



StimSwitch Stimulation System

Hardware Manual

StimSwitch Manual

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1 Introduction



Figure 1: StimSwitch System - Typical Configuration

The IonOptix StimSwitch system permits stimulation and, optionally, perfusion of multiple wells in a multi-well tissue culture plate. The system consists of a switch box, cabling, and a stimulation lid. There are several options for cabling depending on the microscope you are using. The table below lists the individual parts that comprise the entire system.

Part	Part #	Description	Image
StimSwitch-6M	STS-6M.100	Manual 6 Channel HV Stimulation Multiplexer	
StimSwitch 6-24 Stimulation Lid	MUW-6/24.100	6 Channel Stimulation and Perfusion Lid for 24 well format tissue culture plates.	
StimSwitch Standard Cable	STS-6M-CAB-S1/INV	DB9-MM10, 5 feet long.	
StimSwitch CytoCypher Cable Set	STS-6M-CAB-KIT-S2	Multipart cable set consisting of: <ul style="list-style-type: none"> • DB9-MM10 8" long • DB9 Male/Female, 2m long • DB9/Perfusion CytoCypher HTS Microscope Access Port 	

2 StimSwitch-6M



Figure 2: StimSwitch-6M Manual High-Voltage Multiplexor

The StimSwitch-6M is a manual, high voltage, multiplexer box. It takes the high voltage output of a stimulator and allows the user to manually redirect that voltage to one of 6 different wells in a multi-well plate. All the controls for this device are located on the top and back panels which we will now look at more closely.

Top Panel



Figure 3: StimSwitch-6M Top Panel Buttons

The top panel has 6 buttons that allow you to switch the electrical stimulation from one well to another. The meaning of Well 1, Well 2, etc is defined by the stimulation lid you are using. If a button has a green center, that well is receiving stimulation as shown for well 6 in figure 3. Otherwise, the well is not being stimulated. Push a button to toggle its state from stimulating to not stimulating or vice-versa.

Back Panel



Figure 4: StimSwitch-6M Back Panel Connectors

The back panel has three connectors that connect the between the button box, the stimulator, and the stimulation lid.

The two, 2mm pin jacks labeled **HV Input**, seen on the right in figure 4, receive the stimulation voltage from the stimulator. Any stimulator can be used so long as you have an appropriate cable to connect between the StimSwitch-6M and the stimulator. The red jack should connect to the positive output from the stimulator and the black jack should connect tot he negative (ground) output from the stimulator.

The 9-pin DSUB (DB9) female connector, labeled **Stim Output**, routes the stimulation voltage from the StimSwitch-6M to the stimulation electrodes as selected by the buttons on top of the box. There are a variety of different cabling options depending on the stimulation electrode set and the microscope you are using.

Switching Wells



Figure 5: Left - No Wells Selected For Stimulation; Right - Well 1 Selected For Stimulation

To switch wells, push the currently active well's button to turn off that well. At this point, all buttons will have black centers indicating that no wells are being stimulated. Now push the button associated with the new well you would like to stimulate. This will make the center of the button green, indicating that well is now being stimulated.

Stimulating Multiple Wells

You can stimulate more than one well at a time by having more than one button with the green center. However, please be aware that this will double the current requirement of the stimulator.

If your stimulator does have enough current to drive multiple wells, this can be useful for staging your wells. By this we mean start stimulating the next well you wish to investigate several minutes before you actually start acquiring data from it. This lets the cells in that chamber stabilize after the transition from a

state of rest to being stimulated. If in doubt, we recommend stimulating only one well at a time.

