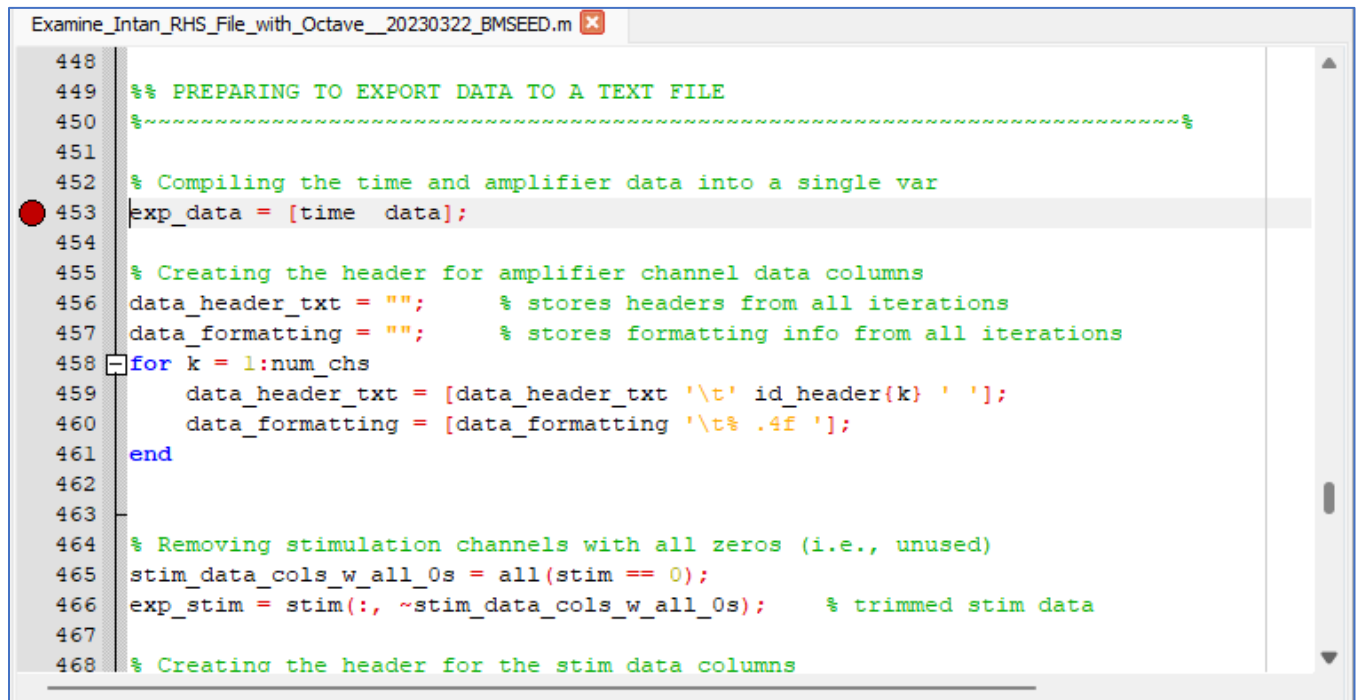
Fig. 131: Section of the Matlab/Octave code to define the plotted electrode signals.

b. Adding "stop" markers (i.e., red circles) to stop the code execution:
"Stop" markers may be added anywhere in the code to pause the code execution, allowing the user access to all the variables to further examine or debug the code. To place the "stop" markers simply left-click next the command line where you want to add the pauses.

After placing the "stop" marker, you may type code in the Command Window to make changes the code. For instance, you may want to create a new figure (e.g., Figure 10), in which you zoom in into only of the electrode's signal (e.g., Electrode 3). For example, you may type the following commands

```
>> figure(10);              % creates a "Figure 10"
>> plot(time, data(:,3))    % plot the time vs data for electrode 3
>> xlim([5 10]);            % zoom in to 5 to 10 on the x-axis
>> ylim([-2000 4000])       % zoom in to -2000 to 4000 on the y-axis
```

Note: To access previously executed commands, use the up and down arrow keys.

Fig. 132: Example of a stop marker being used. Here, it was placed on command line 453 where the code will start the process of exporting the data as a text file.

15 sMEA and glass MEA Electrode Layouts

BMSEED provides sMEAs with one of three layouts for the electrodes' recording sites:

    sMEA **gen4a,d**   **28** recording electrodes and **2** reference electrodes
    sMEA **gen4e**   **28** recording electrodes and **4** reference electrodes
    sMEA **gen6**   **56** recording electrodes and **6** reference electrodes

For all sMEA layouts, the sample must be placed in the interface board as shown in **Fig. 15** (i.e., with the "BMSEED SMEA" label on the PCB positioned on the side with the engraved arrow).

15.1 sMEA gen4a, gen4b

sMEA gen4a electrode arrays have recording sites arranged following an "x" layout. The 28 rec. sites cover an area of 1.6mm × 1.6mm, have an exposed area of 80µm x 80µm, and are irregularly spaced with 100µm being the minimum distance between adjacent sites.

(a)   (b)
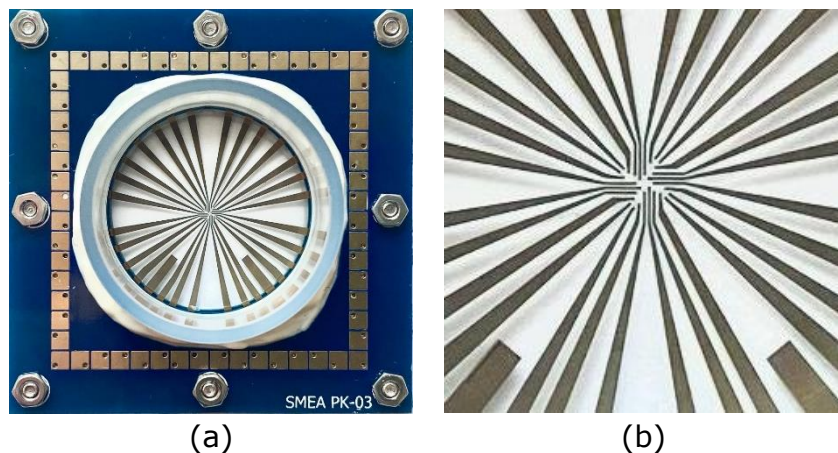
Fig. 133: (a) Macro and **(b)** zoomed-in photos of a gen4a sMEA depicting its layout.

(a)   (b)

Fig. 134: (a) Schematic showing the gen4a sMEA layout. **(b)** Mapping between recording sites at the center of a gen4a sMEA and its outer pads on the PCB. Note: Representative schematic only. Not drawn to scale.

15.2 sMEA gen4d

sMEA gen4d electrode arrays have recording sites arranged following a square layout, covering an area of 1.7mm × 1.7mm. The 28 recording sites have an area of 100µm x 100µm and are uniformly spaced with 300µm minimum distance between adjacent sites. The gen4d layout has the recording sites somewhat less densely packed than the gen4a layout.

(a)   (b)

Fig. 135: (a) Macro and **(b)** zoomed-in photos of a gen4d sMEA depicting its layout.

(a)   (b)

Fig. 136: (a) Schematic showing the gen4d sMEA layout. **(b)** Mapping between recording sites at the center of a gen4d sMEA and its outer pads on the PCB. Note: Representative schematic only. Not drawn to scale.

15.3 sMEA gen4e

Similar to sMEA gen4d, sMEA gen4e electrode arrays have recording sites arranged following a square layout, covering an area of 1.7mm × 1.7mm. The 28 recording sites have an area of 100µm x 100µm and are uniformly spaced with 300µm minimum distance between adjacent sites. The gen4e layout is the same as the gen4d layout, but with two additional internal reference electrodes, for a total of four reference electrodes.

Note: sMEA gen4e has one reference electrode in each orientation (Sdrd vs 90°) that matches the internal reference electrode of glass MEAs (e57), thus can be used as the internal reference electrode in MultiChannel Systems electrophysiology equipment.

Depending on the orientation of the sMEA (See Section 15.7):

Standard Orientation	**REF3** internal reference
90° Orientation	**REF4** internal reference

(a) (b)
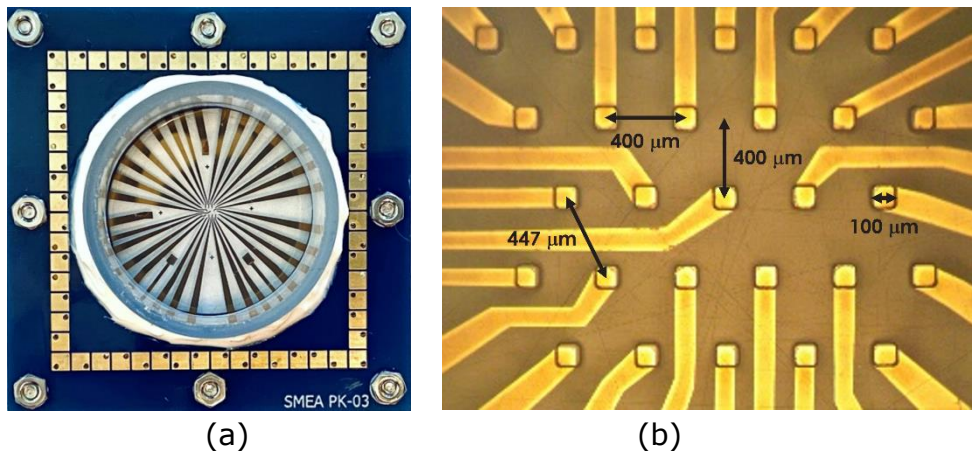
Fig. 100: (a) Macro and **(b)** zoomed-in photos of a gen4e sMEA depicting its layout.

