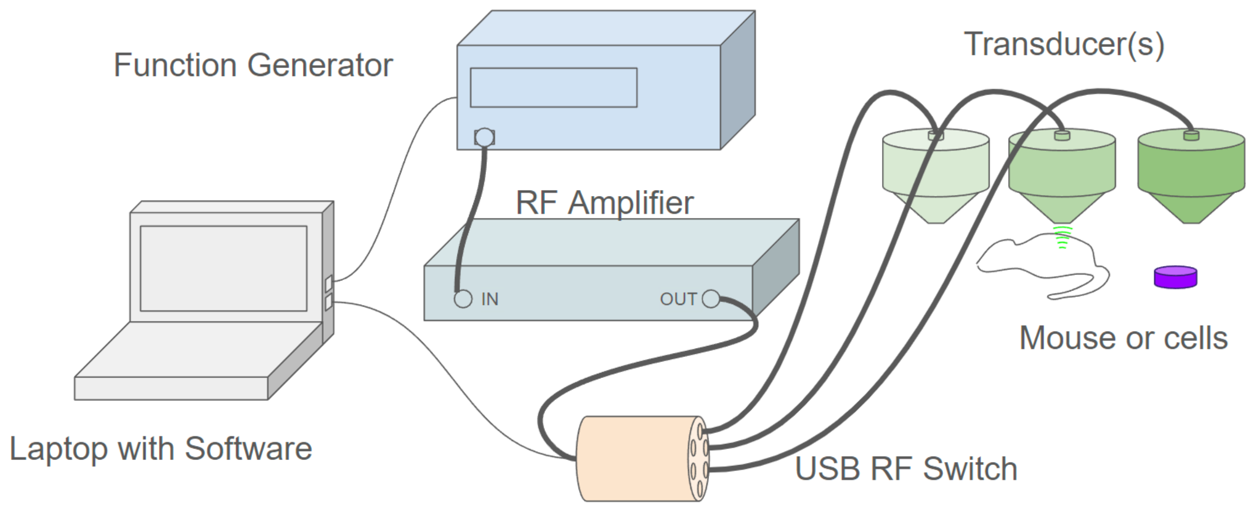
Preclinical Oncolysis System User Manual

Open Water Internet Inc.

Overview

The overall system 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 (laser cut drawing)
- 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)
- (Qty 8) **Clear Plastic Box with Removable Lid** **3-7/16" L x 3-7/16" W x 6-5/16" Hgt., (Item # 55390**)
- 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 (OTS)
- 3D printed tank covers for transducers and thincert holders:
  - **3000-0767**
    - Thincert holder/Laser cut in acrylic (3mm thickness)
  - **3000-0768**
    - Hooks for Inner Tank Cover - 25mm, 3D printed
  - **3000-0769**
    - Hooks for Inner Tank Cover - 30mm, 3D printed
  - **3000-0770**
    - Hooks for Inner Tank Cover - 45mm, 3D printed
  - **3000-0771**
    - Hooks for Inner Tank Cover - 60mm, 3D printed
-

In vivo setup

- Custom Benthowave Transducers (BII7652 with 60mm ROC impedance matched)
  - 100 kHz
  - 150 kHz
  - 200 kHz

- Coupling Cones with 18mm diameter output
  - 3 x (25mm height - 18mm diameter)
    Meant for 100, 150, and 200kHz transducers
- Coupling Cones
  - 1 x (20mm height - 4mm diameter)
    Meant for 100kHz frequency transducers

  - 2 x (30mm height - 4mm diameter)
    Meant for 150kHz and 200kHz frequency transducers
- Gooseneck camera mount (model)
  - 1 x 61mm 3D printed transducer mount (for 150kHz transducer)
  - 1 x 70mm 3D printed transducer mount (for 100 and 200 kHz transducers)
  - ¼-20 screws and nuts

- Stereotactic frame (model)
    - Nose and teeth bars
    - 2x ear bars
    - 2x 8mm diameter rods
    - 1x Aluminum clamp for 8mm diameter rod
    - 10/32 screws and nuts
    - ¼ -28 screws (for attachment of Manipulator X, Y, Z to the side of the main frame)
    - 1 x 61mm 3D printed transducer mount (for 150kHz transducer)
    - 1 x 70mm 3D printed transducer mount (for 100 and 200 kHz transducers)
    - ¼-20 screws

Hydrophone measurements

- Aquarium Tank - Small - Molded Plastic - .75 Gallon Capacity - 7" x 6" x 4.25" (Amazon Eisco Store link)
- Low-frequency acoustic absorbing rubber (Precision Acoustics Apltile SF5048)
- Benthowave Hydrophone (model BII-7181)
- Rigol Oscilloscope (model DS1102Z-E)
- 1x 3D printed transducer mount (for 150kHz transducer) and hydrophone
- 1x 3D printed transducer mount (for 100/200kHz transducer) and hydrophone