3 StimSwitch 6-24 Stimulation Lid

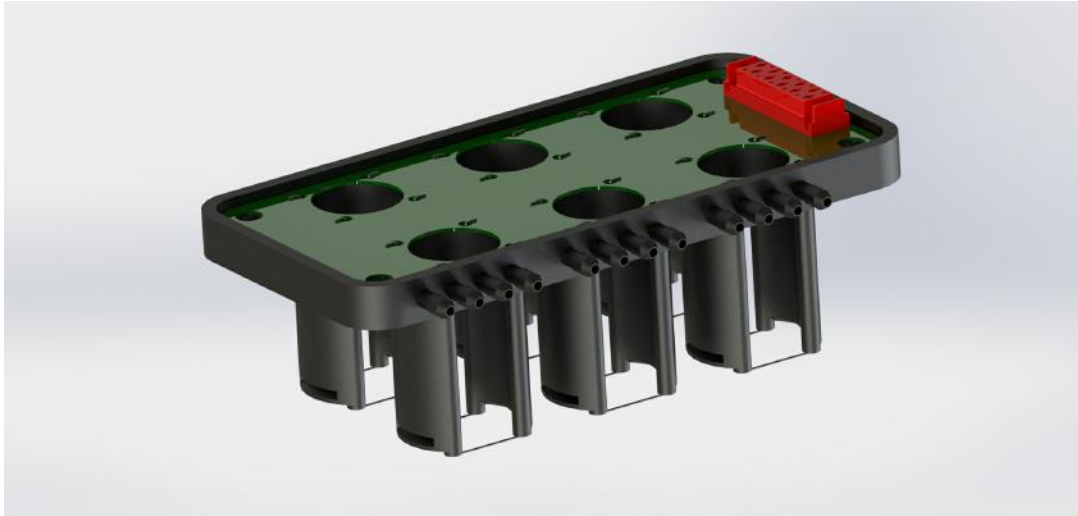


Figure 6: 6-24 Stimulation Lid Overview

The StimSwitch 6-24 Stimulation Lid permits both stimulation and perfusion of 6 wells of a 24 well format tissue culture plate. Each well has its own stimulation electrodes and inlet/outlet perfusion lines so the user can independently control the environment for each well.

The stimulation lid has a 2x3 format which is equivalent to 1/4 of a 24 well plate. This lets the user stimulate all 24 wells by moving the 6-24 Stimulation Lid 4 times, or any other combination as needed by the experiment.

The holes above each well allow for light from the microscope condenser to enter the well for transmitted light imaging of the preparation in each well. You will also notice that the body of the lid is black. This is special paint that prevents the underlying plastic from fluorescing in the presence of UV light so that fluorescent indicator dyes (e.g. Fura 2) can be used.

Fluid Flow Pattern

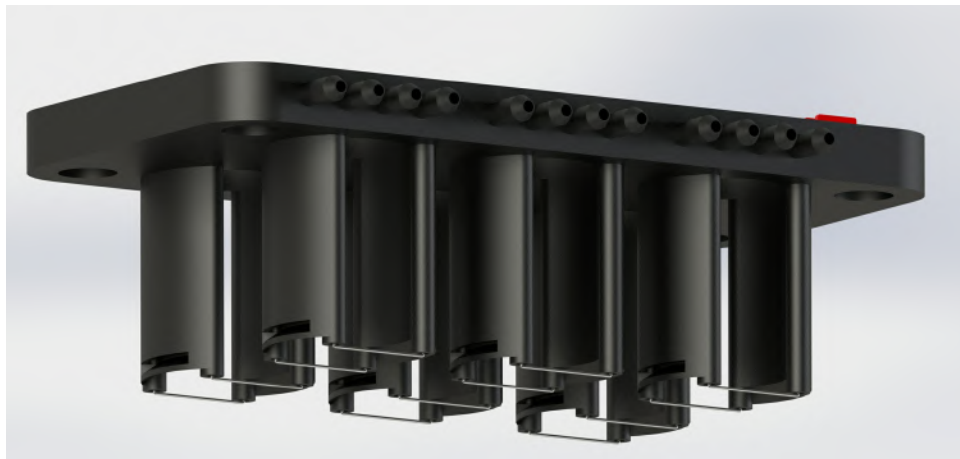


Figure 7: 2 Posts per Well Carry Fluid and Support the Platinum Electrodes

Figure 7 above shows the 6-24 Stimulation Lid from below. Here you can see the semicircular "posts" that extend down into the well. These posts have two functions.

The first is to support the platinum stimulation electrodes you can see in the figure. These electrodes reach up through the post to the circuit board on top of the assembly and then are routed to the connector.

