FLUIDIGM®

400311 Rev 07

Hyperion and Hyperion+ Imaging Systems

User Guide

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About This Guide

IMPORTANT Before using the system, read and understand the safety guidelines in this document. Failure to follow these guidelines may result in undesirable effects, injury to personnel, and/or damage to the system or to property.

Operator Safety for the Hyperion and Hyperion+ Imaging Systems

The Hyperion™ and Hyperion+™ Imaging Systems are classified as Class 1 laser devices.

The laser radiation of the Class 1 laser system is eye-safe under all operating conditions. This product includes lasers of a higher class whose beams are confined within a suitable enclosure so that access to laser radiation is physically prevented.

Safety Alert Conventions

Fluidigm documentation uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
DANGER	Signal word that indicates more severe hazards.
WARNING	Signal word that indicates less severe hazards.

Safety Alerts for Instruments

For hazards associated with instruments, this document uses indicators that include a pictogram and signal words that indicate the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a black triangle-shaped frame. Refer to the system user guide for the applicable pictograms and hazards pertaining to system usage.
DANGER	Signal word that indicates an imminent hazard that results in severe injury or death if not avoided.
WARNING	Signal word that indicates a potentially hazardous situation that could result in serious injury or death if not avoided.
CAUTION	Signal word that indicates a potentially hazardous situation that could result in minor or moderate personal injury if not avoided.
IMPORTANT	Signal word that indicates information necessary for proper use of products or successful outcome of experiments.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Chapter 1: Hyperion and Hyperion+ Imaging Systems Introduction and Specifications

Introduction

The Hyperion™ and Hyperion+™ Imaging Systems are the world's first and only commercially available metal-tagged, antibody-directed Imaging Mass Cytometry™ (IMC™) platforms that allow highly multiplexed imaging with 135 available channels. The systems are designed to detect metal-tagged antibodies bound to proteins in biological samples using standard staining methods. The platforms can simultaneously detect 4 to 37 protein markers in biological samples including fixed tissue sections or cells deposited onto glass slides (liquid biopsies). This allows researchers to investigate cellular subpopulations in various tissue microenvironments. The systems allow for cellular profiling in spatial proximity, enabling subpopulation profiling and exploration of relationships of neighboring cells within the context of the tissue structure.

Figure 1. The Hyperion™ Imaging System

Hyperion and Hyperion+ Imaging Systems Technology

The Hyperion and Hyperion+ Imaging Systems use innovative technology based on laser ablation technology coupled with time-of-flight (TOF) mass cytometry of the resulting plume (see Figures 2 and 3). The systems use a precisely directed laser beam focused at 1 µm to collect protein markers stained with metal-tagged Maxpar® antibodies and directs these metal tags for detection using CyTOF® technology. The sample on the slide is ablated and aerosolized. The resulting aerosol plume is delivered through the coupling tube to the Helios™ inductively coupled plasma (ICP) torch through the argon and helium or helium/hydrogen gas mixture flow.

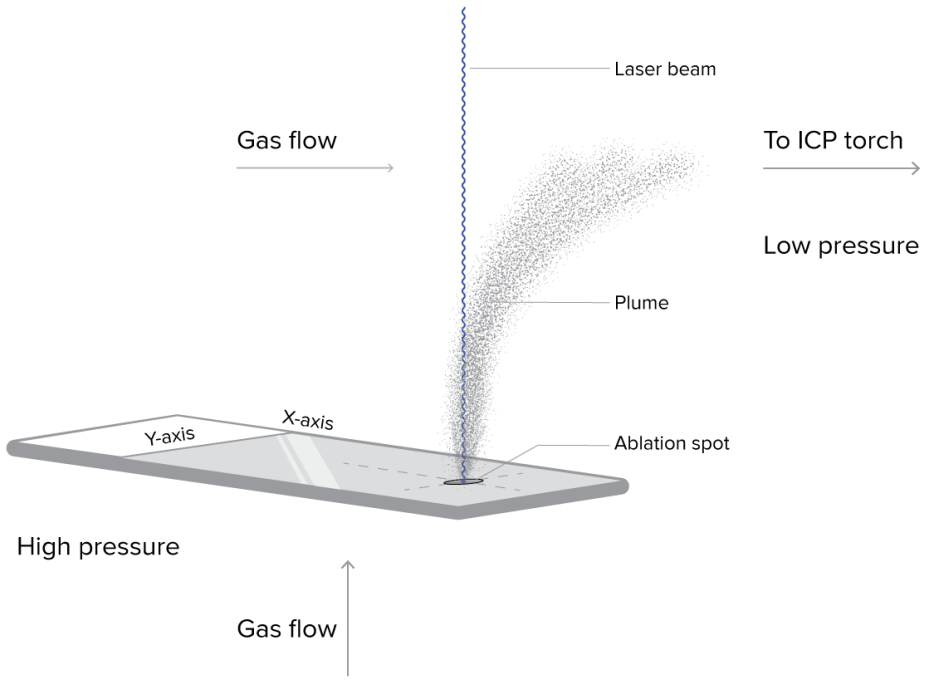


Figure 2. A glass slide containing a biological sample is loaded into the Hyperion and Hyperion+ Imaging Systems. The beam from the solid-state laser is directed to the sample on the slide. The resulting plume is directed through the coupling tube in a stream of argon gas toward the ICP torch for CyTOF detection.

The slide is loaded onto the stage of the ablation chamber and the camera captures the image from the slide. The system directs a laser beam through the optical components of the chamber. The laser beam is focused to a 1 µm spot and ablates sample proteins stained with metal-tagged antibodies on the slide, resulting in aerosol plumes. The plumes are directed to the Helios ICP torch, where they are vaporized, atomized, and ionized in the

plasma. The high-pass ion optics remove the low-mass ions that are not of analytical interest before the ion cloud enters the TOF mass analyzer. The ions enter the TOF mass analyzer in 13 µs intervals (pushes). Ions are separated based on their mass-to-charge ratio and the detector measures the quantity of each isotope for each plume, corresponding to a single laser shot, from the sample based on differences in mass instead of wavelength, and at 1 Da resolution with minimal background.

Acquired data is stored in an MCD file format. In a typical workflow, 1 MCD file is used for 1 sample slide, which can contain multiple regions of interest (ROIs). MCD files can be viewed with MCD™ Viewer software. MCD Viewer can also export acquired data as separate images in a TIFF file format.

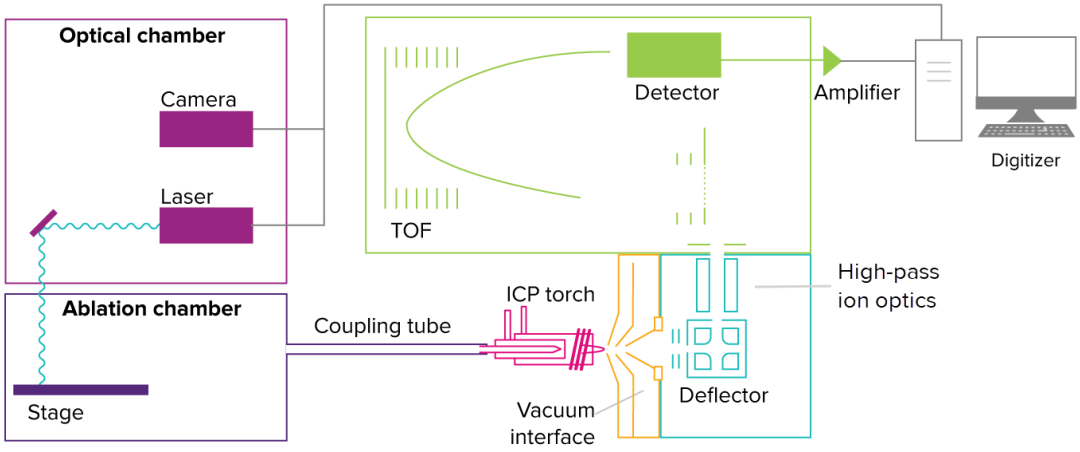


Figure 3. Schematic of the Hyperion or Hyperion+ Imaging System coupled with the Helios system

Plume Transients

The material resulting from the laser shot that is directed to the sample slide is referred to as the plume. The time it takes for a single plume generated as a result of a single laser shot to be transferred to the Helios system, ionized, and then detected by the detector is defined as the plume transient time. The duration of the single plume integration is defined as the plume width. The default value for plume width of the Hyperion Imaging System at a frequency of 200 Hz is 384 pushes (see Figure 4), which is approximately 5 milliseconds in duration. The Hyperion+ Imaging System supports a frequency of 400 Hz and 192 pushes. During manual tuning, the software can generate a transient curve, which is displayed by opening the Transient window in the software. When helium or helium/hydrogen gas mixture flow ramping is set up, the software graphs the dual counts (number of ions) against time to determine the optimal helium or helium/hydrogen gas mixture flow required for effective transient delivery to the Helios instrument with minimal crosstalk (or overlap of

plumes). Autotuning is recommended for optimizing helium or helium/hydrogen gas mixture flow.

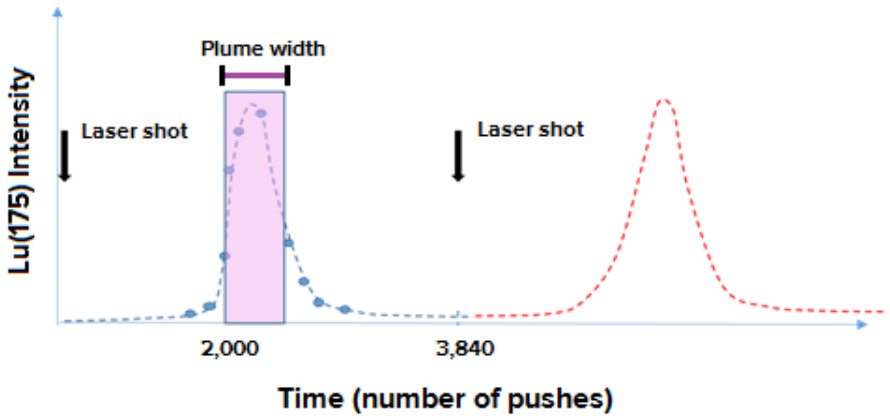


Figure 4. Graph of ^{175}Lu intensity. The plume width of a single plume is represented as 384 pushes during 20 Hz operation. A 20 Hz frequency is used to record and optimize transients during tuning.

