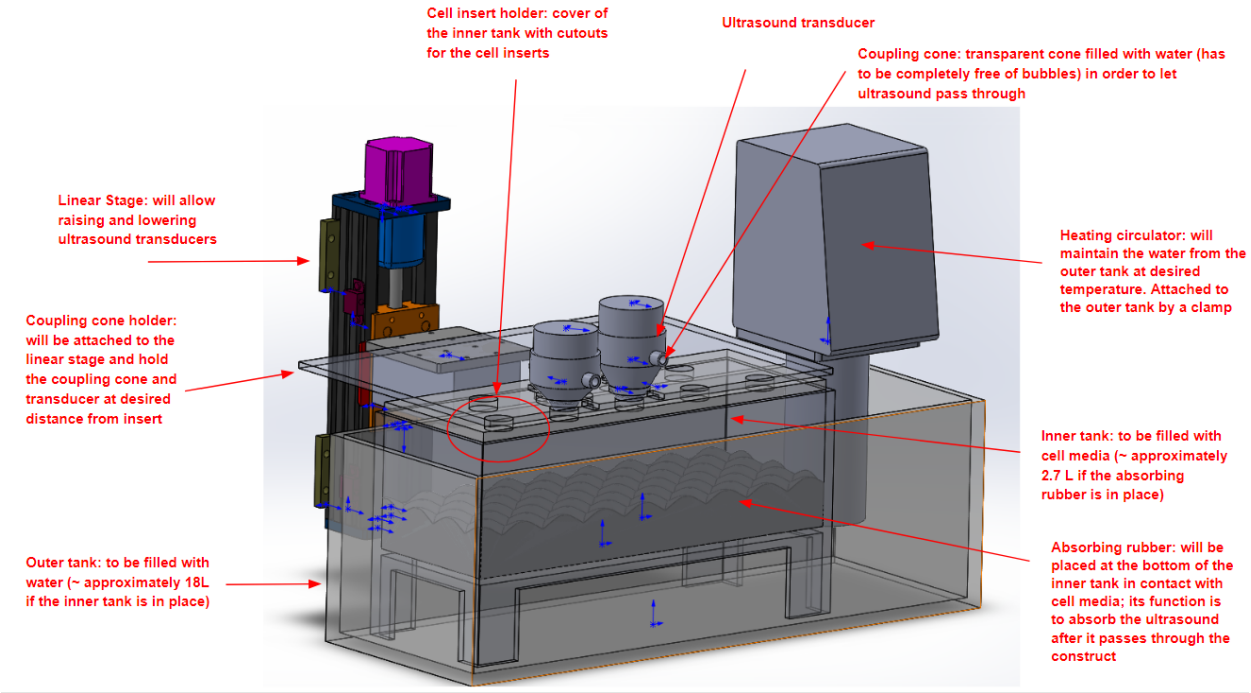
Preclinical *in vitro* Oncolysis System User Manual

Open Water Internet Inc.

Overview

The system details for placement of the is shown in the following drawing:



In general the system comprises the following components (details found in next section):

- Laptop with the oncolysis controller software installed
- Function generator, connected to the laptop via USB cable. Model Rigol DG4162
- 55 dB RF amplifier E&I Model 1040L
- Radiall USB switch 210
- 1 or more ultrasound transducers with coupling cones
- Fixuring for holding transducers in place

Required Materials

Common equipment within experiments

- Windows Laptop with USB hub or 3 open USB ports
- 60mm diameter, 60mm ROC focused transducers

- 2 laser-cut U-shaped acrylic pieces to help separate the coupling cone from the transducer
- O-rings
    - Internal O-rings (two needed for each cone) - 2 9/16” OD
    - External O-rings (one needed to hold saran wrap - 27mm OD
- Saran Wrap
- Electronic Equipment
    - 55dB gain 400W Power Amplifier (E&I Model 1040L)
    - Rigol Function Generator (DG4162)
        - USB-A cable
    - Electronic switch with 8 SMA ports and BNC connector (Radiall USB switch Model R573F11801)
        - SMA to BNC connectors