The second function is to carry the fluid down into the well via internal channels. There is an opening on

the outside of the post that points toward the wall of the chamber. This orientation requires that the fluid reverse direction, flow under the inlet post, across the well, under the outlet post, reverse direction again, and finally flow into the outlet opening. This flow pattern, illustrated below, spreads the fluid out as much as possible to reduce dead spots in the well.

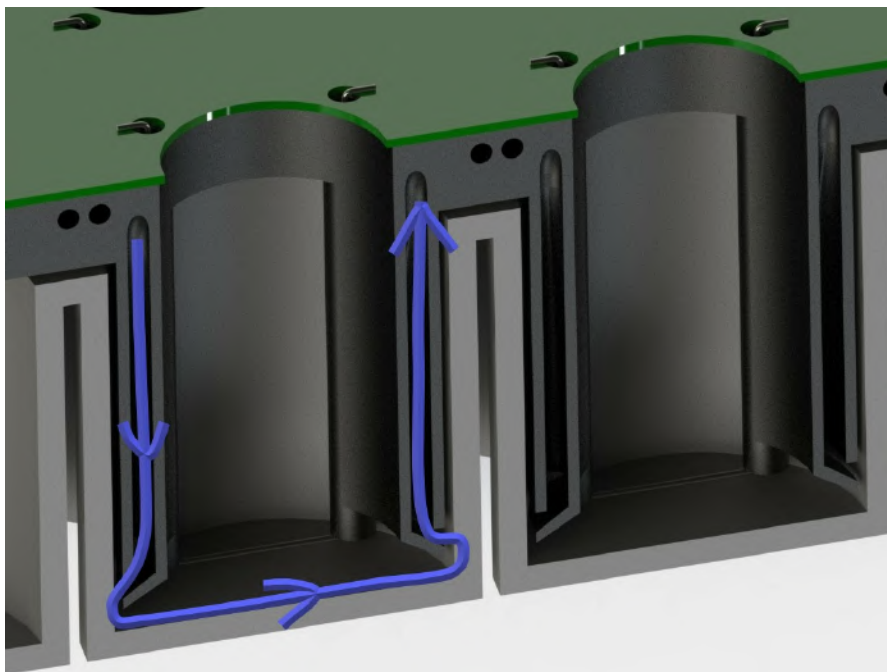
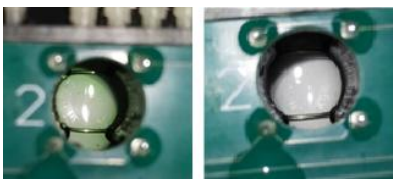
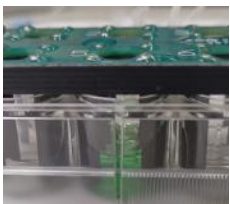


Figure 8: Fluid Flow Pattern. Notice the Flow Is Parallel to the electrodes.

Our tests have shown that there are still some dead spots on the very periphery of the wells for some types of well plate. This means that you should keep perfusion flowing if you are doing a rinse to assure that the small remaining amount of compound does not diffuse into the central section of the well.

The table below summarizes our test results with several different 24 well plates. Our test was to fill the well with green colored solution and then wash it with clear solution.

Plate Type	Result	Visual
Ibidi	Middle is clear after 1ml, completely clear after 10ml	
Falcon	Middle is clear after 2ml, but sides never clear completely and rim of glass bottom has a very light green shade	None captured.
Costar	Middle is clear after 2ml, but sides never clear completely. Possibly because fluid rises up along the posts as shown at right.	