Solid-State Laser Specifications

Table 1. Performance specifications for the solid-state laser

Instrument	Description	Performance Specifications
Hyperion Imaging System (PN 108700)	Laser type	Nd:YAG
	Wavelength	213 nm
	Energy output	≤3 µJ
	Repetition rate (frequency)	20/100/200 Hz
Hyperion+ Imaging System (PN 119001)	Laser type	Nd:YAG
	Wavelength	213 nm
	Energy output	≤3 µJ
	Repetition rate (frequency)	20/200/400 Hz

Hyperion and Hyperion+ Imaging Systems Specifications

Table 2. System specifications for the Hyperion and Hyperion+ Imaging Systems

Instrument	Description	System Specification
Hyperion Imaging System (PN 108700)	Tissue thickness (full ablation)	≤7 µm thickness
	Maximum addressable sample size	15 mm x 55 mm
	Maximum recommended ROI area	2.25 mm^2 (2250000 $µm^2$)
	Ablation spot size	≤1 $µm^2$
	Recommended operating temperature	18–25 °C
	Optimal operating temperature	21 ± 1 °C
Hyperion+ Imaging System (PN 119001)	Tissue thickness (full ablation)	≤7 µm thickness
	Maximum addressable sample size	15 mm x 55 mm
	Maximum recommended ROI area	2.25 mm^2 (2250000 $µm^2$)
	Ablation spot size	≤1 $µm^2$
	Recommended operating temperature	18–25 °C
	Optimal operating temperature	21 ± 1 °C

Table 3. Electrical specifications for the Hyperion and Hyperion+ Imaging Systems

Instrument	Description	Electrical Specifications
Hyperion Imaging System (PN 108700)	Power	200 VA
	Operating voltage	100–240 V AC
	Operating frequency	50/60 Hz
	Operating mode	Single-phase operation
	Power dissipation, full load	<75 W
Hyperion+ Imaging System (PN 119001)	Power	200 VA
	Operating voltage	100–240 V AC
	Operating frequency	50/60 Hz
	Operating mode	Single-phase operation
	Power dissipation, full load	<75 W

Table 4. Gas specifications for the Hyperion Imaging System (PN 108700)

Gas Specifications	Purity	Pressure (psi)	Flow Rate (L/min)	Impurities (ppm)
Argon	≥99.996%	80 ± 1	20	Oxygen <5 Nitrogen <20 Hydrogen <1 Water <4
Helium	≥99.9993% Grade 5.3	30 ± 1	NA	Oxygen <0.1 Nitrogen <0.5 Hydrogen <0.4 Water <0.2

Table 5. Gas specifications for the Hyperion+ Imaging System (PN 119001)

Gas Specifications	Purity	Pressure (psi)	Flow Rate (L/min)	Impurities (ppm)
Argon	≥99.996%	80 ± 1	20	Oxygen <5 Nitrogen <20 Hydrogen <1 Water <4
Helium/hydrogen gas mixture (2% hydrogen in the balance of helium)	Hydrogen ≥99.99% Helium ≥99.999%	30 ± 5	<0.3 (normal operation) <5 (chamber flushing only)	Total impurities <12

Table 6. Instrument dimensions for the Hyperion and Hyperion+ Tissue Imagers (HTI™ and HTI+™)

Instrument	Description	Instrument Dimensions
Hyperion Imaging System (PN 108700)	Height	134 cm (53 in)
	Width	56 cm (22 in)
	Depth	56 cm (22 in)
	Weight	159 kg (350 lb)
Hyperion+ Imaging System (PN 119001)	Height	134 cm (53 in)
	Width	56 cm (22 in)
	Depth	56 cm (22 in)
	Weight	159 kg (350 lb)

Table 7. Instrument dimensions for the Helios system

Description	Instrument Dimensions
Height	132 cm (52 in)
Width	103 cm (41 in)
Depth	87 cm (35 in)
Weight	320 kg (705 lb)

Table 8. Input and output connections for the Hyperion and Hyperion+ Imaging Systems

Instrument	Description	Input and Output Connections
Hyperion Imaging System (PN 108700)	Power	AC power
	Gases	Argon gas, helium gas
	Sample	Sample slide, ablated sample
	Digital I/O	USB 2.0 communication cable, USB 3.0 camera, trigger (data acquisition card)
Hyperion+ Imaging System (PN 119001)	Power	AC power
	Gases	2% hydrogen in the balance of helium
	Sample	Sample slide, ablated sample
	Digital I/O	USB 2.0 communication cable, USB 3.0 camera, trigger (data acquisition card)

Table 9. Workstation specifications for the Hyperion and Hyperion+ Imaging Systems

Instrument	Description	Workstation Specifications
Hyperion Imaging System (PN 108700)	Operating system	Win10 Enterprise
	Processor	EMB CORE i7-6700 @ 3.4 GHz
	Memory	32 GB DDR4
	Monitor	LG® 34” Class 21:9 UltraWide® LED Monitor
	Motherboard	Intel® H110
	Data storage	OS: 1 x 240 GB SSD Storage: 2 x 8 TB (RAID10)
Hyperion+ Imaging System (PN 119001)	Operating system	Win10 Enterprise
	Processor	EMB CORE i7-6700 @ 3.4 GHz
	Memory	32 GB DDR4
	Monitor	LG® 34” Class 21:9 UltraWide® LED Monitor
	Motherboard	Intel® H110
	Data storage	OS: 1 x 240 GB SSD Storage: 2 x 8 TB (RAID10)

Standard Equipment

Table 10. Standard parts included in shipment of the Hyperion and Hyperion+ Imaging Systems

Part Number	Standard Equipment
108700	Hyperion Imaging System, Helios system, Universal computer, keyboard and mouse, UltraWide LG monitor
108701	Hyperion Imaging System Package (Upgrade with Hyperion Tissue Imager)*
119001	Hyperion+ Imaging System (Helios system, Universal computer, keyboard and mouse, UltraWide LG monitor)

* Upgrade for Helios customers only

Recommended Slide Dimensions

Table 11. Recommended slide dimensions for the Hyperion and Hyperion+ Imaging Systems

Instrument	Dimensions	(mm)*
Hyperion Imaging System (PN 108700)	Width	24.8–26.2
	Length	71.5–76.5
	Thickness	0.8–1.7
Hyperion+ Imaging System (PN 119001)	Width	24.8–26.2
	Length	71.5–76.5
	Thickness	0.8–1.7

* Recommendations are based on the size of the Hyperion and Hyperion+ Imaging Systems slide stage.

Consumables and Reagents

Hyperion and Hyperion+ Imaging Systems

Table 12. Consumables available for the Hyperion and Hyperion+ Imaging Systems

Part Number	Standard Equipment
108147	O-Ring, Coupling Tube—5 Pack
108480	Coupling Tubing Kit—Hyperion™ and Hyperion+™ Tissue Imager
108174	Injector—Hyperion and Hyperion+ Tissue Imager
108168	Injector Nut
108286	Gas Connection Kit—Hyperion and Hyperion+ Tissue Imager
108650	Front and Back Ferrule Set—Hyperion and Hyperion+ Tissue Imager—5 Pack
132123	Wheel retraction switch access tool 1 (held in the palm of the hand)*

* Use with Hyperion and Hyperion+ Tissue Imagers that require a Wheel Retraction Switch Access Tool.

Table 13. Reagents available for the Hyperion and Hyperion+ Imaging Systems

Part Number	Standard Equipment
201088	3 Element Full Coverage Tuning Slide

Helios

Refer to the Helios, a CyTOF System User Guide (400250).

Consumables Ordering

North America

Customers in the US and Canada who have a Fluidigm account are already registered for online ordering. Go to fluidigm.com/catalog. New customers can set up an online account to place orders, view past order history, and see current order confirmations.

Phone: Toll-free (US/CAN) 866 358 4354

Email: salesadmin@fluidigm.com

Outside North America

To reorder parts and reagents, contact your regional Fluidigm sales representative or distributor. Go to fluidigm.com/contact.

Chapter 2: Instrument Overview

Hyperion and Hyperion+ Tissue Imagers

WARNING Laser instruments generate potentially hazardous UV radiation. Do not remove the top cover of the Hyperion™ or Hyperion+™ Tissue Imager. Only a Fluidigm Field Service Engineer should remove the top cover and perform maintenance.

