AGILE PLATFORM FOR DROPLET ANALYSIS AND MANIPULATION

BEND TIME IN HIGH-THROUGHPUT SCREENING

USER MANUAL

Styx

Droplet Genomics

YOUR GROUND CONTROL IN HIGH-THROUGHPUT BIOLOGY

DROPLETGENOMICS.COM

CONTENTS

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LIMITED LIABILITY

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WARRANTY TERMS

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SYSTEM OVERVIEW

STYX INSTRUMENT COMPONENTS

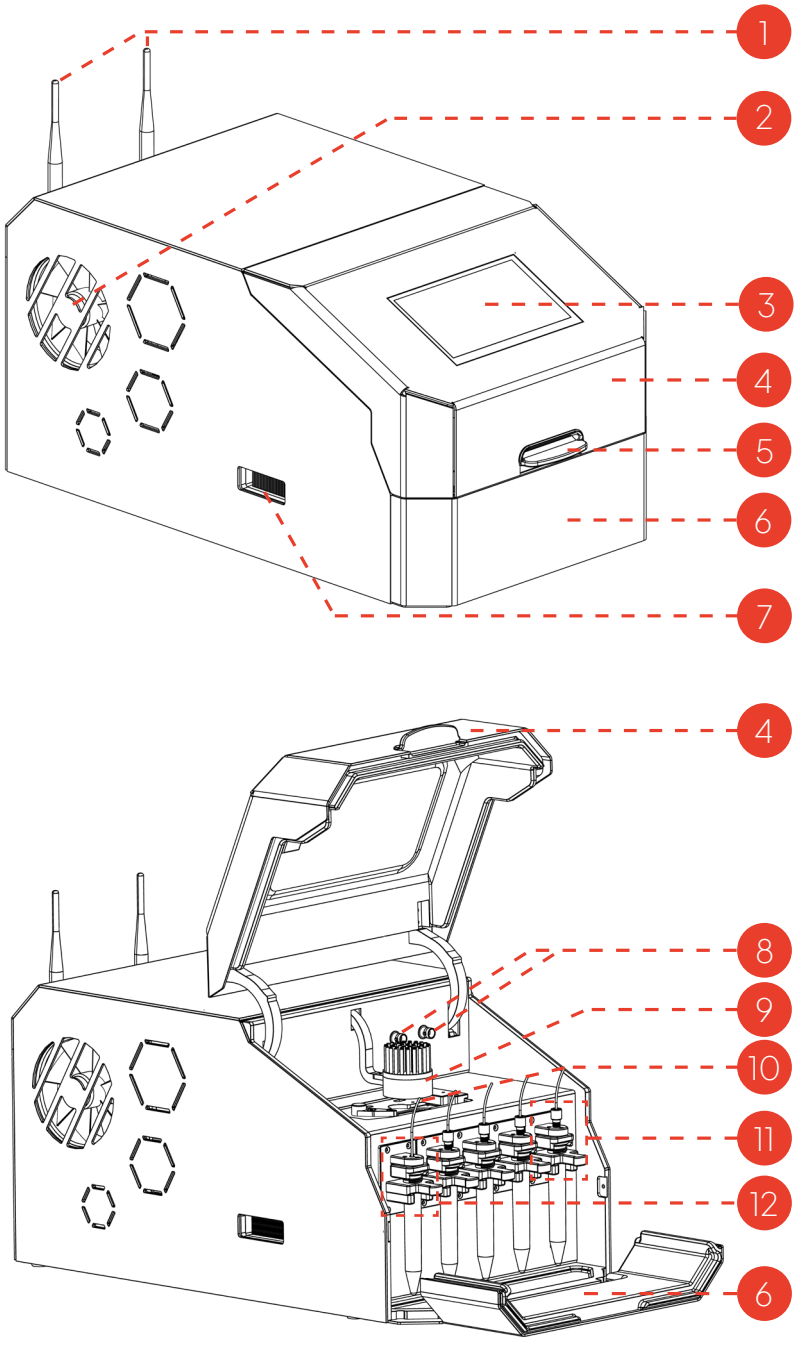

Figure 1: Instrument components

1. Wi-Fi antennas
2. Fan intake
3. LCD screen
4. Upper lid
5. Lid handle
6. Lower lid
7. Fan outlet
8. Electrode ports
9. Illuminator
10. Slide holder
11. Pressurisable channel
12. Outlet
13. USB port
14. Ethernet port
15. Information panel
16. Power entry socket
17. Fuse box
18. Power on/off button

STYX INSTRUMENT COMPONENTS

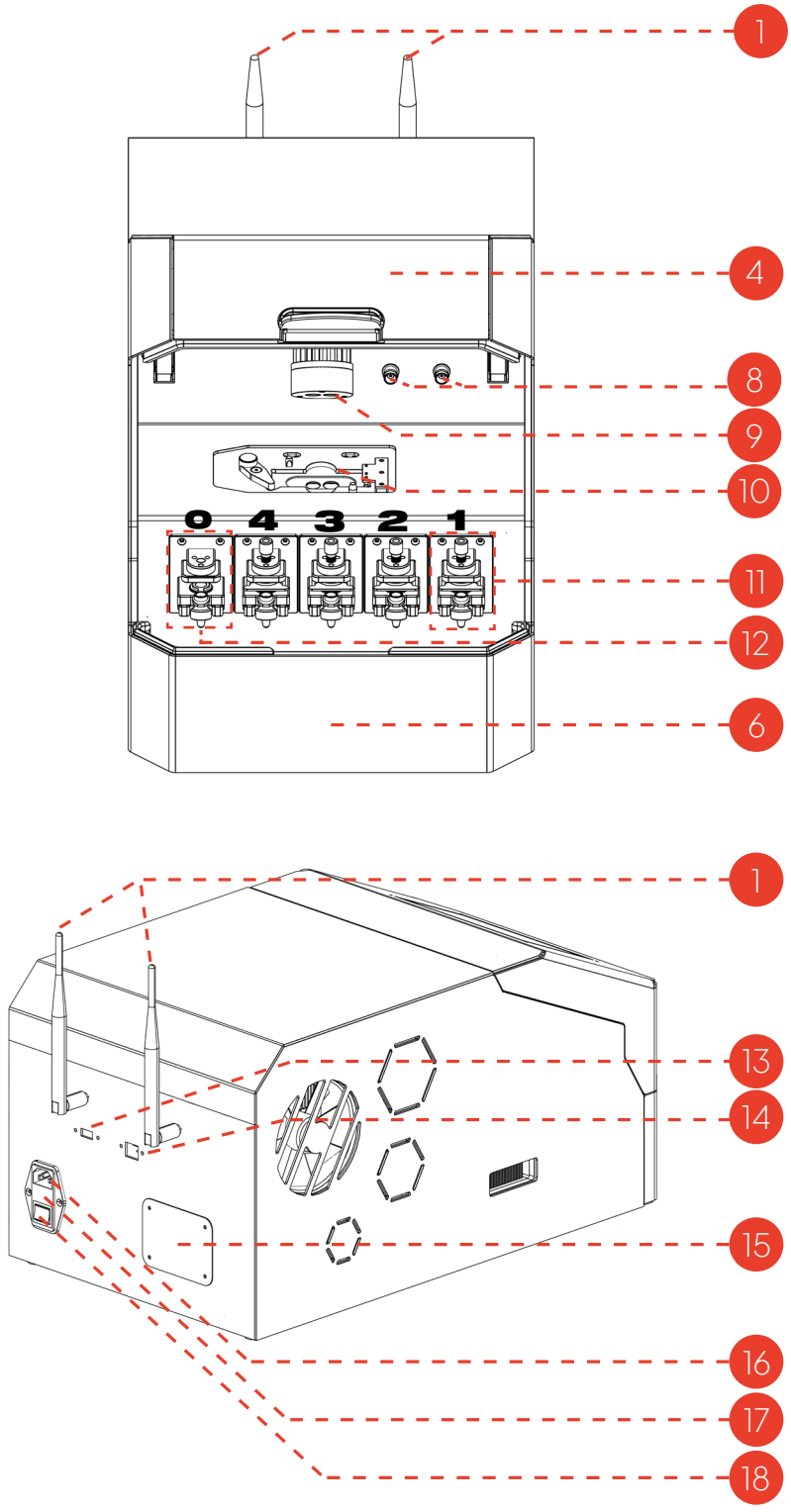

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Figure 2: Instrument components

STYX MODULES' EXPANDED VIEW

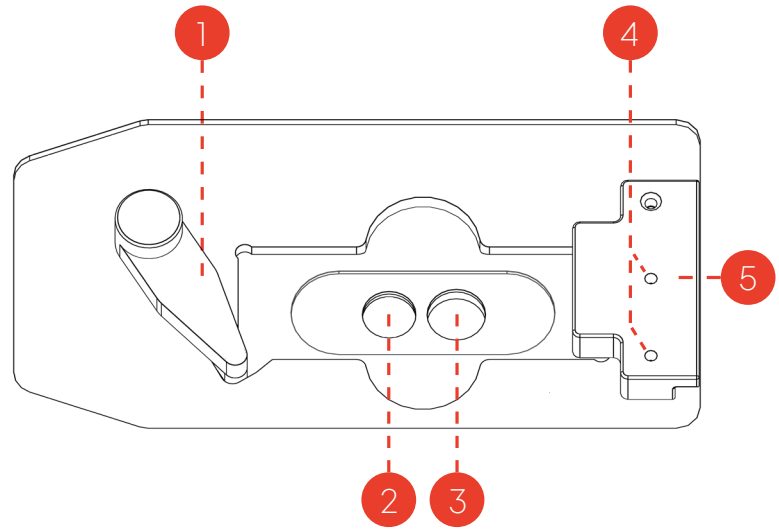


Figure 3: Slide holder components

Slide holder

1. Slide clamp
2. Left objective
3. Right objective
4. Adjustable electrode holder screws
5. Electrode holder

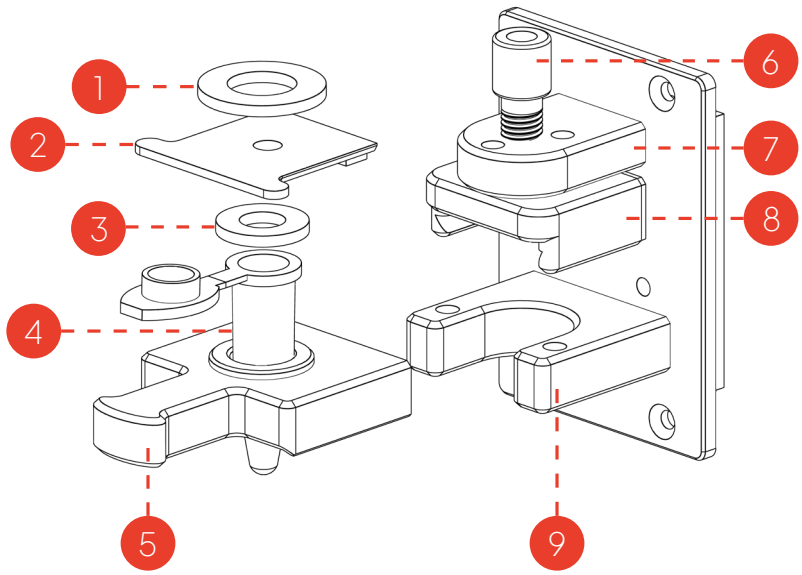


Figure 4: Pressurizable channel components

Pressurizable channel

1. Large seal
2. Small tube adapter
3. Small seal
4. Small tube (1.5mL)
5. Small tube bracket
6. Screw nut
7. Screw nut bracket
8. Tube sealing plate
9. Small tube bracket

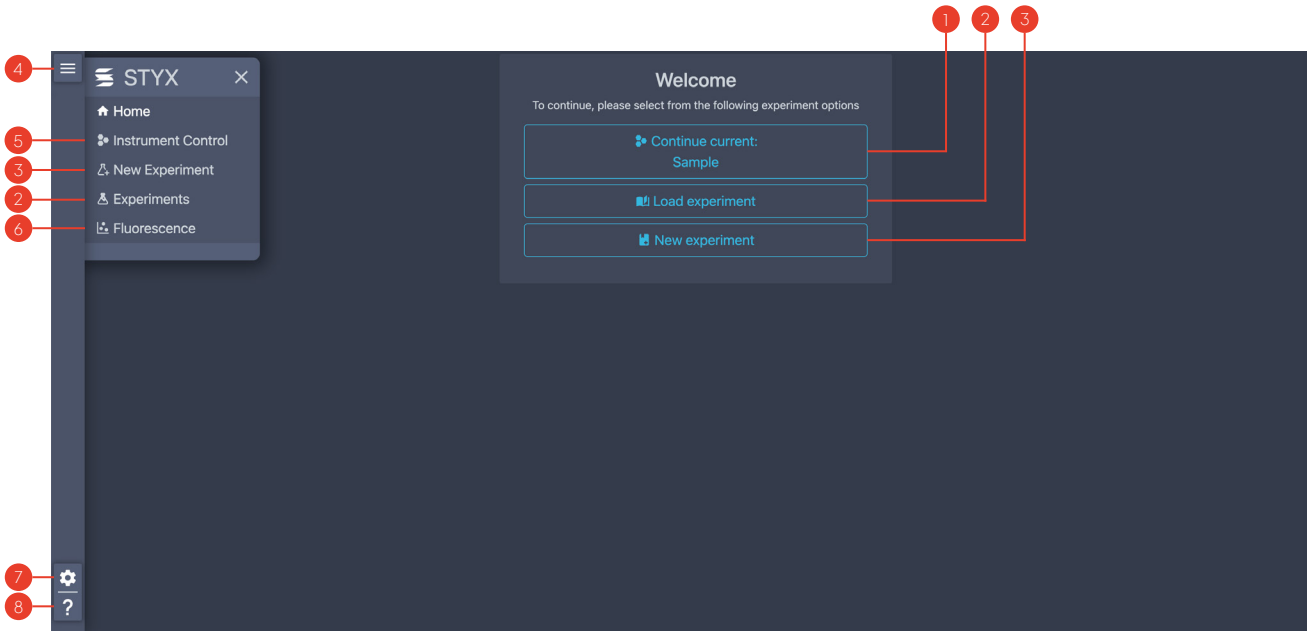
STYX USER INTERFACE

The listed User Interface controls are applicable to the software version **3.24.2.0**. In case of software updates please contact Droplet Genomics for the most up to date User Interface controls.

HOME WINDOW

SELECT EXPERIMENT OPTIONS

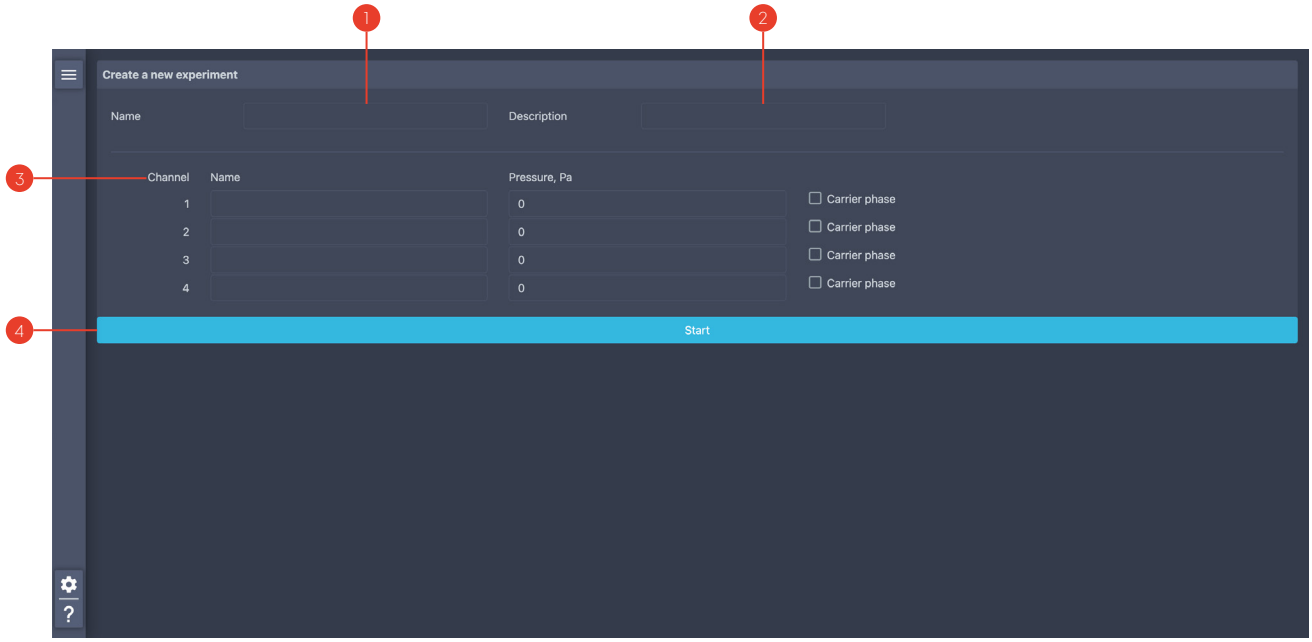
1. Continue the previous experiment. *Continue the last loaded experiment.*
2. Load experiment. *List of previously saved experiment settings.*
3. New Experiment. *Set up a new experiment.*
4. Control Menu.
5. Instrument Control. *Main window to monitor and control experiments.*
6. Fluorescence dashboard. *Analyze detected droplets and set up triggers.*
7. Settings. *List of adjustable instrument settings.*
8. Shortcuts, Updates and File names. *List of additional customizable options and other functions.*