Electrical and Fluid Connection

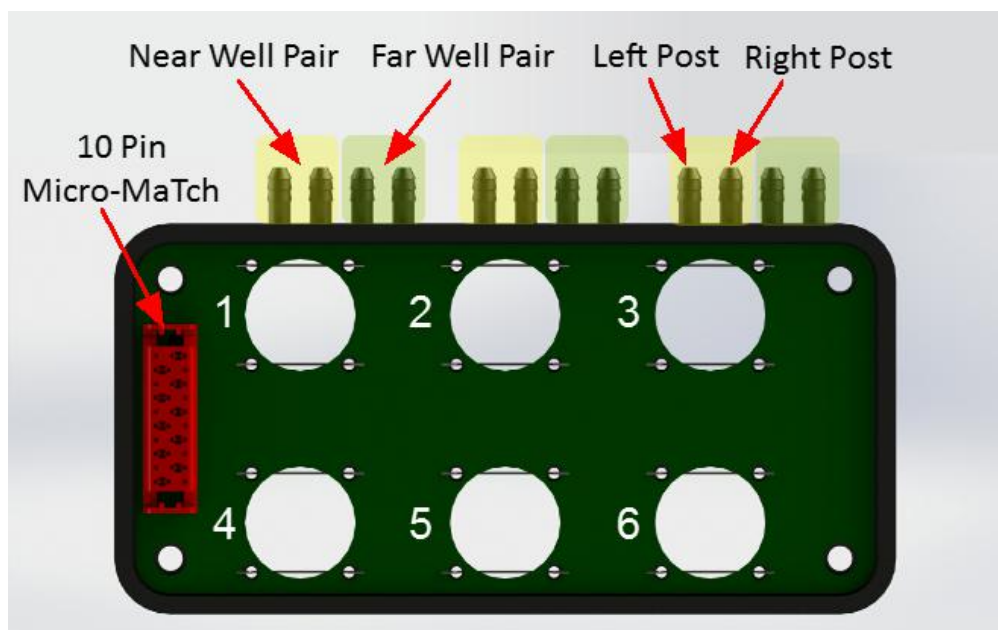


Figure 9: 6-24 Electrical and Fluid Connections and Well Format

Figure 9 shows the Stimulation Lid from above. You can see 6 wells numbered 1-3 left to right along the top row and 4-6 along the bottom row. These numbers correspond to the numbers under the buttons on the StimSwitch-6M multiplexer box and indicate which well will be stimulated when a particular button is selected.

Along the back (top) you can see twelve, 1/16" barb connectors that allow connections from a pump system. If you have a multiplexed pump system like the CytoCypher CY-FLO, you can individually program perfusion through each well one at a time. Other pump systems will permit other perfusion protocols.

There are 3 groups of 4 connectors. Each group has 2 pair of barb connectors highlighted in yellow and green in figure 9. Each group feeds the two wells below it. The left (yellow) pair of connectors feeds the nearest wells, namely wells 1, 2, and 3. The right (green) pair of connectors feeds the furthest wells, namely wells 4, 5 and 6.

Within each pair of barb connectors, there is a left and right connector. The left connector is connected to the left post that extends into the well while the right connector is connected to the right post. Thus, if you connect the left connector in each well to the solution source, and the right connector to the solution evacuation, the flow would be from left to right.

You can also see on the left hand side of figure 9 the 10 pin Micro-MaTch connector that connects to the StimSwitch-6M multiplexer box. This brings stimulation waveforms into the circuit board mounted on the top of the lid where they are connected to the platinum electrodes that extend down into the wells.

Cleaning

There are two considerations when cleaning the lid. The first is that the plastic used for the main body of the lid is rated for 56C/132F. This precludes autoclaving. The second consideration is that the body is painted to prevent the plastic from fluorescing. So care should be taken to avoid solvents (e.g. acetone) that would dissolve the paint. **Recommended cleaning solutions are ethanol or weak detergents.**

Cleaning is best accomplished in 2 steps. First clean the internal channels by running cleaning solution through the tubing using the perfusion pump. It is important to do this as a separate step because the internal channels are too small to get clean with a general soaking. When the channels are clean, soak and agitate the lid in cleaning solution to remove contaminants from the posts, electrodes, and remaining parts of the lid.

4 Cabling Options

There are two cable options for the StimSwitch system depending on whether you are using a regular research microscope or a CytoCypher HTS microscope.

Cabling for Standard Research Microscope



Figure 10: Standard Research Microscope cable.

For a standard microscope you need a DB9 to Micro-MaTch 10, 5 foot cable, part number STS-6M-CAB-S1/INV. This directly connects between the DB9 connector on the back of StimSwitch multiplexer box and the Micro-MaTch 10 connector on the 6-24 Stimulation Lid.