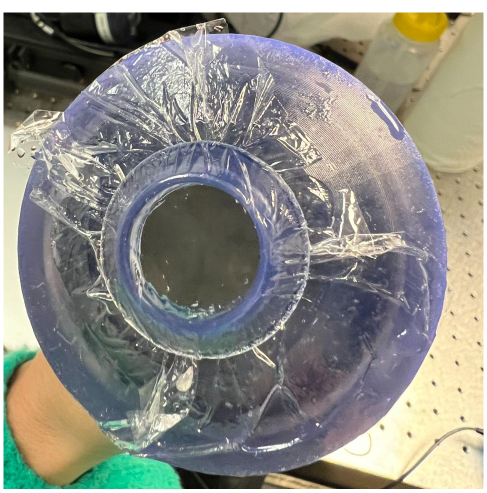
Setting up experiments

Testing the transducer in the small water tank

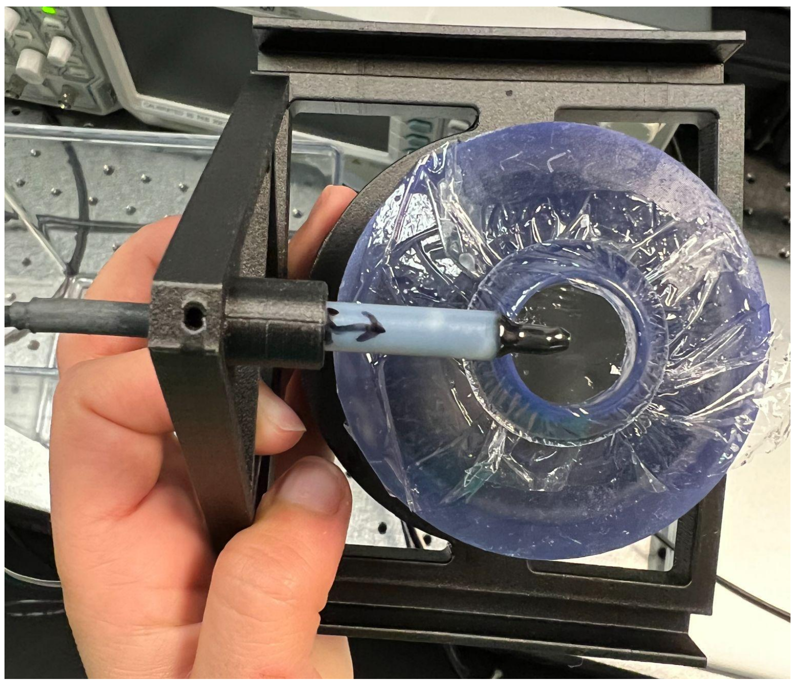
I. Turn on the computer and connect to the PC the USB-A cables from the Rigol Function Generator and from the electronic switch.
The password of the computer should be in a post it above the keyboard. If you have questions, please contact Sarah or Chris.

II. Heat some water until 80ºC 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.

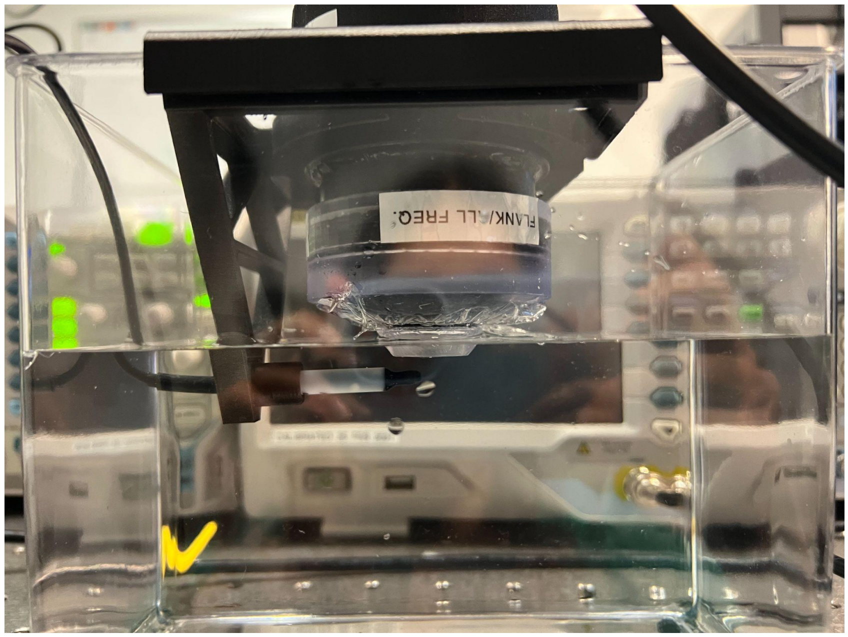
III. Submerge the cone in water. Place big O-rings internally in the coupling cone. While still underwater, push the transducer inside until it's flush. Use saran wrap and the small O-ring to seal the cone with water, ensuring no bubbles are visible.



IV. Use the hydrophone mount that is labeled to be used with your transducer. Place the hydrophone such as, when you're holding the mount with the hydrophone opening facing upwards, you can see that the marked arrow in the hydrophone is facing up and the horizontal marker line delimitates the part of the hydrophone that is exposed (picture below)



V. Place the hydrophone mount in the small water tank and fill with enough water to submerge the tip of the coupling cone. Connect the hydrophone to the Rigol Oscilloscope in Channel 1, and connect the transducer to the appropriate switch according to the transducer and experiment used.



Transducer	Type of Experiment	Electronic Switch
100 kHz	In vivo - flank/brain	1
150 kHz	In vivo - flank/brain	3
230 kHz	In vivo - flank/brain	5

VI. Turn on the Power Amplifier and launch the OW application on the PC
The current shortcut on the Desktop is for the Flank Tumor Application. Each type of experiment (in vivo-flank, in vivo-brain, and in vitro) has a specific Launch App. Each Launch app corresponds to a setting associated with the specific coupling cone transducer combination for the experiment, so it is important to use the correct App link.