NEW EXPERIMENT WINDOW

PROVIDE THE REQUIRED INFORMATION TO SET UP A NEW EXPERIMENT

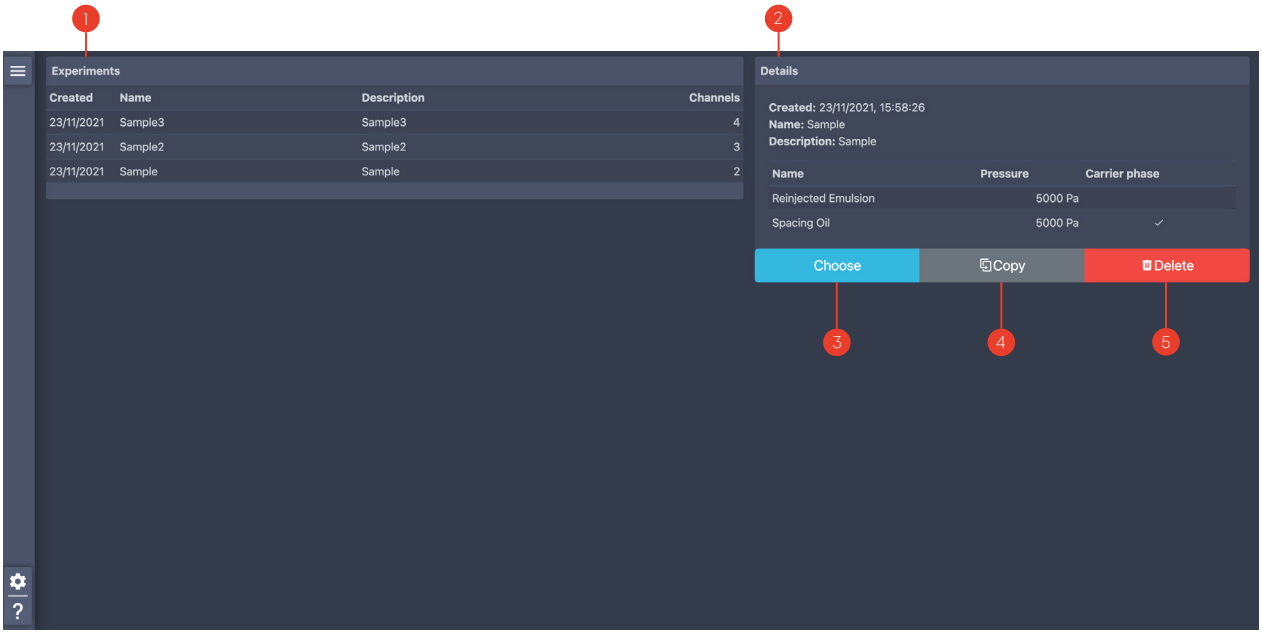
1. Experiment name.
2. Experiment description.
3. Experiment details. *Name channels used in the experiment and set pressures.*
   NOTE: Carrier phase selection is optional and is only used in reporting.
4. Submit all information and proceed to experiment



EXPERIMENTS/LOAD EXPERIMENT WINDOW

CHOOSE AND CONTINUE OR COPY/DELETE PREVIOUS EXPERIMENTS

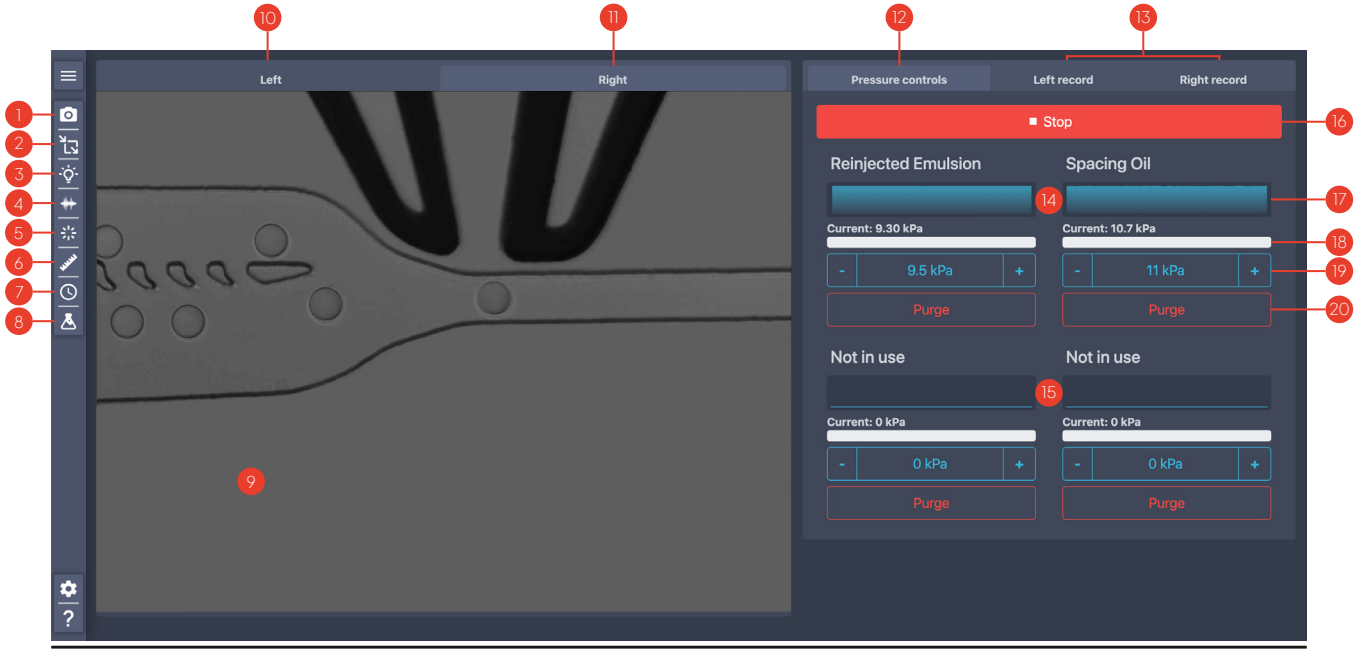
1. A list of all saved experiments. Choose a specific experiment on click.
2. Experiment details. Appears after selecting a specific experiment.
3. Choose an experiment. Continue to instrument control window with preset experiment details.
4. Copy the experiment. Create a new experiment entry with parameters inherited from the selected experiment.
5. Delete the experiment.



INSTRUMENT CONTROL

MONITOR AND CONTROL CURRENT EXPERIMENT

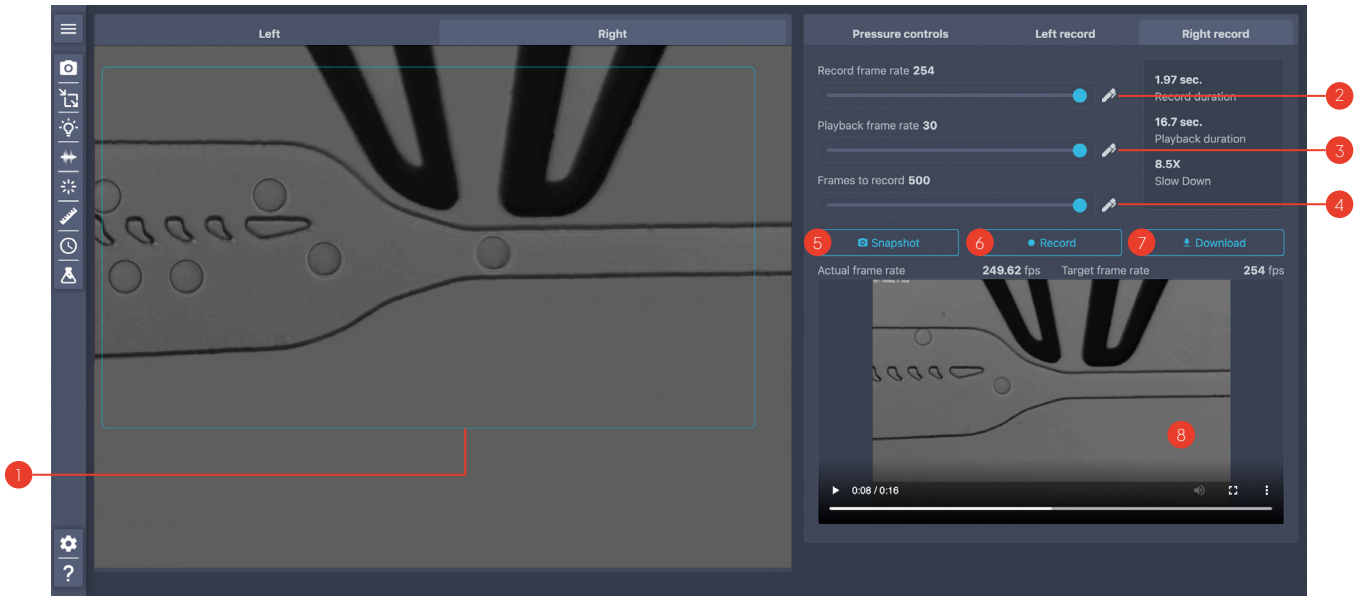
1. Camera settings
2. XYZ stage motion controls.
3. Illumination settings.
4. High-voltage module controls.
5. Laser controls.
6. On-screen measurements. *Draw a line or a box and measure droplet volume and diameter.*
7. Timer. *Keep track of experiment run time or set a timer and stop the experiment when the timer expires.*
8. Experiment. *Change experiment name, description and save/copy the experiment entry.*
9. Live camera view.
10. Switch to Left Camera view.
11. Switch to Right Camera view.
12. Pressure controls window.
13. Recording dashboard.
14. Channels that are currently used in the experiment.
15. Channels that are currently inactive.
16. Start/stop all channels in the experiment.
17. Pressure measurement display.
18. Current pressure value and error indication. *Blue colour indicates normal operation; red colour – actual pressure is different from set pressure.*
19. Adjust pressure setpoint. *Click to enter pressure manually.*
20. Temporary pressure increase for priming.
    IMPORTANT: Use with caution – sample loss may occur.



RECORDING DASHBOARD

TAKE A SNAPSHOT OR RECORD A CLIP OF MICROSCOPE VIEW

1. Select recording area. *Select by clicking the left mouse button on the camera view area and dragging it.*
2. Set the recording frame rate value. *The maximum frame rate depends on the recording region.*
   NOTE: Select values by dragging a slider or press the pen icon to enter the value manually.
3. Set playback frame rate value. *Determines the speed of playback for the recorded video.*
4. Set the number of frames to record. *Determines the length of the recorded video.*
   NOTE: The characteristics (record duration, playback duration and magnitude of slow down) of the recorded video will be displayed in the table on the right after setting the parameters.
5. Take a snapshot of the entire field of view.
6. Record a video with selected parameters.
7. Download recorded video.
   IMPORTANT: Videos are not stored in the internal memory of the device. Make sure to download it every time after recording if you intend to save it.
8. Recorded video preview.



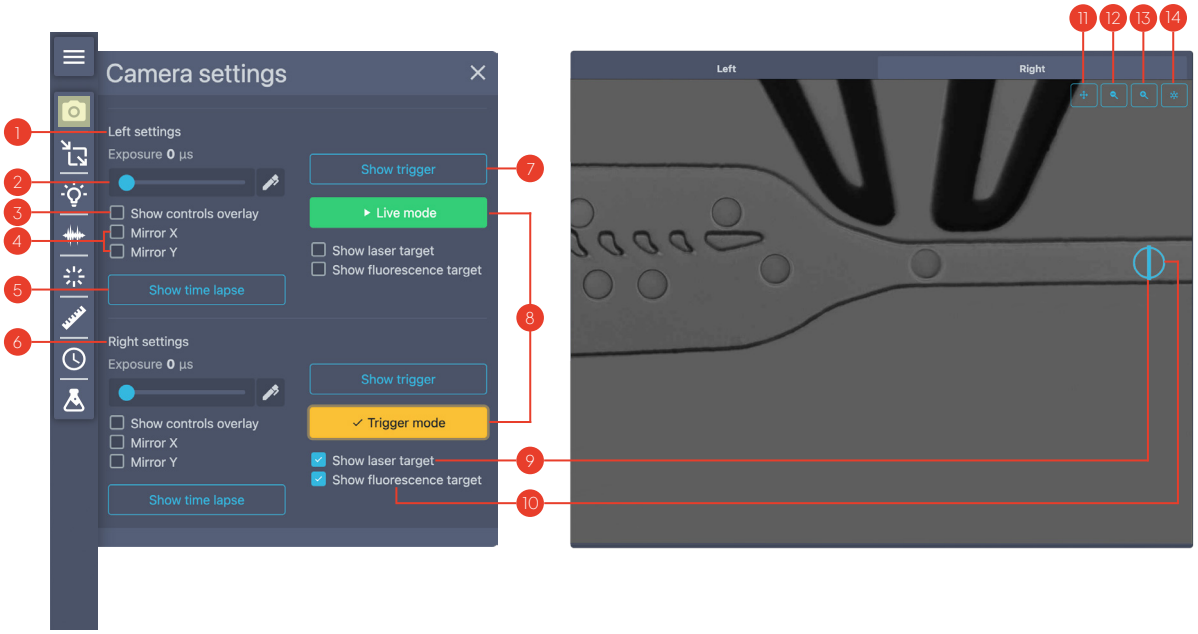
CAMERA SETTINGS

CONTROL CAMERA SETTINGS

1. Left camera settings.
2. Adjust camera exposure (µs).
3. Enable additional options (see 11–14).
4. Control the camera view direction.
5. Record time lapse (refer to the section “RECORD A TIME LAPSE VIDEO”).
6. Right camera settings.
7. Show camera trigger controls (refer to the section “MAKE A SYNCHRONISED VIDEO CLIP ”)
8. Switch between Live and Trigger modes (refer to the section "MAKE A SYNCHRONISED VIDEO CLIP).
9. Show laser target on the camera view. *Estimated laser line position.*
10. Show fluorescent target on the camera view. *Estimated fluorescence detection zone position.*

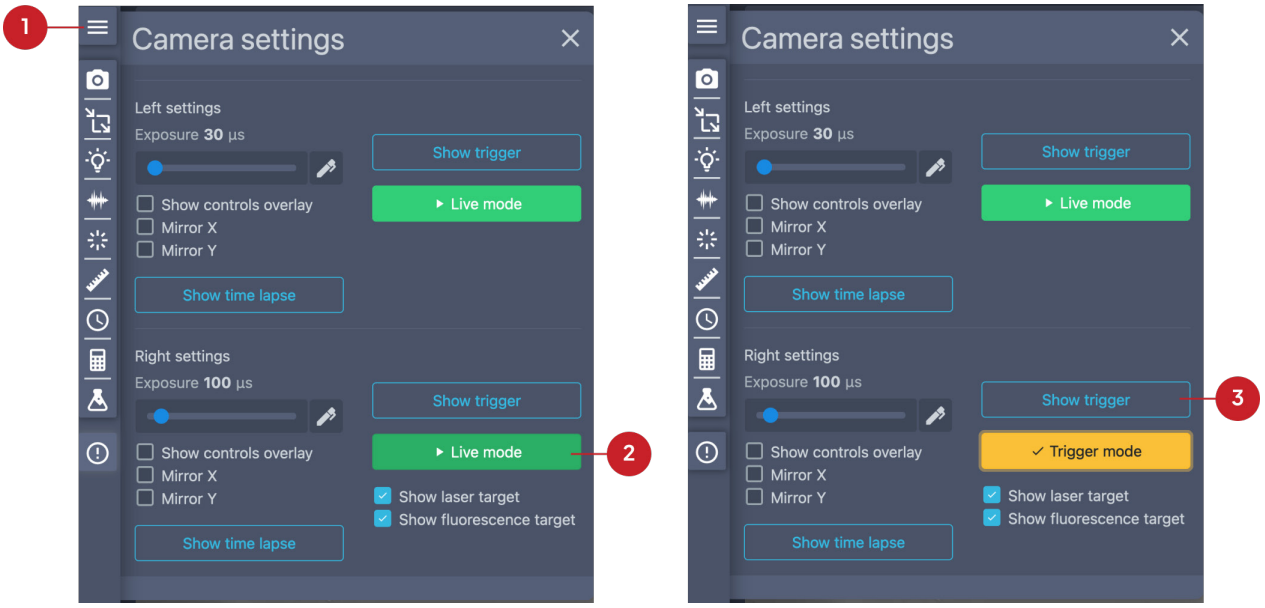
Settings accessible when “Show controls overlay” is selected:

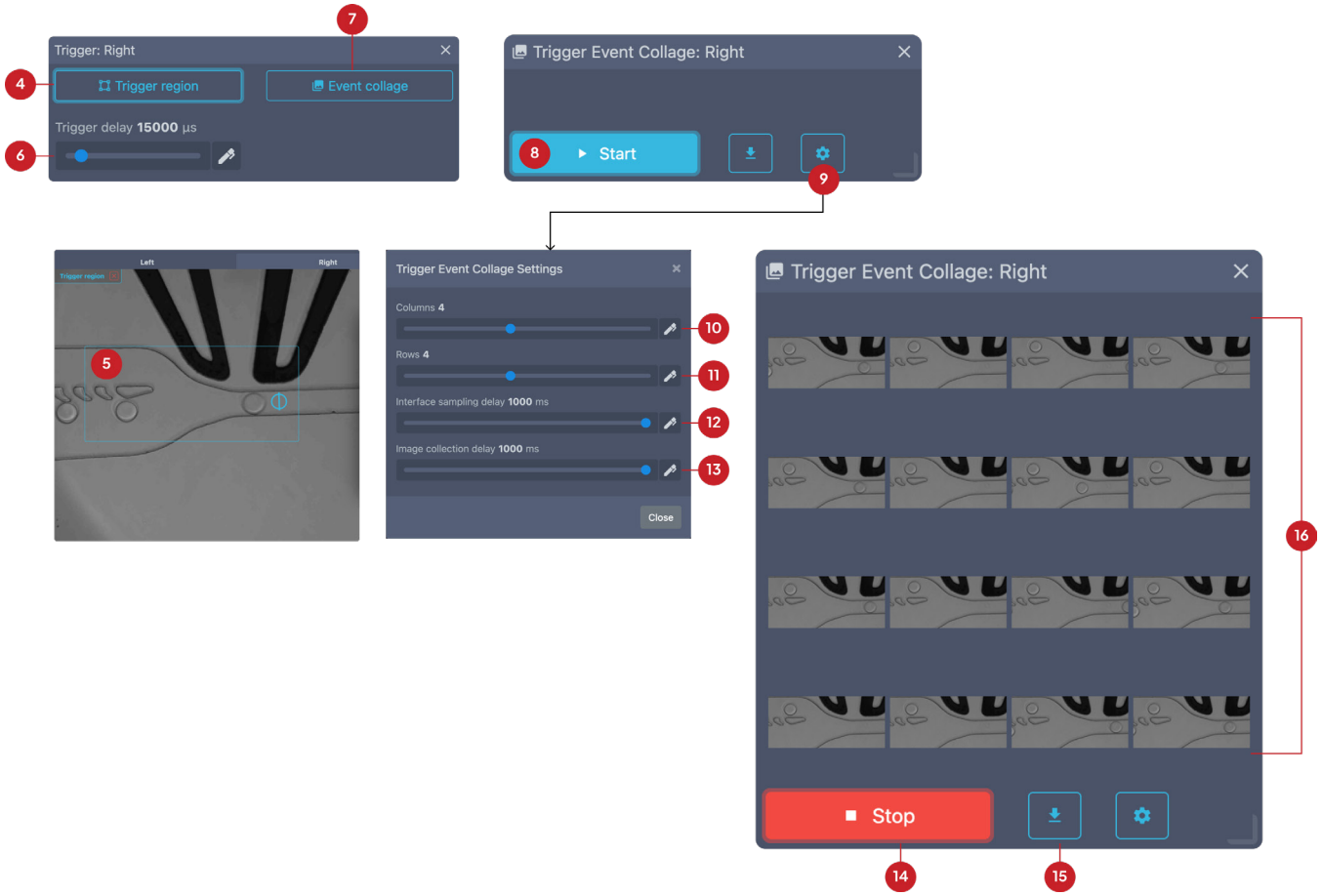
11. Drag camera view.
12. Zoom out the camera view.
13. Zoom in the camera view. *Zoom in by clicking the left mouse button on the camera view area and dragging it.*
14. Freeze/Unfreeze camera view. *Alternatively, keyboard shortcut “F” can be used.*



CREATE SYNCHRONIZED IMAGES

1. Show Camera settings.
2. Click to switch to Trigger mode. The current mode is shown on the button.
   Note: Trigger mode needs to remain active for the synchronized images to be generated. If Live mode is switched on, the image generation will be paused.
3. Click to open synchronized image creation controls.

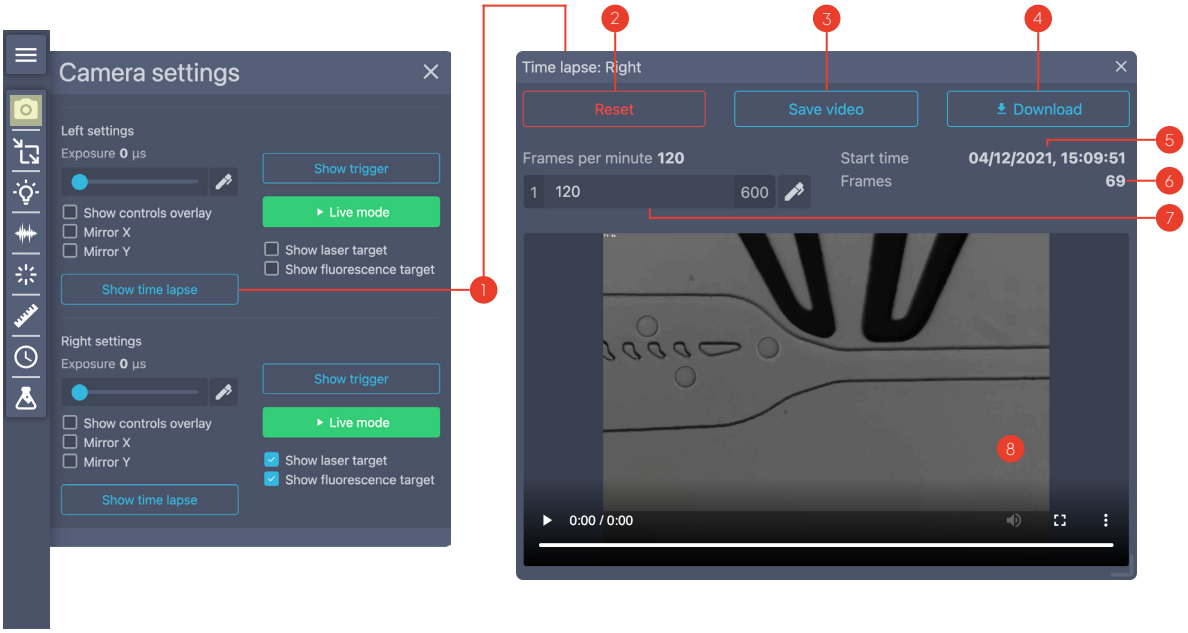




4. Click to select the region of which the synchronized images will be taken in the camera view.
5. To select the region of interest, left-click and drag on the camera view.
6. Adjust trigger delay. This reflects how much time passes from the triggering of positive event to the time of the photo is taken. Depending on the delay and pressure settings droplets will be photographed at different spots. Note: if you see no droplet in the image taken, the droplet was likely outside the selected region of interest.
7. Click to open the Event collage.
8. Click to Start creating synchronized snapshots.
9. Synchronized image generation settings
10. Adjust the number of columns in the generated synchronized image (event) collage.
11. Adjust the number of rows in the generated synchronized image (event) collage.
12. Adjust interface sampling delay (ms). How often event collage (16) is updated.
13. Adjust image collection delay (ms). How often a synchronized image is recorded.
14. Stop generating synchronized images.
15. Download the synchronized images.

RECORD A TIME LAPSE VIDEO

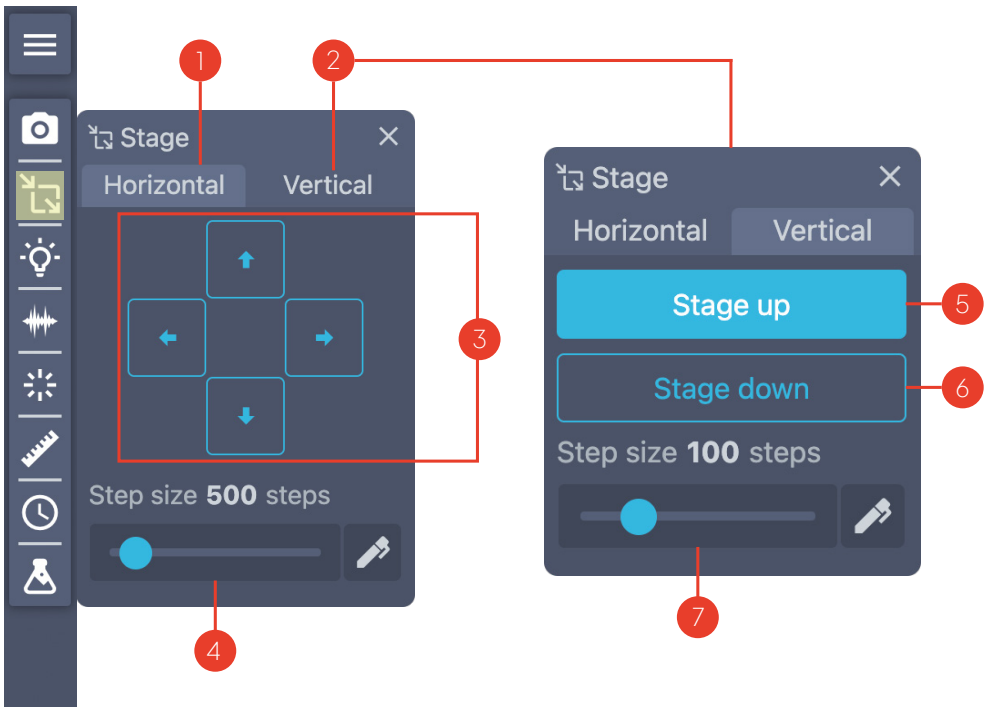
1. Show time lapse video controls.
2. Reset accumulated frames.
   NOTE: Time lapse is constantly active from the moment device is turned on. If you wish to start the recording from a current time point, you have to reset the recording first.
3. Save video. *The video will only be displayed in the preview after saving it.*
4. Download video.
   IMPORTANT: Videos are not stored in the internal memory of the device. Make sure to download it every time after recording if you intend to save it.
5. Time lapse start time.
6. Recorded frame count.
7. Set the recording speed (frames per minute).
8. Recorded time lapse preview.



XYZ STAGE MOTION CONTROL

CONTROL STAGE MOTION

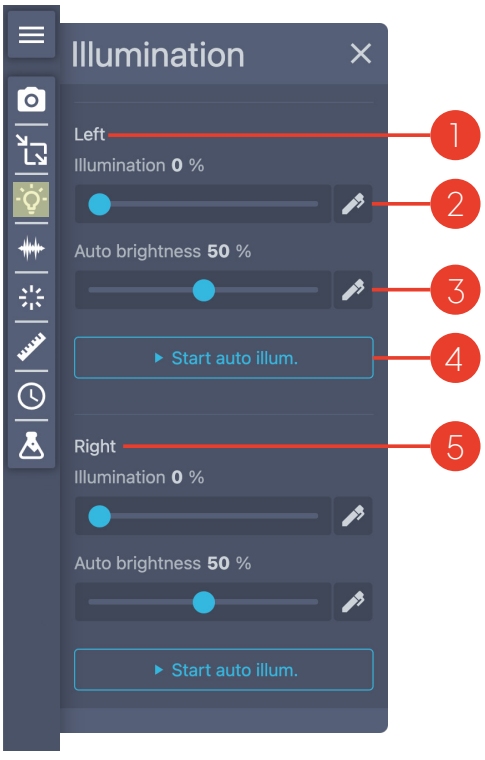
1. Stage motion controls in a horizontal direction (X and Y-axis).
2. Stage motion controls in a vertical direction (Z-axis).
3. Move stage in different directions horizontally.
4. Select step size for stage motion in a horizontal direction.
5. Move stage up vertically (Z-axis).
6. Move stage down vertically (Z-axis)
7. Select step size for stage motion in a vertical direction.



ILLUMINATION

CONTROL ILLUMINATION SETTINGS

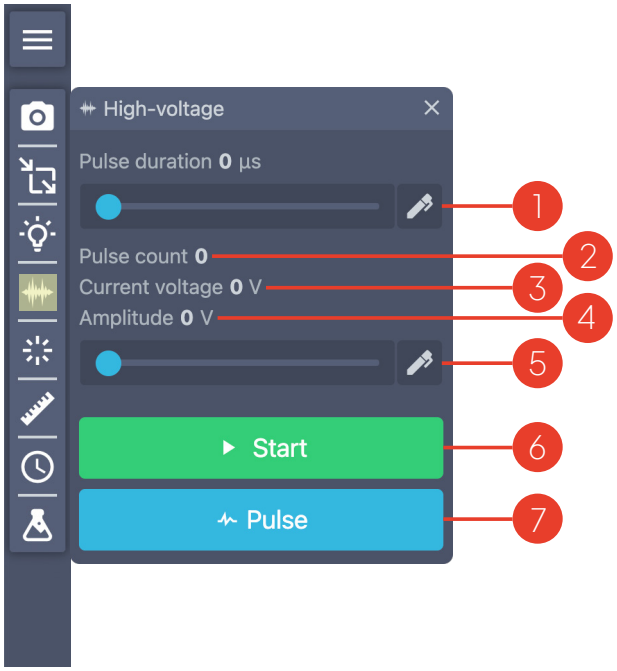
1. Left camera illumination settings.
2. Adjust camera illumination (%).
   NOTE: Auto illumination has to be turned off to make illumination settings active.
3. Adjust auto-brightness (%). *100% corresponds to fully white view, 0% - fully black view.*
4. Enable/Disable auto illumination. *When started the instrument will automatically try to adjust illumination to keep it at the selected brightness level.*
5. Right camera illumination settings.



HIGH-VOLTAGE MODULE

CONTROL HIGH-VOLTAGE SETTINGS FOR ON-DEMAND DROPLET MANIPULATION

1. Set high-voltage pulse duration (µs). *Electrical pulse length that is triggered after fluorescent event detection.*
2. Detected pulse count. *Pulses received by the high-voltage module.*
3. Measured voltage value (V).
4. Set signal amplitude (V). *High-voltage pulse amplitude setpoint.*
5. Enable/disable high-voltage electrical field generation.
6. Create a pulse manually with selected settings.



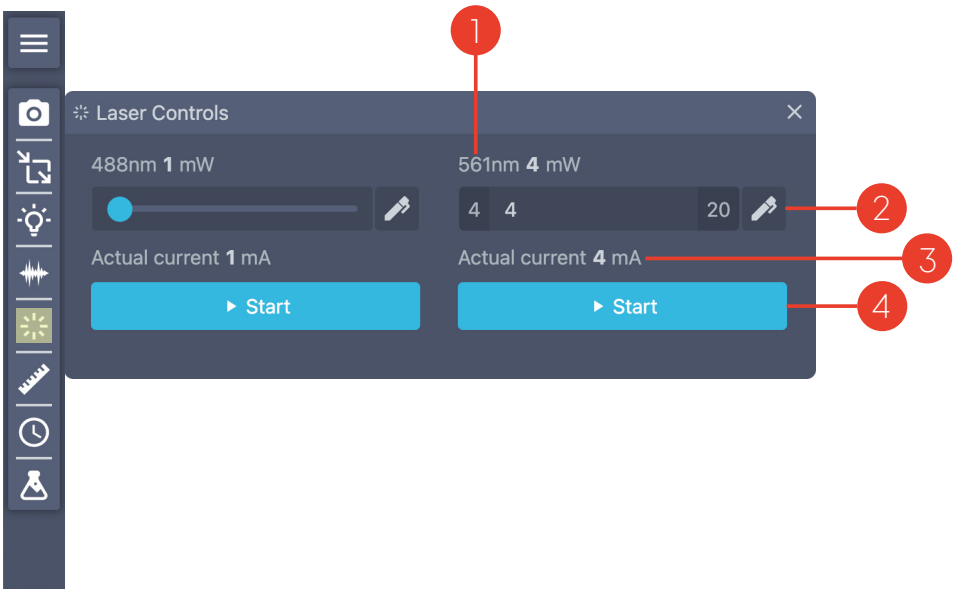
SAFETY NOTICE!

USING A HIGH-VOLTAGE POWER SUPPLY MIGHT RESULT IN ELECTROCUTION IF HANDLED IN-CORRECTLY. MAKE SURE THAT APPROPRIATE SAFETY PRECAUTIONS HAVE BEEN MET BEFORE PROCEEDING.