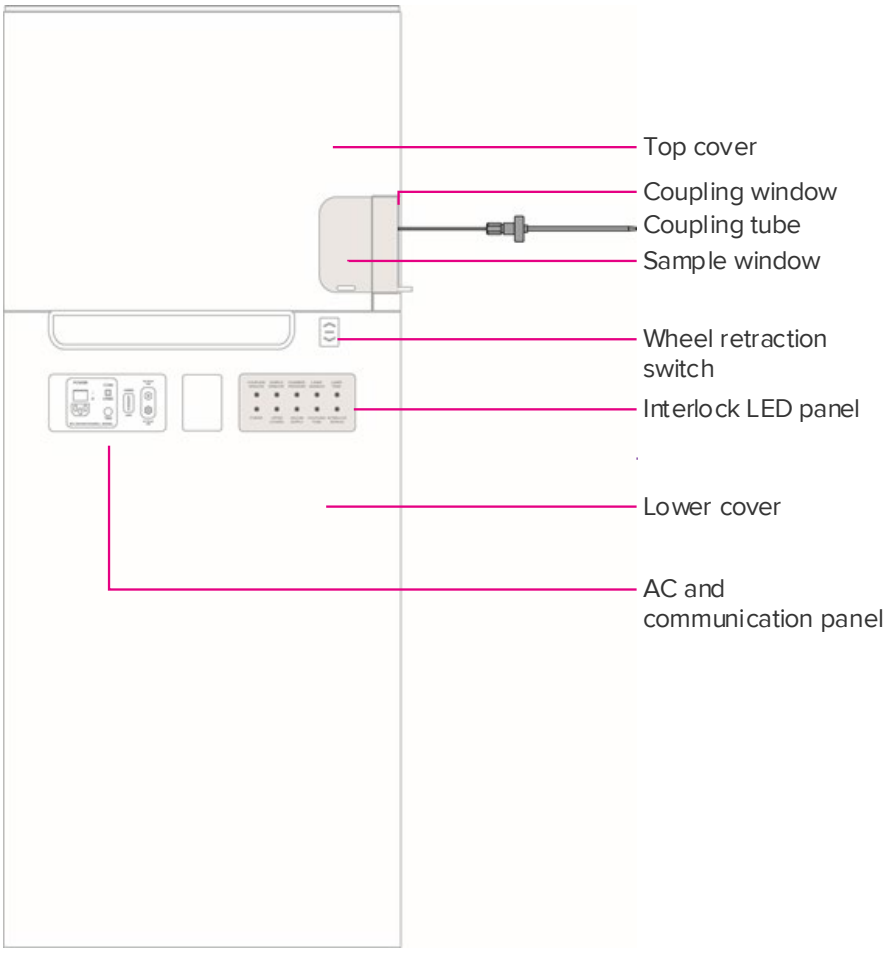


Figure 5. Side-view schematic of the Hyperion and Hyperion+ Tissue Imagers

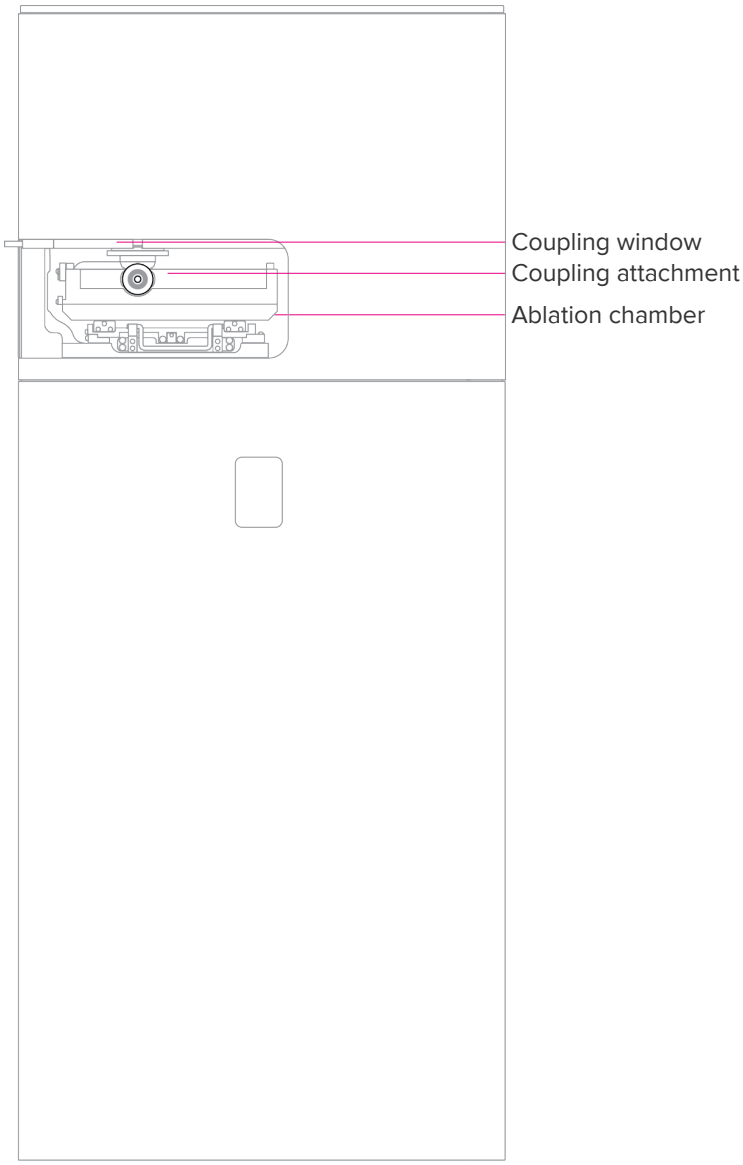


Figure 6. Back-view schematic of the Hyperion and Hyperion+ Tissue Imagers

Sample Window and Coupling Window

Closed Position

When the sample window or the coupling window is in the closed position, the light corresponding to that window on the LED panel on the right side of the instrument is GREEN.

Open Position

When the sample window or the coupling window is in the open position, the light corresponding to that window on the LED panel on the right side of the instrument is NOT LIT and the user cannot perform tuning or acquisition on the system.

Hyperion and Hyperion+ Tissue Imagers LED Panel

Table 14. Instrument Interlock LEDs on the LED panel of the Hyperion and Hyperion+ Tissue Imagers

Instrument Interlock LED	Description
COUPLING WINDOW	When the light is green, the coupling window is closed. When the light is not lit, the coupling window is open and the interlock has been triggered.
SAMPLE WINDOW	When the light is green, the sample window is closed. When the light is not lit, the sample window is open and the interlock has been triggered.
CHAMBER PRESSURE	When the light is lit, the chamber is pressurized.
LASER ENABLED	When the light is amber, the laser is firing and ready to ablate, or ablation is in progress.
LASER TEMP	When the light is red, the laser has exceeded safe operating temperature. The laser will be disabled.
POWER	When the light is continuously green, the system power supplies are on. When light is not lit, the system power supplies are off.
UPPER COVERS	When the light is not lit, the upper cover housing the optics and the laser has been removed.
HELIUM SUPPLY	When the light is green, the pressure in the helium or helium/hydrogen gas mixture supply line is within operating range.
HELIUM/HYDROGEN GAS MIXTURE *	
COUPLING TUBE	When the light is lit, the coupling tubing has been connected to the ablation chamber. The wheel retraction system is not activated.
INTERLOCK BYPASS	When the light is flashing green, all safety interlocks have been bypassed. (The interlock bypass is for the Field Service Engineering team.)

* HELIUM/HYDROGEN GAS MIXTURE – ONLY when using the Hyperion+ Imaging System

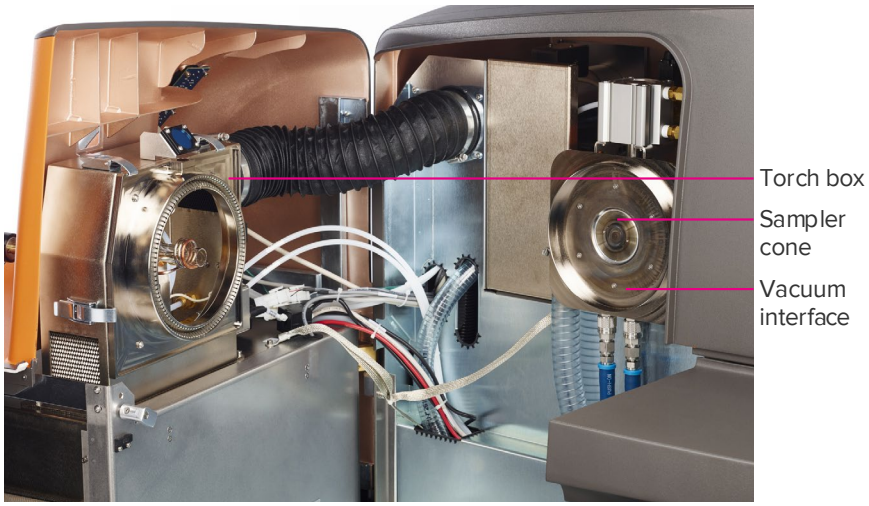


Figure 7. Interior view of the Helios™ instrument with front access door open

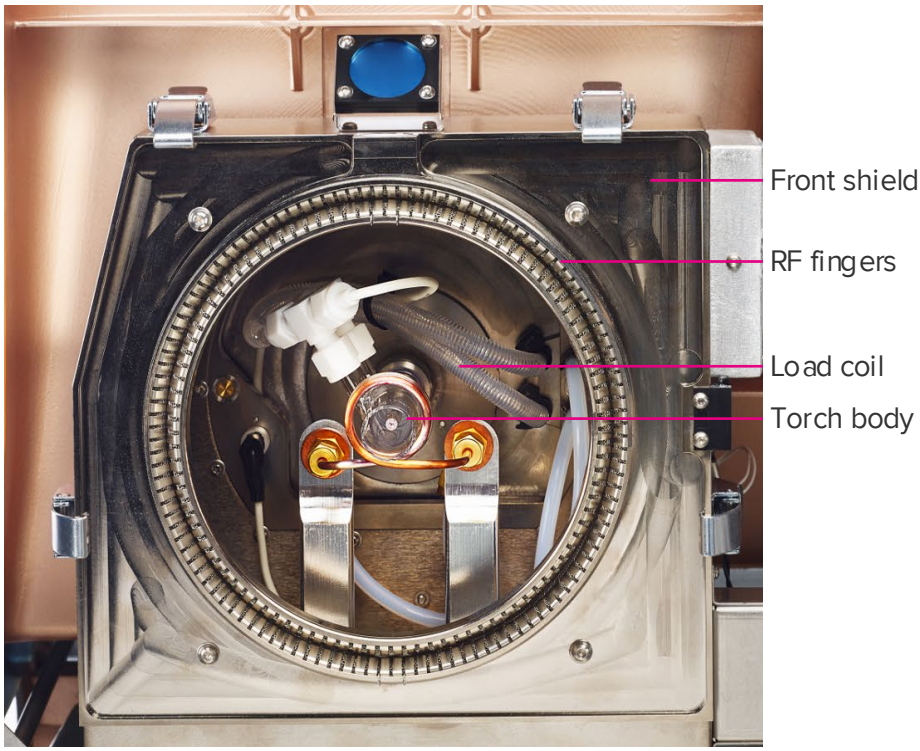
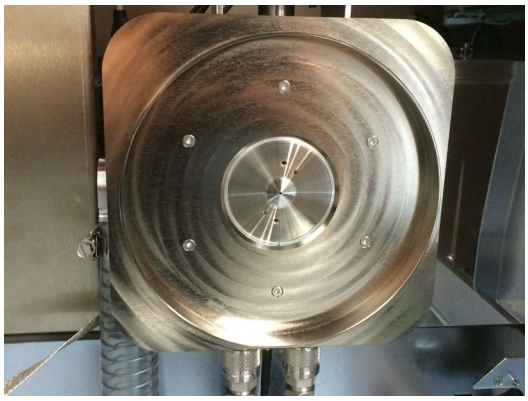


Figure 8. Torch box

Other Components

Table 15. Other Helios components

Parts	Image	Location
AC distribution box		Right side of Helios system
Sampler cone		In the vacuum interface

Parts	Image	Location
Cooling fans		Inside lower front door

Chapter 3: Operation

This section describes how to

- connect the Hyperion™ or Hyperion+™ Tissue Imager to Helios™
- start the Hyperion or Hyperion+ Imaging System.
- disconnect the Hyperion or Hyperion+ Tissue Imager from Helios.

NOTE See CyTOF® Software v7.0 Help for procedures related to instrument control, tuning, and data acquisition.

Connect the Hyperion or Hyperion+ Tissue Imager to Helios

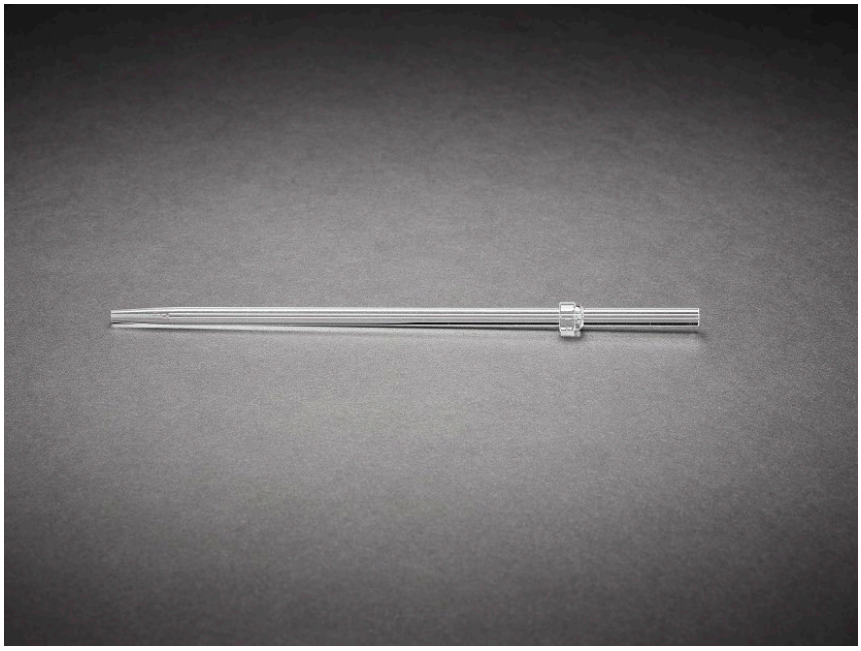
IMPORTANT Place the Hyperion and Hyperion+ Imaging Systems in a vibration-free environment.