Cable for CytoCypher HTS Microscope



Figure 11: CytoCypher HTS Microscope Access Ports: Series 1 DSUB cutout on the left and Series 2 access port on the right.

The cabling set you need for your CytoCypher HTS Microscope depends on your microscope series. If you have a Series 1 microscope, there is a DSUB connector cutout in the diagonal side of the microscope. If you have a Series 2 or later microscope, there is a rectangular access port in the diagonal side of the microscope.

Series 1 Cables

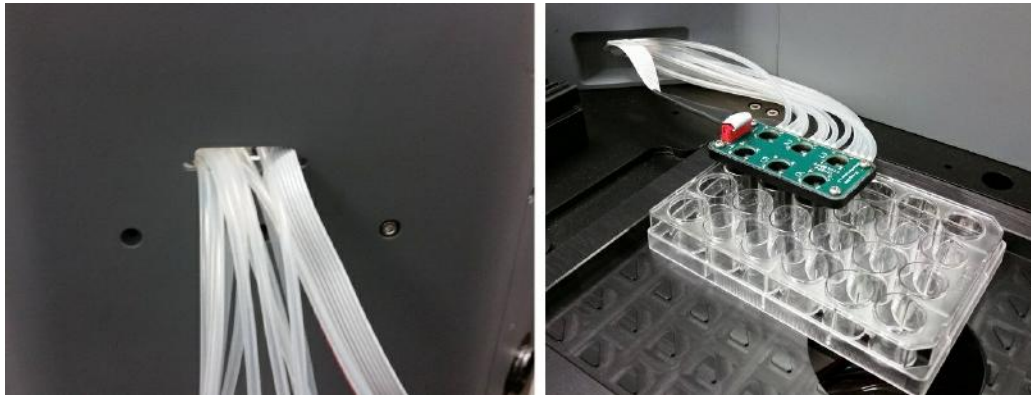


Figure 12: Series 1 Cable Feedthrough Examples, Exterior on Left, Interior on Right

For a Series 1 microscope you need to use the Standard Research Microscope cabling set part number STS-6M-CAB-S1/INV, as described above. You need to simply feed the ribbon cable through the 25 pin cutout as shown in figure 12.

Series 2+ Cables

For Series 2 and greater microscopes you need the CytoCypher HTS Series 2+ Cable Set, part number STS-6M-CAB-KIT-S2. This set has three parts.



Figure 13: CytoCypher
HTS Microscope
Series 2 StimSwitch 6
Access Plate

The first part is the the DB9/Tubing Access Plate shown. This plate fits in the CytoCypher HTS Microscope Access Port and has a DB9 pass through connector and a hole for tubing to pass through.



Figure 14: StimSwitch DB9 to Micro-MaTch 10
8 Inch Cable

The second part is the DB9-MM10-8i cable. This 8 inch cable connects between the DB9 female connector in the inside of the access plate and the Micro-MaTch 10 connector on the Stimulation Lid.



Figure 15: DB9 Male to DB9 Female, 2 Meter Cable

The third part is a standard DB9 male to female cable. This connects between the DB9 female connector on the back of the StimSwitch-6M and the DB9 male connector on the outside of the CytoCypher HTS Microscope Access Port.



Figure 16: Series 2 Cable Access Port, Exterior on Left, Interior on Right

The images above show the full cabling solution from both inside and outside the Series 2 CytoCypher HTS Microscope.

5 Accessories

CytoCypher 24 Well Fixed Stage



Figure 17: CytoCypher HTS Microscope 24 Well Fixed Stage

To use a 24 well plate on the CytoCypher HTS Microscope, you will need a 24 Well Fixed Stage, part number CY-STG-24, shown in figure 17. This plate replaces the standard 6 Well Fixed Stage that ships with the microscope. It lets you position any individual well in the 24-well plate over the objective.

Perfusion Tubing



Figure 18: 0.9" Barbs For Perfusion Tubing Connections

The 6-24 lid has 0.09" (2.3mm) OD barb connectors. These are suitable for use with 1/16" ID (0.0625", 1.6mm) tubes or 1.5mm ID (0.06") tubes. These barbs can accept tubing as small as 1mm ID (0.04"), but it is not recommended as it is a very tight fit.