LASER CONTROLS

SET LASER OPTICAL POWER AND ENABLE/DISABLE LASER OUTPUT

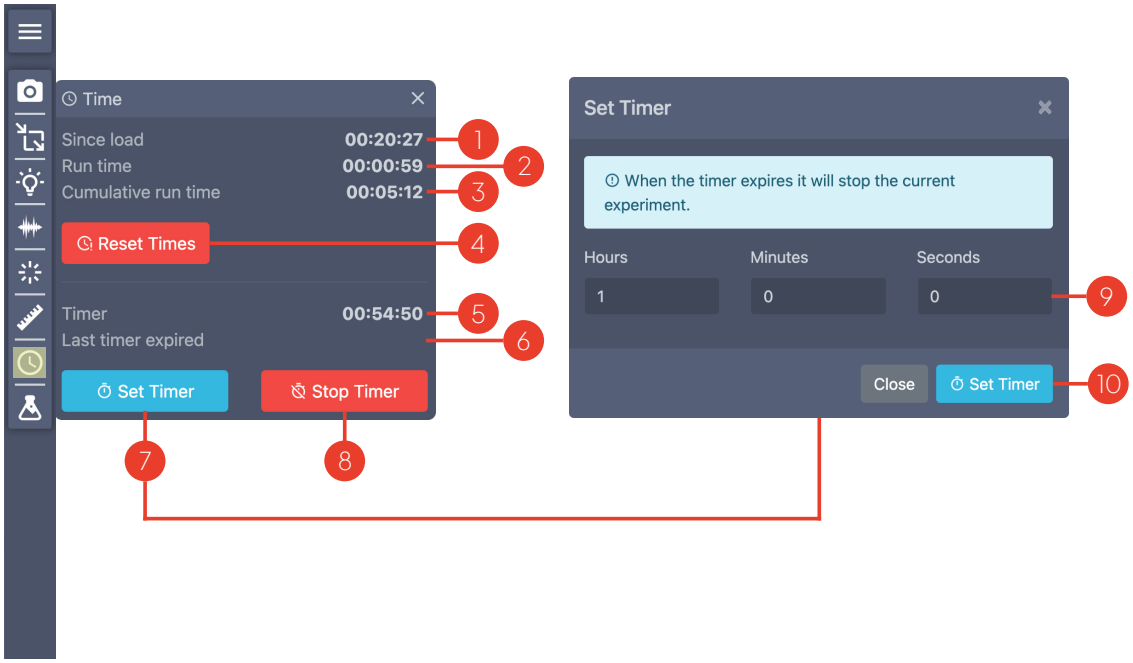
1. Available lasers. *The contents of this window will depend on your STYX laser configuration.*
2. Adjust laser optical power setpoint (mW).
3. Measured laser current (mA) proportional to optical power.
   IMPORTANT: some lasers might take up to 5 minutes to warm up before they can be turned on.
4. Enable/disable laser output.



TRACKABLE TIME PERIODS & TIMER CONTROLS

TRACK DIFFERENT TIME PERIODS & SET A TIMER

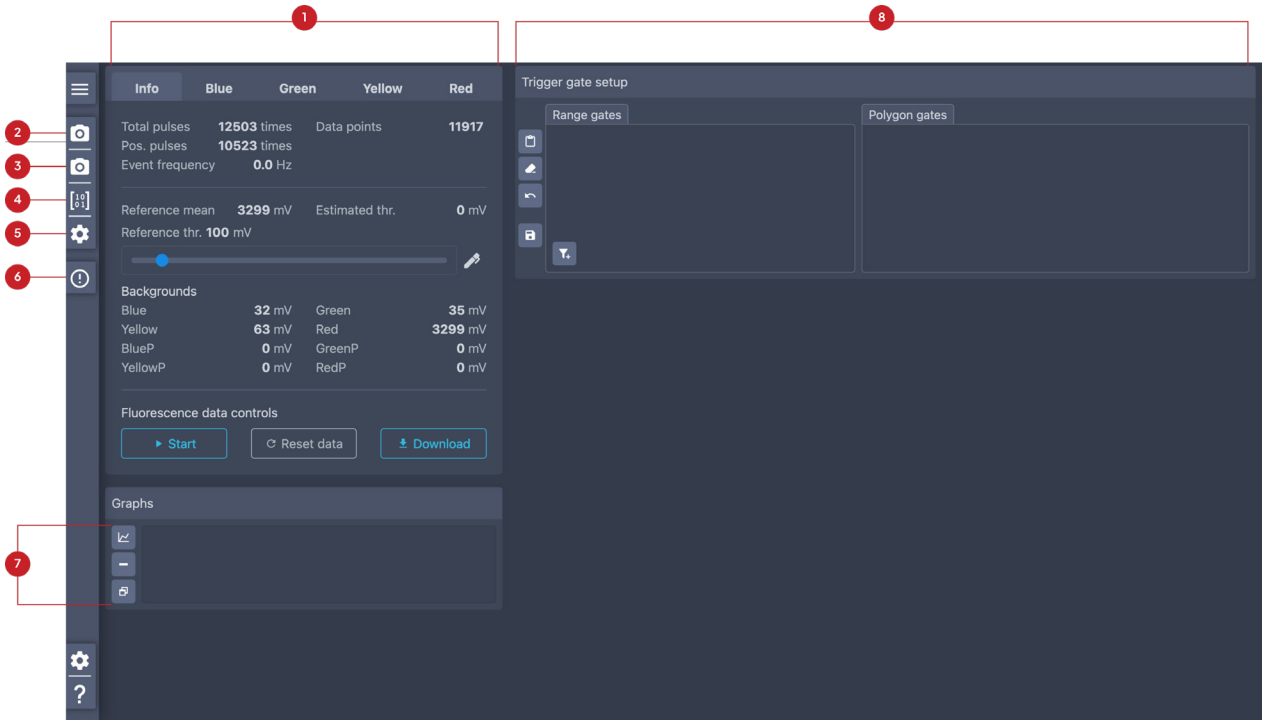
1. Time elapsed since creating/loading an experiment.
2. Current run time. *Active pump time until stopped.*
3. Cumulative run time. *Active pump time in the loaded experiment.*
4. Reset trackable time period values.
5. Time remaining in the active timer.
6. Time since the last timer expired. *Displayed only after expiration of the set timer.*
7. Show timer set-up window.
8. Stop the current timer.
9. Set the timer duration (hours/minutes/seconds).
10. Start the timer.



FLUORESCENCE DASHBOARD

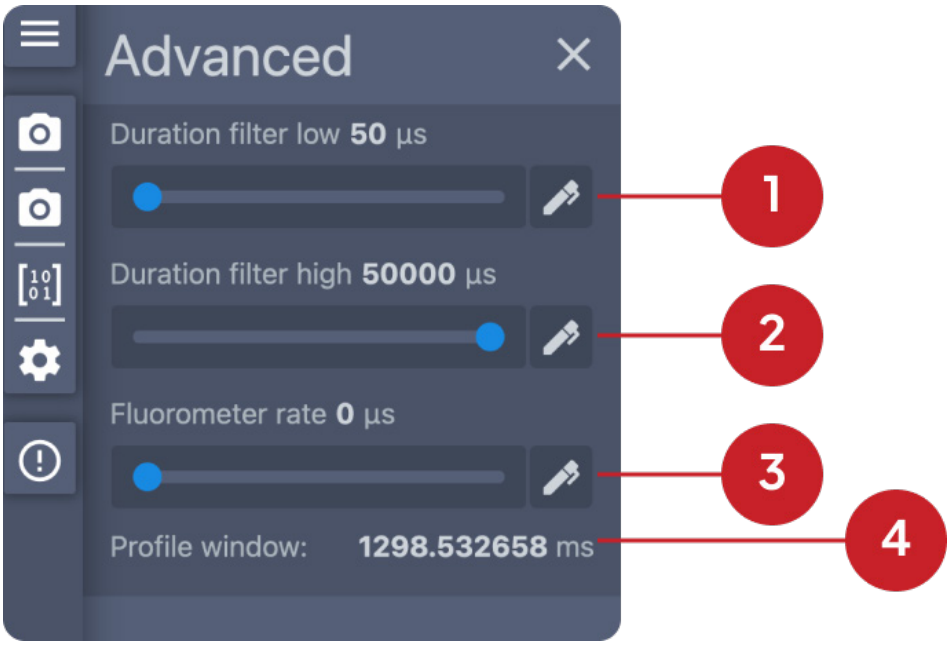
ANALYZE & VISUALIZE DETECTED EVENTS

1. General & channel-specific information.
2. Open Left camera view in a floating window.
3. Open Right camera view in a floating window.
4. Compensation settings.
5. Advanced settings.
6. Notifications.
7. Controls of graphs for fluorescence data visualization. All created graphs are also listed here.
8. Trigger set-up controls.



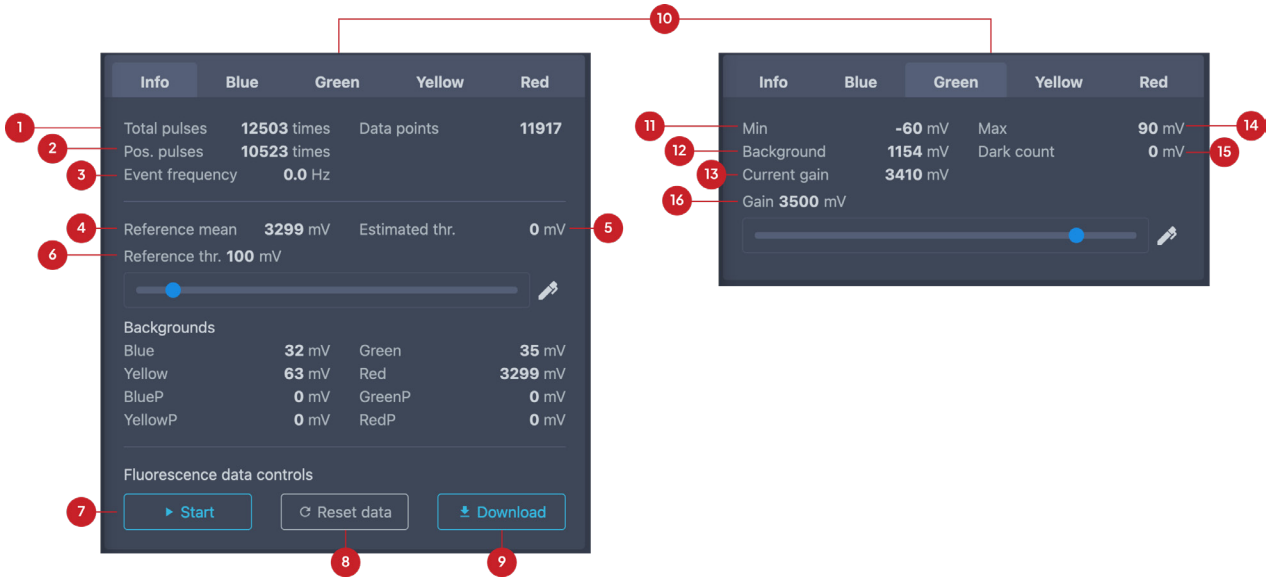
ADVANCED FLUORESCENCE SETTINGS

1. Duration filter low. Set minimum duration of fluorescence signal to be registered as a fluorescence event.
2. Duration filter high. Set maximum duration of fluorescence signal to be registered as a fluorescence event.
3. Fluorometer rate. How often Profile graph is refreshed.
4. Profile window. The full timescale of Profile graph.



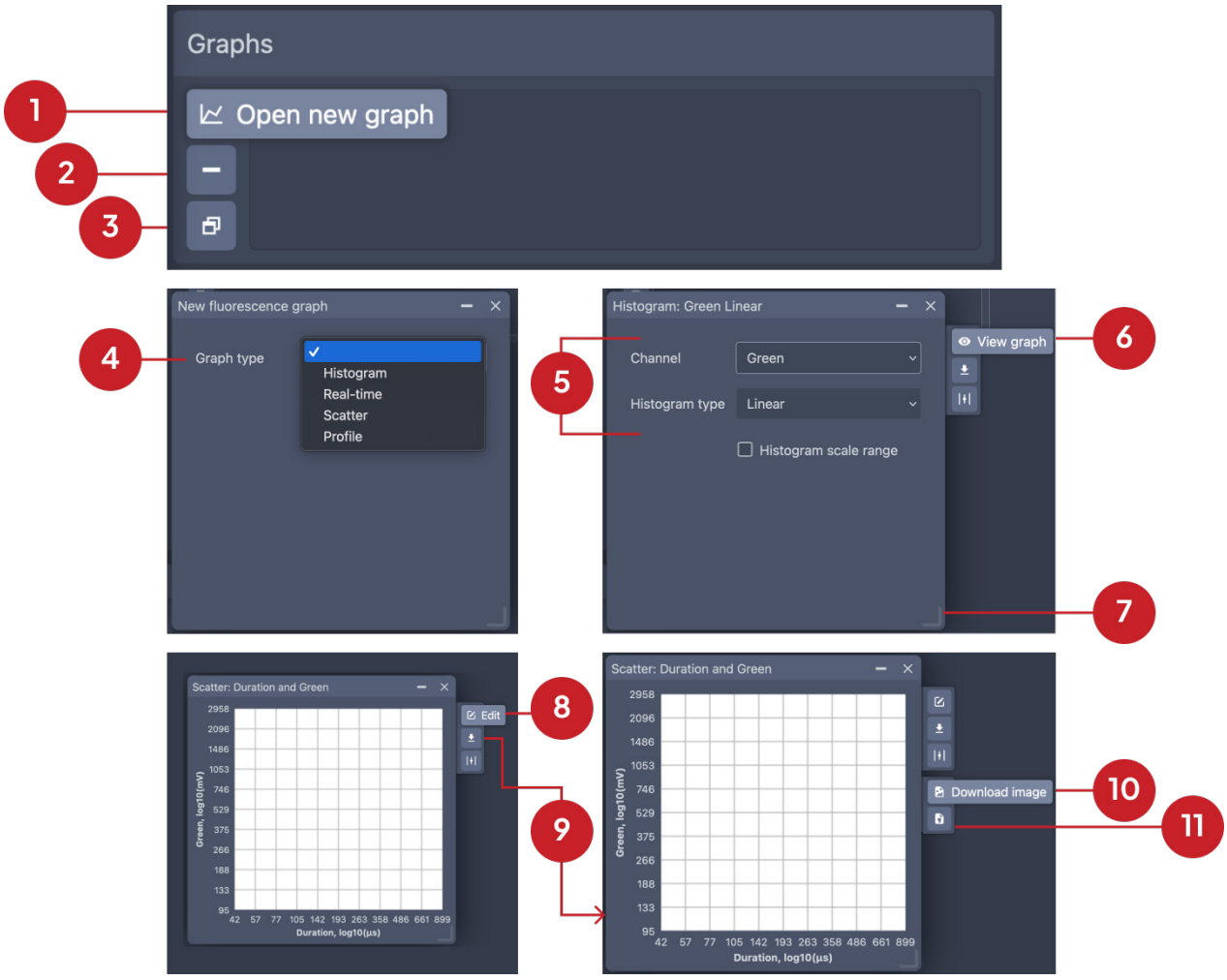
VIEW GENERAL INFORMATION & ADJUST MODULE OPERATION

1. The total number of detected pulses. The number of pulses with values over set reference threshold (6) and having minimal set duration.
2. The number of positive pulses. The number of registered triggered events.
3. Event frequency (Hz). Measured pulse rate before gating.
4. Reference mean (mV). Measured optical background level.
5. Estimated threshold (mV).
6. Set reference threshold value (mV) to filter out noise. Adjust the value by dragging the slider or clicking the pen icon and entering the value.
7. Start collecting fluorescence data. Starts populating scatter plot and histogram graphs.
8. Reset accumulated fluorescence event data. Permanently deletes all collected fluorescence event data points.
9. Download accumulated data in .fsc file format.
10. Switch to channel-specific information & gain controls.
11. Minimum. Smallest measured signal value.
12. Background. Mean measured signal value.
13. Current Gain. Current measured gain.
14. Maximum. Highest measured signal value.
15. Dark count. Estimated theoretical sensor background.
16. Set gain value (mV). Sensor gain setpoint; default value: 3500mV.



VISUALIZE FLUORESCENCE DATA

1. Create a new graph. Graphs are created in floating windows.
2. Minimize all graphs.
3. Restore all graphs.
4. In the graph window, select graph type. Available types: histogram, real-time, scatter, profile.
5. Select the parameters for display. Different parameters are available depending on graph type.
6. Click “View graph” to display the graph with selected parameters.
7. Graphs can be resized by dragging the bottom right corner.
8. Edit the graph parameters.
9. Download the graph.
10. Download the graph as image.
11. Download the graph as .csv file.



Examples of different graphs:

12. Histogram.
13. Real-time graph. All fluorescence event readings in time.
14. Scatter plot.
15. Profile graph. A snapshot of raw sensor readings in time.