(a) (b)
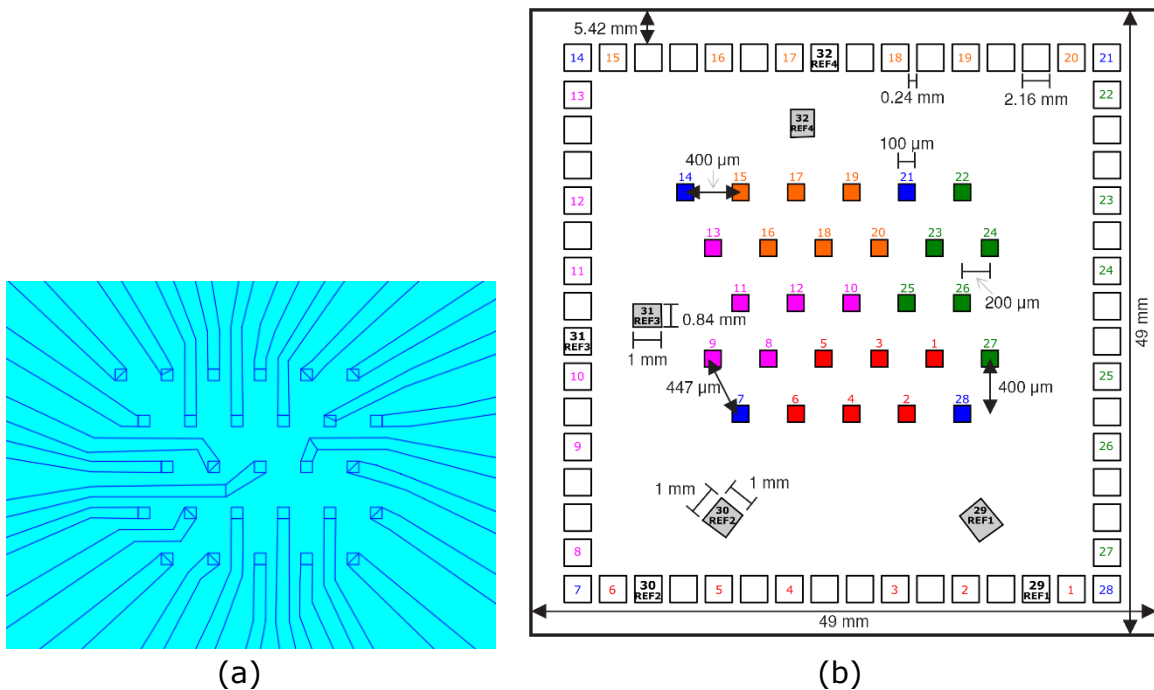
Fig. 101: (a) Schematic showing the gen4e sMEA layout. **(b)** Mapping between recording sites at the center of a gen4e sMEA and its outer pads on the PCB. Note: Representative schematic only. Not drawn to scale.

15.4 sMEA gen6a

sMEA gen6 electrode arrays have recording sites arranged following a hexagonal layout, covering an area of 2.64mm × 2.3mm. The 56 round recording sites have a diameter of 70µm and are uniformly spaced with 260µm minimum distance between adjacent sites. In contrast to its gen4 counterpart, gen6 sMEAs have 4 reference electrodes, one on each side.

Note: sMEA gen6a has one reference electrode in each orientation (Sdrd vs 180°) that matches the internal reference electrode of glass MEAs (e57), thus can be used as the internal reference electrode in MultiChannel Systems electrophysiology equipment.

Depending on the orientation of the sMEA (See Section 15.8):

|	
Standard Orientation	**REF4** internal reference
180° Orientation	**REF2** internal reference

(a) (b)
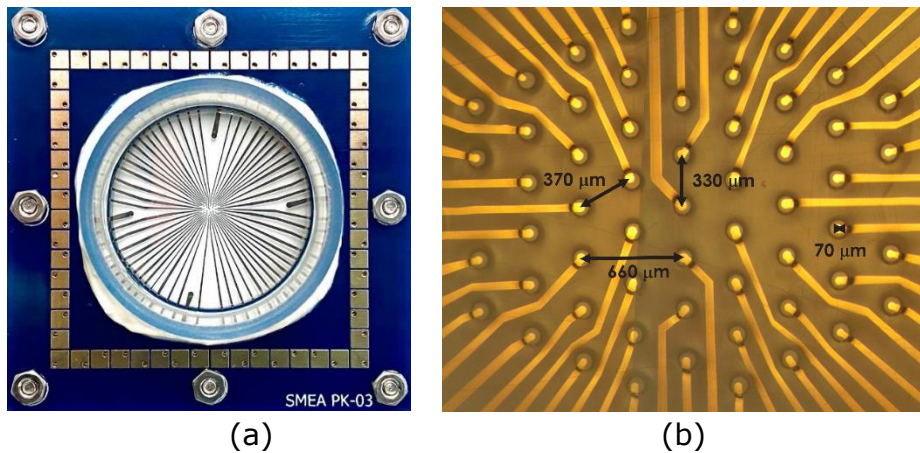
Fig. 137: (a) Macro and **(b)** zoomed-in photos of a gen6 sMEA depicting its layout.
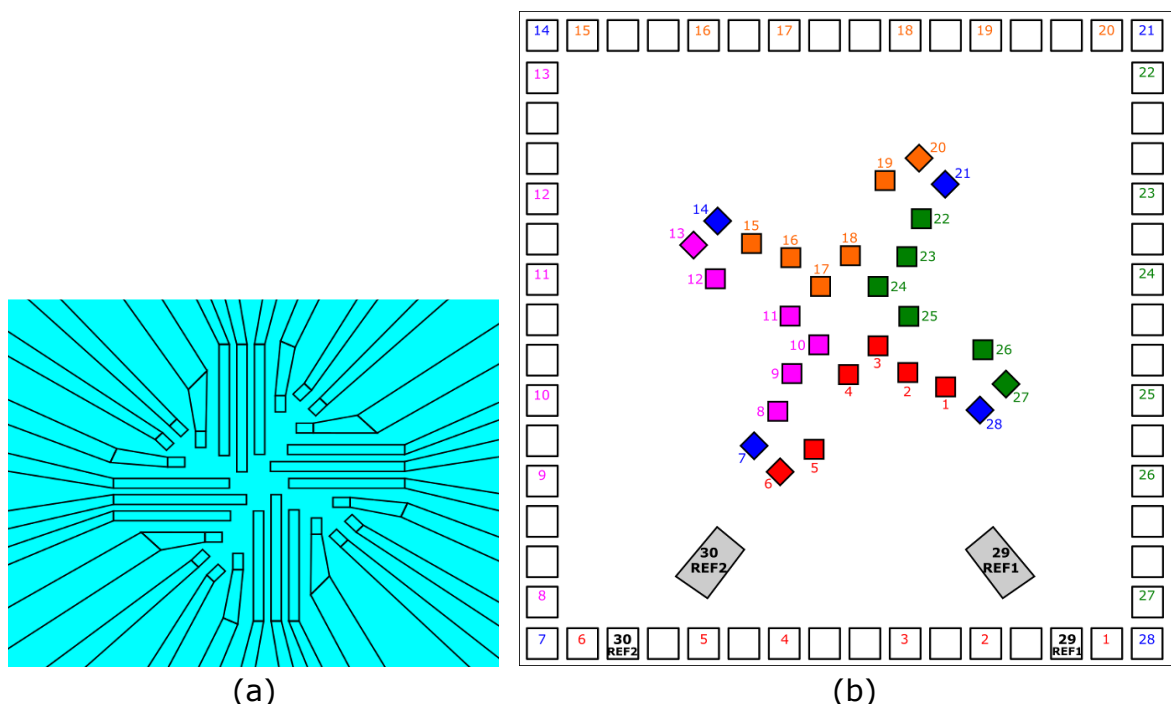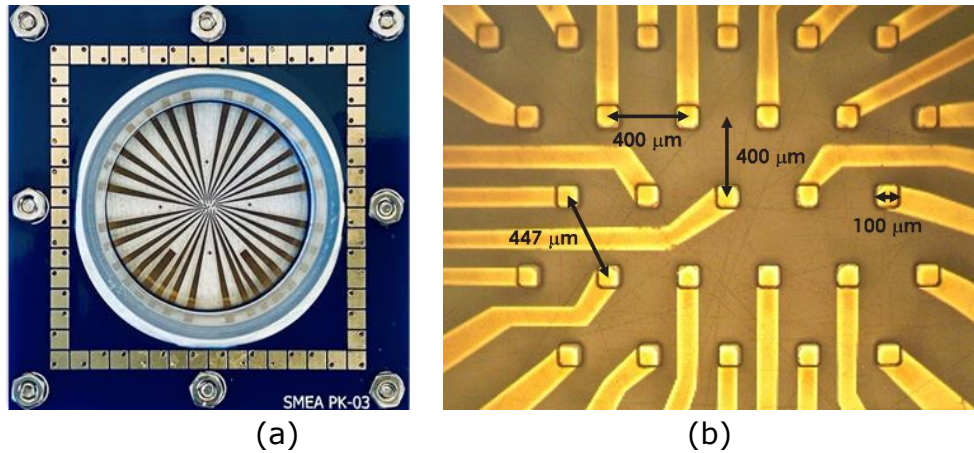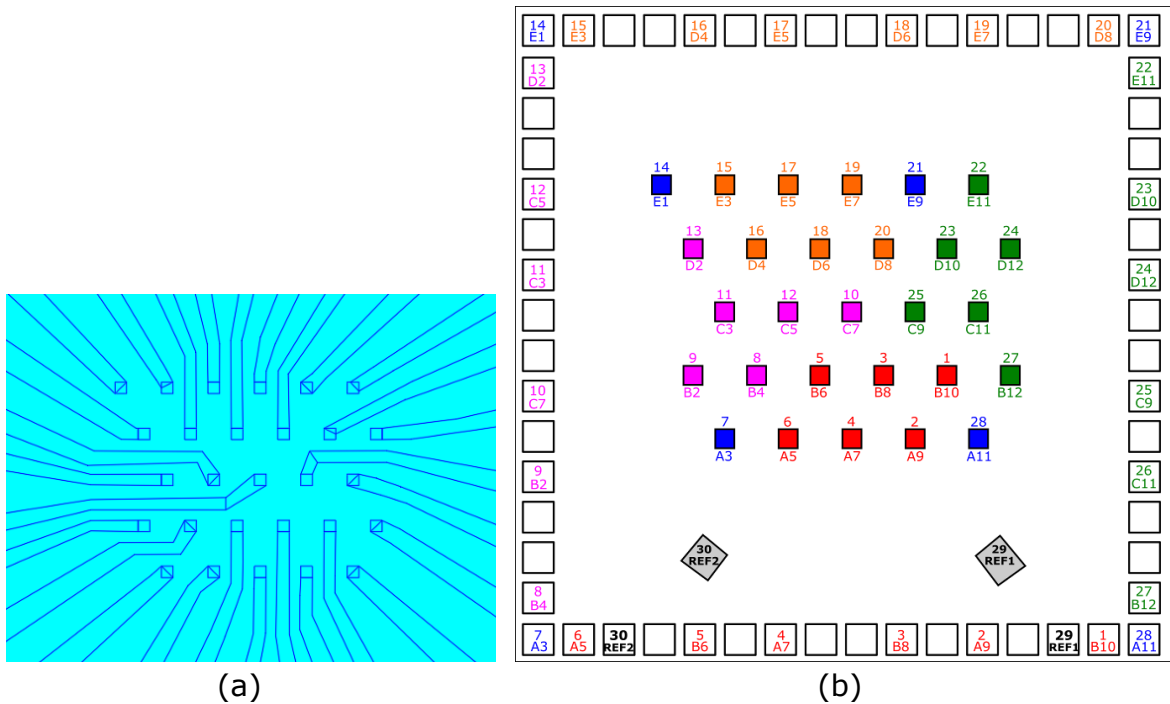