In vitro setup

- Custom Benthowave Transducers (BII7652 with 60mm ROC impedance matched)
    - 70kHz
    - 100 kHz
    - 150 kHz
    - 200 kHz
    - 300 kHz
    - 500 kHz
    - 1000 kHz
- Precision Acoustic 670kHz Focused Transducer (PA1798)
- Two water tanks:
    - **Outer tank with cover (cutouts for inner tank and heating circulator)**
        - Inner/working dimensions are 60.5 (L) x 21.0 (W) x 17 (H) cm. Will hold approximately 16.5L of water
    - **Inner tank with cover (cutouts for cell inserts)**
        - Inner/working dimensions are 42 (L) x 15.5 (W) x 9.2 + 8.7 (tank legs) (H) cm. Will hold approximately 2.7L of cell media

      Other relevant dimensions

        - 2cm distance between inner tank and outer tank walls, 3cm to heating circulator
        - 4cm from expected transducer focus to inner tank walls
        - Approximately 3 cm between absorbing rubber and insert bottom
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- Low-frequency acoustic absorbing rubber (Precision Acoustics Apltile SF5048)
- Coupling Cones
  - Internal O-ring for all the cones (OTS)
  - External O-ring for 25mm and 30mm cones (OTS)
  - External O-ring for 45mm and 60mm cones (OTS)
  - **3000-0772**
    - 25mm cone used for 70,100 and 150kHz. 3D printed in 3rd party manufacturer (Xometry - Process: Stereolithography (SLA) Material: Accura ClearVue, Clear, Standard Resolution)
  - **3000-0773**
    - 30mm cone used for 230 and 300kHz. 3D printed in 3rd party manufacturer (Xometry - Process: Stereolithography (SLA) Material: Accura ClearVue, Clear, Standard Resolution)
  - **3000-0774**
    - 45mm cone used for 500 and 1000kHz. 3D printed in 3rd party manufacturer (Xometry - Process: Stereolithography (SLA) Material: Accura ClearVue, Clear, Standard Resolution)
  - **3000-0775**
    - 60mm cone used for 670kHz. 3D printed in 3rd party manufacturer (Xometry - Process: Stereolithography (SLA) Material: Accura ClearVue, Clear, Standard Resolution)
  -
- Thincert cell culture inserts for 6 well plates (Greiner Bio-one Thincert for 6 well plate)

- Heating immersion circulator (CORIO CD Heating immersion circulator)

- Velmex bislide mounted on optical board and calibrated to position transducers

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Hydrophone measurements

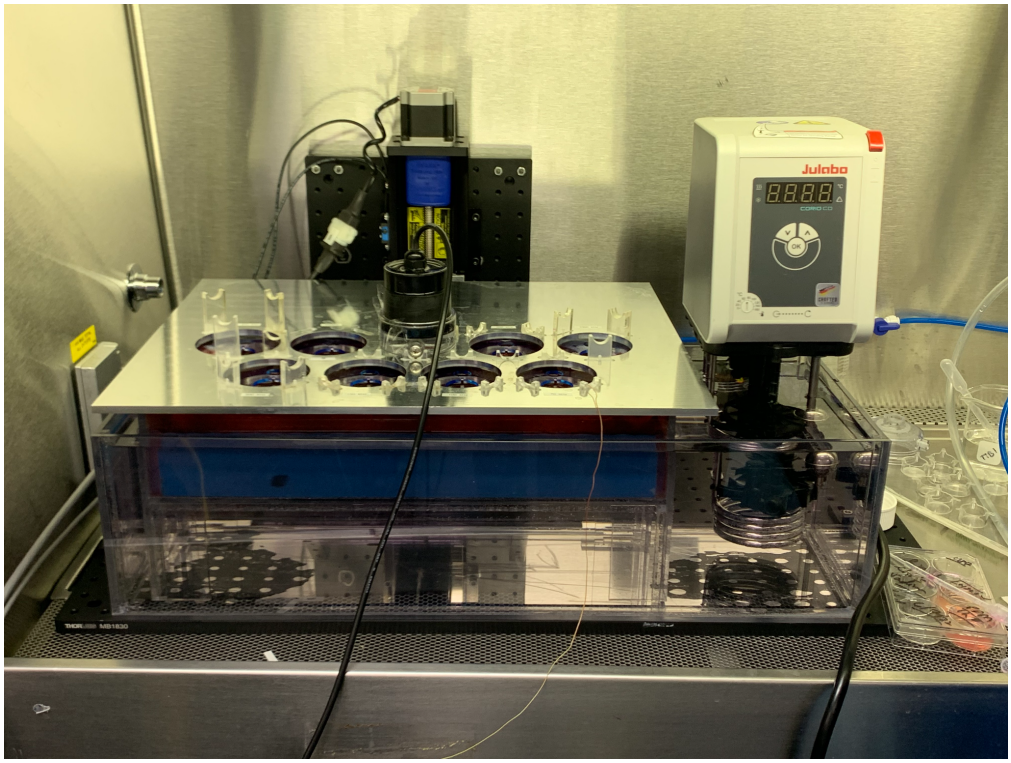
- Benthowave Hydrophone (model BII-7181)
- Rigol Oscilloscope (model DS1102Z-E)
- 3D printed hydrophone mount for in