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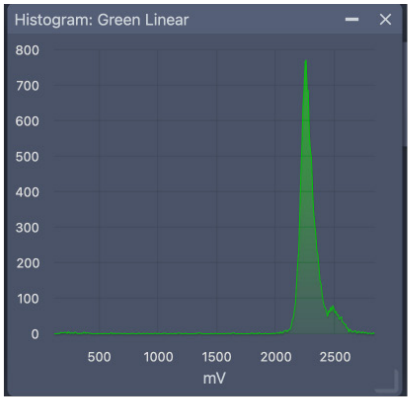

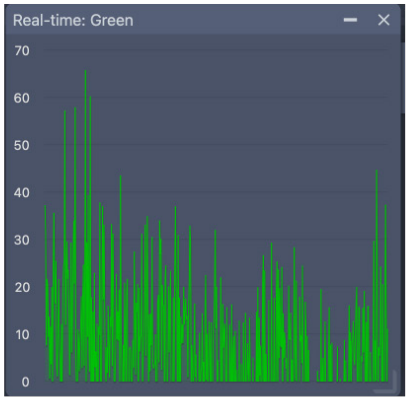

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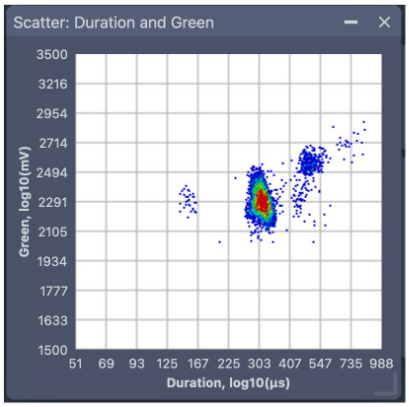

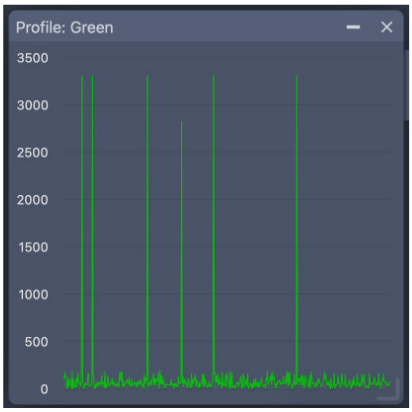

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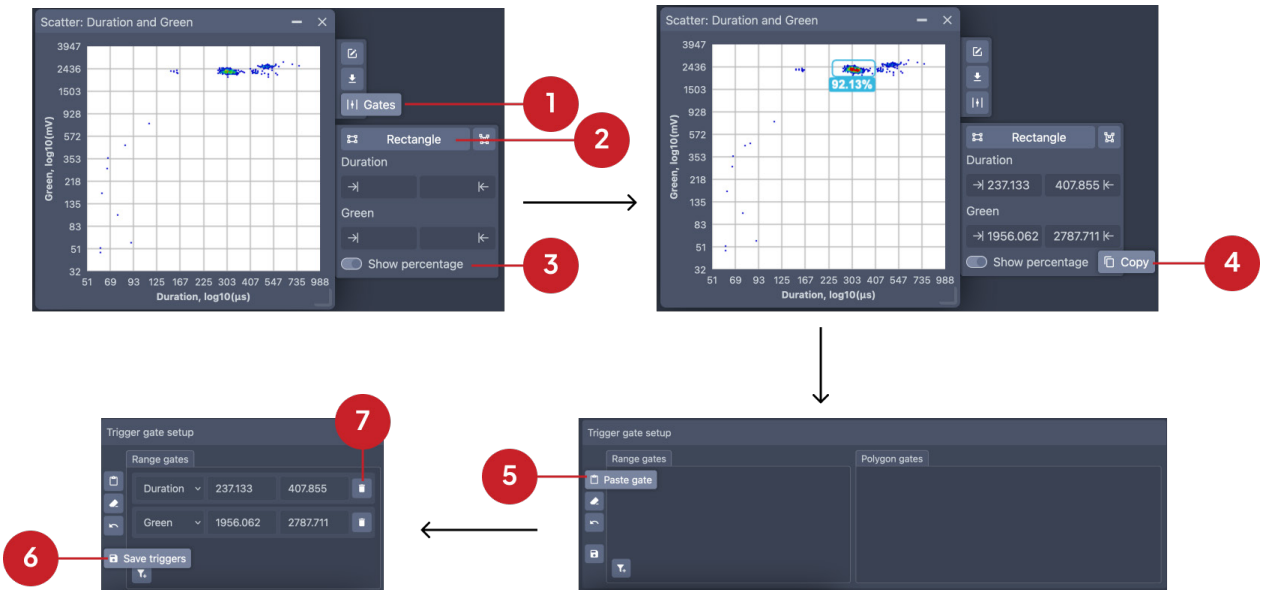
SET UP SORTING GATES (TRIGGERS)

Note: Sorting gates (triggers) can be set up in a few different ways. In addition, sorting according to multiple triggers created in different ways is possible. If multiple triggers are created, a droplet must satisfy ALL conditions to be selected as positive.

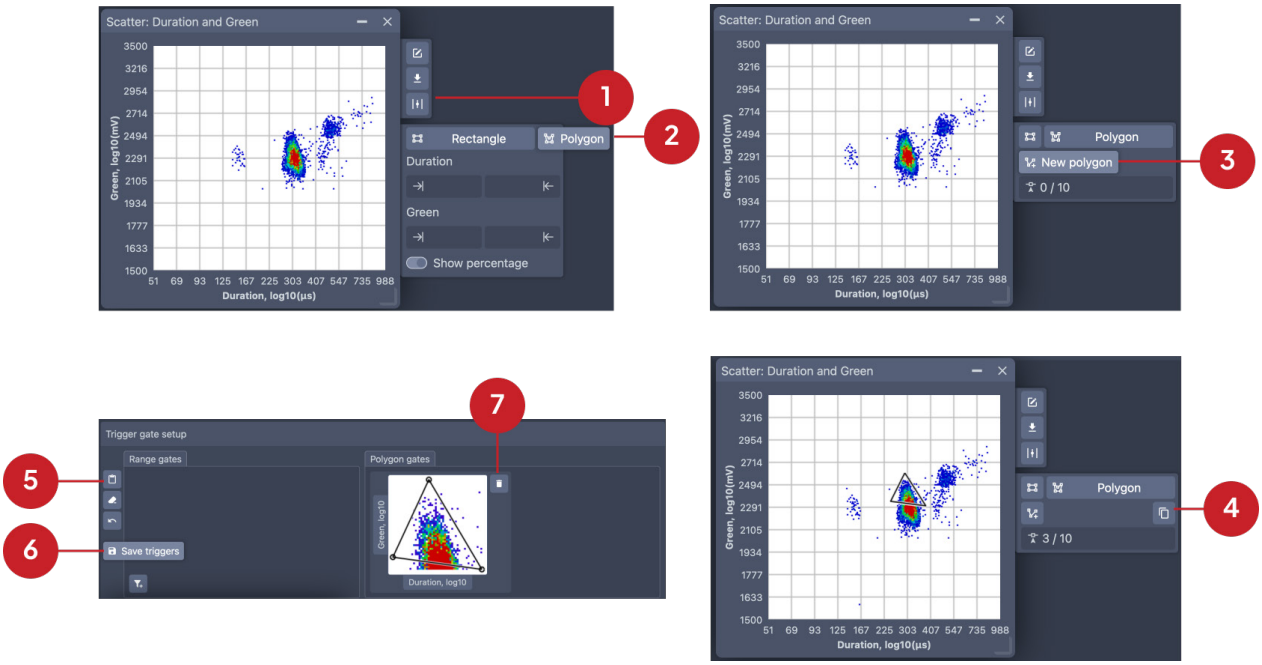
Set up rectangle-shaped sorting gates from a scatter plot

1. Click to open gating controls.
2. Create a rectangle-shaped gate. To create the gate, left-click and drag on the scatter plot.
3. Enable to show the percentage of events (data points) selected by the gate.
4. Copy the gate values.
5. Paste the gate values into the Trigger Setup control window.
6. Save triggers to activate them for droplet selection. Note: after saving the triggers, positive pulse count should start increasing.
7. Delete trigger.



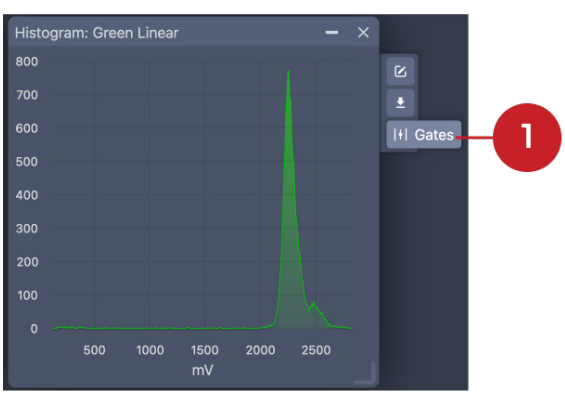
Set up rectangle-shaped sorting gates from a scatter plot

1. Click to open gating controls.
2. Click to open Polygon-shaped gating controls.
3. Click to create a new polygon gate. To create the gate, left-click on the scatter plot at each corner of the desired gate. The polygon can have up to 10 corners.
4. Copy the gate values.
5. Paste the gate values into the Trigger Setup control window.
6. Save triggers to activate them for droplet selection. Note: after saving the triggers, positive pulse count should start increasing.
7. Delete trigger.



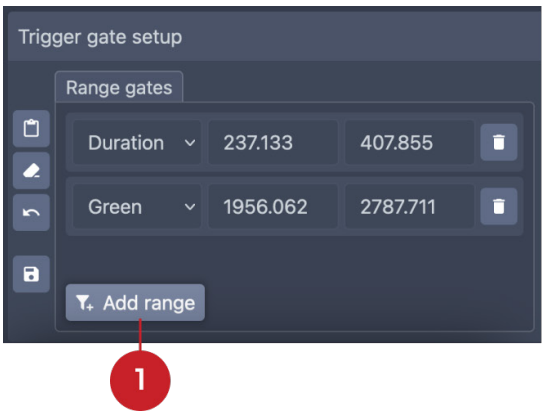
Set up a single range trigger from a histogram plot

1. Click to open gating controls.
2. Left-click and drag on the histogram to select the gating range.
3. Copy the gate values.
4. Paste the gate values into the Trigger Setup control window. (Refer to the preceding section.)
5. Save triggers to activate them for droplet selection. (Refer to the preceding section.)



Manual trigger set-up and other trigger controls

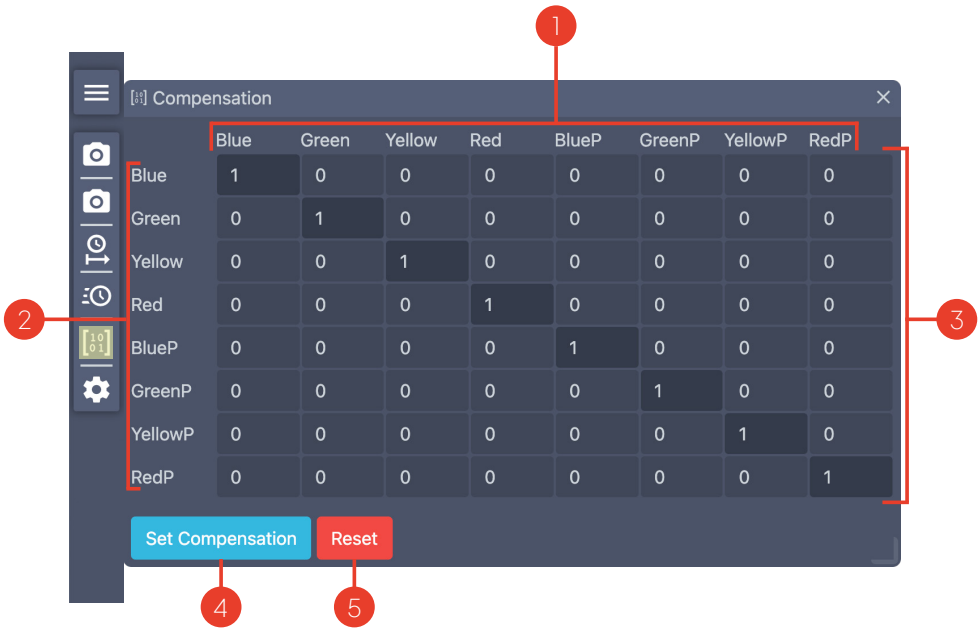
1. Add a trigger (gate) range manually.
2. Select the parameter for gating.
3. Enter the parameter values for gating.
4. Save triggers.
5. Undo changes.
6. Delete all gates.
7. Delete selected gate.



SET UP COMPENSATION

CORRECT FOR INTERCHANNEL FLUORESCENCE BLEEDING

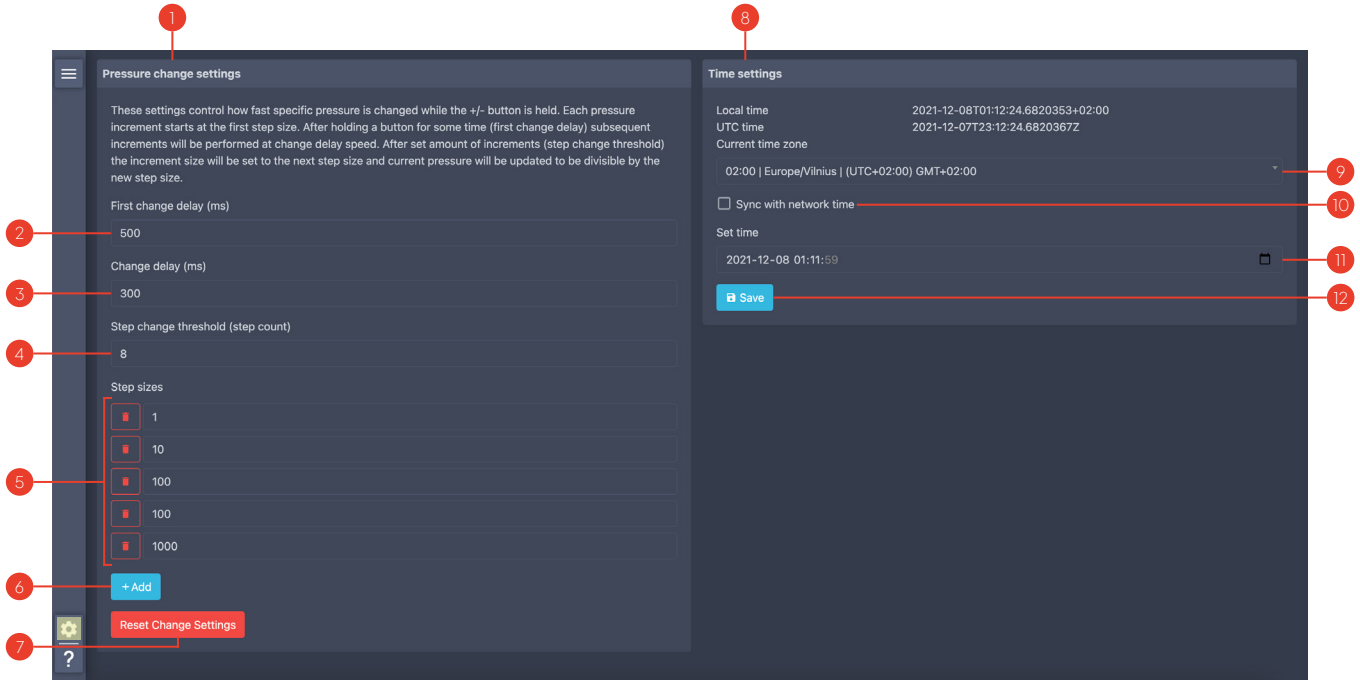
1. Channel names in columns.
2. Channel names in rows.
3. Interchannel multiplier values. *Range: 0 - 1,000; smallest step – 0.001.*
4. Enable compensation.
5. Reset to defaults.



PRESSURE STEP CHANGE & TIME SETTINGS

ADJUST PRESSURE CHANGE (+/-) BUTTON CONTROL SETTINGS & TIME

1. Pressure change settings window.
2. First change delay (ms).
3. Change delay (ms). *Adjust the delay speed that subsequent increments will be performed at.*
4. Step change threshold (step count). *Set the number of increments.*
5. Preset step sizes.
6. Add step sizes.
7. Reset to defaults.
8. Time settings window.
9. Select time zone.
10. Sync the device with network time.
    NOTE: We recommend checking this box if your STYX has internet access.
11. Set the date and time manually. *Visible only when sync with network time box is unchecked.*
12. Save all changes.



SHORTCUTS, UPDATES & FILE NAMES

MANAGE KEYBOARD SHORTCUTS

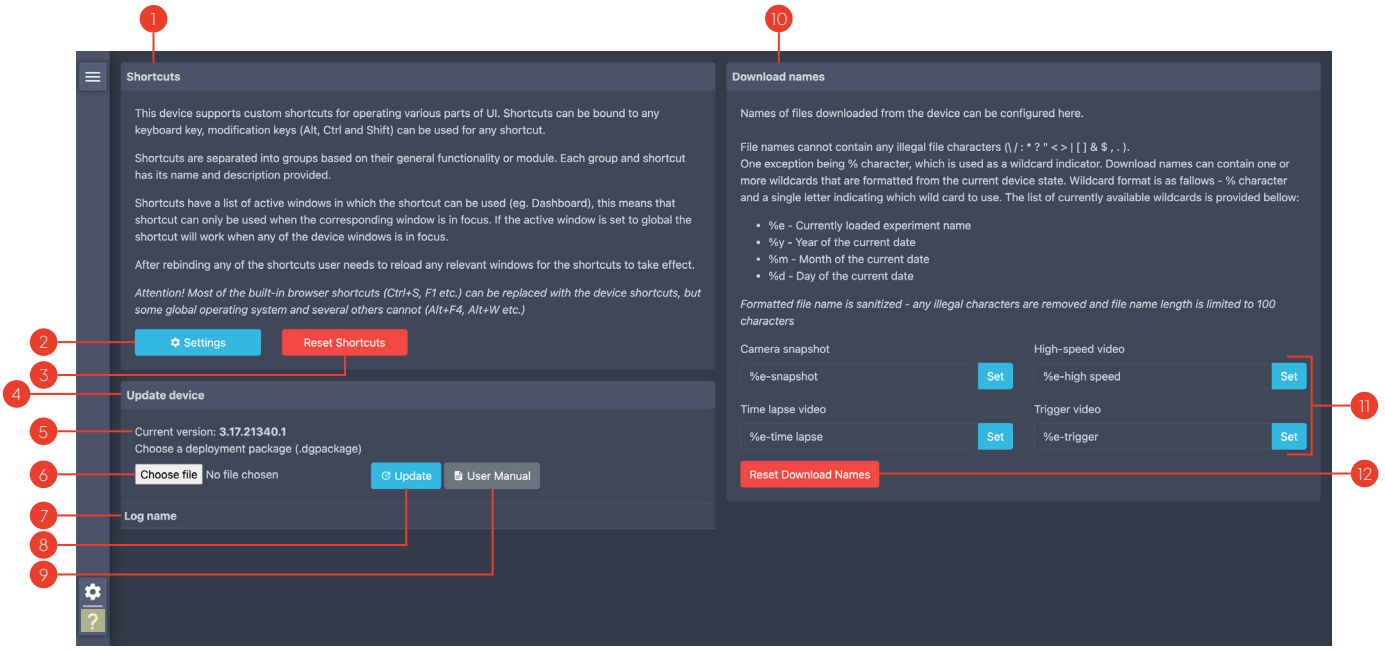
1. Keyboard shortcuts configuration window.
2. Shortcut settings. *Set device control keyboard shortcuts.*
3. Reset assigned shortcuts to defaults.