(a) (b)
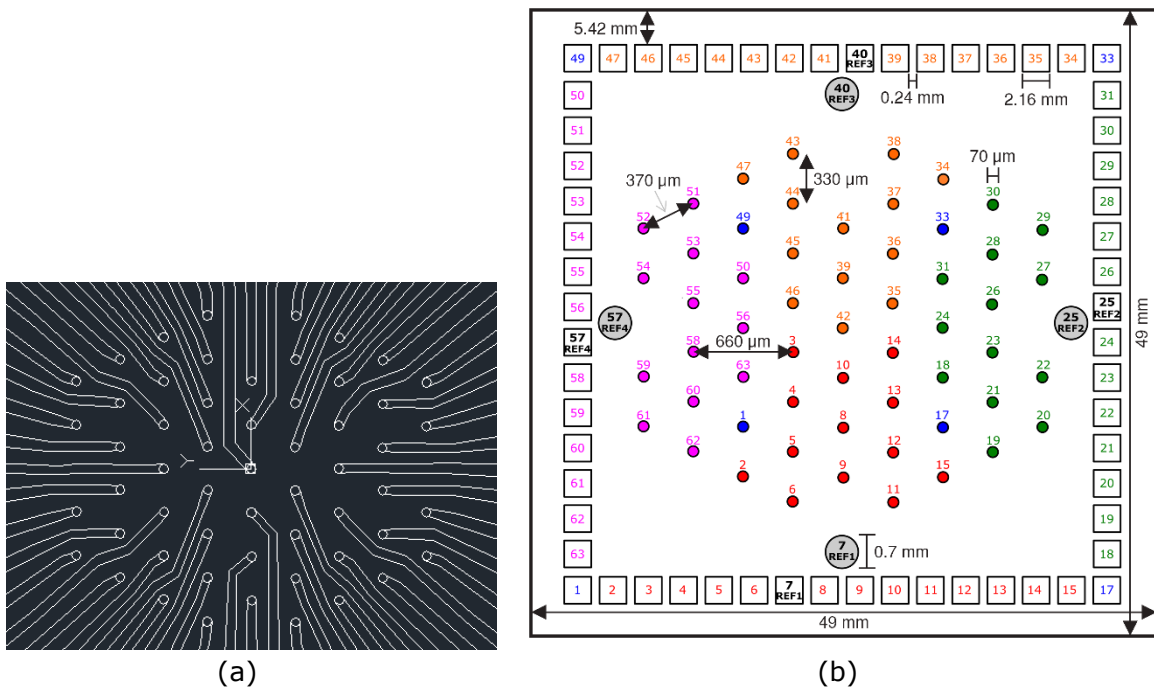
Fig. 138: (a) Schematic showing the gen6 sMEA layout. **(b)** Mapping between recording sites at the center of a gen6 sMEA and its outer pads on the PCB. Note: Representative schematic only. Not drawn to scale.

15.5 sMEA gen7aF

sMEA gen7aF electrode arrays have recording sites arranged following a circular layout, covering an area of 5.0mm × 5.0mm. The 30 square recording sites have a diameter of 200µm and are uniformly spaced with _µm minimum distance between adjacent sites. Similar to gen6 sMEAs, gen7aF sMEAs have 4 reference electrodes, two on each side of the microchannel location.
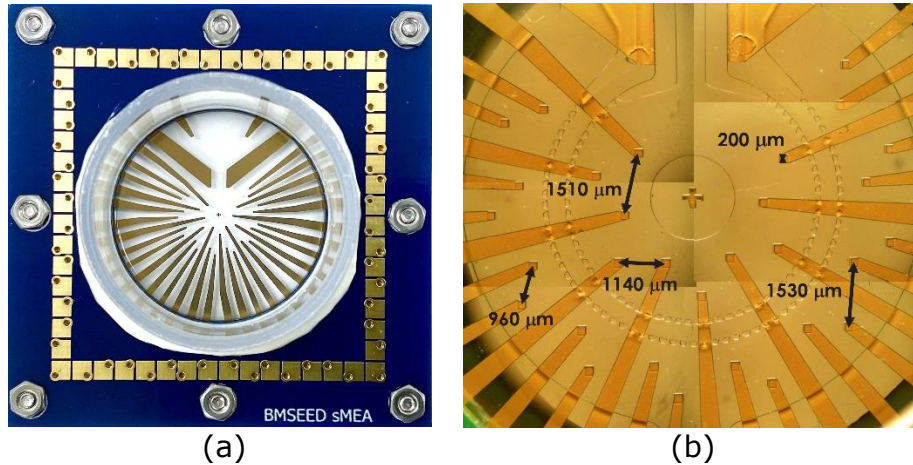
Fig. 139: (a) Macro and **(b)** zoomed-in photos of a gen7aF sMEA depicting its layout.
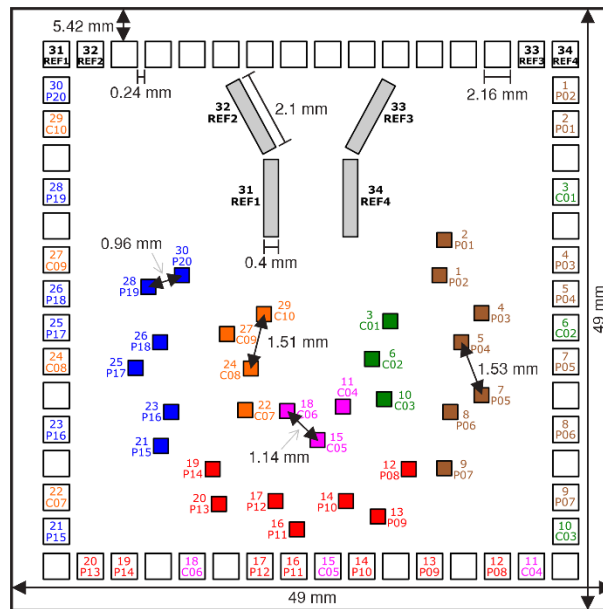
Fig. 140: Mapping between recording sites at the center of a gen7aF sMEA and its outer pads on the PCB. Note: Representative schematic only. Not drawn to scale.

15.6 Glass MEA (60MEA200/30iR-Ti)

BMSEED's glass MEAs are the same as those from MCS. The mapping of the electrodes is the same as well. For the 60MEA200/30iR-Ti, the electrode array has 60 recording sites with a diameter of 30µm spaced at a distance of 200µm center-to-center.