Setting up experiments

Prior to all experiments all components of the system must be sterilyzed. The entire setup should be placed in a biological hood so that UV treatment is possible.

Prepping the water tanks for in vitro experiments

The first step is to fill the outer tank with water and begin heating it with the water circulator. Please set the temperature to 37 degrees Celsius and allow 30 minutes for the water to come to temperature.



The second step is to fill the inner tank with cell media. This should be done so that the media comes to within 2mm of the top of the tank. This will allow for the samples to sit in the media submerged and for the coupling cones to be be able to properly couple to the sample.

Finally, ensure that the motor stage is functioning properly and that the transducer holder is properly attached and aligned. This can be done by placing samples in the bath as shown below and making sure the transducer's coupling cones are able to go inside and out of the insert unimpeded.

Photos below is the inner tank cover where the inserts are placed with the cell samples

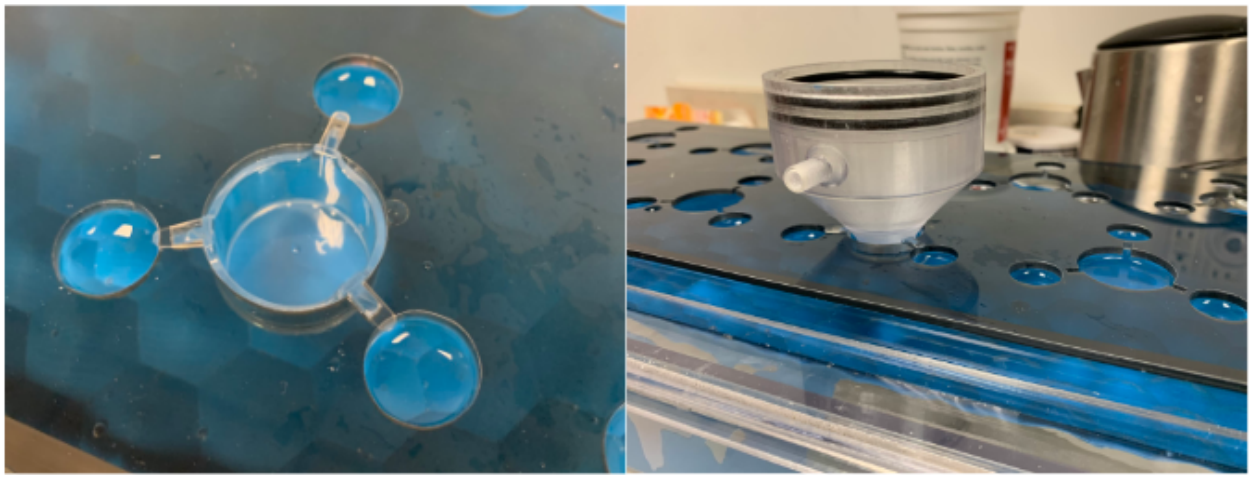
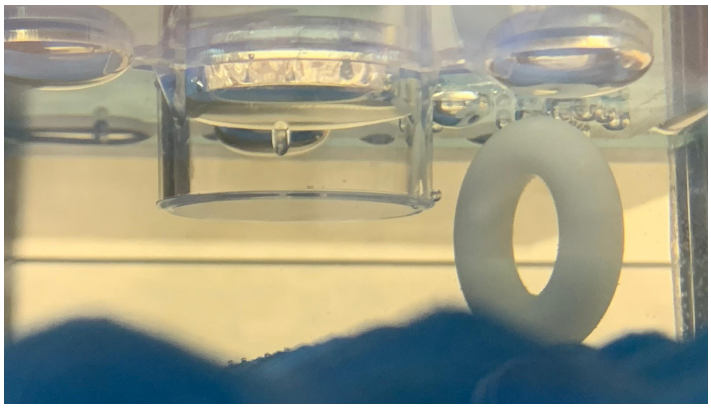