UPDATE DEVICE SOFTWARE

4. Device update window.
5. Current software version display.
6. Choose a device update file (.dgpackage).
7. Software update log section. *Information appears only after the software update procedure.*
8. Start the update procedure.
9. Download up-to-date STYX user manual.

CONFIGURE FILE NAMES

10. File names configuration window.
11. File download names. *Configure names of files downloaded from the device.*
12. Reset assigned download names to defaults.



OPERATING INSTRUCTIONS

BEFORE STARTING:

1. Read these instructions to familiarize with the workflow.
2. Remove any dust from the microfluidics instrument.
3. Inspect microfluidic chips for any potential defects
4. Make sure to have all the necessary consumables.

SETTING UP

Estimated time: 5–7 min.

1. Before first use attach the Wi-Fi antennas to the back of the instrument (refer to Instrument Components section; Fig. 2).
2. Use the provided power cable to plug the instrument into a power outlet.
   IMPORTANT: the instrument is susceptible to power surges, thus, consider using a surge protector or uninterrupted power supply (UPS).
3. Power on the instrument.
4. Turn on the computer or tablet.
5. Connect to the "STYX-serial_number" Wi-Fi access point (password: droplets).
   NOTE: the ethernet cable can also be used to access the instrument via a wired connection. Please contact Droplet Genomics for set-up instructions.
6. Find your device IP address on the LCD screen (Fig. 5).
7. Type in the displayed IP address in the browser and launch the STYX user interface.
   NOTE: We recommend using the Google Chrome browser.

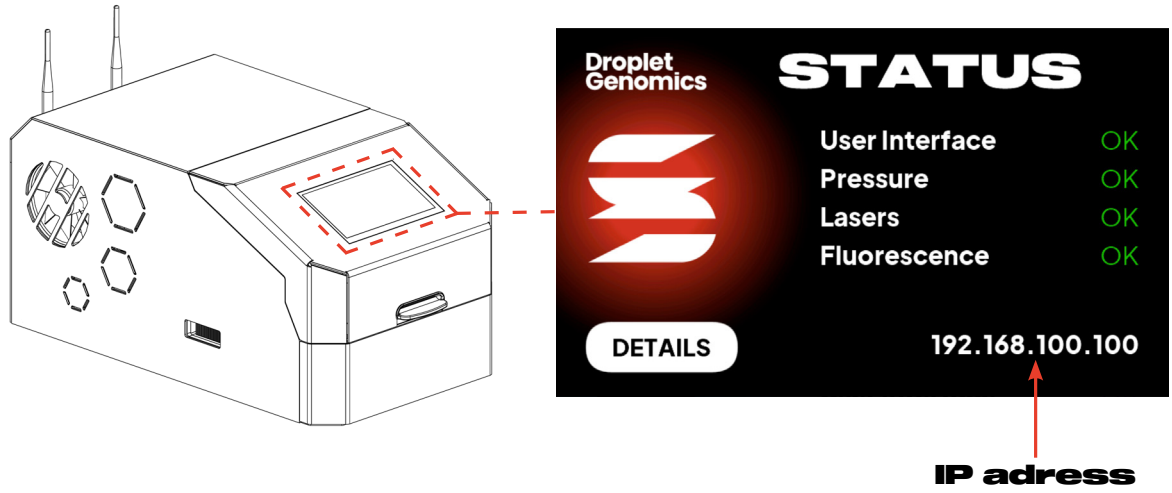


Figure 5: STYX LCD display

ATTACHING TUBES TO PRESSURE CHANNELS

Estimated time: 15 min.

1. Load your generated emulsion & spacing oil in the desired volume tubes (1.5mL, 5mL, 15mL).
   NOTE: Non-standard tubes may require additional spacers to seal properly.
2. Prepare & attach your tubes to pressure channels:

If 5mL or 15mL tubes are used (refer to Fig. 6A):

1) Add a large seal (a) at the bottom of the tube sealing plate (b).
NOTE: The seal should stay in place if mounted correctly.
2) Mount the tube (c) with loaded liquid/emulsion into the tube bracket (d).
3) Place the tube bracket (d) on the tube holding bracket (e).
4) Attach screw nut (f) and ferrule (g) on microfluidic tubing (h) as depicted in Fig. 6A.
5) Run microfluidic tubing (h) with screw nut-ferrule assembly (f,g) through the hole in the sealing plate (i) until it nearly touches the bottom of the tube (c).
IMPORTANT: Make sure the tubing does not touch the bottom of the tube.

If 1.5mL tubes are used (refer to Fig. 6B):

1) Attach a large seal (a) at the bottom of the tube sealing plate (b).
2) Attach a small seal (d) at the bottom of the small tube adapter (c).
NOTE: Seals (a, d) should stay in place if mounted correctly.
3) Place the small seal-small tube adapter assembly (c, d) on the tube sealing plate.
4) Mount the tube (e) with loaded liquid/emulsion into the small tube bracket (f).
5) Place the small tube bracket (f) with tube (e) on the tube holding bracket (g)
6) Attach screw nut (h) and ferrule (i) on microfluidic tubing (j) as depicted in the Fig. 6B.
7) Run microfluidic tubing (j) with screw nut-ferrule assembly (h,i) through the hole in the sealing plate (k) until it nearly touches the bottom of the tube (e).
IMPORTANT: Make sure the tubing does not touch the bottom of the tube.

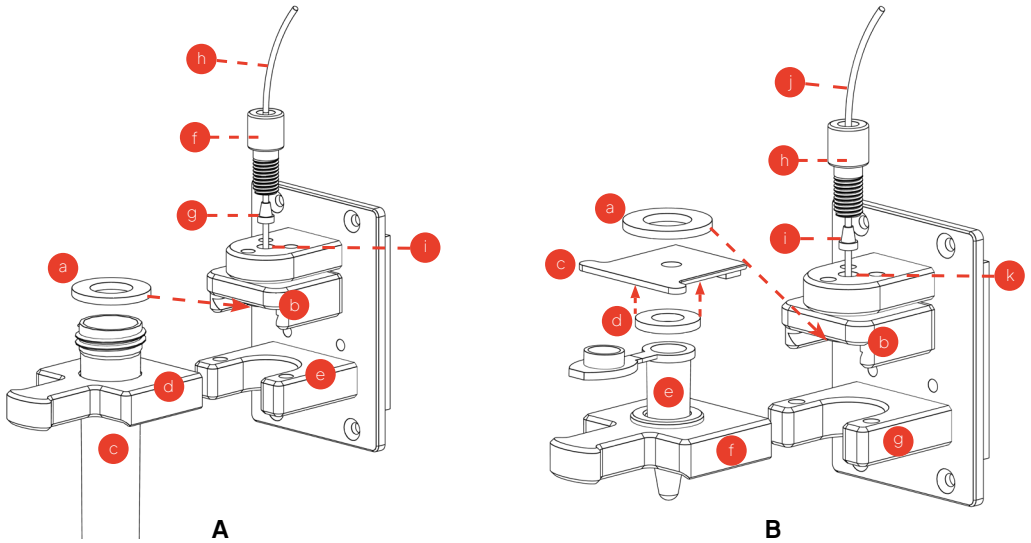


Figure 6: Pressurizable channel components; A - 5 and 15mL tubes, B - 1.5mL tube.

ADJUSTING CAMERA VIEW

Estimated time: 5min.

1. Place a DG-SRT-STX (droplet sorting) chip in the slide holder. Make sure the ITO coated tracks are facing the electrode adapter (the right side of the instrument).
2. Click on "New Experiment" in Home window and provide the required information to set up a new experiment.
3. Use the XYZ motion controls (Fig. 7) to adjust the stage position to bring the microfluidic device into camera view.
   1) Select the step size (Fig. 7a). Start with maximum step size and decrease it as you approach the desired position in microfluidic device.
   NOTE: You may observe stage backlash using small step sizes (<100).
   2) Move to a desired position in a microfluidic device (Fig. 7b).
   NOTE: If the camera is out of focus, you can use the vertical tab (Fig. 7c) to adjust position in Z axis.
4. Select illumination for left (Fig. 8a) and right (Fig. 8b) cameras to reach the desired image quality.
   NOTE: Alternatively, use auto illumination (Fig. 8c) with set auto brightness (Fig. 8d).

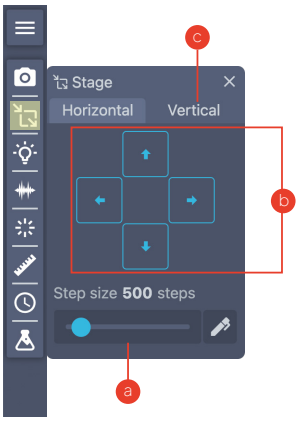


Figure 7: XYZ stage motion controls.

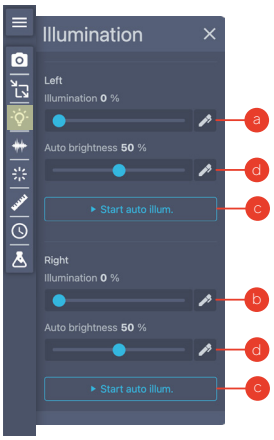


Figure 8: Illumination & camera settings.

STARTING THE EXPERIMENT

Estimated time: 5–10 min.

1. Carefully prime reinjected emulsion and droplet spacing oil channels. To speed up priming use the "Purge" function (Fig. 9b) in the Pressure controls window (Fig. 9a) and wait until liquid/emulsion reaches the end of the tubing.
   IMPORTANT: Use with caution – sample loss may occur.
2. Use your fingers or forceps to insert each tubing into the corresponding microfluidic chip ports (Fig. 10).
3. Connect a piece of microfluidic tubing to both of the outlet ports and make sure they are placed in separate collection tubes.
4. Tighten the adjustable screws on the electrode adapter and plug-in wires into electrode ports (refer to Instrument Components section; Fig. 3).
   NOTE: Polarity is not important.
5. Select the correct pressure values (Fig. 9c) and click Start (Fig. 9d) to start all active channels in the experiment.
6. Observe the reinjected droplets, their spacing and adjust pressures as necessary to achieve the desired function.

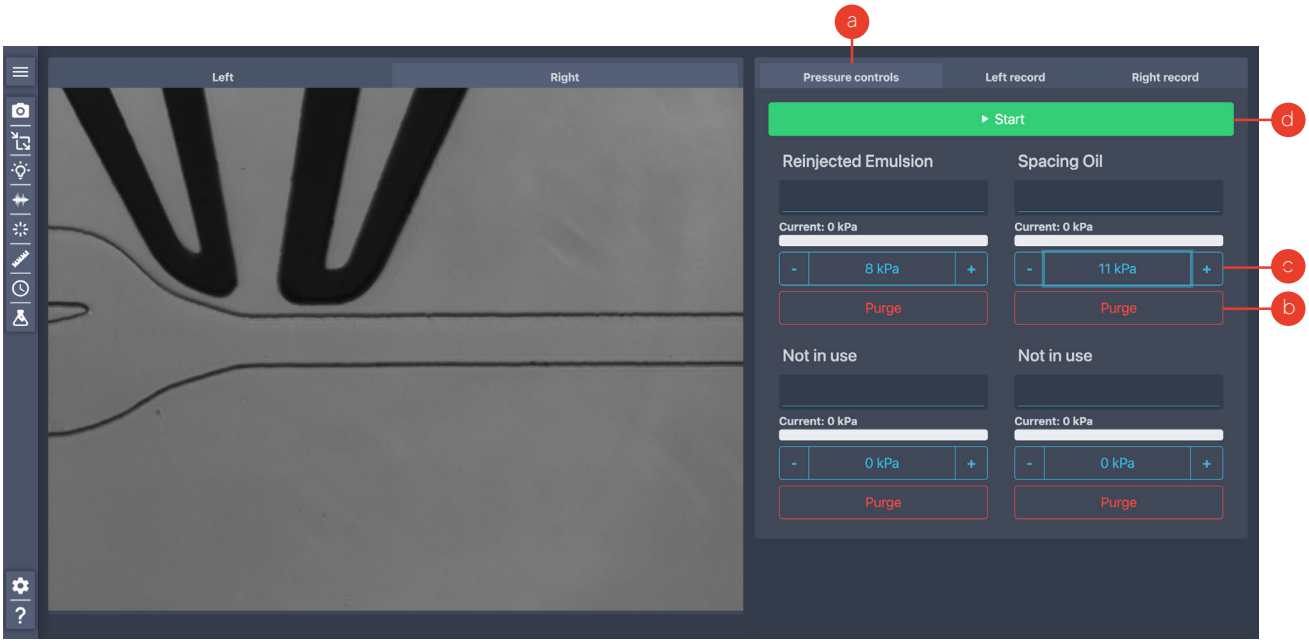


Figure 9: Instrument control dashboard.

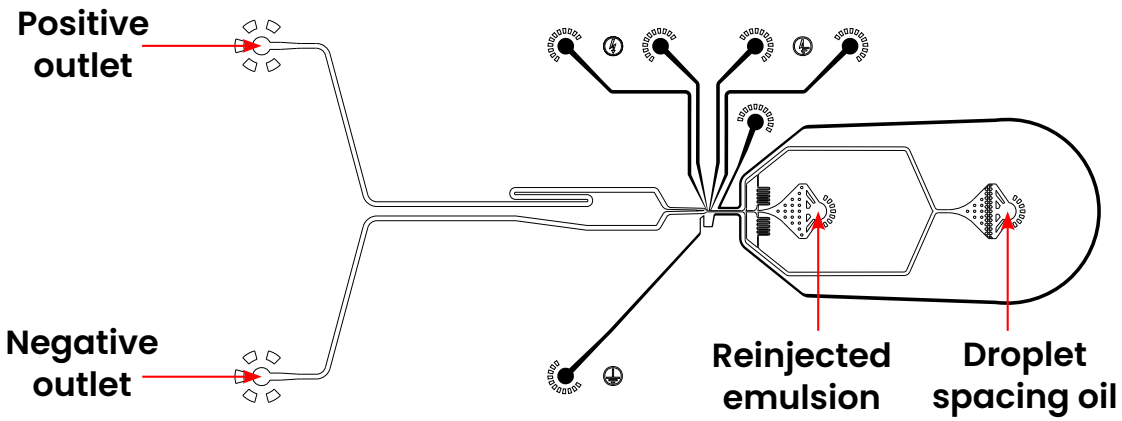


Figure 10: MCY-R1 chip design.

SETTING UP DETECTION

Estimated time: 5 min.

1. Turn on laser target (Fig. 11a) and fluorescence target (Fig. 11b) display for the right camera in Camera Settings.
2. Using XYZ stage motion controls, place the targets in the desired detection area. Generally, this area is in the middle of the microfluidic chip channel and to the right of the electrodes.
3. Select the desired laser optical power and enable laser output in Laser Controls.
   NOTE: check the actual laser current measurement to make sure laser power is consistent between experiments.