IMPORTANT The following procedure outlines the coupling of the Hyperion or Hyperion+ Tissue Imager to Helios. Plumes of ablated material are transported through the coupling tube to Helios for mass cytometry analysis. Connect the Hyperion or Hyperion+ Tissue Imager to the AUXILIARY power connection on the right panel of the Helios instrument to allow for raising and lowering of the instrument on its wheels and for efficient coupling to Helios.

WARNING The system must be correctly connected to a suitable electrical supply For more details, see Helios, a CyTOF System Site Requirements Guide (400252).

1 Inspect both ends of the Hyperion or Hyperion+ Tissue Imager (HTI™ or HTI+™) Injector to check for debris or material prior to beginning this procedure. Inspect under a microscope (if available).

NOTE Use compressed air to remove any debris from the injector.

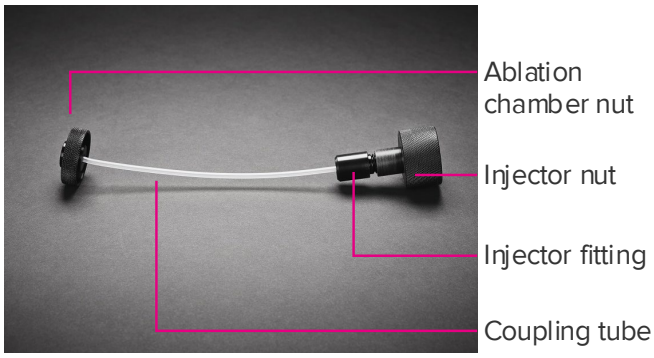


2 Insert the injector into the torch assembly. Gently rotate the injector to push it past the O-rings until it is fully seated into the torch assembly.

WARNING Finger cut hazard. Broken glass may cause injury or cutting of fingers or hands.

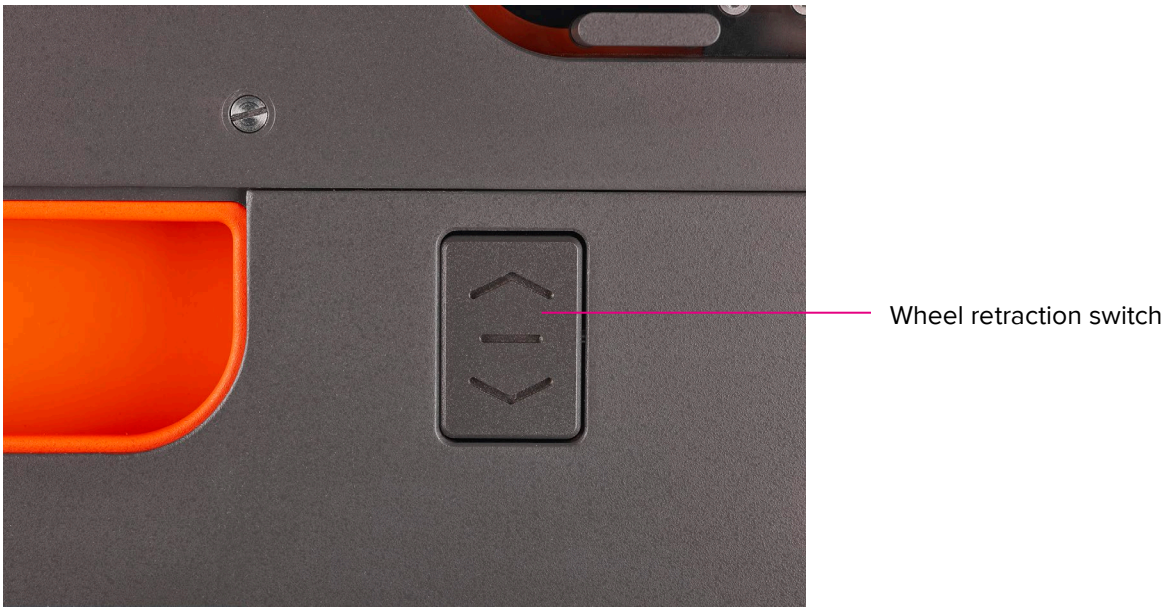
IMPORTANT Use care when inserting the injector.

3 Inspect the coupling tube to ensure that there is no debris or dust (use compressed air to remove any dirt from the coupling), and then connect the larger-diameter nut to the injector.



Raise the Hyperion Tissue Imager That Does Not Require a Wheel Retraction Switch Access Tool

Press the wheel retraction switch on the right side of the instrument to lift the system so that the castors are exposed for movement of the system.



Raise the Hyperion or Hyperion+ Tissue Imager That Requires a Wheel Retraction Switch Access Tool

Raise the instrument using the wheel retraction switch access tool so that the castors are exposed for movement.

IMPORTANT The access tool unlocks the wheel retraction switch. Contact the designated person in your lab for access to the wheel retraction switch access tool.

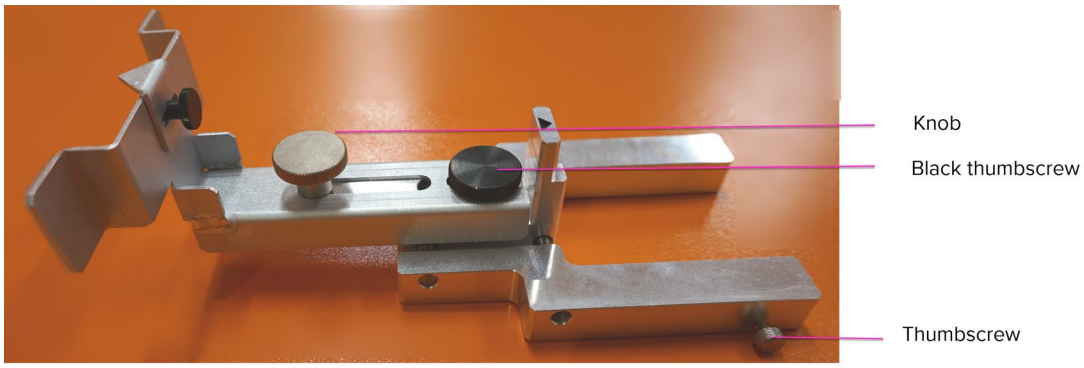
1 Locate the wheel retraction switch located on the right side of the HTI or HTI+ instrument.

2 Insert the access tools into the slot of the wheel retraction switch.

3 Push the access tool up such that the top of the wheel retraction switch tilts inside the instrument frame. The wheel retraction switch unlocks, allowing the HTI or HTI+ to raise for movement.

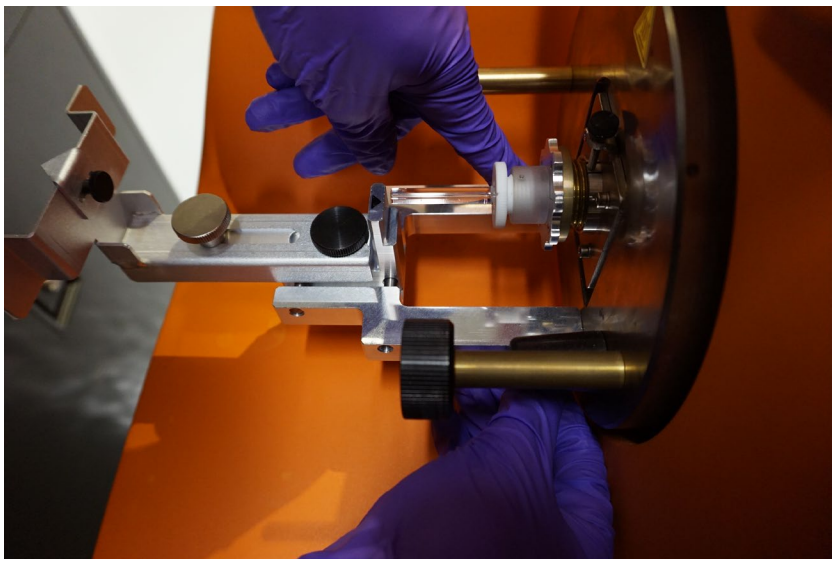
Align the Hyperion or Hyperion+ Tissue Imager with Helios

1 Loosen the knob of the alignment tool and push the arm in all the way.

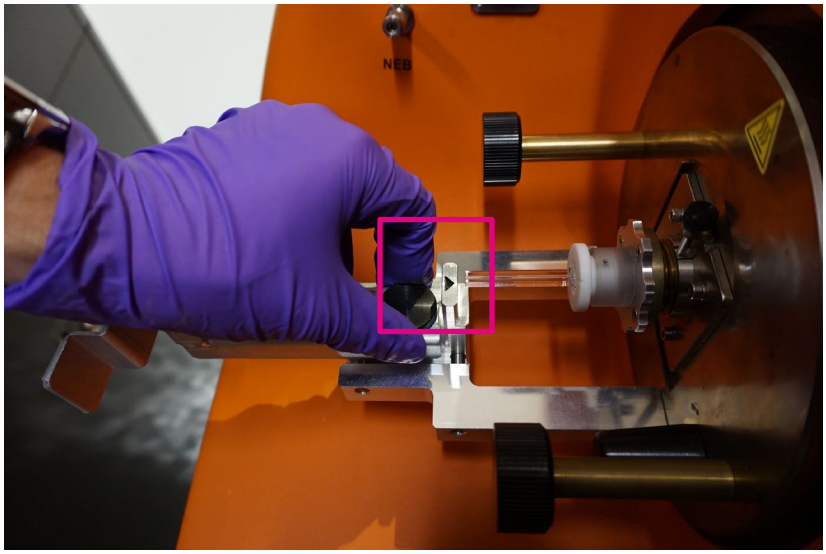


2 Carefully slide the alignment tool onto the heater guide pins of Helios, ensuring that the back of the tool is flush against the torch assembly. Secure the tool in position with one hand and tighten the right-side thumbscrew.