(a)   (b)
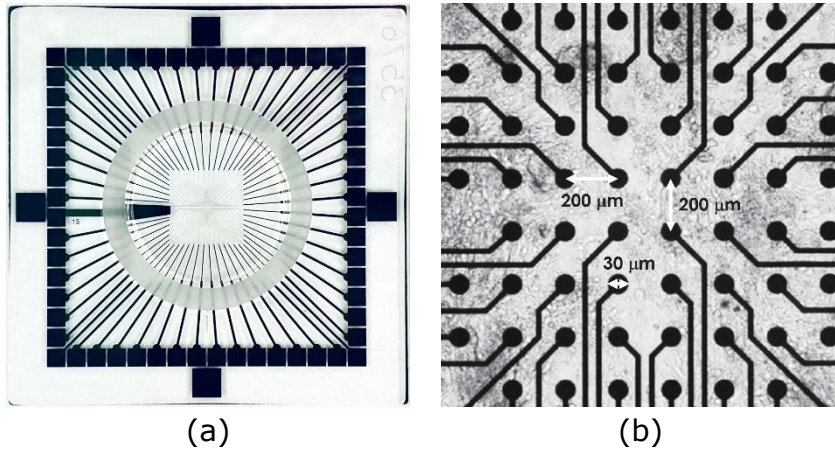
Fig. 141: (a) Macro and **(b)** zoomed-in photos of 60 electrode glass MEAs.

BMSEED uses the numbering scheme shown in yellow circles below to map the electrodes.
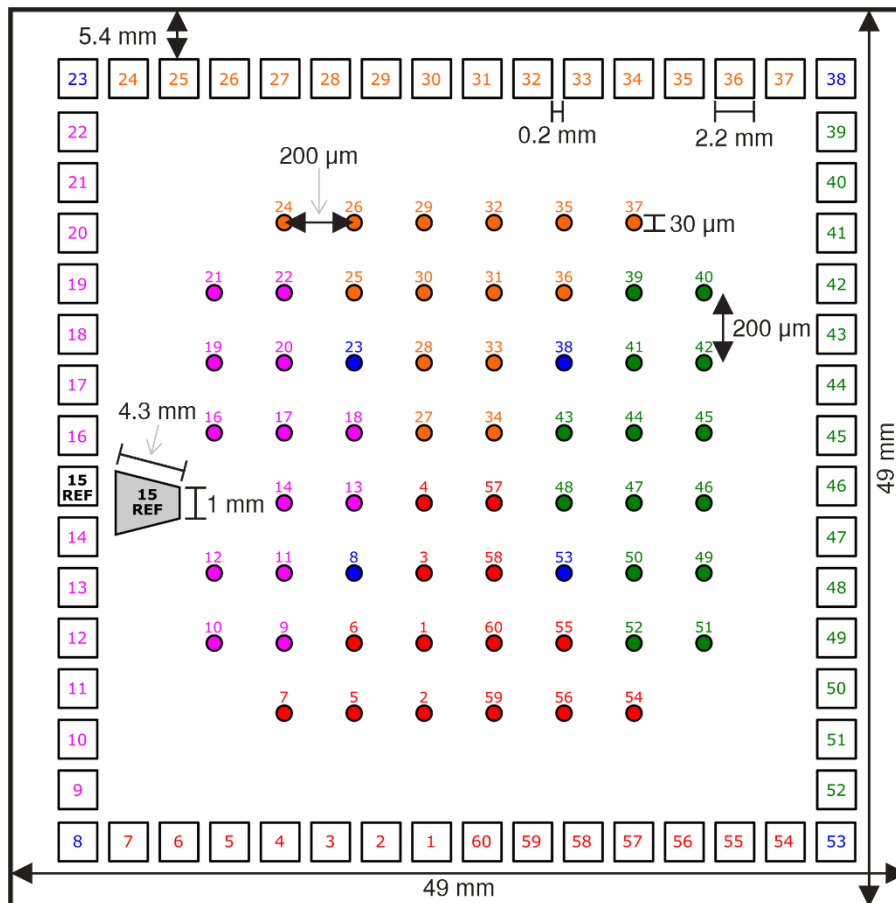
Fig. 142: Mapping between recording sites at the center of a glass MEA and its outer pads.
Note: Representative schematic only. Not drawn to scale.

15.7 Comparing gen4e sMEA and glass MEA Reference Electrodes

Depending on its orientation when placed inside the interface board, two gen4e sMEA reference electrodes could be in the same location as the sole reference channel of the glass MEA (**Fig. 143**:).

- When placing the gen4e sMEA with the <u>white "SMEA" label located along the engraved arrow side of the interface board</u> (i.e., recommended "standard" orientation as given in **Fig. 15a** of this document):
  - **Reference electrode #3** (highlighted in red in **Fig. 143**:**b,c**) will match the standard glass MEA reference channel (highlighted in red in **Fig. 143**:**a**) as they will both be located on pin 57 of the interface board.
- When placing the gen4e sMEA with the <u>white "SMEA" label located along the opposite side of the engraved arrow side of the interface board</u> (i.e., rotated 180° from the "standard" orientation recommended in Fig. 15a of this document):
  - **Reference electrode #4** (highlighted in **green** in **Fig. 143**:**b,c**) will match the standard glass MEA reference channel (highlighted in red in **Fig. 143**:**a**) as they will both be located on pin 57 of the interface board.

(a)   (b)   (c)
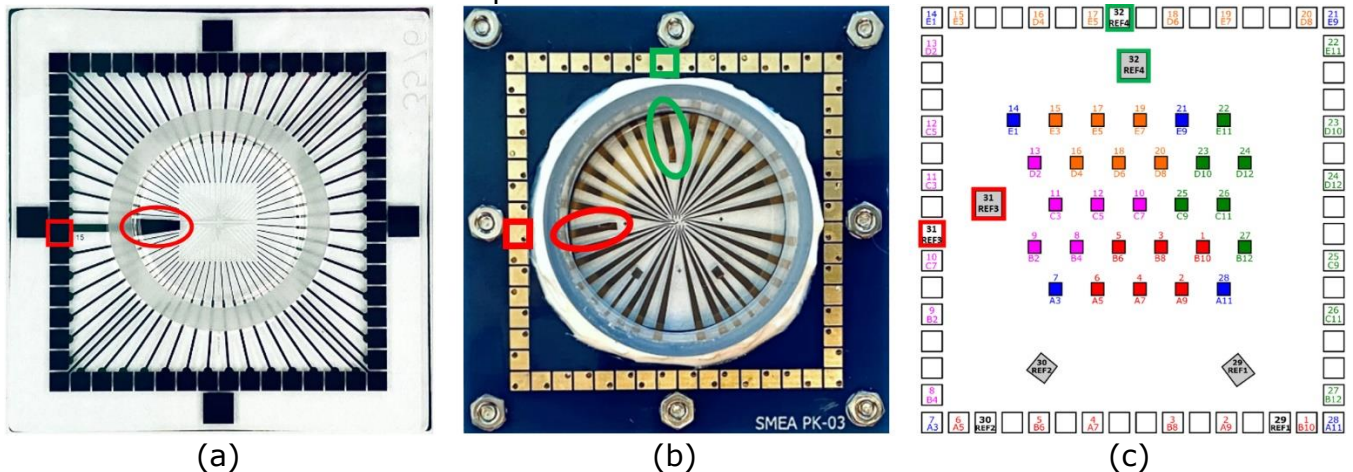
Fig. 143: (a) the sole reference channel of the standard glass MEA compared to **(b)** a photo of a gen4e sMEA and **(c)** gen4e sMEA pad layout showing which of its reference electrodes could be located in the same position as the glass MEA (i.e., Pin 57 of the board).

15.8 Comparing gen6 sMEA and glass MEA Reference Electrodes

Depending on its orientation when placed inside the interface board, two gen6 sMEA reference electrodes could be in the same location as the sole reference channel of the glass MEA (**Fig. 144**).

- When placing the gen6 sMEA with the <u>white "SMEA" label located along the engraved arrow side of the interface board</u> (i.e. recommended "standard" orientation as given in **Fig. 15a** of this document):
  - o **Reference electrode #4** (highlighted in red in **Fig. 144b,c**) will match the standard glass MEA reference channel (highlighted in red in **Fig. 144a**) as they will both be located on pin 57 of the interface board.

- When placing the gen6 sMEA with the <u>white "SMEA" label located along the opposite side of the engraved arrow side of the interface board</u> (i.e. rotated 180° from the "standard" orientation recommended in **Fig. 15a** of this document):
  - o **Reference electrode #2** (highlighted in green in **Fig. 144b,c**) will match the standard glass MEA reference channel (highlighted in red in **Fig. 144a**) as they will both be located on pin 57 of the interface board.