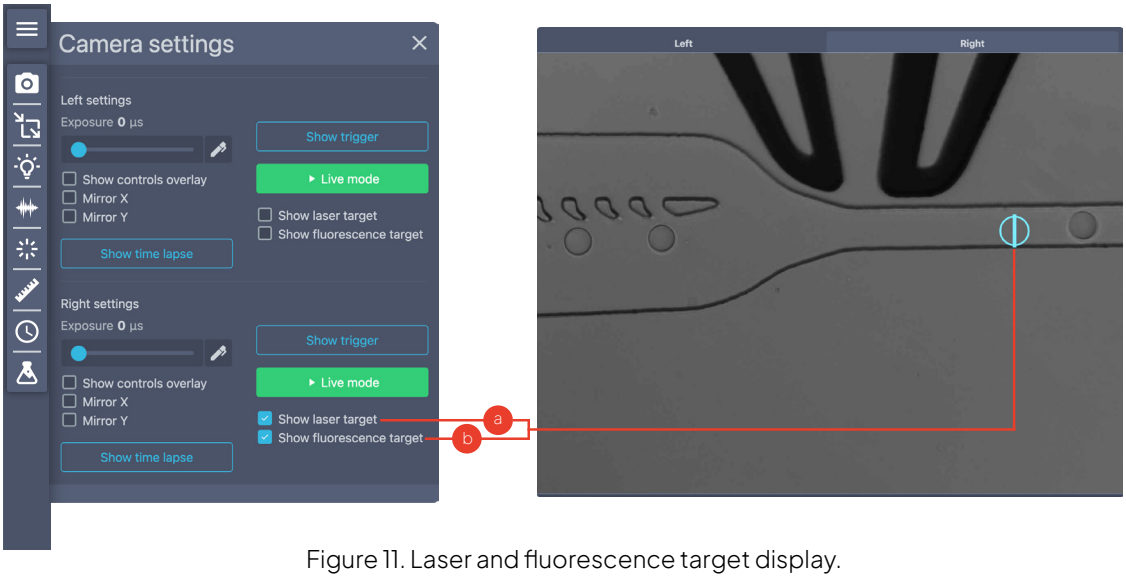


Figure 11. Laser and fluorescence target display.

SETTING DETECTION THRESHOLD

Estimated time: 5 min.

1. Open fluorescence dashboard.
   NOTE: After setting up detection in the previous section, you should see the number of pulses increasing (Fig. 13a).
2. In the graph creation window, select a Profile graph type in the desired channel of detection.
3. Based on the profile graph (Fig. 12), determine the background and set reference threshold (Fig. 13c).
   NOTE: after decreasing the pulse reference threshold, you should see the number of positive pulses increasing (Fig. 13b).
4. Reset accumulated fluorescence data (Fig. 13d).
   OPTIONAL: If your signal is saturating the detector, you can decrease channel gain (Fig. 13f) in the channel-specific information window (Fig. 13e).

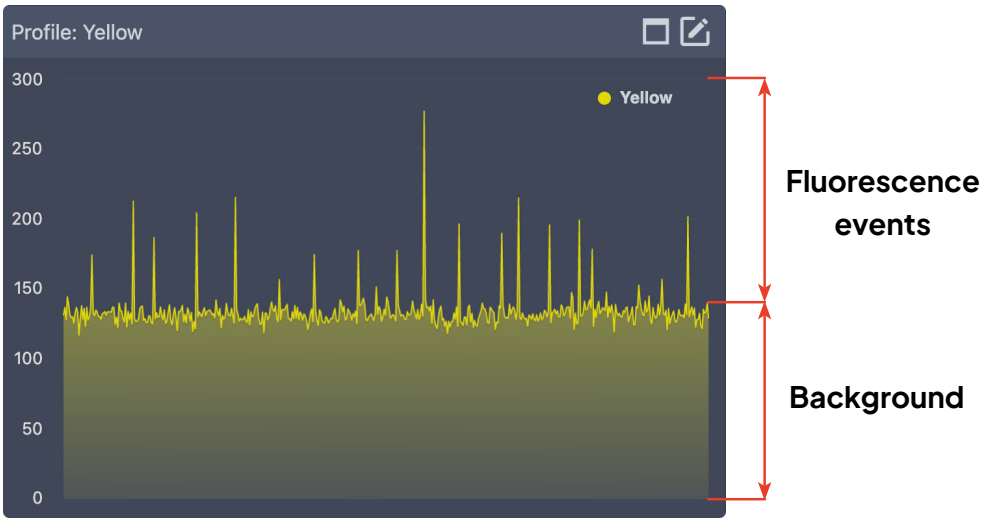


Figure 12: Profile graph.

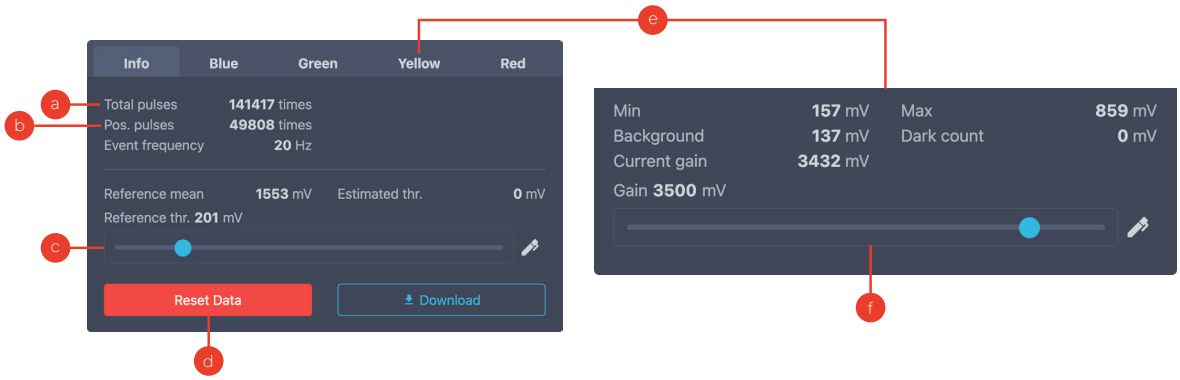


Figure 13: General & channel specific detection information.

SETTING UP TRIGGERS

Estimated time: 5 min.

Refer to Fluorescence Dashboard section on setting up triggers – See Page 22.

SETTING UP ELECTRICAL FIELD GENERATION

Estimated time: 5 min.

1. Open high-voltage module controls.
   NOTE: After correctly setting-up triggers, the pulse count (Fig. 15a) should be increasing.
2. Select pulse duration (Fig. 15b) and amplitude (Fig. 15c). Exact values will depend on your setup.
3. Enable high-voltage electrical field generation (Fig. 15d).
   NOTE: After enabling the HV module, current voltage reading (Fig. 15e) should update.

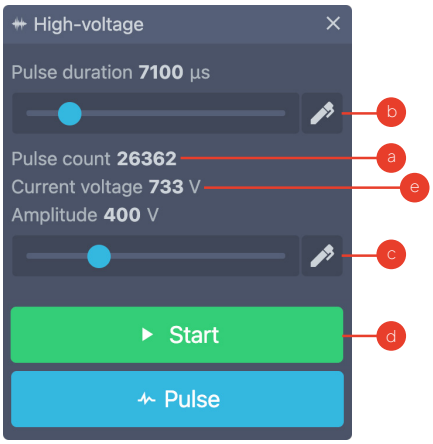


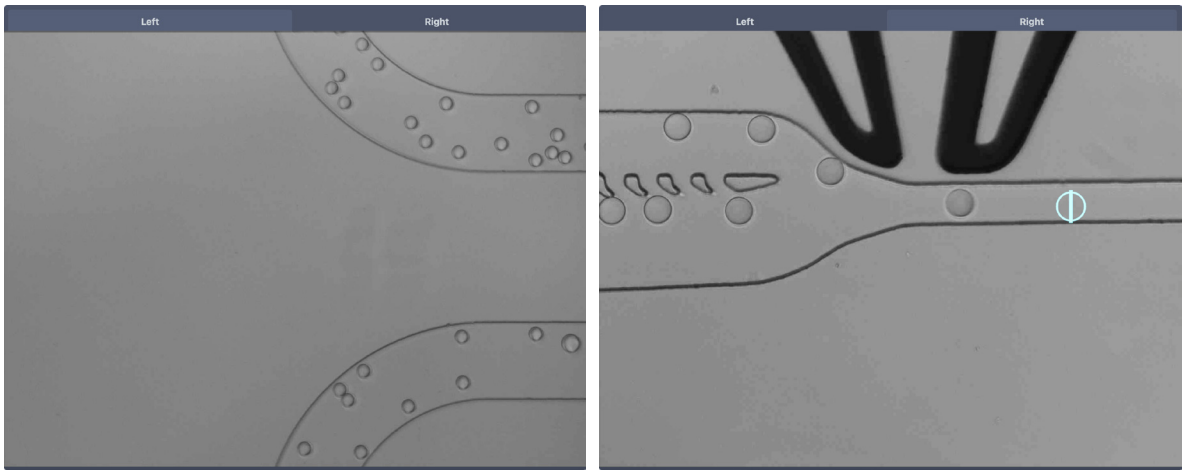
Figure 15: High-voltage module controls.

VISUALISING DROPLET SORTING

Estimated time: 2 min.

After completing all the previous steps, the reinjected droplets should be sorted based on the selected triggers.

1. Use Left camera view (Fig. 16A) to visualise positive and negative outlets.
2. Use Right camera view (Fig. 16B) to visualise the sorting junction.
3. Alternatively, use trigger mode to make a synchronised video clip (refer to section "MAKE A SYNCHRONISED VIDEO CLIP").



A **B**

Figure 16: Left (A) and right (B) camera views during droplet sorting.

TECHNICAL SPECIFICATIONS

STYX SYSTEM TECHNICAL SPECIFICATIONS

PRESSURE PUMPS

Type: piezo-electric
Independent channels: 4
Pressure range: 0.5–25kPa
Pressure change response time: <2.5s
Sample reservoir: compatible with standard 1.5mL, 5mL and 15mL laboratory tubes

INTEGRATED MICROSCOPY

Independent viewing areas: 2
Optical magnification: 0.513µm/px (viewing area 1), 1.04µm/px (viewing area 2)
Illumination source: 0 – 0.25W LED 760nm, monochromatic
Illumination type: infrared
Microfluidic chip dimensions: 25 x 75mm
Stage: motorized 3 direction stage (XYZ)

FLUORESCENCE EXCITATION AND DETECTION

Lasers: up to 4 separate wavelengths 488nm (base model), 405nm, 561nm, 633nm
Laser power: 0–40mW (depending on laser model)
Detection channels: 4 independent channels centred at 450nm, 520nm, 600nm and 670nm
Detection sensitivity: 100µs – 100ms duration light pulses, with lower limit for photon flux at 1 photon/µs at 25°C
Measurement throughput: >1000 samples/s
Trigger latency: <100µs with jitter <10µs
Trigger synhronization: HV pulses, camera

HIGH-SPEED CAMERAS

Number of independent cameras: 2
Resolution: 1440 x 1080
Exposure time: 25µs - 1.5ms
Recording framerate: 10 - 3500 frames/s (region size dependent)
Maximum recording buffer size: 500 frames
Video playback rate: 1 – 30 frames/s

HIGH-VOLTAGE PULSE GENERATOR

Pulse amplitude range: 100 - 1000Vpp
Duty cycle: up to 50%
Frequency tuning: automatic
Pulse duration: 500µs - 50ms
Pulse synchronization delay: 100µs - 10ms with 10µs jitter

ARTIFICIAL INTELLIGENCE SYSTEM

GPU: 512-core NVIDIA Volta, 32 TOPs
CPU: 8-core NVIDIA Carmel Arm®v8.2 64-bit CPU 8MB L2 + 4MB L3
Memory: 32 GB 256-bit LPDDR4x 136.5GB/s

CONNECTIVITY

Wired connectivity: 1x Ethernet, 1x USB
Wireless connectivity: 2.4GHz Wi-Fi hotspot with DHCP server
Device control software: integrated into the on-board computer

ADDITIONAL INFORMATION

SAMPLE REQUIREMENTS

Sample type: liquids & stable suspensions
Sample volume: 0.02–15mL

OPERATING CONDITIONS & PHYSICAL INSTRUMENT DATA

Operating temperature: +4°C to 30°C (non-condensing)
Dimentions (L x W x H): 580×330×290mm
Weight: 19.7kg

POWER SUPPLY ELECTRICAL DATA

Input voltage range: 110 ~ 240VAC (Withstands 300VAC surge for 5sec. without damage)
Maximum current rating: 2A @ 115VAC, 1.2A @ 230VAC
Frequency: 47 ~ 63Hz
Maximum power rating: 246W
Electric fuses: 2A, slow blow
Applicable safety standards: UL60950–1, TUV EN60950–1

APPLICATION PROGRAMMING INTERFACE

The instrument can be controlled via an application programming interface (API), which is implemented over the HTTP protocols. Using the instrument‘s IP address, any programming language capable of making HTTP requests can be used to control the instrument.

The commands outlined here contain only the basic instrument control functionality. Additional functionality commands can be provided free of charge upon request from Droplet Genomics. All control endpoints are available over the scheme:

http://(IP address)/api/(Controller)/(Action)?(parameters)

Commands that modify device state are called as a POST HTTP method and commands that only read information are called as GET HTTP method. All results are returned as JSON text. When no return information is available, for example when processing POST methods, an HTTP Response code of 200 will show that the request succeeded)].

Parameters can be formatted as both query strings in the URL or passed in the request body. It is recommended (but not required) to use the query string parameters for GET requests and the request body parameters for POST requests.

The listed commands are applicable to software version 3.17. In case of software updates contact Droplet Genomics for the most up to date list.

TABLE 1: VARIOUS CONTROLS

ACTION	METHOD	PARAMETERS	DESCRIPTION
Illumination	GET	id –0 or 1	Gets the current state for illumination control.
Illumination	POST	id – 0 or 1 illumination – an integer value for the percentage of illumination power (0–100)	Sets the illumination power percentage. Note – if auto illumination is turned on this value might change.
IlluminationTarget	POST	id – 0 or 1 target – an integer value for the percentage of camera view brightness	The device will automatically adjust illumination to meet the desired image brightness set by the target.
StartAutoIllumination	POST	id – 0 or 1	Starts the auto illumination process.
StopAutoIllumination	POST	id – 0 or 1	Stops the auto illumination process
Exposure (Camera)	POST	cameraId – 0 or 1 exposure – an integer value for camera exposure duration (µs) 25–1500	Sets the exposure of the camera.

TABLE 2: EXPERIMENT CONTROLS

ACTION	METHOD	PARAMETERS	DESCRIPTION
Current	GET	n/a	Returns currently loaded experiment or null if none is loaded currently.
GetAll	GET	n/a	Returns a list of all available experiments.
Choose	POST	id (GUID/UUID) – an existing experiment identification as plain text, for example: 0980cf2b-50e3-480a-b40b-fa04f2066314	Chooses one of the existing experiments and loads it for future operations.
Experiment	GET	id (GUID/UUID) – an existing experiment identification as plain text, for example: 0980cf2b-50e3-480a-b40b-fa04f2066314	Returns detailed information about a specific experiment.
Ids	GET	n/a	Returns a list of all available experiment identifications
Start	POST	n/a	Starts the experiment by starting all the pumps which have set pressure above 0.
Stop	POST	n/a	Stops the experiment by stopping all the pumps that were running.

ADDITIONAL INFORMATION

NOTES ON DEVICE SAFETY

General

The Styx platform is intended solely for the analysis and manipulation of fluorescent droplets. It is designed to be used in laboratory settings for research purposes. Any unauthorized or unintended use of the Styx platform, including but not limited to its use for diagnostic or therapeutic applications, is strictly prohibited. The user acknowledges and agrees that they are responsible for ensuring that their use of the Styx platform complies with all applicable laws and regulations.

The system utilizes lasers whose beams are shaped into a focused line and directed onto the microfluidic chip channel, where they interact with the flowing droplets. Laser line ensures complete coverage of the microfluidic channel where the droplets are flowing and the line shape provides the necessary spatial resolution to distinguish signals from discrete components within the droplet content, such as cells, beads, or particles.

The manufacturer will not assume liability for any malfunction or damage caused by anything other than the intended use of the Styx platform or individual modules or parts of it, nor by any repair or other service operation performed or attempted by persons other than duly authorized service staff. Any such action will invalidate any claim under warranty, including parts not directly affected by such action.

Styx instrument belongs to laser hazard class 1. Class 1 laser instruments are designed to be inherently safe for users under normal operating conditions. To achieve this, several protective measures are implemented. The laser beam is completely enclosed within the instrument's housing. This prevents accidental exposure to the laser radiation. Styx instrument incorporates interlock system that prevent the laser from operating if the enclosure is opened. Instrument is clearly labeled with a warning symbol and information about the laser class to inform users and servicing personnel of the potential hazard.

Regulations

Styx instrument was designed, build and tested in conformity with the following regulations and guidelines:

- DIN EN 61010–1 (IEC 61010–1) "Safety requirements for electrical equipment for measurement, control and laboratory use",
- DIN EN 60825–1 (IEC publication 60825–1) "Safety of laser equipment",
- 21 CFR §1040.10: "Performance Standards for light emitting products – laser products",
- DIN EN 61326: "Electrical equipment for control technology and laboratory use – EMC requirements",
- Low voltage directive: 2006/95/EG,
- EMC directive: 2004/108/EG,
- RoHS.

Atrandi company operates under an ISO 14001–certified environmental management system. The Product was developed, tested and produced in accordance with the valid regulations and guidelines for environmental law of the European Union. The product and

its accessories have been classified as instrument category 9 (laboratory equipment or comparable standard). The product and its accessories agree with the EU-regulations 2002/95/EG (RoHS) and 2002/96/EG (WEEE), if applicable for the product. For details on the disposal and recycling please refer to your relevant Atrandi sales or service organization. The product must not be disposed in the household waste or through the municipal disposal organizations. In case of resale the seller is obliged to inform the buyer that the product must be disposed according to the said regulations.