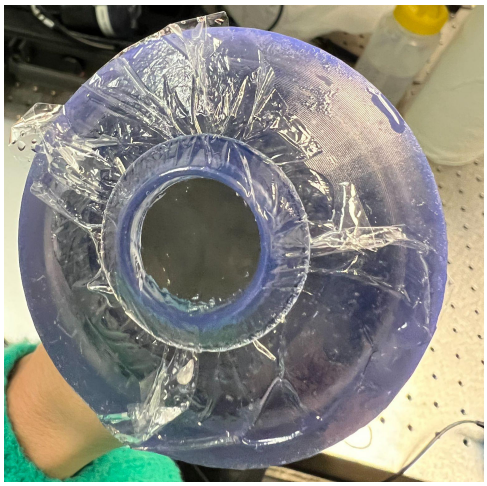
Photo of an insert in the tank with the coupling cone inside of it in contact with the liquid.



Attaching the coupling cones to the transducers

I. Heat some water until 80°C or higher and let it cool until it's comfortable to submerse a hand. This step is necessary to reduce the number of bubbles inside the coupling cone. You should use distilled water.

II. Submerge the cone in water. Place big O-rings internally in the coupling cone. While still underwater, push the transducer inside until it's flush. Use saran wrap and the small O-ring to seal the cone with water, ensuring no bubbles are visible.



Oncolysis Controller Software

Installation

See the installation instruction in the README

Starting the Controller

The controller can be started by double clicking `startapp.bat`, or directly from the command line using `python runapp` from the environment containing 32 bit Python. `runapp` accepts two optional flags:

- `runapp.py --simulate` or `runapp.py -s` launch in simluation mode for practice or debugging
- `runapp.py --config configuration` launch in a specific configuration. Also possible is
- `runapp.py –—config *defaultconfig,optionalconfig1,optionalconfig2`: Start app in the configuration marked with an asterix, and make the other configurations available.

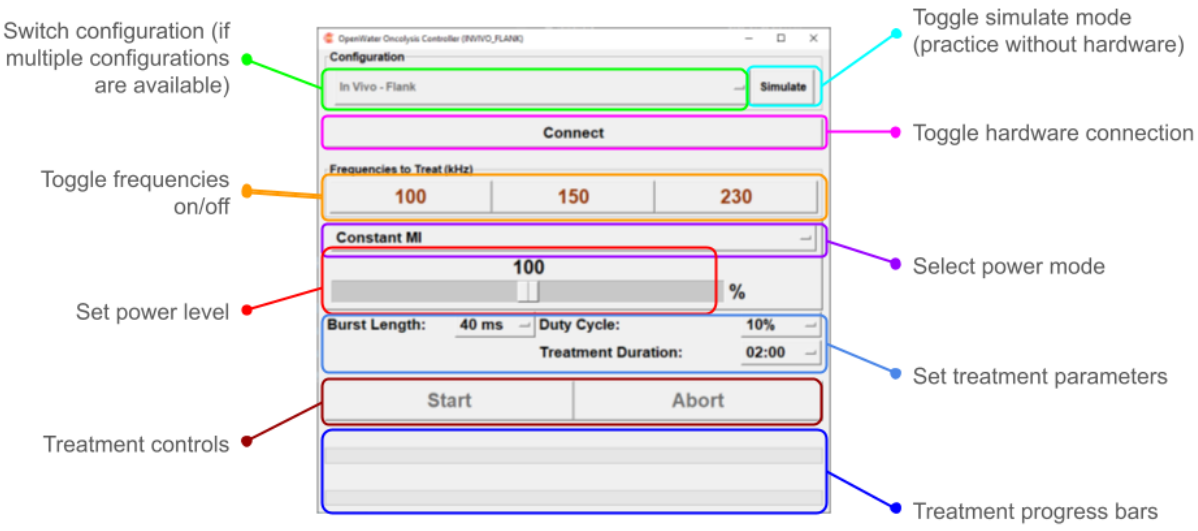
Typically, the configuration options wrapped up in a `startup_<description>.bat` file, so that the user can directly launch with the correct options for their application

Configuration Constants

Configurations are the constants that describe what options are available, and how the system connects to and controls hardware. Configurations are stored in `oncolysis_ctrl/configurations/constants_<id>.py`. Because these are Python files, they can import one another if certain constants are re-used across multiple configurations. `constants_global.py` is the default configuration constants, while the other available files include application-specific constants that override the constants set in `constants_global.py`. If no configuration options are passed to the launcher, the ID in `CONFIG_ID.txt` will be used (if available, otherwise `config.DEFAULT_CONFIG` is used) as the default, and the configurations listed in `config.CONFIG_IDS` will be made available. When the user switches configurations, `CONFIG_ID.txt` gets updated with the selected configuration so that by default, the app will launch in the last configuration that was used.