VII. Click ‘ Connect’ on the app to ensure the connection is successful.

VIII. Take hydrophone measurements at 50% and 100% Treatment Pressures, using the oscilloscope in the Manual trigger function at CH1. Compare readings with the measurements taken at Openwater described in the table below:

IN VIVO FLANK			
Transducer /Frequency	**Coupling Cone**	**Input Pressure**	**Oscilloscope Reading**
#4/100 kHz	25mm height/18mm diameter	50%	2.12V Range: 1.78-2.28V
#4/100 kHz	25mm height/18mm diameter	100%	4.12V Range: 3.68-4.24V
#6/150 kHz	25mm height/18mm diameter	50%	810mV Range: 760-860mV
#6/150 kHz	25mm height/18mm diameter	100%	1.5V Range: 1.45-1.7V
#8/230 kHz	25mm height/18mm diameter	50%	536mV Range: 484-688mV
#8/230 kHz	25mm height/18mm diameter	100%	1.1V Range: 0.956-1.42V

IN VIVO BRAIN			
Transducer /Frequency	**Coupling Cone**	**Input Pressure**	**Oscilloscope Reading**
#4/100 kHz	20mm height/4mm diameter	50%	818mV Range: 670-940mV
#4/100 kHz	20mm height/4mm diameter	100%	1.70V Range: 1.50-1.85V
#6/150 kHz	30mm height/4mm diameter	50%	470mV Range:420-544mV
#6/150 kHz	30mm height/4mm diameter	100%	670mV Range: 650-850mV
#8/230 kHz	30mm height/4mm diameter	50%	450mV Range: 360-504mV
#8/230 kHz	30mm height/4mm diameter	100%	840mV Range: 800-936mV

IX. After getting hydrophone measurements, decouple the cone from the transducer by pulling the cone. Use the laser-cut U-shaped piece as a lever to facilitate the process.

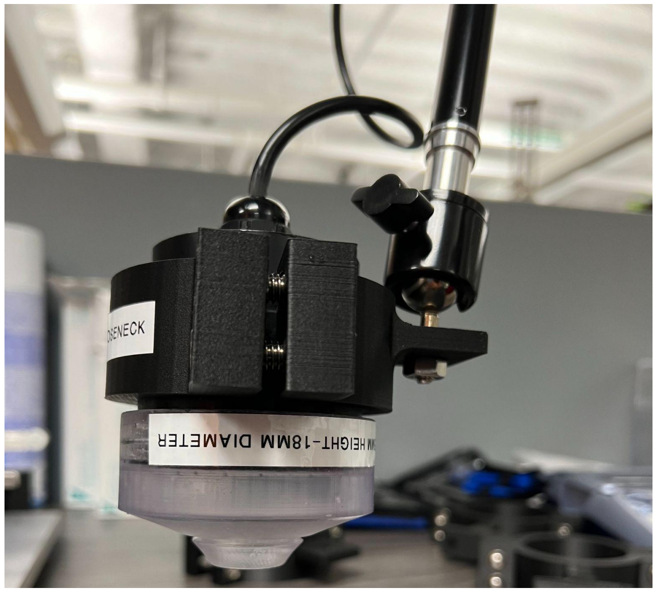
Setup flank tumor treatment

I-III. The same steps as described in section 2.1

IV. Use the 3D-printed transducer mount that is labeled for the specific transducer frequency you're using. Place the coupling cone and transducer inside it and tighten the four ¼-20 screws on the fixture to secure the transducer within the half circumferences

V. Mount the gooseneck holders on a flat surface (e.g. table) and use its knobs to position the ¼ screw end on your preferred treatment position

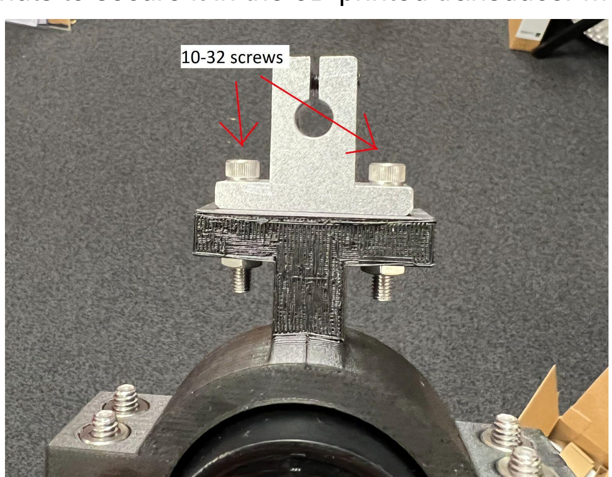
VI. Use the ¼ nut to secure the 3D printed fixture to the ¼ screw end. Using the last knob, position your transducer on top of your flank tumor



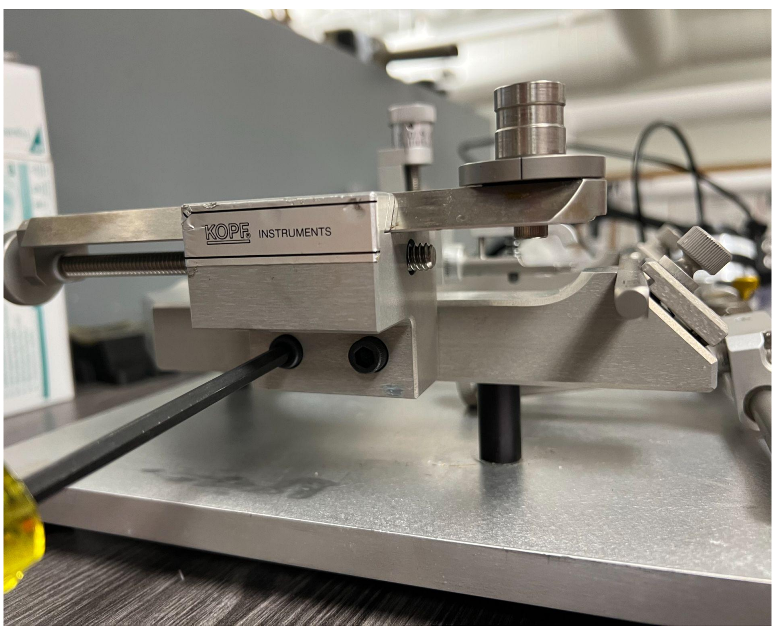
VII. Connect the transducer to the appropriate switch according to the transducer and experiment used.
VIII. Turn on the Power Amplifier and launch the OW application on the PC
IX. The current shortcut on the Desktop is for the Flank Tumor Application. Each type of experiment (in vivo-flank, in vivo-brain, and in vitro) has a specific Launch App.
X. Click ‘ Connect’ on the app to ensure the connection is successful.
XI. Start Treatment.