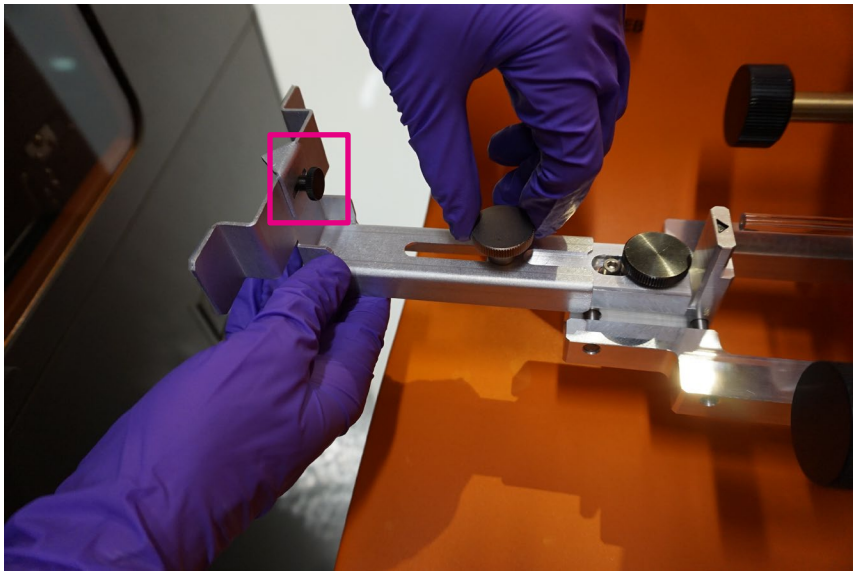
3 With the first thumbscrew secure, reach down from above with your other hand and tighten the thumbscrew on the left. After both screws are secure, ensure that the alignment tool is level on the heater guide pins and that the back of the tool is against the torch assembly.



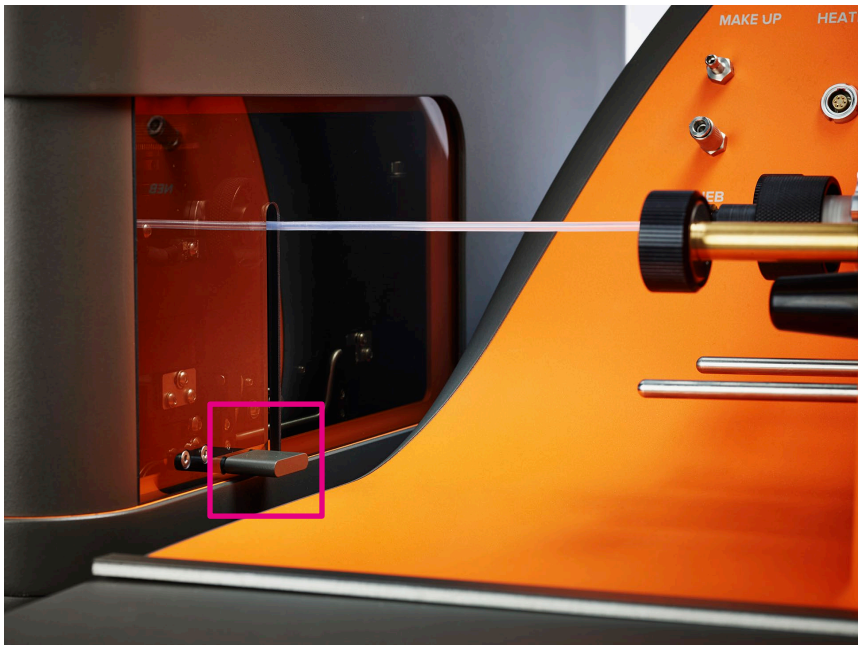
4 Loosen the black thumbscrew on the top of the alignment tool and horizontally line up the arrow of the alignment tool with the center of the injector. Tighten the black thumbscrew to lock the position of the alignment tool.



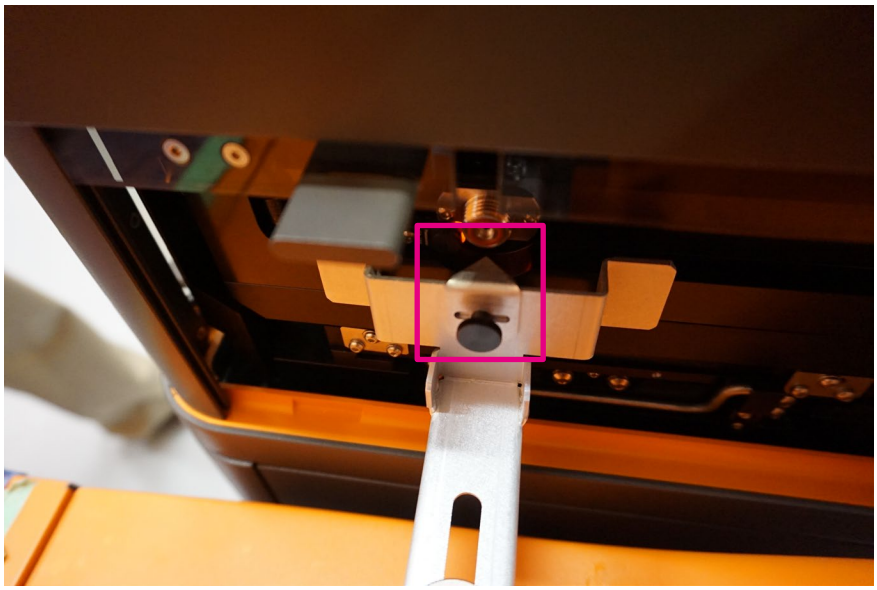
5 Loosen the knob and extend the arm of the alignment tool fully. Tighten the knob to lock the arm in place.



6 Ensure that the Hyperion or Hyperion+ Tissue Imager is raised completely. Lift the coupling window using the gray tab.



7 Carefully move the Hyperion or Hyperion+ Tissue Imager toward the tool so that the ablation chamber gently touches the 2 extended pads of the alignment tool arm. This helps ensure that the tissue imager is parallel with Helios. The arrow on the alignment tool (pink box in the photo) should point toward the center of the coupling insertion on the Hyperion or Hyperion+ Tissue Imager.



8 Loosen the knob on the alignment tool and push in the arm of the alignment tool all the way back toward the Helios instrument.

9 With an arm over the top of the Hyperion or Hyperion+ Tissue Imager, do 1 of the following:

- Press down on the access tool while it is inserted in the wheel retraction switch to lower the module until it is seated on the ground.
- Press down on the wheel retraction switch to lower the module until it is seated on the ground.

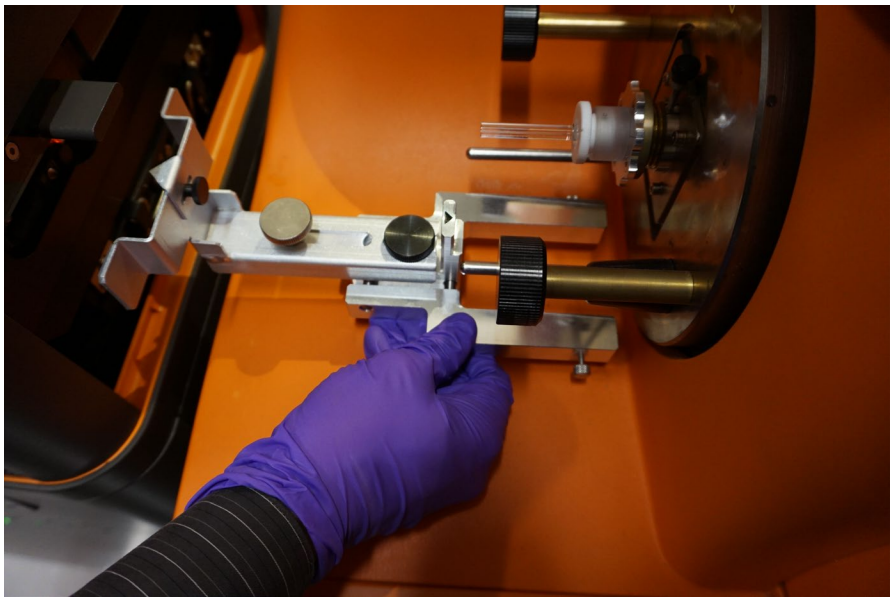
Watch for any shifting of the Hyperion or Hyperion+ Tissue Imager as it settles.

IMPORTANT Ensure that the Hyperion or Hyperion+ Tissue Imager is not sitting on any cords or gas lines before lowering into position.

10 If the Hyperion or Hyperion+ Tissue Imager settles significantly to the left or right of the Helios instrument, loosen the small black thumbscrew at the end of the arm and offset the alignment arrow right or left to adjust for the settling. The alignment should be both vertical and horizontal so that the output of the ablation chamber lines up with the coupling tube and Injector.

11 Repeat Steps 6 through 10 as necessary.

12 Carefully remove the alignment tool from the heater guide pins. Lift the tool off the guide pins and then slide it out and away from the torch assembly horizontally below the pins.