System Operation

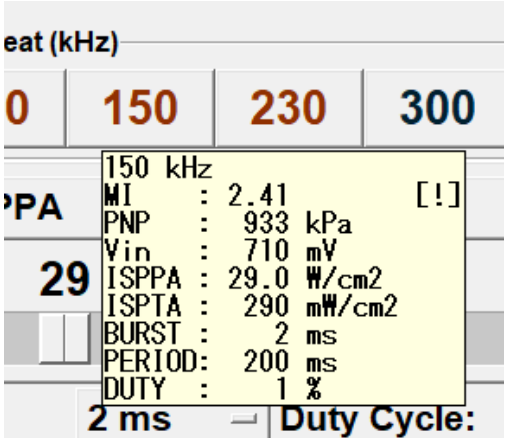
A screenshot of the controller software is shown in the following diagram:



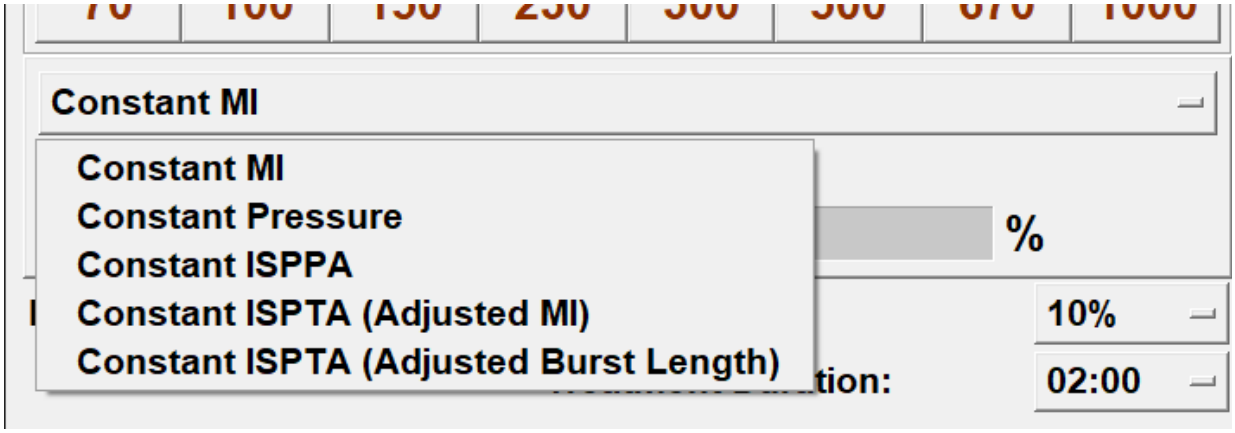
The top row controls the configuration. Switching the configuration will cause the app to reboot, because the configuration includes the frequencies and options for other controls that are created on boot.

The Connect button toggles the connection to the hardware. The configuration cannot be changed while the hardware is connected.

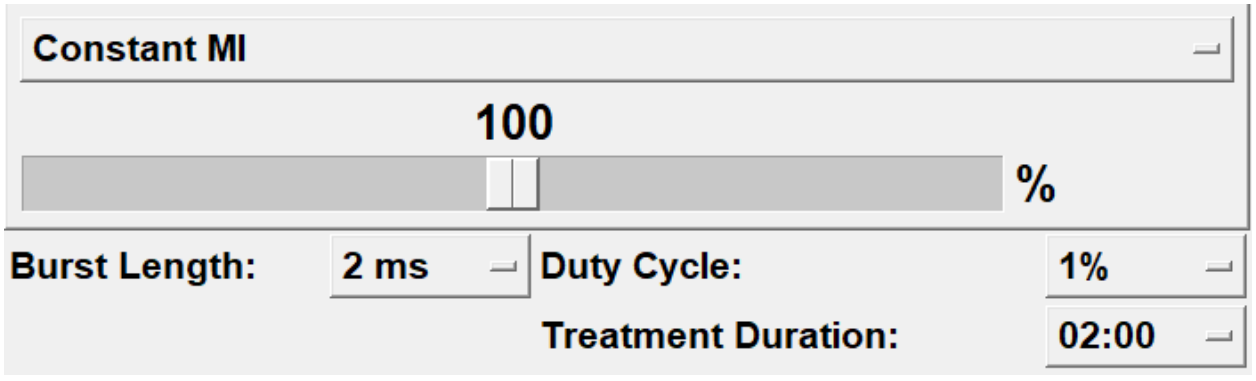
The Frequencies row contains one toggle button for each frequency in the configuration. At least one frequency must be selected to initiate treatment. If more than one frequency is selected, they will be sequentially run, left-to-right. Hovering the mouse over a button will provide additional information about the pulse parameters for that frequency. If any of the parameters (MI, ISPPA, ISPTA) are above the diagnostic limits, the button text will be shaded red and the offending parameter will be marked with a [!].