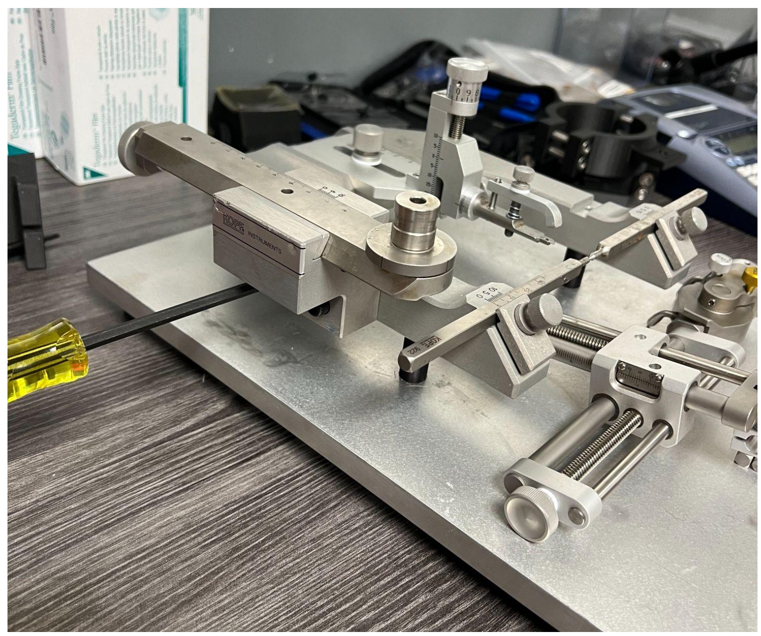
Setup brain tumor treatment

I-III. The same steps as described in section 2.1
IV. Use the 3D-printed transducer mount labeled for the specific transducer frequency you’re using. Place the coupling cone and transducer inside it and tighten the four ¼-20 screws on the fixture to secure the transducer within the half circumferences
V. Remove the thumb screw from the 8mm diameter rod aluminum clamp and use the 10-32 screws and nuts to secure it in the 3D printed transducer mount

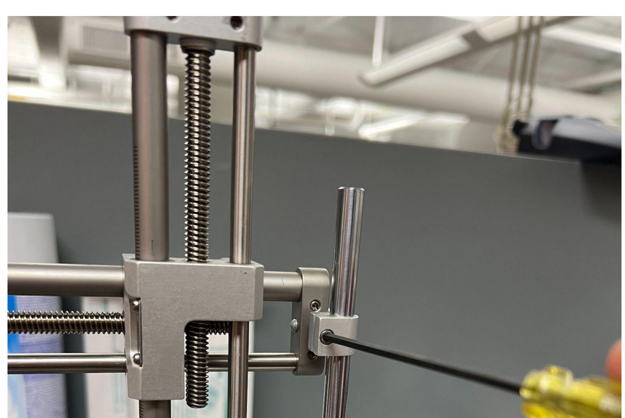


VI. In the stereotactic frame, attach the X-Y-Z manipulator base mount to the main frame using two ¼-28 screws

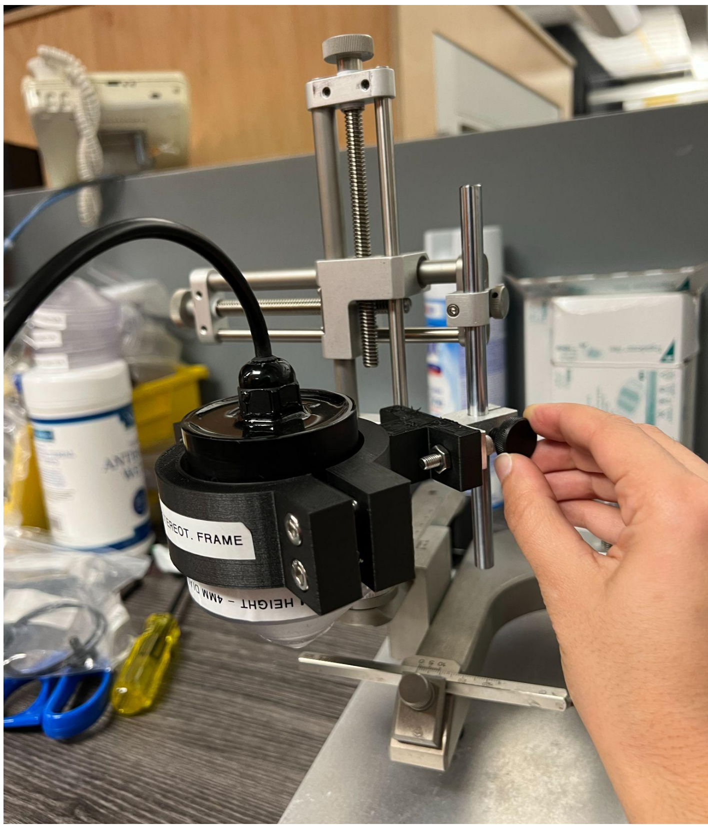




VII. Place the remaining X-Y-Z manipulator structure on the top of the base and lock it in place. Secure the 8mm rod in the X-Y-Z manipulator tip