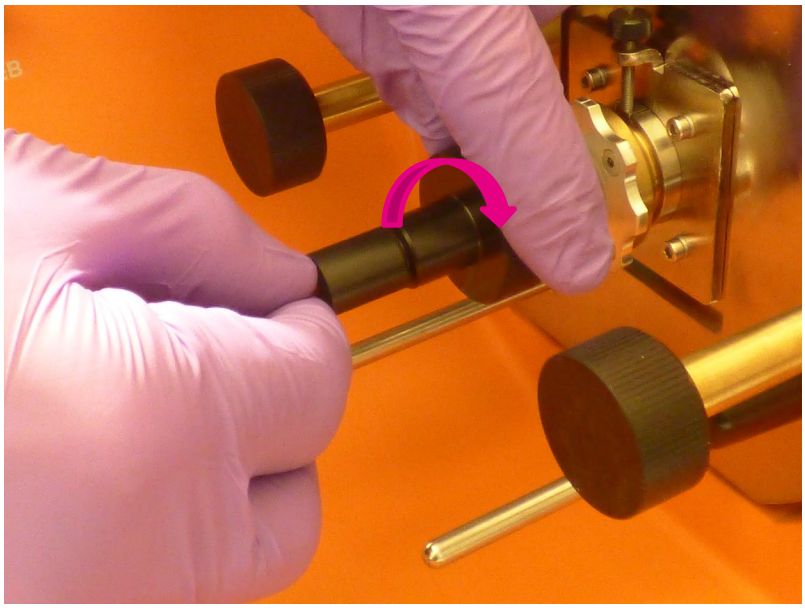


Connecting the Coupling Tube

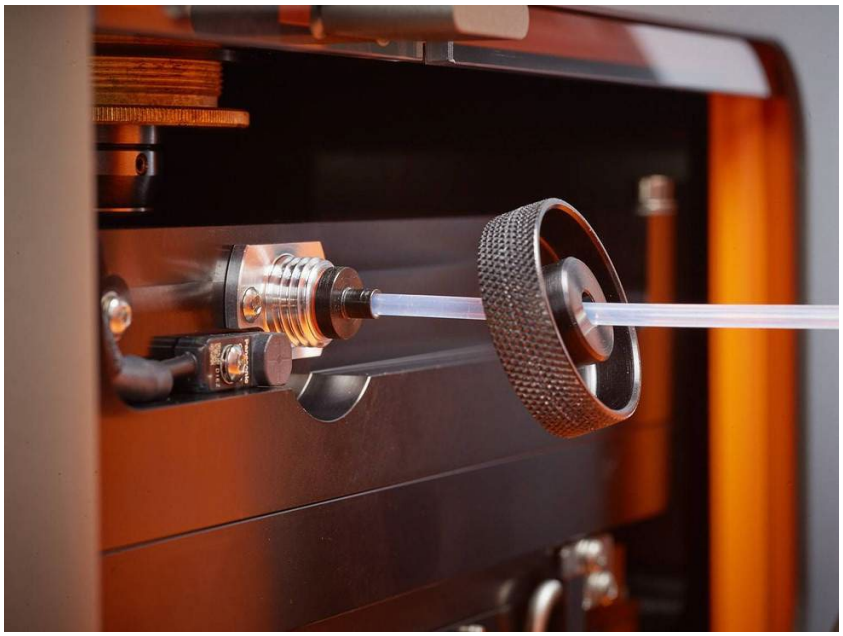
1 Slide the injector fitting over the back end of the injector in Helios. Grip the back nut with one hand while tightening the injector fitting in the clockwise direction. Hold the injector fitting so that the orientation of the coupling tube is maintained.

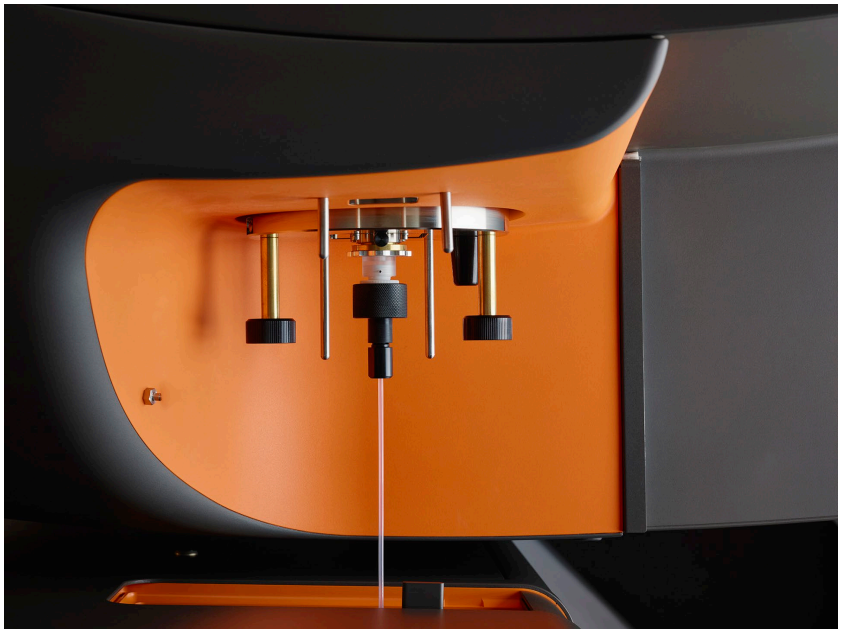
2 Do not overtighten.

NOTE Do not grip the coupling tube. Gripping the tube may result in damage.



3 Insert the coupling tube end into the coupling attachment in the ablation chamber.

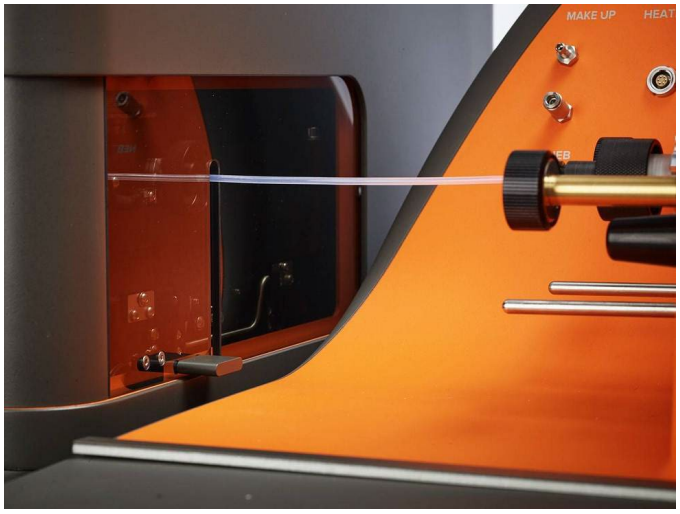




IMPORTANT The tube must be as straight as possible. If the tube is not straight then raise the instrument so that the castors are exposed. Adjust the instrument position and check that the tube is fully inserted. Lower the instrument back to the ground for operation. Check that the tube is correctly positioned as straight as possible.

4 Tighten the ablation chamber nut to the ablation chamber in the clockwise direction.

5 Close the coupling window.



a Ensure that all the communication cables Have been connected.

- Connect the USB 2.0 cable connects to the computer for software control.
- Connect the USB 3.0 cable connects to the computer for the camera.
- Connect the BNC trigger line connects to the data acquisition board.

- Connect the gas supply lines.

NOTE The Hyperion Tissue Imager uses helium as its gas supply, and the Hyperion+ Tissue Imager uses helium/hydrogen gas mixture as its gas supply.

- Connect the helium or helium/hydrogen gas mixture gas line to the external helium or helium/hydrogen gas mixture supply.
- Connect the argon gas line to the makeup gas supply on the front cover of the Helios instrument.

6 Open the helium or helium/hydrogen gas mixture and argon gas valves.

7 Turn the instrument on by clicking the I/O power switch on the communication panel to the I position.

The Hyperion or Hyperion+ Tissue Imager is now connected to Helios and is ready for operation.

WARNING TRIP HAZARD. Watch your step to avoid falling over objects.

IMPORTANT The electrical cables and communications are a potential trip hazard. Be careful when walking close to the Hyperion or Hyperion+ Tissue Imager.

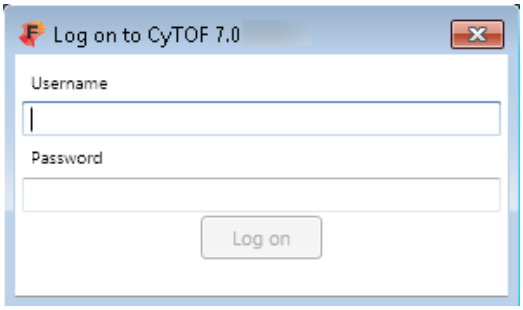
Start the Hyperion or Hyperion+ Imaging System

To start the Hyperion or Hyperion+ Imaging System, log on to the acquisition computer connected to the system and open CyTOF Software v7.0.

1 Start and log on to CyTOF Software.

a Double-click CyTOF Software icon on the desktop.

b If you already have a CyTOF user account, enter your username and password. Click **log on**.



c If you are a new user, in the **username** text box, enter Administrator. Leave the Password text box empty. Click **log on**.

The system takes approximately 30 sec to initialize.

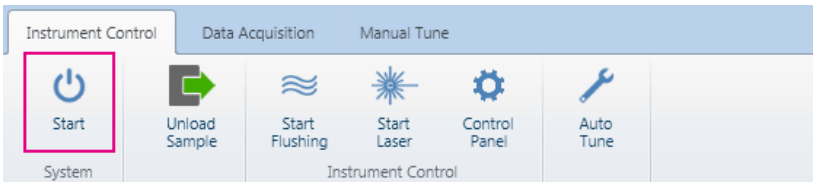
- If CyTOF Software is in cell suspension mode, set the Acquisition Mode to Imaging.
  - Under **system**, click **control Panel**.

- On **control Panel**, click the **devices** tab, and under **acquisition Mode**, click **imaging**.
- On **control Panel**, click **set Configuration**. CyTOF Software restarts and the log on dialog box is displayed. Enter your account credentials to log on to the software and continue to Step 3.

2 Click **Start Flushing** to displace any air in the system.

3 After initialization is complete, start the system. On the **instrument Control** tab, click **start**.

NOTE It takes approximately 7 min for the system startup sequence to complete. During this time the ablation chamber is flushed with helium or helium/hydrogen gas mixture, plasma is ignited, and hardware components are initialized.



NOTE Allow the system at least 30 min to warm up before tuning. Refer to the software Help for information about tuning, acquisition, and instrument control. Help is started from the upper right corner of the screen—click .

NOTE The button now displays Cancel. If you want to cancel the system startup sequence, click Cancel.

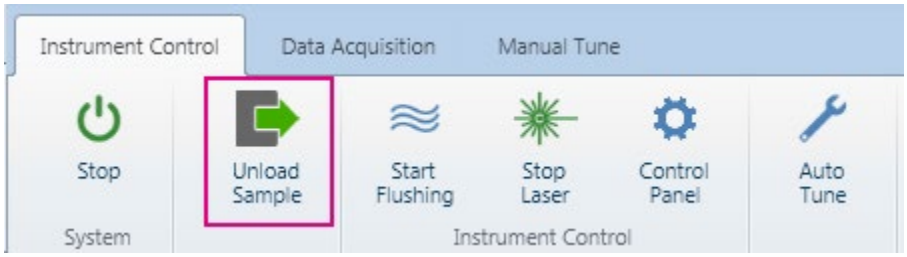
Unload and Load the Tuning Slide or Sample Slide

CAUTION Finger cut hazard. Broken glass may cause injury or cutting of fingers or hands. Caution when loading and unloading the sample slides.

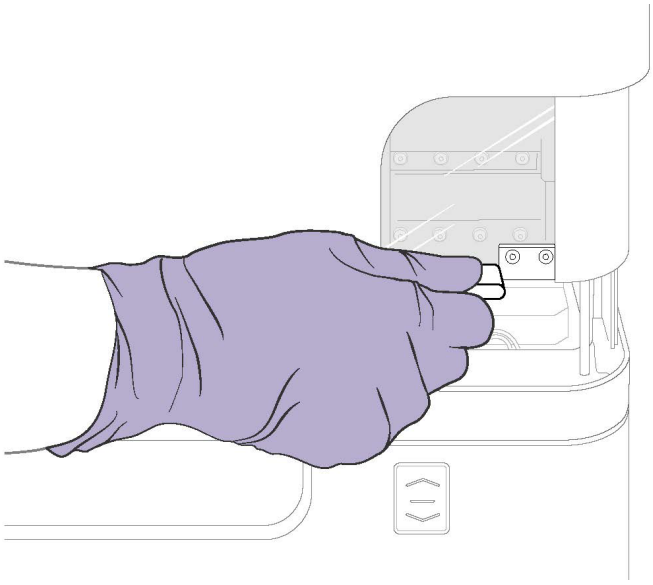
1 Obtain the 3 Element Full Coverage Tuning Slide (201088).