Electrical safety
The manufacturer of the unit cannot be held liable for damage resulting from operating errors, negligence or unauthorized tampering with the device system, particularly as the result of removal or replacement of parts of the unit or as the result of the use of unsuitable accessories. Any such action will render all warranty claims null and void and in case of tampering with the optical system – laser safety is no longer warranted.

Styx instrument should only be disassembled by a trained technician. There are no user-serviceable parts inside.

The instrument allows for user-replaceable fuses. Refer to the Fig. 1 which shows a power entry module which can be located on the back panel of the instrument (Fig. 3, on the bottom left).

Fuse replacement procedure:

1. **Disconnect Power:** Ensure the instrument is unplugged from the mains power source and the instrument.
2. **Remove Fuse Drawer:** Use a small, flat-head screwdriver to gently pry out the fuse drawer.
3. **Inspect Fuses:** Examine the fuses within the drawer. If any are visibly damaged or blown, or fails electrical continuity test with a multimeter, replace them. Use a replacement ceramic time lag 5×20 mm 250V 3.15A fuses which are included in the instrument accessory box.
4. **Reinsert Drawer:** Carefully slide the fuse drawer back into the power entry module, ensuring it is fully inserted and flush with the panel.
5. **Reconnect Power:** Once the drawer is securely in place, plug the mains power cord back into the instrument.

Fig. 1. Fused power entry module with fuse drawer removed.

Power requirements
Styx instrument come with a mains power supply cord and plug, which dependent on the region can be with BS 1363 (Type G) (UK), CEE 7/7 (EU) or NEMA 5–15 grounded (Type B) (US) plug. The mains socket outlet must be equipped with a fuse having minimum tripping characteristic C according to IEC/EN 60898. Continuity of the grounding connection must not be affected by the use of extension leads. Main instrument power requirements are listed in the 1 table.

Table 1. Instrument mains power supply information.

Line voltage	1/N/Ground 240/120 V AC (±10 %)
Line frequency	50…60 Hz
Max. current	1.25A
Max power consumption	150W
Class of protection	I
Type of protection	IP 20
Overvoltage category	II
Pollution degree	2

Environmental Requirements
Do not set up the unit in the proximity of heat sources such as radiators or direct sunlight. To avoid heat build-ups, the ventilation slots on the instrument system must not be covered up. The system must not be set up in areas with potential danger by explosives. Main environmental requirements are listed in the Table 2.

Table 2. The list of main environmental requirements

1. Operation with specified performance	T=22 °C ± 3 °C
2. Operation with reduced electronics and laser lifetime	T = 15 °C to 35 °C, any conditions different from item 1. and 4.
3. Storage	T = -20 °C to 55 °C
4. Temperature gradient	± 1 °C/h
5. Warm up time, including lasers	15 min
6. Relative humidity	< 65 % at 30 °C
7. Operation altitude	max. 2000 m

Location of safety, warning and information labels
The warning and information labels attached on the Styx instrument must be observed. All labels should be affixed to the instrument and should not be removed. Location of labels concerning laser safety and information are indicated in Fig. 2 and 3. Fig. 4 shows illuminator's heatsink label.

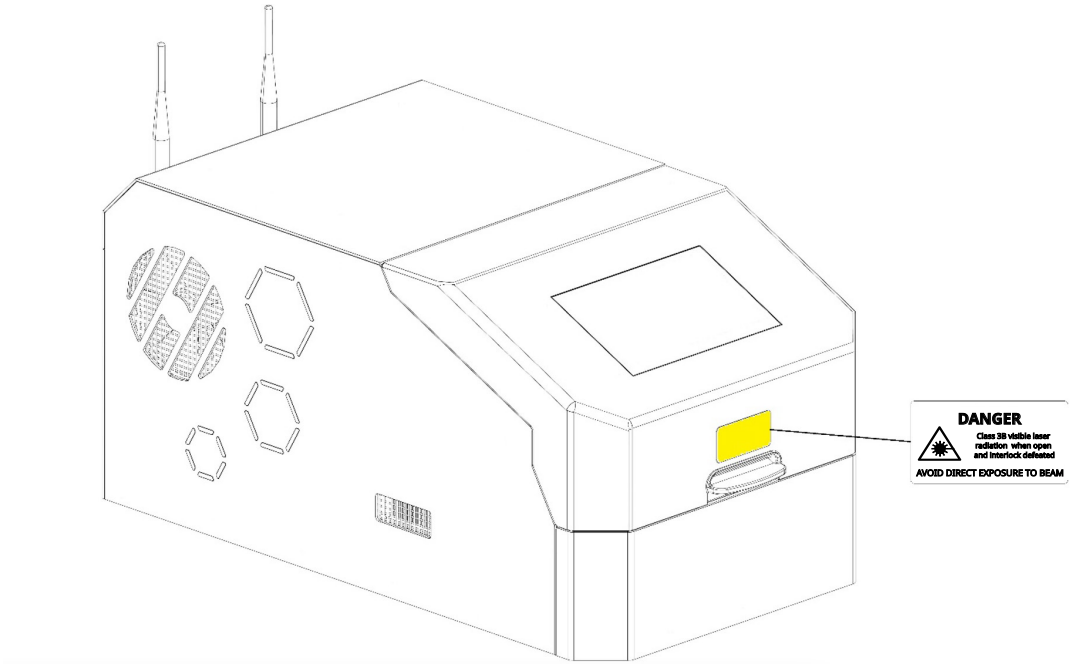


Fig. 2. Laser warning label on the front of the Styx instrument.

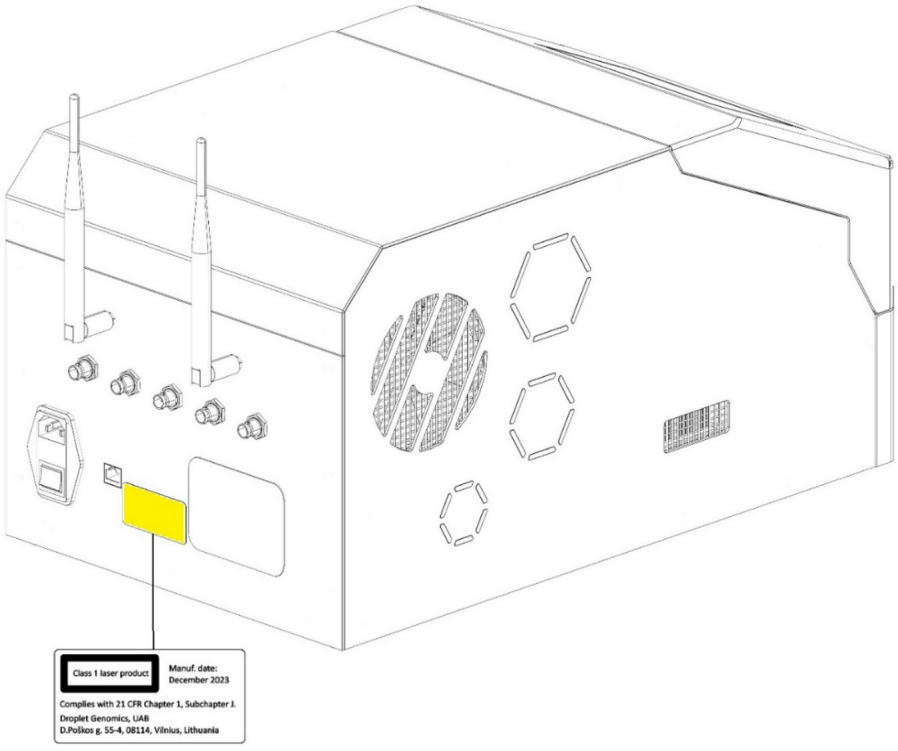


Fig. 3. Laser system information and registration label on the back of the Styx instrument.

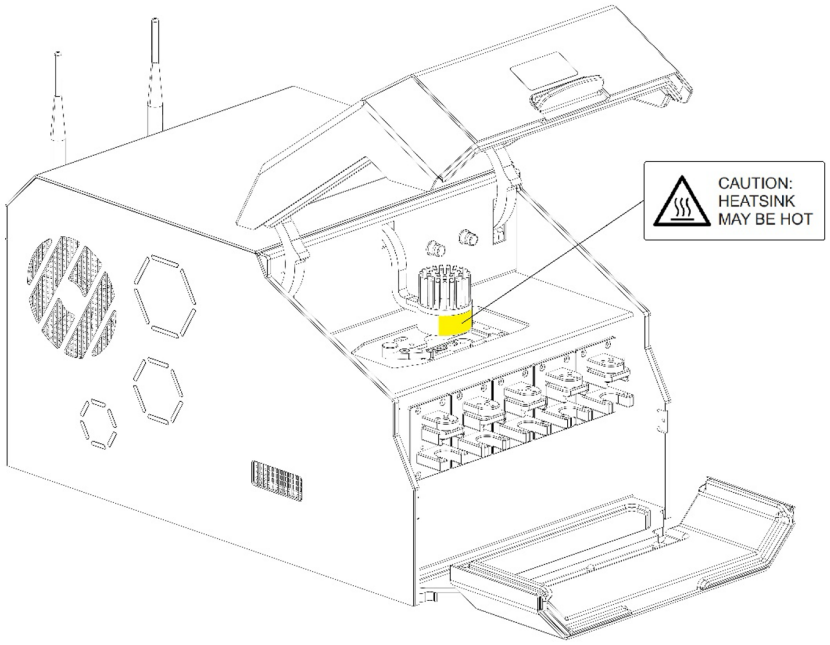


Fig. 4. Illuminators heatsink label.

Notes on handling the laser components and illumination systems
Styx instrument is a laser hazard class 1 instrument. User is not exposed to a laser radiation during normal use. The instrument lid is equipped with an interlock system that automatically disables laser power and droplet manipulation voltage pulses when opened, ensuring user safety and preventing accidental exposure or electrical shock. The instrument's **laser safety interlock is designed to be bypassed only during servicing procedures** and necessitate partial disassembly of the instrument.

Instrument is intended to incorporate laser types listed in Table 3, the combination of these lasers depends on the customer requirement.

During maintenance or repair carried out by the service personnel the customer is requested to stand aside and wear a pair of laser safety goggles (not provided by the service personnel).

Table 3. The list of main environmental requirements

LASER	CLASS	NOMINAL POWER
Integrated optics Matchbox 405 nm	3B	120 mW, electronically limited to max 20 mW
Integrated optics Matchbox 488 nm	3B	40 mW, electronically limited to max 20 mW
Integrated optics Matchbox 638 nm	3B	130 mW, electronically limited to max 20 mW
Coherent OBIS CORE LS 561 nm	3B	20 or 50 mW version, electronically limited to max 20 mW

DECONTAMINATION METHODS

ULTRAVIOLET (UV) IRRADIATION ✓

Compatible.

NOTE: prolonged exposure to a very intensive UV light might degrade plastic parts.

ISOPROPANOL/ETHANOL SPRAY ✓

Compatible.

NOTE: avoid getting liquid inside the instrument (use as little spray as possible).

OZONE STERILIZATION ✓

Compatible.

CHEMICAL DISINFECTANTS ✗

Incompatible.

Chemical disinfectants like hydrogen peroxide, iodophor, bleach and other compounds might oxidise metal parts or leave residue on optics.

GAMMA RADIATION ✗

Incompatible.

Gamma radation might damage electronics.

AUTOCLAVING ✗

Incompatible.

Autoclaving will damage electronics and deform plastic parts.

DRY HEATING ✗

Incompatible.

Dry heating will damage electronics and deform plastic parts.

ETHYLENE OXIDE ✗

Incompatible.

Ethylene oxide might damage electronics.

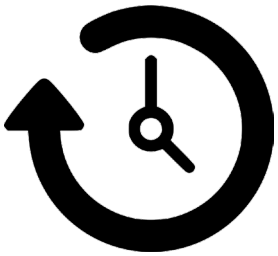
MAINTENANCE

MAINTAIN THE CLEANLINESS OF THE SYSTEM

1. Wipe the surface of the instrument with a lint-free tissue. Use 2–propanol for cleaning if needed.

2. Wipe the protective glass with lint-free tissue.

3. Clean the screw nut bracket and tube sealing plate using cotton swab. Use 2–propanol if needed.



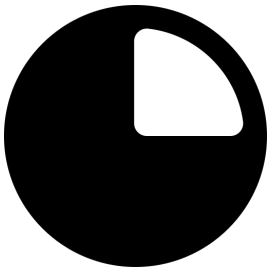
Before/after every use

CHECK FOR WORN PARTS

1. Check for torn or imprinted rubber gaskets. Replace if needed.

2. Check for damaged metal tube adapters. Replace if needed.

NOTE: replace the parts earlier if constant pressure in individual channels cannot be maintained.

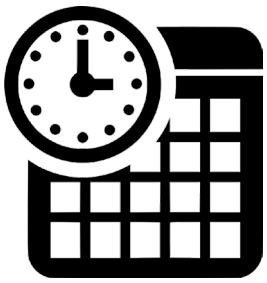


Every quarter

SERVICE THE INSTRUMENT

Contact DG representative

Service includes:
Calibration of pressure pumps
Alignment of optical system
Cleaning of internal components
Calibration of lasers and detectors
Calibration of high-voltage pulse generator
Software & firmware updates



Once a year

TROUBLESHOOTING

A LIST OF FAILURE MODES LISTED BY FREQUENCY OF OCCURENCE

ERROR INDICATION	POSSIBLE CAUSES	SOLUTIONS
NO SIGNS OF INSTRUMENT OPERATION	No power.	Check if there is AC power in your power outlet. Check fuses in the back panel located near the AC connector (refer to STYX instrument components section). If fuses are intact – contact Droplet Genomics for servicing.
PRESSURE IS NOT REACHING THE SETPOINT	Bad sealing.	Check that the tubes are inserted correctly, and the screw nut is tightened sufficiently.
LIQUID IS NOT FLOWING IN THE TUBING AFTER STARTING CHANNELS	Bad sealing.	Check measured pressure values. If pressure is not reaching the setpoint, refer to the previous error indication.
	Tubing touches the bottom of the tube.	Loosen the screw nut and lift the tubing upwards so that a gap can be seen between the bottom of the tube and the tubing.
REINJECTED DROPLETS ARE DETECTED, BUT NOT SORTED	High-voltage (HV) module is not enabled.	Check that the HV module is enabled and current voltage display value is changing as new events are detected.
	Electrode holder is not properly connected to the electrodes on the chip.	Verify that adjustable screws are tightened sufficiently on the electrode holder and it is properly connected. A proper connection can be tested with a multimeter by measuring the resistance between electrode pins and electrode ports.
	HV module is not receiving pulses from the fluorometer.	Check that the high-voltage module pulse count is increasing. If not, refer to the next error indication.

ERROR INDICATION	POSSIBLE CAUSES	SOLUTIONS
REINJECTED DROPLETS ARE NOT BEING DETECTED	Fluorescence thresholds are incorrect or not saved.	Check that the fluorometer pulse count is increasing. If not, verify that the thresholds are set up correctly and the "Save Triggers" button is pressed.
	Fluorescence signal is too weak.	Use different fluorescent dye/fluorophore or redesign your fluorescence assay.
	Analyzed particles are too small.	If your particles are smaller than 1 µm they may not be reliably detected.
XYZ STAGE OR ILLUMINATION IS NOT RESPONDING	Internal electronics failure.	Refresh the dashboard page. If that does not help, contact Droplet Genomics for servicing.
VIDEO IS LAGGING	Wi-Fi Interference.	Connect the instrument to a router via a wired connection. If router supports, use 5GHz Wi-Fi to avoid crowded 2.4GHz wireless bands. Alternatively, connect a computer to this router via a wired connection.
ARTIFACTS ON THE VIDEO, E.G. SPOTS, DUST, CRACKS	Microscope window became dirty or scratched.	Clean the camera window with a microfiber cloth soaked in rubbing alcohol. If the microscope window is badly scratched or broken, contact Droplet Genomics for a replacement.
	CCD matrix degradation.	Contact Droplet Genomics for instrument servicing.

DECLARATION OF CONFORMITY

Droplet Genomics, UAB declares that the system:

Droplet microfluidic system STYX, consisting of four pressure channels, integrated high-speed microscope with dual cameras, lasers, four-channel fluorescence detectors, high-voltage pulse generator and an integrated computing system with embedded instrument control software, to which this declaration relates is in conformity with the following regulations.

EMC Directive Standards:
EN 61326l1:2006 – electrical equipment for laboratory use
IEC 61000312 (emission)
IEC 61000l313 (emission)

IEC 61000l412 Electrostatic discharge immunity
IEC 61000l413 Radiated RF Electromagnetic Field Immunity
IEC 61000l414 Electrical Fast Transient Immunity

FCC EMC Compliance:
Emissions comply with the Class B Limits of FCC Code of Federal Regulations 47, Par 15, Subpart B.

EC declaration of conformity, Low Voltage:
Low Voltage Directive 2006/95/EC (Replaces 73/23/EEC, amended by 93/68/EEC)
EN 61010l1:2001 Safety Requirements for Electrical Equipment for Laboratory use. Part 1: General requirements.

RoHS:
All components and manufacturing procedures are compliant with RoHS standards.

A copy of the technical file is available upon request from the company.

VERSION	UPDATE DATE
V2.2	2024 – November 15