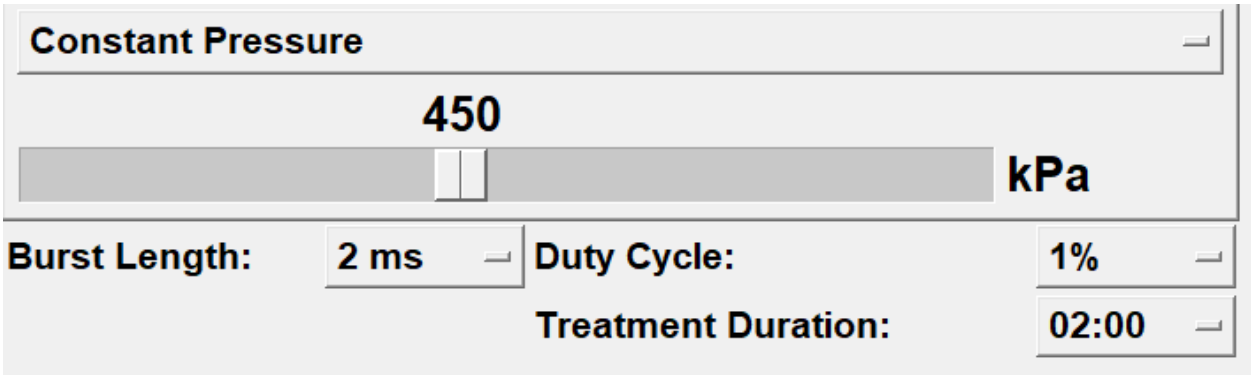
The Power Mode dropdown provides (as listed in the configuration), options for what parameter will be used as a target for each frequency. Available options are:



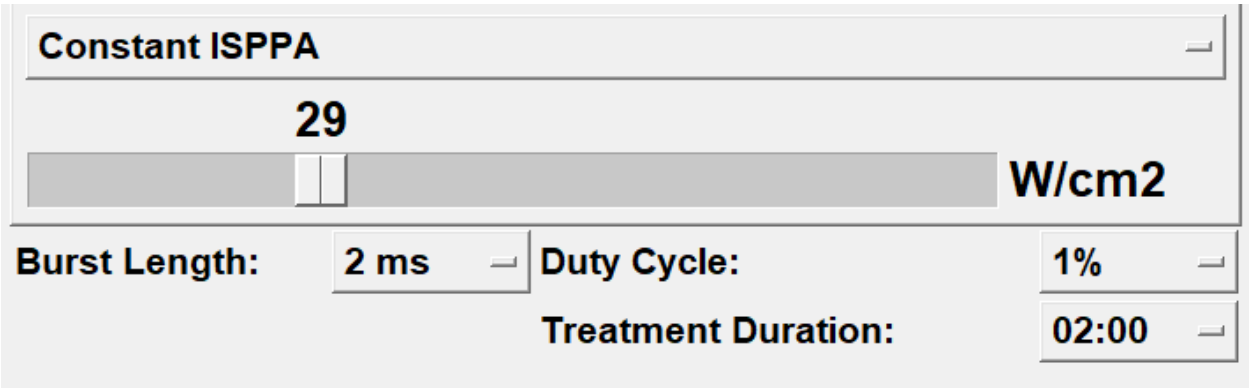
- Constant MI: scale the pressure for each frequency as a percentage of MI=1.9