(a)   (b)   (c)
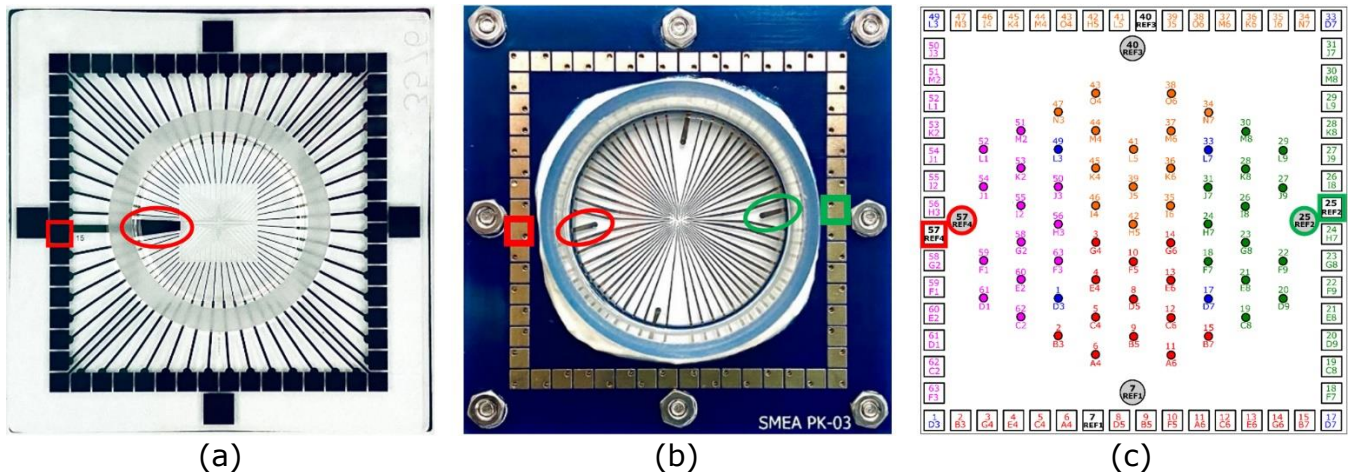
Fig. 144: (a) the sole reference channel of the standard glass MEA compared to **(b)** a photo of a gen6 sMEA and **(c)** gen6 sMEA pad layout showing which of its reference electrodes could be located in the same position as the glass MEA (i.e., Pin 57 of the board).

16 BMSEED EIB Mapping

16.1 EIB and Intan Headstage Mapping
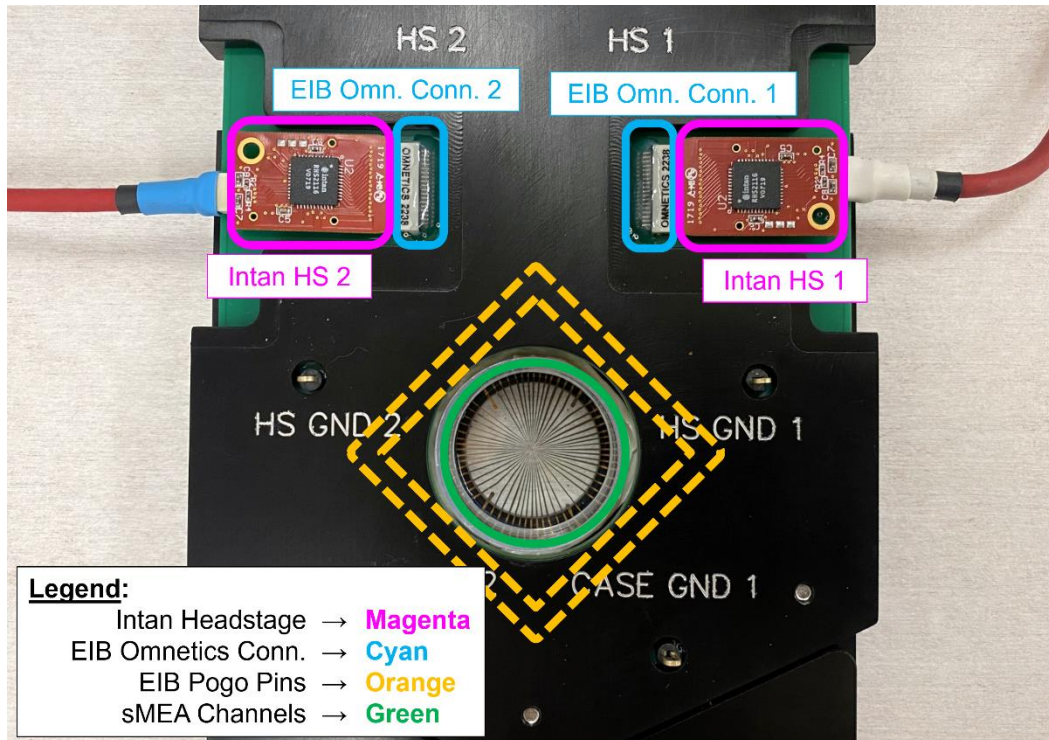
Fig. 145: Different components of the electrophysiology module connected to the BMSEED electrophysiology interface board.
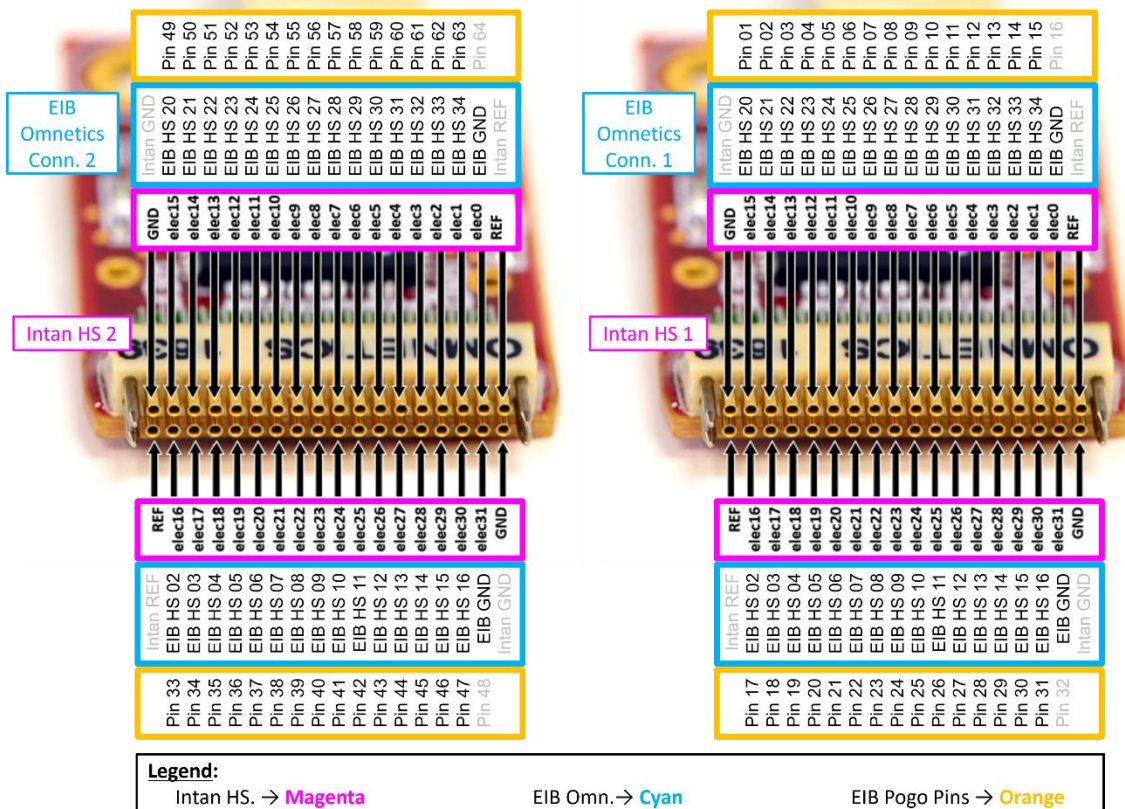
Fig. 146: From the headstage perspective: mapping between the Intan headstages, Omnetics connector on the interface board, and board pins.
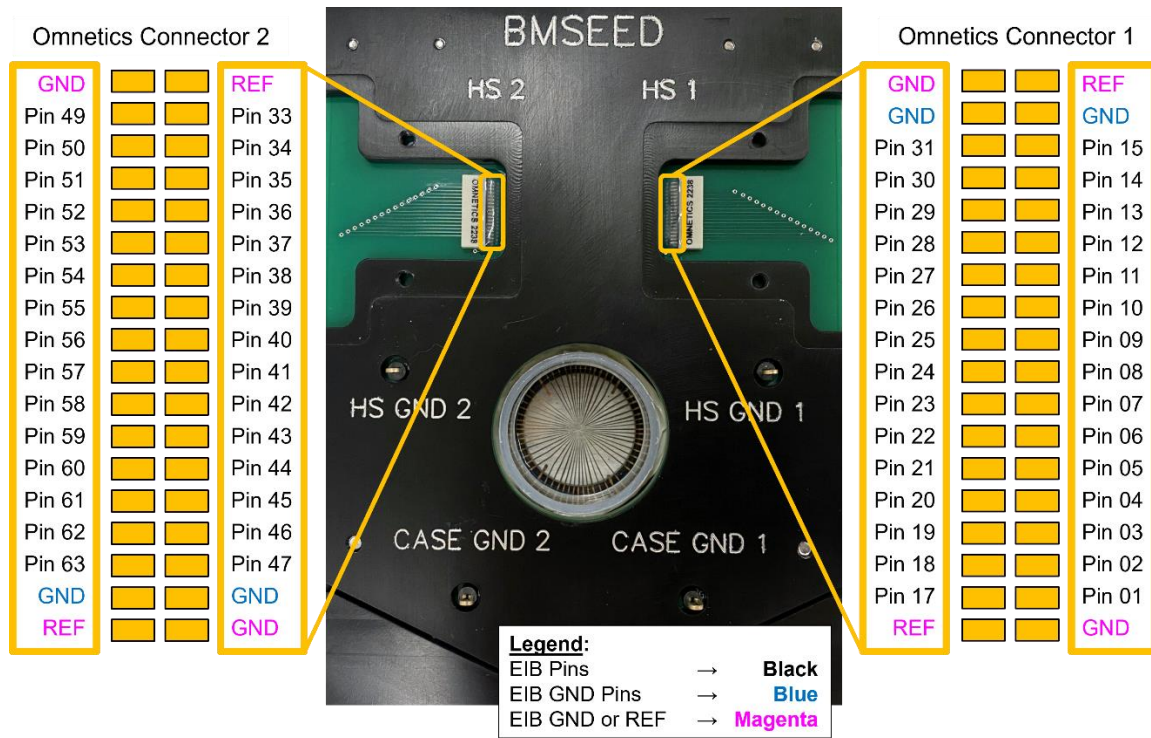
Fig. 147: Pad layout of the Omnetics connector resting on EIB. Pads are mapped to their corresponding EIB pin number.
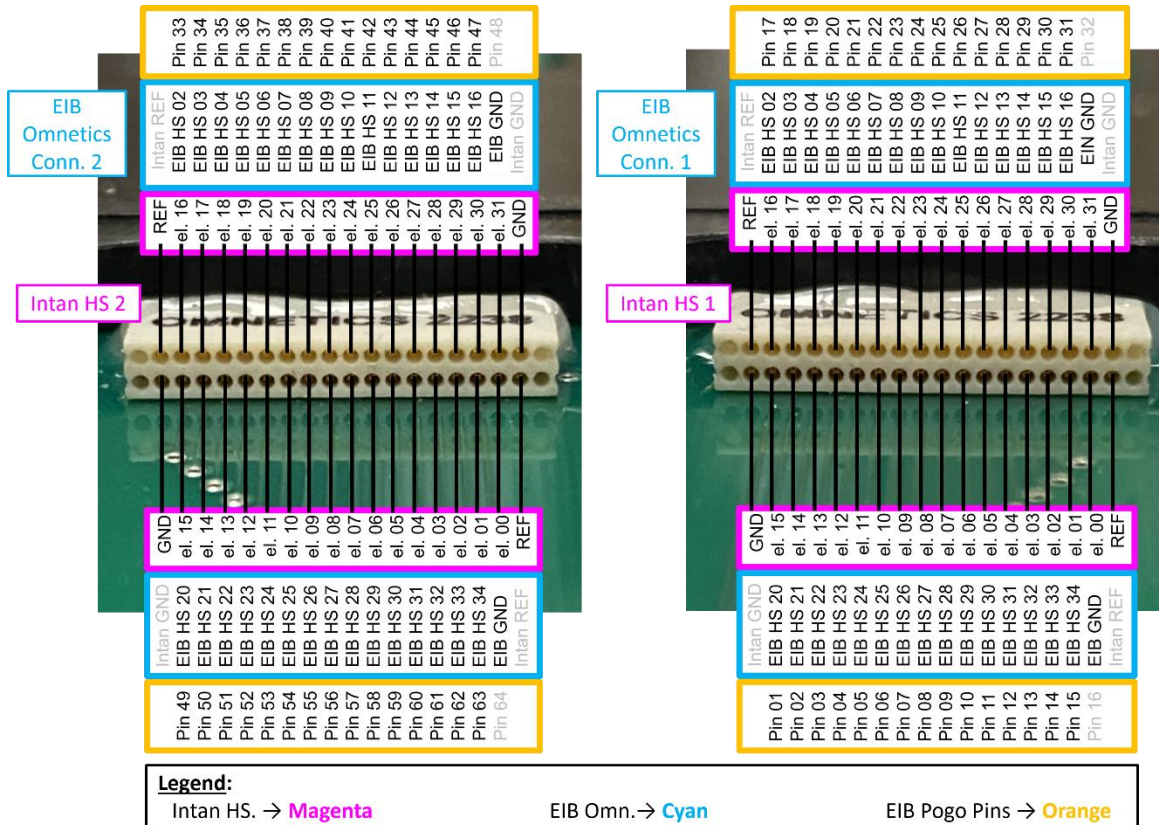
Fig. 148: From the connector perspective: mapping between the Intan headstages, Omnetics connector on the interface board, and board pins.