VIII. Secure the aluminum clamp in the 8mm rod using the thumb screw.



IX. Connect the transducer to the appropriate switch according to the transducer and experiment used.

X. Turn on the Power Amplifier and launch the OW application on the PC

XI. The current shortcut on the Desktop is for the Flank Tumor Application. Each type of experiment (in vivo-flank, in vivo-brain, and in vitro) has a specific Launch App.

XII. Click ‘ Connect’ on the app to ensure the connection is successful.

XIII. Start Treatment.

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.

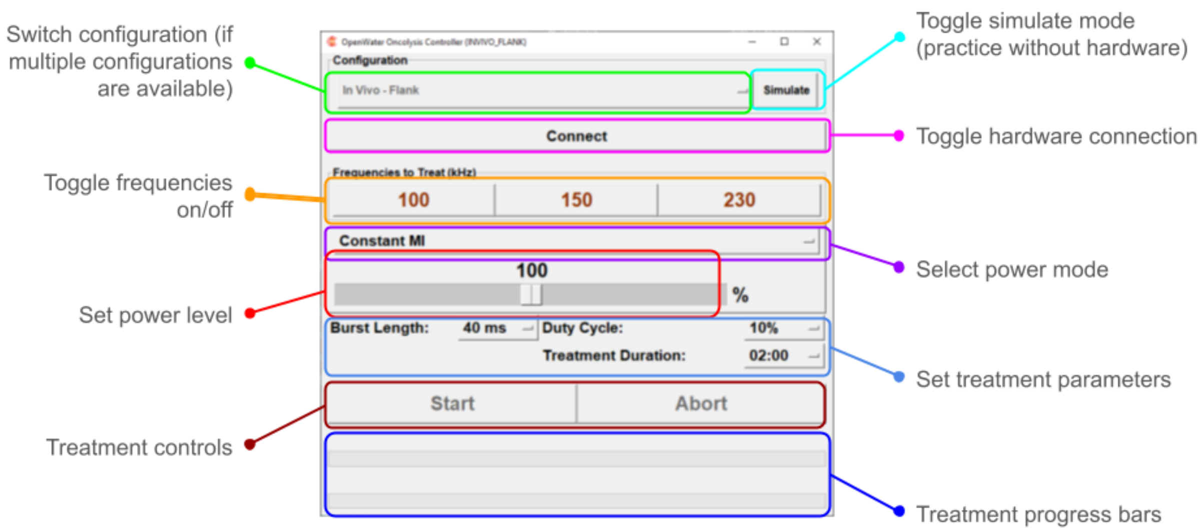
Typically, the configuration options wrapped up in a `startup_<description>.bat` file, so that the user can directly launch with the correct options for their application

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

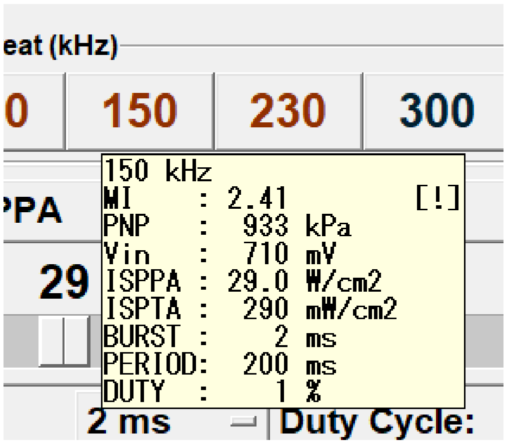
A screenshot of the controller software is shown in the following diagram:



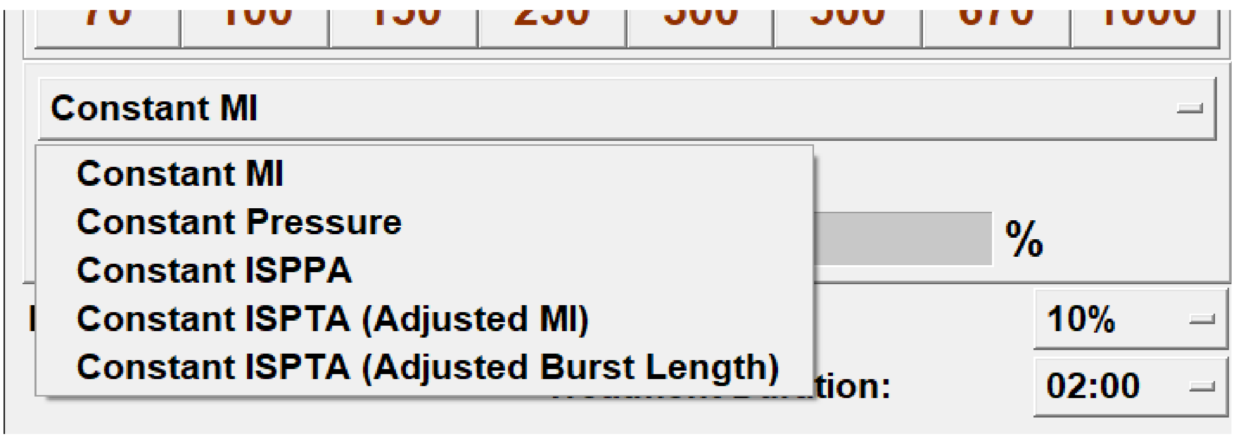
The top row controls the configuration. Switching the configuration will cause the app to reboot, because the configuration includes the frequencies and options for other controls that are created on boot.