IMPORTANT It is recommended that you store the tuning slide and the sample slide in a slide holder between uses to prevent dust buildup.

2 On the Instrument Control tab, click Unload Sample.



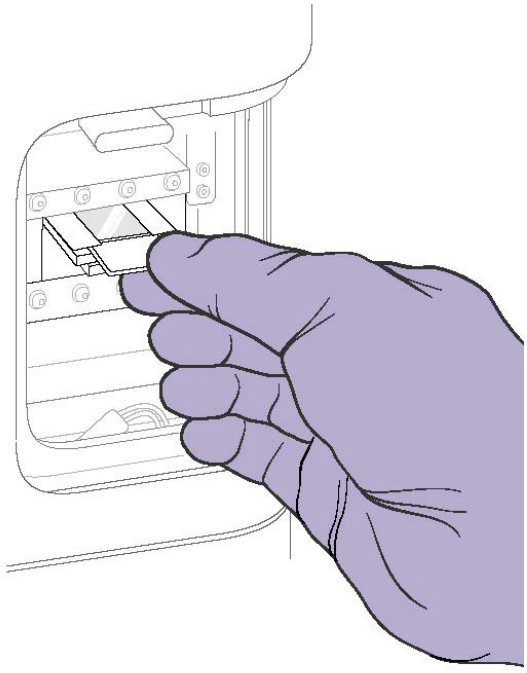
3 After the stage on the Hyperion or Hyperion+ Tissue Imager fully extends, open the sample window and, if necessary, remove the previously loaded slide.



4 When the stage is fully extended, carefully insert the slide into the ablation chamber stage with tuning film facing up.

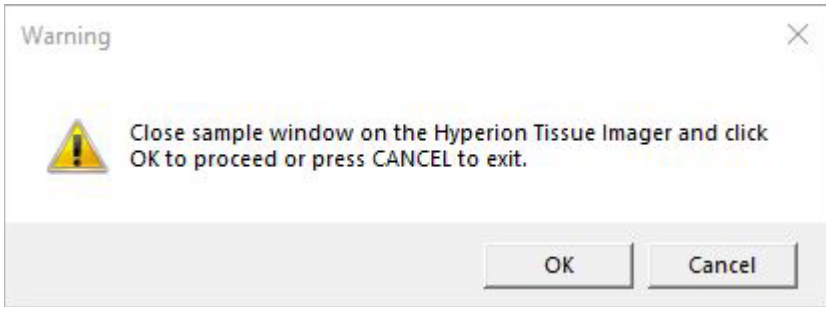
IMPORTANT Gently grip the slide with your thumb and index finger to ensure that you are not pushing down on the slide. Place the slide in the grooves on the stage and insert.

5 Push the slide until it is firmly seated. A loud noise occurs if the slide is inserted incorrectly.



6 Click **Load**.

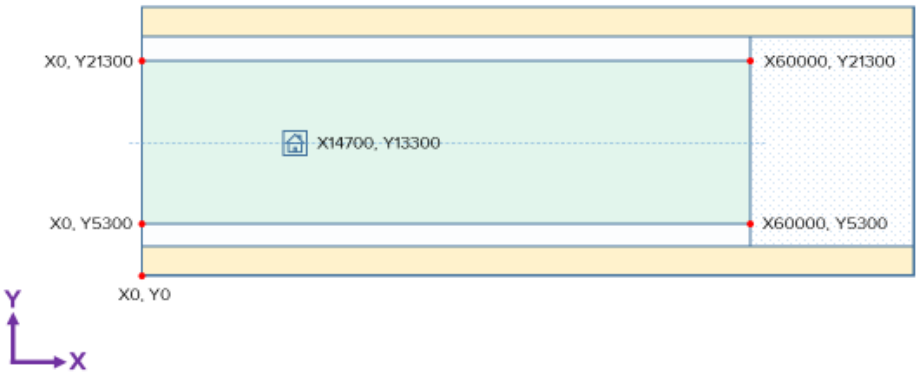
7 If the instrument sample window is open, a dialog box confirms that a Cover Door is Closed. Close the sample window. Click **OK**.



8 Load the slide onto the stage and close the sample window.

9 After the sample is loaded, click **Load Sample**.

NOTE The slide moves to the home position immediately after it is loaded. The home position is set to 14,700 µm from the left side of the inserted microscope slide and 13,300 µm from the bottom of the slide, which is considered the midpoint of the slide. The slide coordinate system starts on the bottom left-hand corner of the slide, which represents X=0 and Y=0.



NOTE The area available for ablation is approximately 16,000 µm in the Y direction and 60,000 µm in the X direction.

10 The chamber automatically flushes for approximately 5 min (unless the default flushing time has been changed in the HTI Settings). Check the HTI Status Panel to confirm that the chamber has been flushed (blue circle).

Start the Laser

The laser is automatically started when the system is started. To conserve the laser between acquisitions without stopping plasma, click Stop Laser. The chamber begins flushing and the button toggles to Start Laser.

1 In the Toolbar click **Start Laser** to turn on the laser power. Check the HTI Status Panel to confirm that the laser is on (blue circle).

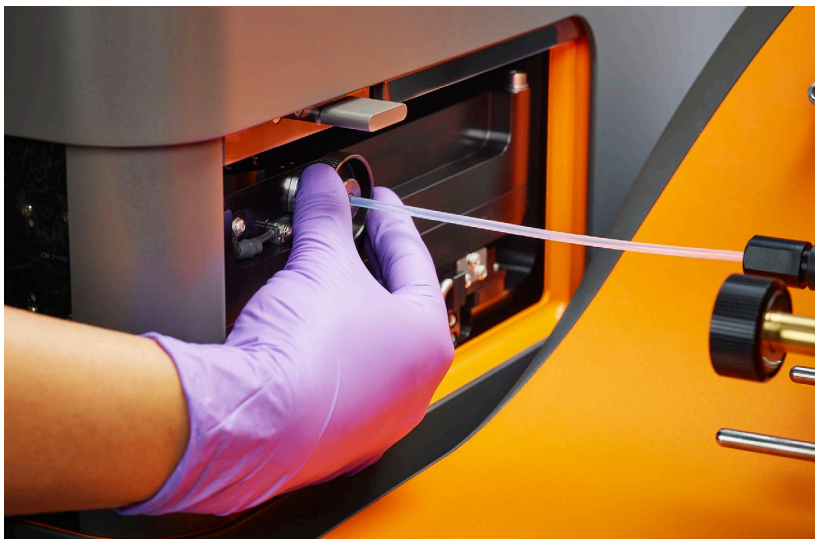
NOTE See CyTOF Software v7.0 Help for procedures related to instrument control, tuning, and data acquisition.

Disconnect the Hyperion or Hyperion+ Tissue Imager from Helios

Disconnect Cables and Coupling Tube from HTI That Does Not Require a Wheel Retraction Access Tool

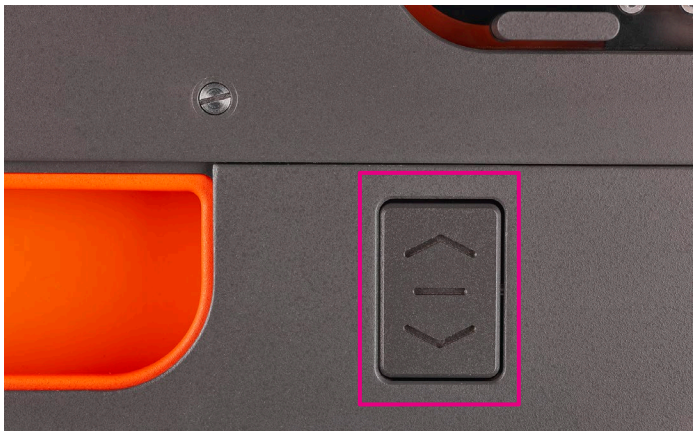
1 Disconnect communication cables and gas lines including USB 2.0 connection, USB 3.0 connection, trigger, argon, and helium or helium/hydrogen gas mixture gas lines.

2 On the Hyperion Tissue Imager ablation chamber, loosen the black nut on the coupling tube. Disconnect the coupling interlock.



3 Using the wheel retraction switch on the right side of the instrument, raise the instrument.

NOTE The wheel retraction switch is disabled when the coupling tube is connected to the ablation chamber.



4 Turn off the radio frequency (RF) generator circuit breaker on the right side of the Helios instrument. Disconnect the AC power cord from the AUXILIARY connection panel.

5 Carefully roll the Hyperion Tissue Imager to a safe area in the laboratory using the instrument castors.

6 Reconnect the instrument power to a wall receptacle and use the instrument wheel retraction switch to lower the instrument and secure it in place.

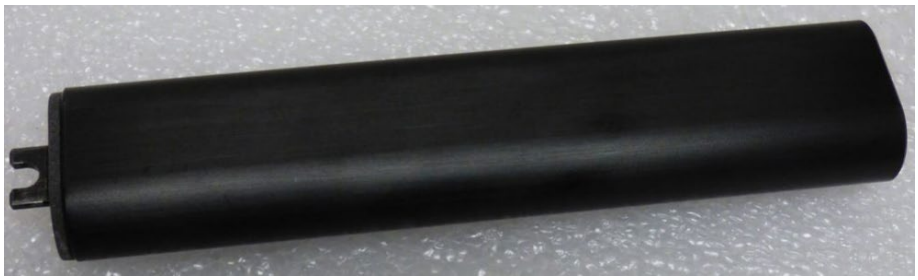
Disconnect Cables and Coupling Tube from HTI or HTI+ that Requires a Wheel Retraction Switch Access Tool

1 Disconnect communication cables and gas lines including USB 2.0 connection, USB 3.0 connection, trigger, argon, and helium or helium/hydrogen gas mixture gas lines.

2 On the Hyperion or Hyperion+ Tissue Imager coupling window, loosen the black nut on the coupling tube. Disconnect the coupling interlock.