16.2 BMSEED EIB, Intan, and gen4e sMEA Electrode Mapping
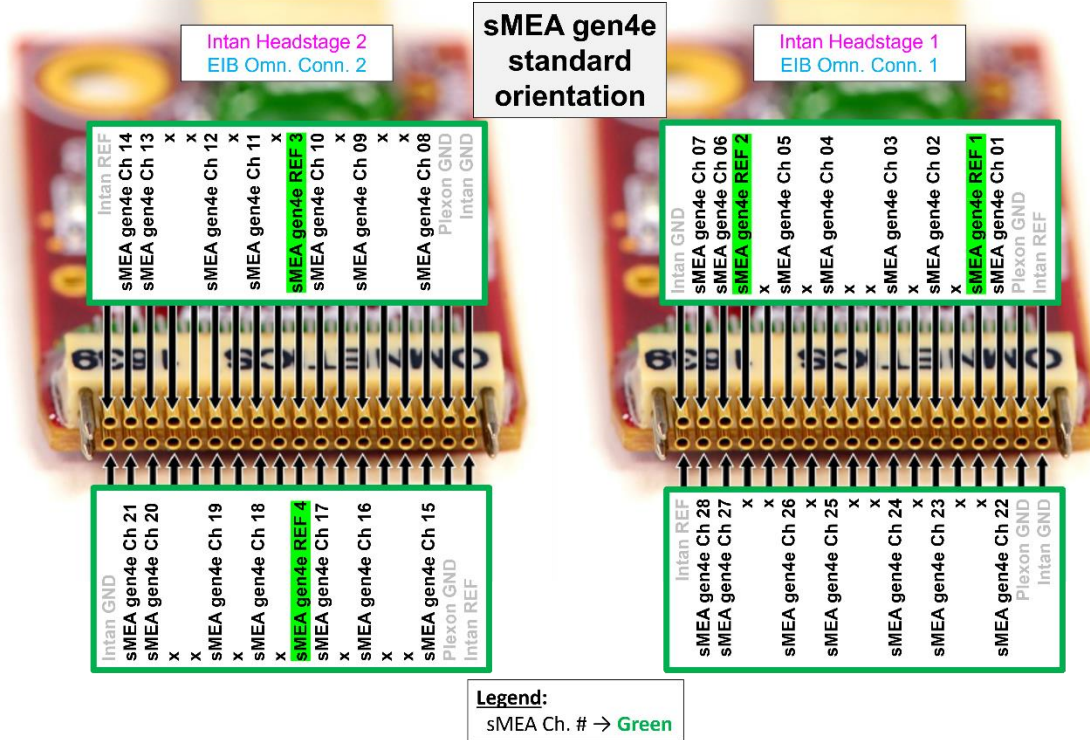
Fig. 149: Mapping between the Intan headstage and the channels of a gen4 sMEA placed in the interface board with standard orientation. The two reference channels are highlighted in green.
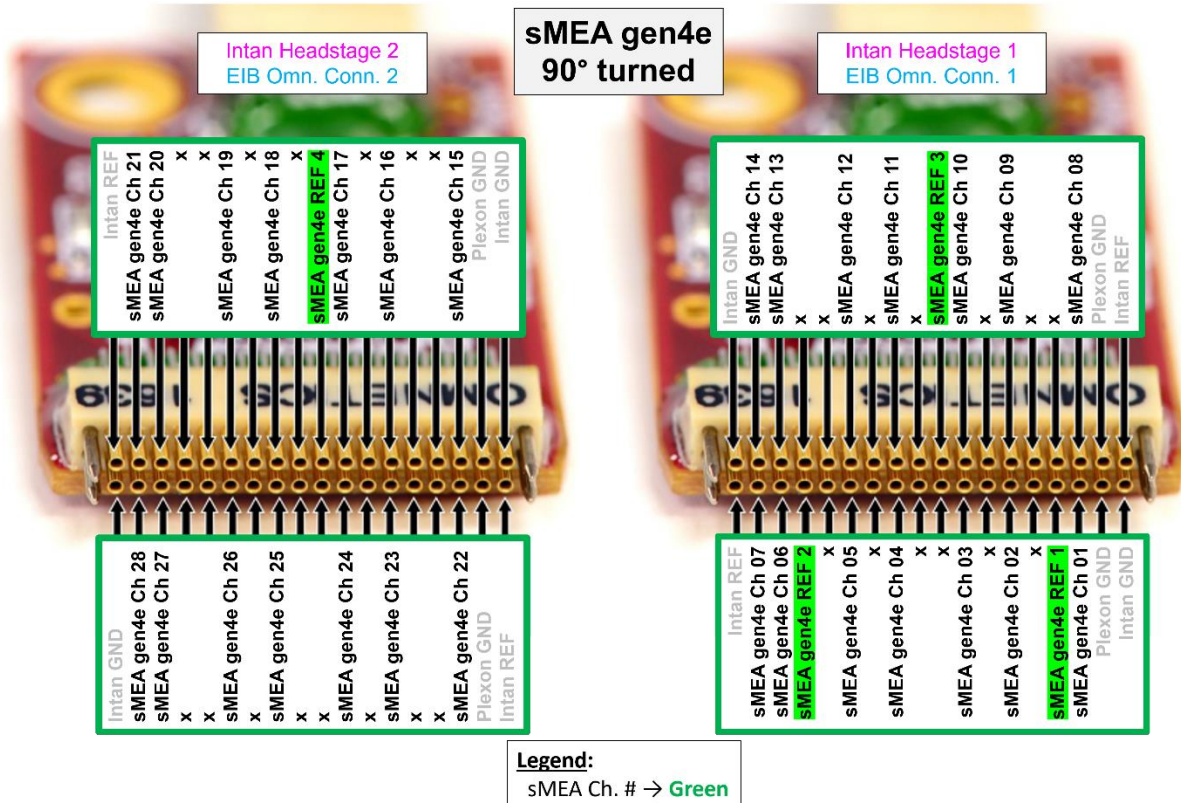
Fig. 150: Mapping between the Intan headstage and the channels of a gen4 sMEA placed in the interface board 90° turned (counter-clockwise from standard orientation). The two reference channels are highlighted in green.