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

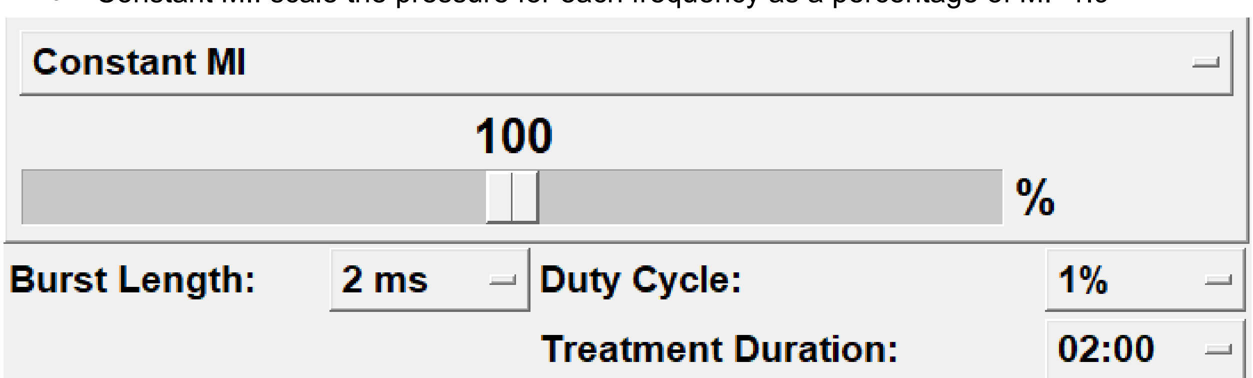
The Frequencies row contains one toggle button for each frequency in the configuration. At least one frequency must be selected to initiate treatment. If more than one frequency is selected, they will be sequentially run, left-to-right. Hovering the mouse over a button will provide additional information 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 [!].



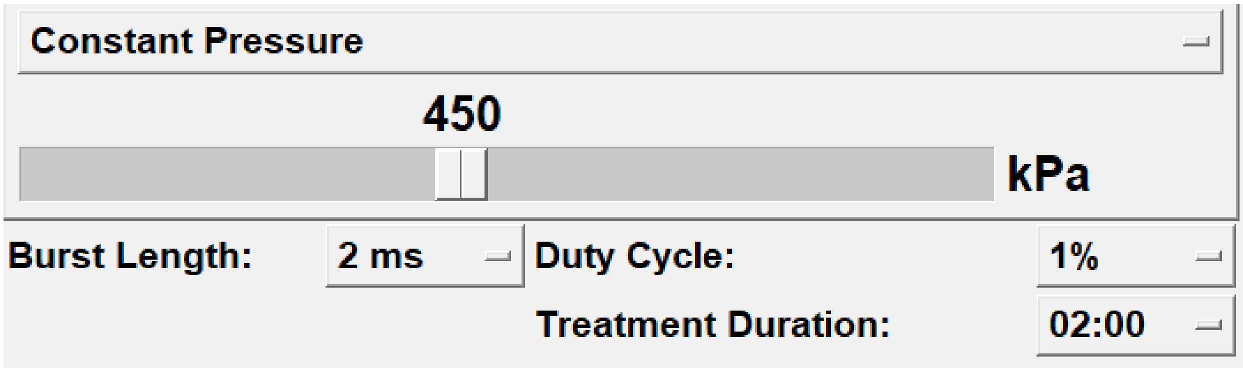
The Power Mode dropdown provides (as listed in the configuration), options for what parameter will be used as a target for each frequency. Available options are:



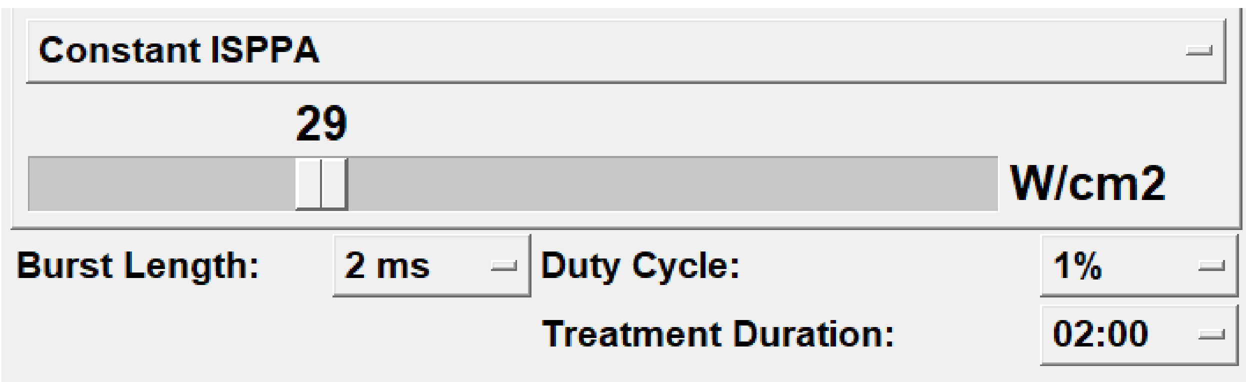
- Constant MI: scale the pressure for each frequency as a percentage of MI=1.9