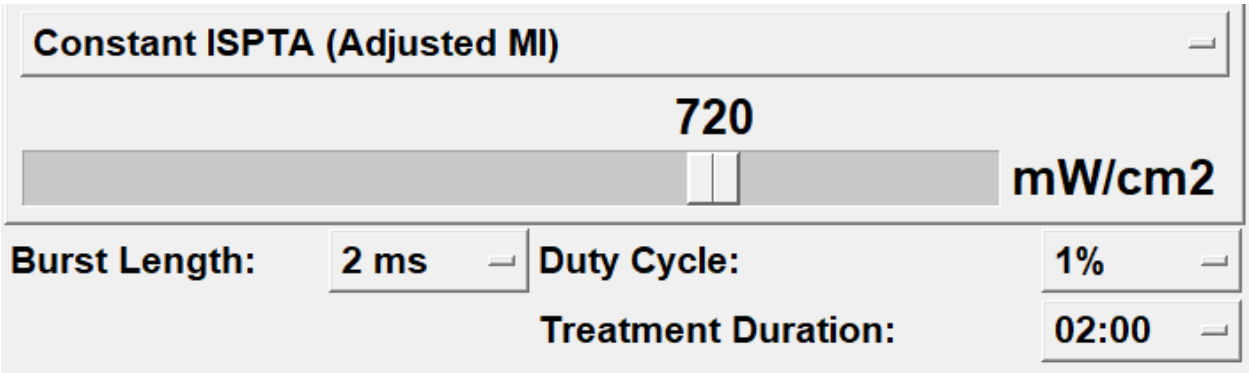
- Constant Pressure: set a fixed pressure for each frequency (kPa)



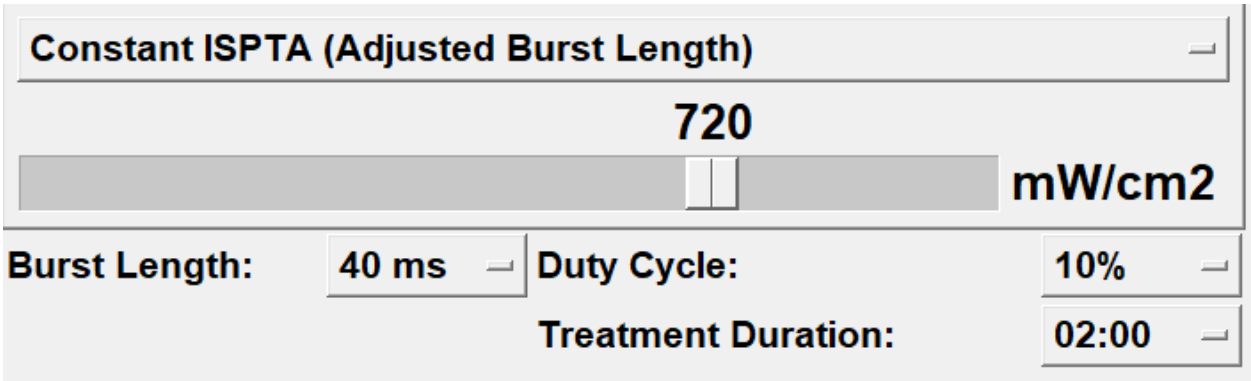
- Constant ISPPA: set a constant intensity for each frequency (W/cm2)



- Constant ISPTA (Adjusted MI): Adjust MI/Pressure for each frequency to hit the target intensity (mW/cm2) for the specified Burst Length and Duty Cycle