16.3 Interface board, Intan, and gen6 sMEA Electrode Mapping

Fig. 151: Mapping between the Intan headstage and the channels of a gen6 sMEA placed in the interface board with standard orientation. The four reference channels are highlighted in green.

16.4 Interface board, Intan, and glass MEA Electrode Mapping

Fig. 152: Mapping between the Intan headstage and the channels of a glass MEA placed in the interface board with standard orientation. Reference channel is highlighted in green.

16.5 Interface Board Connections (Omnetics connector, Intan headstage, pogo pins, sMEA #, glass MEA #)

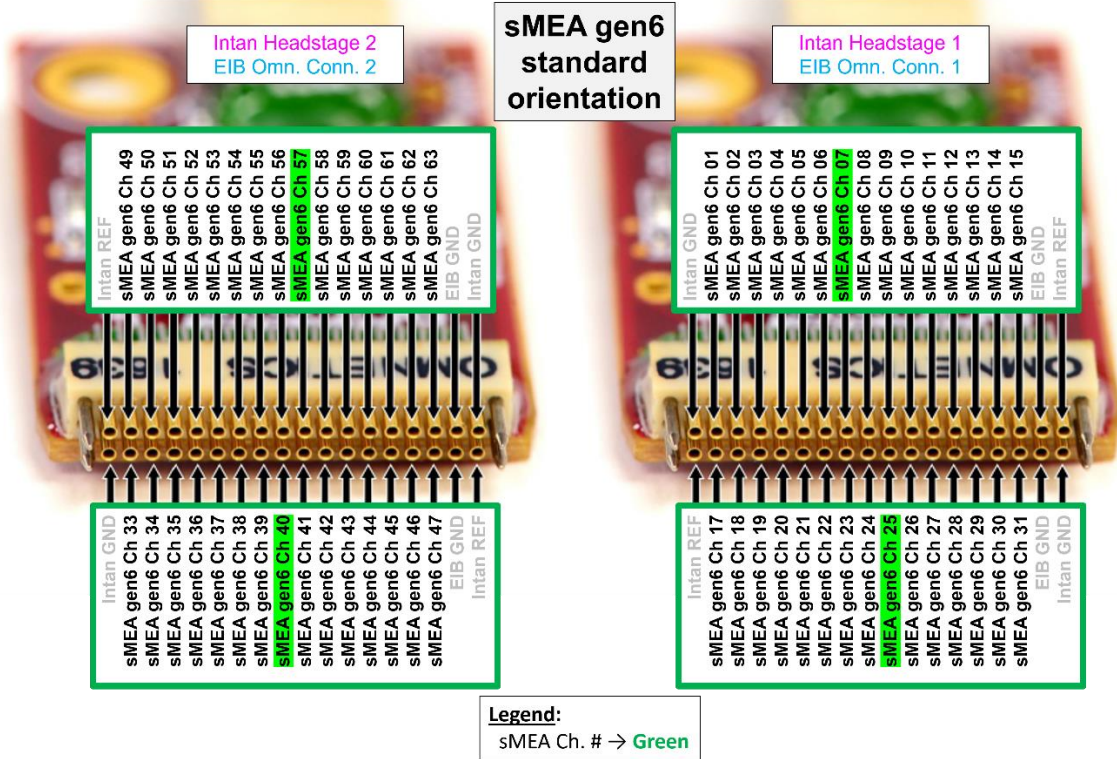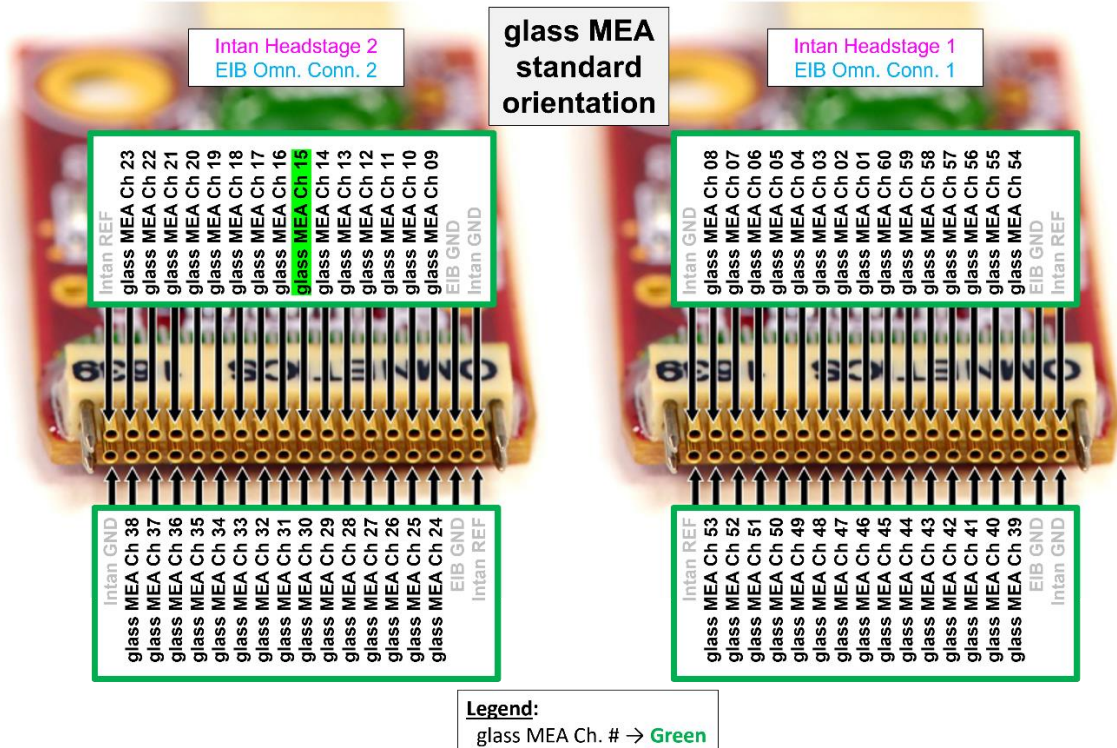| Intan Port	sMEA Interface Board Pogo Pin #	sMEA Int. Board Omnetics Connector	InTan Headstage RHS2000 (RED)	gen4 sMEA channel # - standard orientation	gen4 sMEA channel # - 90deg turned	gen4e sMEA channel # - standard orientation	gen4e sMEA channel # - 90deg turned	gen6 sMEA channel # - standard orientation	gen6 sMEA channel # - 90deg turned	gen7a sMEA channel # - standard orientation	labels, original layout	labels, flipped layout on wafer	glass MEA channel # - standard orientation	glass MEA channel # - 90deg turned
Port A or C	1	20	in 015	7	14	7	14	1	49				8	23
	2	21	in 014	6	13	6	13	2	50	20	C07	P13	7	22
	3	22	in 013	REF_2	__	REF_2	__	3	51	19	P13	P14	6	21
	4	23	in 012					4	52				5	20
	5	24	in 011	5	12	5	12	5	53	18	P12	C06	4	19
	6	25	in 010					6	54				3	18
	7	26	in 009	4	11	4	11	REF_1	55	17	P11	P12	2	17
	8	27	in 008					8	56	16	C06	P11	1	16
	9	28	in 007	__	__	__	REF_3	9	REF_4	15	P10	C05	60	REF
	10	29	in 006	3	10	3	10	10	58	14	P09	P10	59	14
	11	30	in 005					11	59				58	13
	12	31	in 004	2	9	2	9	12	60	13	C05	P09	57	12
	13	32	in 003					13	61				56	11
	14	33	in 002	REF_1	__	REF_1	__	14	62	12	P07	P08	55	10
	15	34	in 001	1	8	1	8	15	63	11	P08	C04	54	9
	16	GND	in 000											
	17	2	in 016	28	7	28	7	17	1	__	__	__	53	8
	18	3	in 017	27	6	27	6	18	2	10	P06	C03	52	7
	19	4	in 018	__	REF_2	__	REF_2	19	3	9	C04	P07	51	6
	20	5	in 019					20	4				50	5
	21	6	in 020	26	5	26	5	21	5	8	P05	P06	49	4
	22	7	in 021					22	6				48	3
	23	8	in 022	25	4	25	4	23	REF_1	7	C03	P05	47	2
	24	9	in 023					24	8	6	P04	C02	46	1
	25	10	in 024	__	__	__	__	REF_2	9	5	P03	P04	45	60
	26	11	in 025	24	3	24	3	26	10	4	C02	P03	44	59
	27	12	in 026					27	11				43	58
	28	13	in 027	23	2	23	2	28	12	3	P02	C01	42	57
	29	14	in 028					29	13				41	56
	30	15	in 029	__	REF_1	__	REF_1	30	14	2	C01	P01	40	55
	31	16	in 030	22	1	22	1	31	15	1	P01	P02	39	54
	32	GND	in 031											
Port B or D	33	2	in 016	21	28	21	28	33	17	REF_4	REF_4	REF_4	38	53
	34	3	in 017	20	27	20	27	34	18	REF_3	REF_3	REF_3	37	52
	35	4	in 018					35	19				36	51
	36	5	in 019					36	20	pad dne	pad dne	pad dne	35	50
	37	6	in 020	19	26	19	26	37	21	pad dne	pad dne	pad dne	34	49
	38	7	in 021					38	22	pad dne	pad dne	pad dne	33	48
	39	8	in 022	18	25	18	25	39	23	pad dne	pad dne	pad dne	32	47
	40	9	in 023	__	__	__	__	REF_3	24	pad dne	pad dne	pad dne	31	46
	41	10	in 024	__	__	REF_4	__	41	REF_2	pad dne	pad dne	pad dne	30	45
	42	11	in 025	17	24	17	24	42	26	pad dne	pad dne	pad dne	29	44
	43	12	in 026					43	27	pad dne	pad dne	pad dne	28	43
	44	13	in 027	16	23	16	23	44	28	pad dne	pad dne	pad dne	27	42
	45	14	in 028					45	29	pad dne	pad dne	pad dne	26	41
	46	15	in 029					46	30				25	40
	47	16	in 030	15	22	15	22	47	31	REF_2	REF_2	REF_2	24	39
	48	GND	in 031											
	49	20	in 015	14	21	14	21	49	33	REF_1	REF_1	REF_1	23	38
	50	21	in 014	13	20	13	20	50	34	30	P19	P20	22	37
	51	22	in 013					51	35	29	P20	C10	21	36
	52	23	in 012					52	36				20	35
	53	24	in 011	12	19	12	19	53	37	28	C10	P19	19	34
	54	25	in 010					54	38				18	33
	55	26	in 009	11	18	11	18	55	39	27	P18	C09	17	32
	56	27	in 008					56	REF_3	26	P17	P18	16	31
	57	28	in 007	__	__	REF_3	REF_4	REF_4	41	25	C09	P17	REF	30
	58	29	in 006	10	17	10	17	58	42	24	P16	C08	14	29
	59	30	in 005					59	43				13	28
	60	31	in 004	9	16	9	16	60	44	23	P15	P16	12	27
	61	32	in 003					61	45				11	26
	62	33	in 002					62	46	22	P14	C07	10	25
	63	34	in 001	8	15	8	15	63	47	21	C08	P15	9	24
	64	GND	in 000											