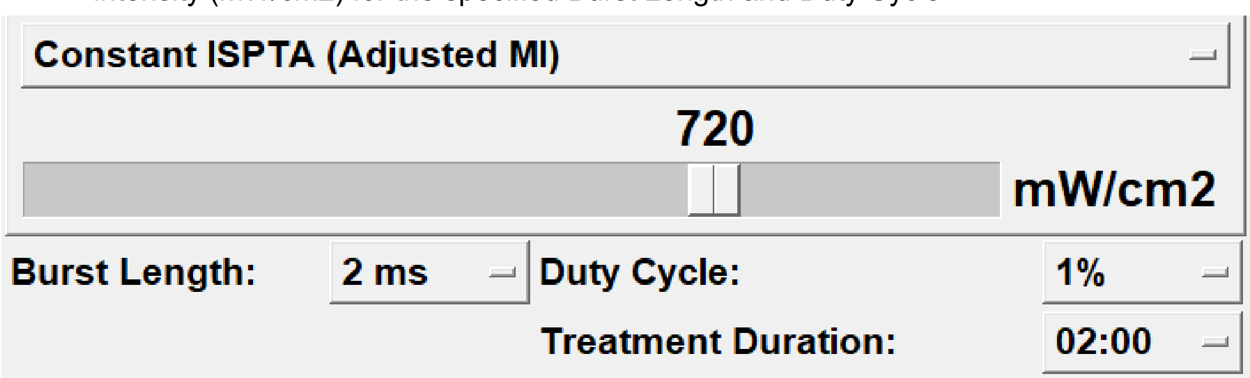
- Constant Pressure: set a fixed pressure for each frequency (kPa)



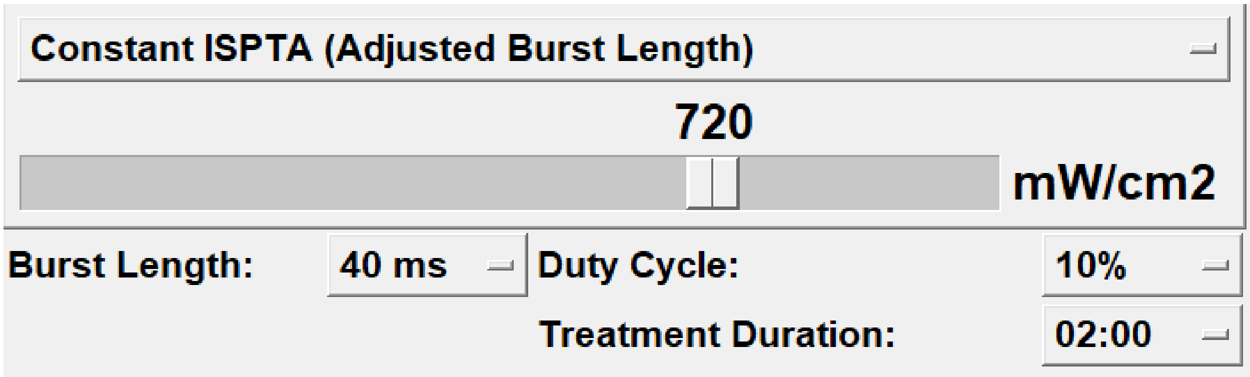
- Constant ISPPA: set a constant intensity for each frequency (W/cm2)



- 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

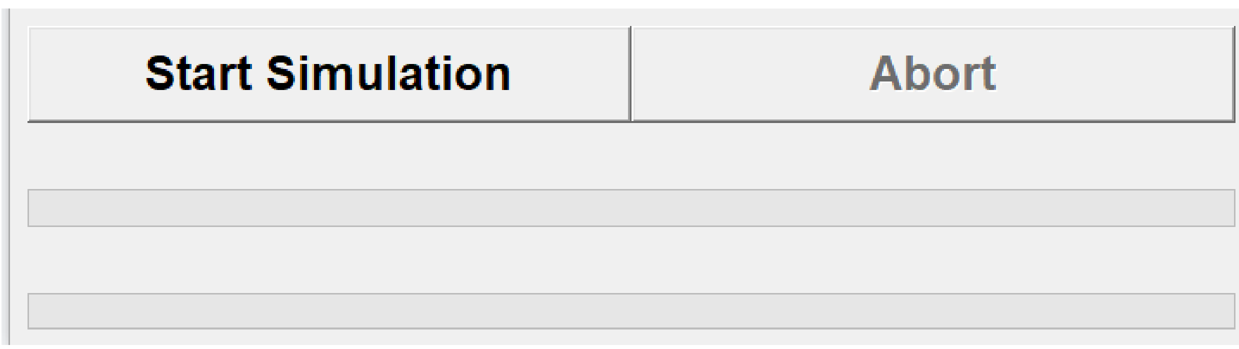


- Constant ISPTA (Adjusted Burst Length): Use 100% MI to set voltage, set the period based on the chosen (nominal) burst length and duty cycle, and then adjust the burst length for each frequency to hit the target ISPTA (mW/cm2)