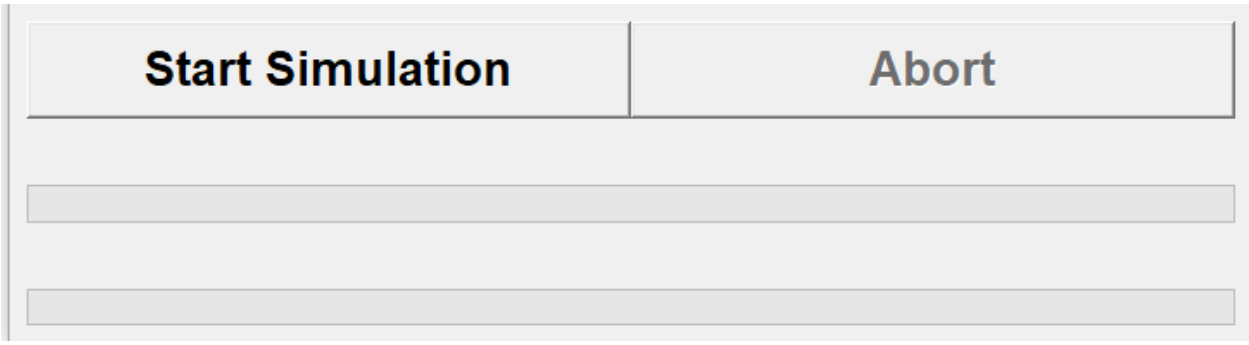


- Constant ISPTA (Adjusted Burst Length): Use 100% MI to set voltage, set the period based on the chosen (nominal) burst length and duty cycle, and then adjust the burst length for each frequency to hit the target ISPTA (mW/cm2)

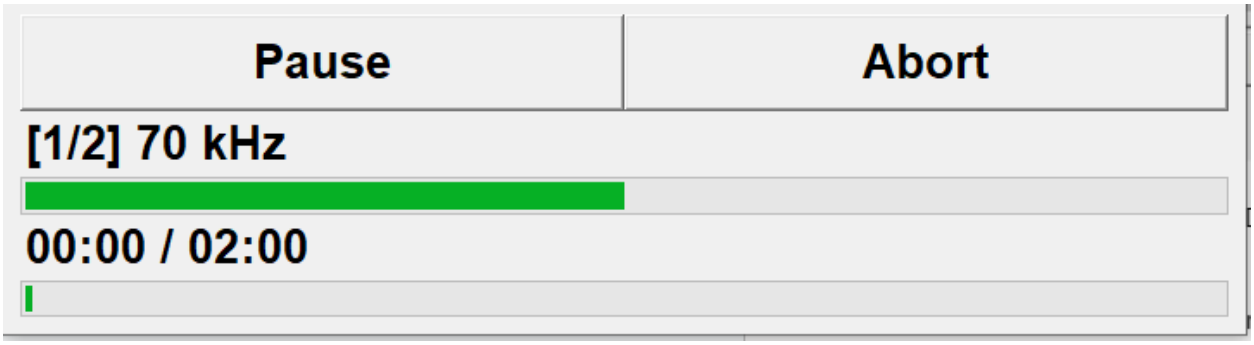


The options for Burst Length, Duty Cycle, and Treatment Duration are all specified in the configuration file

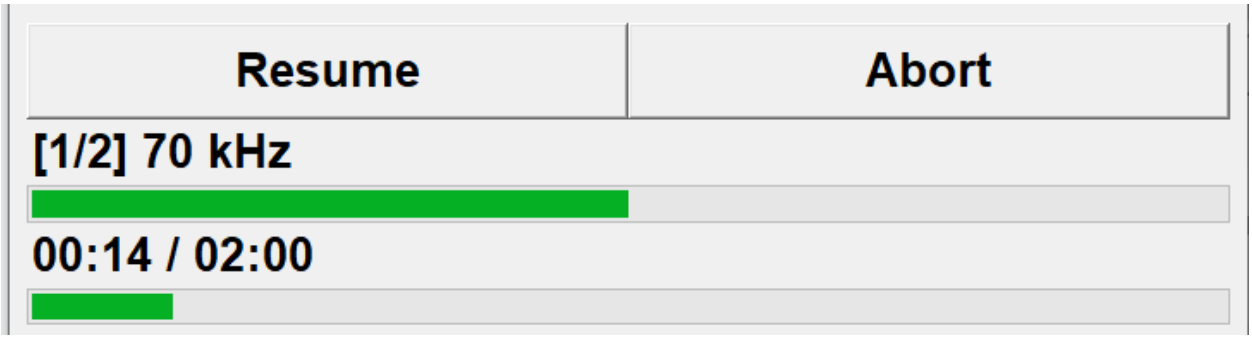
The Start and Abort Buttons are contextually enabled per the connection and running status. Hitting “Start” or “Start Simulation” will initiate treatment:



When the treatment is running, the top bar will show progress through frequencies, and the bottom bar will show progress through the active frequency.



The Start button becomes a Pause button, which will halt transmission and become a “Resume” button if pressed.



The Abort button will immediately stop transmission and cancel the treatment.

Shutting Down

To shut down the software, ensure that a treatment is not running, disconnect the hardware, and close the application.

Logs

During operation, all of the activity in the application will be saved to a timestamped .log file in the `logs/` folder.