17 Troubleshooting

a) Impedances and noise level are high and unstable

i. Check for bubbles on the recording sites (i.e., exposed areas)

If bubbles are present, place the sMEA with solution under vacuum inside a desiccator for at least 10 minutes. Inspect under the microscope. If bubbles are still present, you can place it under vacuum for an extra 20 minutes or use a pipette to carefully blow or suction the bubbles off the recording sites.

ii. Check that the sMEA or MEA is inserted in the interface board in the correct orientation

The standard orientation is defined as:

- For sMEAs: The two larger reference electrodes and white label "SMEA" on the PCB should be aligned with the side of the interface board with the engraved arrow pointing to it (Fig. 15a+b). The reference electrodes of the gen4 sMEAs must contact pins on this side of the interface board.

- For MEAs: The side of the MEA with the large reference electrode (Electrode #57) should be aligned with the side of the interface board to the left of the side with the engraved arrow pointing to it (Fig. 15c).

In addition, make sure that the proper Intan mapping file (".isf" file extension) is being used. Pay attention that the physical placement of the sMEA in the interface board matches the label in the Intan mapping file in the following ways:

- Array: "sMEA-gen4" corresponds to stretchable 28-channel sMEAs and "glass MEA" corresponds to the rigid 60-channel glass MEAs.

- Ports: Make sure that the headstages connected to the interface board are connected to the appropriate ports on the front panel of the Intan controller. The Intan mapping filename should list the ports in order: the first port listed should connect to the interface headstage labeled "HS1"; the second port listed should connect to the interface headstage labeled "HS2."

- Orientation: If no orientation is mentioned in the Intan mapping file, the sMEA should be placed in the interface board using the standard orientation. If "90degTilt" appears in the mapping file, the sMEA should be placed with the two larger reference electrodes and white label "SMEA" on the PCB aligned with the side of the interface board to the right of the side with the engraved arrow pointing to it.

iii. Check that the external or internal reference electrode properly grounded

The use of an external platinum counter electrode is the preferred method for defining the reference electrode. Use an IC clip (or IC hook) wire to connect one of the four "Ground" pins on the interface board to the platinum electrode. The electrode should be dipped into the saline solution in the sMEA/MEA well. During experiments, the electrode might come out of the well. You may use electrical tape to hold the IC clip wire in place to prevent this.

iv. Check shielding of electromagnetic interferences (EMI)

The shielding cover should minimize the EMI around the interface board. If necessary, use a wire to connect one of the four "Ground" pins on the interface board to the shielding cover via electrical tape.

> Make sure that the end of the USB cable connected to the PC loops around the ferrite core (solid, 23mm 19Ω) at least eight times. This decreases the EMI affecting the Intan signals.

v. Check issues with the interface board

Issues with the interface board are not common but could result in noisy signals.

- Changing the orientation that the sMEA is placed in the board allows to distinguish between issues of the electrode array and the interface board. Follow these steps:

    1. Measure impedance and recording noise with the sMEA in the standard orientation, i.e., the sMEA is placed in the interface board such that the reference electrodes (gen4) connect to pins on the side with the engraved arrow pointing to it.

    2. Rotate the sMEA counter-clockwise by 90° so that reference electrodes (gen4) connect to pins on the side to the right of the side with the engraved arrow pointing to it.

    3. Change the Intan mapping (".isf" file) to the corresponding 90° rotated orientation mapping file.

    4. Measure impedance and recording noise with the sMEA in this rotated orientation.

    5. Compare the electrodes with high noise and impedance.

        o If the same electrodes have high noise or high impedance in both orientations, then the issue is likely caused by the sMEA.

        o If the electrodes with high impedance are different in the standard and the rotated orientation, then the issue is likely caused by the interface board.

- If one channel displays a high amplitude, high frequency signal (i.e. an almost completely blue window) as shown in Fig. 153, this could indicate an issue with the Intan headstage or Omnetics connector.
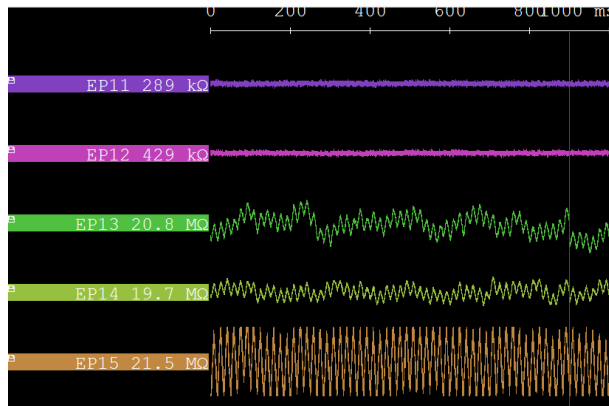
Fig. 153: Screenshot of the Intan signals when there could be an issue with an Intan pin.

One simple way to, at least temporarily, resolve this issue is to unplug and reconnect the headstages into the interface board Omnetics connectors.

A more in-depth protocol to find the cause of the issue is:

1. Note the sMEA channel number and EIB pin ID where the signal is noisy. In our example above, this would be channel *15* and pin ID *D-027*.

2. Swap the headstages, i.e. remove both headstages from the Omnetics connectors and cables connected to the Intan controller ports; plug in the headstage initially connected to the Omnetics connector labeled "HS 2" into the Omnetics connector labeled "HS 1", and vice versa; and reconnect to the appropriate SPI cable.

3. Run the Intan controller with the swapped headstages.

4. Note the sMEA channel number and EIB pin ID with the noisy signal. Compare the sMEA channel number and EIB pin ID with those previously noted.

   o If the same sMEA channel and EIB pin have this high noise with the swapped headstages, then the issue is likely with the Omnetics connector fixed to the interface board.

   o If different sMEA channels and EIB pins have this high noise with the swapped headstages, then the issue is likely caused by the headstages.

b) The Intan software crashes whenever the VCA is enabled

The VCA generates a large amount of EMI. The crashing of the Intan software is a symptom indicating that the electromagnetic interference from the VCA is greatly affecting the Intan signals. While the Intan software is running, the Intan software will crash immediately after the VCA is enabled or if the computer gets too close to the VCA. Try the following:

1. Make sure that the end of the USB cable connected to the PC loops around the ferrite core (solid, 23mm 19Ω) at least eight times. If this fails, perform more loops around the ferrite core or try a larger ferrite core.

2. A temporary solution is to decrease the input voltage to MEASSuRE's control drive and VCA. Software crashes are more likely when applied voltage is large. Thus, if the target strain and strain rate permit, decrease the applied voltage until the software crashes disappear.

18 Glossary

PDMS:	Polydimethylsiloxane
EMI:	Electromagnetic Interferences
EIB:	Electrophysiology Interface Board
PCB:	Printed Circuit Board
PBS:	Phosphate Buffered Solution
sMEA:	Stretchable Microelectrode Array
GUI:	Graphic User Interface
CPU:	Central Processing Unit
ISF:	Intan Setup File

Comments for Reid:

1) The loaded mapping file should be displayed somewhere on the GUI.
2) When selecting a channel by clicking on it, the "enable" box on the left of the GUI gets checked, which allows to set reference channels and stimulation parameters. However, when unchecking this box, the channel disappears. I think this is confusing. Also, how does one get the disabled channel back?