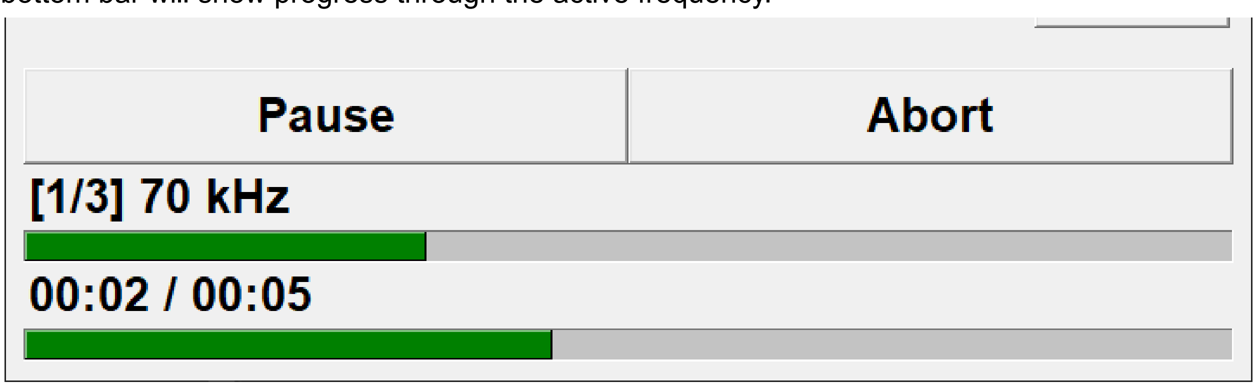


The options for Burst Length, Duty Cycle, and Treatment Duration are all specified in the configuration file

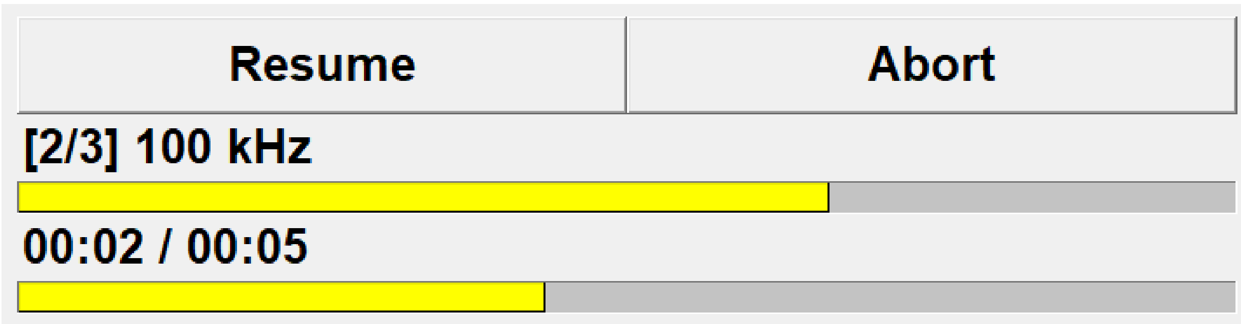
The Start and Abort Buttons are contextually enabled per the connection and running status. Hitting “Start” or “Start Simulation” will initiate treatment:



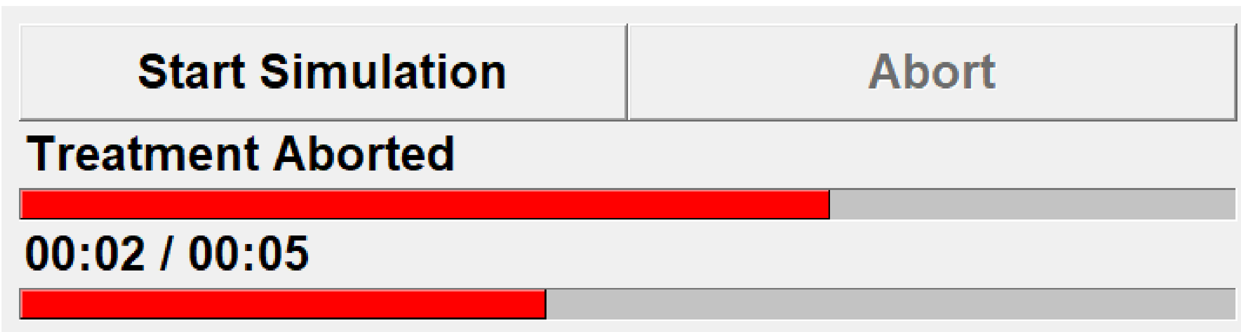
When the treatment is running, the top bar will show progress through frequencies, and the bottom bar will show progress through the active frequency.



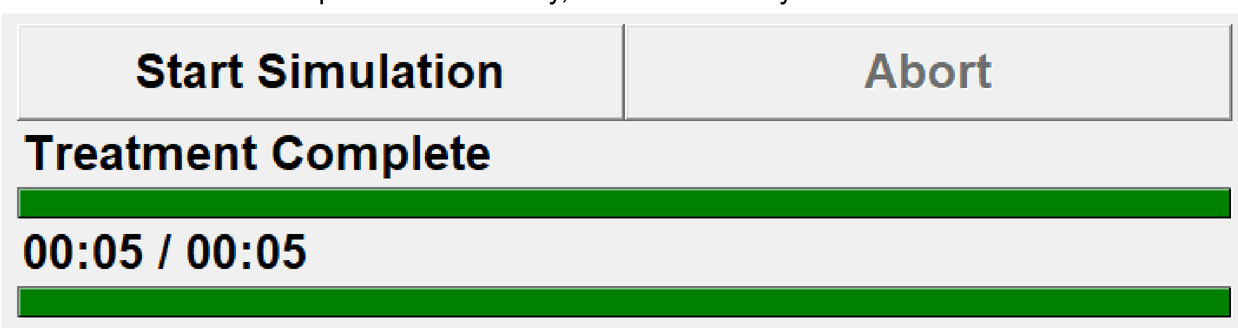
The Start button becomes a Pause button, which will halt transmission and become a “Resume” button if pressed:



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



When the treatment completes successfully, the bars will stay filled:



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.