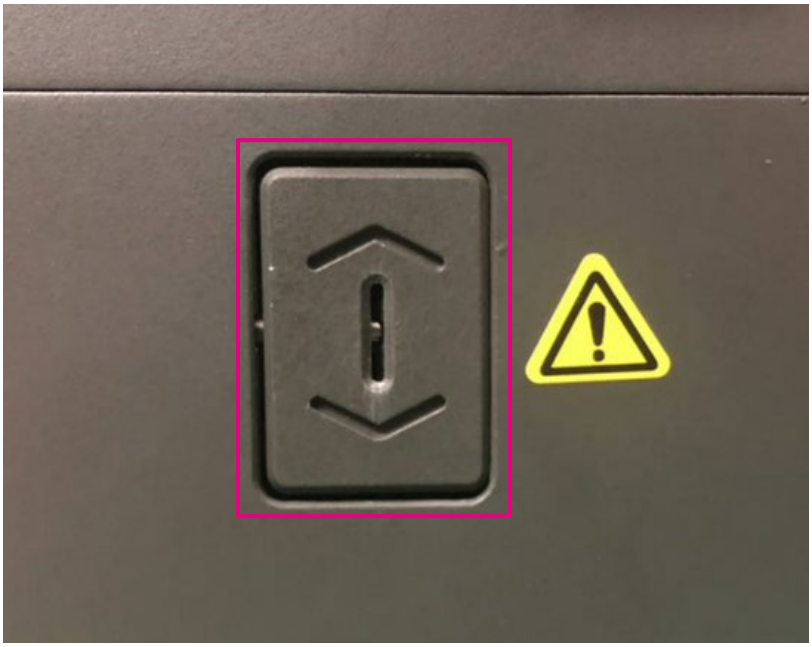
3 Raise the instrument using the wheel retraction switch access tool so that the castors are exposed for movement.

IMPORTANT The access tool unlocks the wheel retraction switch. Contact the designated person in your lab for access to the wheel retraction switch access tool.



NOTE The wheel retraction switch is disabled when the coupling tube is connected to the ablation chamber.

a Locate the wheel retraction switch located on the right side of the HTI or HTI+ instrument.



b Insert 1 of the access tools into the slot of the wheel retraction switch.

c Push the access tool up such that the top of the wheel retraction switch tilts inside the instrument frame. The wheel retraction switch unlocks, allowing the instrument to raise for movement.

4 Turn off the radio frequency (RF) generator circuit breaker on the right side of the Helios instrument. Disconnect the AC power cord from the AUXILIARY connection panel.

5 Carefully roll the Hyperion or Hyperion+ Tissue Imager to a safe area in the laboratory using the instrument castors.

6 Reconnect the instrument power to a wall receptacle. Insert the access tool into the instrument wheel retraction switch. Push down on the access tool such that the bottom of the wheel retraction switch tilts inside the instrument frame. The wheel retraction switch unlocks allowing the instrument to be lowered and secured in place.

Switch to Cell Suspension Mode

IMPORTANT Ensure that the nebulizer, torch, injector, spray chamber, cones, and load coil on the Helios system are clean and correctly installed, and aligned on the instrument. Check the condition of the interface pump oil in the visual inspection window of the Helios instrument. Refer to the Helios, a CyTOF System User Guide, Chapter 5: Maintenance (400250) for more details.

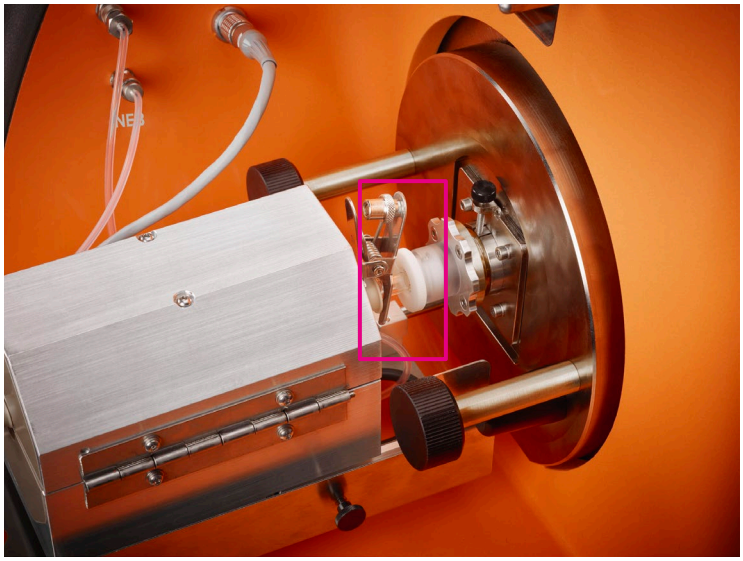
1 Disconnect the coupling tube and move the Hyperion or Hyperion+ Tissue Imager out of the way to access the injector.

2 Gently twist and pull the injector from the torch assembly.

3 Insert the HT Injector (107018) into the torch body and push in until it can go no farther.

4 Reconnect the heater box to the torch assembly. Slide the heater box onto the support pins toward the torch assembly so that the spray chamber ball joint connection meets the injector.

5 Connect the ball joint clamp to secure the spray chamber to the injector.



6 Place the heat shield onto the heater and tighten the 4 thumbscrews, 2 on each side of the shield.

Figure 9. The heat shield on the Helios heater box. There are 4 screws, 2 on each side of the shield, that must be tightened before replacing the shield.

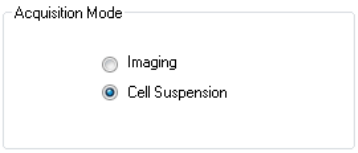
7 Connect the heater connection to the front face of the instrument.

8 Start CyTOF Software. Log on with your user name and password.

9 Set the Acquisition Mode to Cell Suspension.

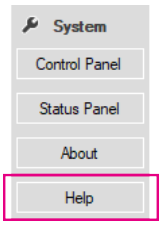
a On the instrument Control tab, click **control Panel**.

b On control Panel, click the **devices** tab, and under acquisition Mode, click **cell Suspension**.



c On **control Panel**, click **set Configuration**. CyTOF Software restarts and the log on dialog box is displayed. Enter your account credentials and click **log on**. The software opens in Cell Suspension mode.

IMPORTANT Helios is now ready to run in cell suspension mode for mass cytometry analysis. Refer to the software Help for information about tuning, acquisition, and instrument control in cell suspension mode. Help is started from the side bar, under **system**, click **help**.



IMPORTANT Run a Full Tuning Protocol before running the instrument in solution mode. In Tuning Manager select Full Protocol from the drop-down.

Chapter 4: Maintenance

The Hyperion™ and Hyperion+™ Imaging Systems are very simple to maintain. You may be required to clean the outside of the instrument, but all internal components including the laser ablation chamber and the cover glass are addressed during preventive maintenance every 6 months.

Cleaning Instructions

Table 16. Cleaning frequency and reagents required for maintenance and cleaning of the Hyperion and Hyperion+ Imaging Systems

Parts	Frequency	Performed By	Agents/Equipment	Company and Part Number
Injector	As required	Operator	10% Contrad® 100 in DIW Glassware brushes	Decon Labs
Torch	As required	Operator	10% Contrad 100 in DIW Glassware brushes	Decon Labs
Skimmer-Reducer	Preventive maintenance	Field Service Engineer		
Sampler cone	Preventive maintenance	Field Service engineer		
Coupling tube	Preventive maintenance	Field Service Engineer		

NOTE For cleaning frequency in solution mode refer to the Helios, a CyTOF® System User Guide (400250), Chapter 5: Maintenance. It is recommended that you clean glassware and instrument cones weekly if you are running the Helios™ instrument in solution mode.

Table 17. Equipment required for maintenance and cleaning of the Hyperion and Hyperion+ Imaging Systems

Parts	Equipment	Company	Product Name	Part Number
Torch	Glassware brushes	Restek®	Nylon Tube Brushes and Pipe Cleaner	20108
Injector	Glassware brush	Gordon Brush	Nylon 0.030 Miniature Single-Spiral brushes with a stainless steel stem and a cut end	01023

Exterior Surfaces

NOTE This procedure may only be done when the Hyperion or Hyperion+ Tissue Imager has been disconnected from Helios and moved out of the way.

1. Disconnect the Hyperion or Hyperion+ Imaging System from the power supply.
2. Wipe the instrument exterior surfaces only using a towel dampened with a lab-grade cleaning agent (for example, isopropanol).
3. Repeat Step 2, using a towel dampened with deionized water.
4. Dry the instrument exterior using a dry towel.

Injector Cleaning

1. Soak the injector in 10% Contrad for up to 1 hr.
2. Scrub the injector with the recommended glassware brush (Gordon Brush, 01023).
3. Insert the brush into the tip of the injector and pull it in and out a few times to dislodge any buildup around the tip.
4. Rinse thoroughly with DIW.
5. Spray isopropanol on the injector and air-dry.
6. Alternatively, completely dry the injector with a blow dryer or heat gun.

   NOTE Ensure that the parts are completely dry before reinstalling.

Torch Cleaning

See the Helios, a CyTOF System User Guide (400250) for procedure on torch assembly, removal, cleaning, and for reassembly of the injector and torch.

1. Soak in the torch in 10% Contrad for up to 1 hr.
2. Scrub the parts with the recommended glassware brush (Restek, 20108).
3. Rinse thoroughly with DIW.
4. Spray isopropanol on the torch and air-dry.
5. Alternatively, completely dry the glassware with a blow dryer or heat gun.

   NOTE Ensure that the parts are completely dry before reinstalling.

Coupling Tube

IMPORTANT We do not recommend cleaning of the coupling tube. Inspect the tube periodically to ensure that there are no breaks or cracks. It is recommended to apply compressed air on the connectors of the coupling tube to remove the buildup of dust periodically. Replace the Coupling Tube Kit (108480) if necessary.

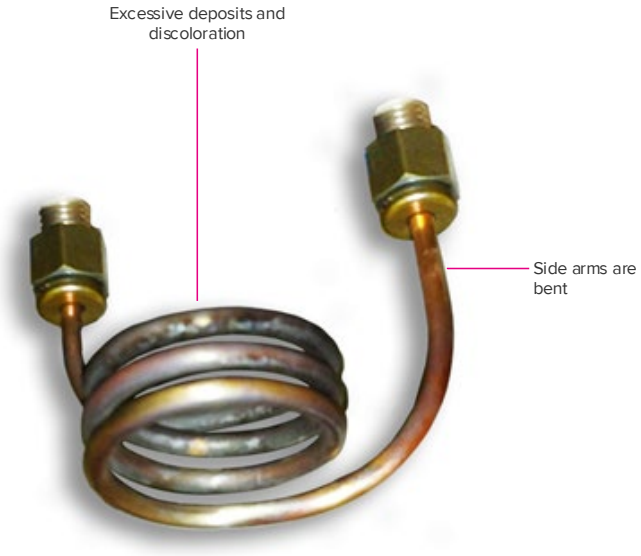
Periodic Maintenance

Vacuum Interface Cones

The vacuum interface cones are inspected and cleaned if necessary during the preventive maintenance of the instrument. If running solution mode, clean the skimmer-reducer and sampler cone weekly. See the Helios, a CyTOF System User Guide (400250) for the procedure to remove, clean, and reinstall the vacuum interface cones.

Replace the Load Coil

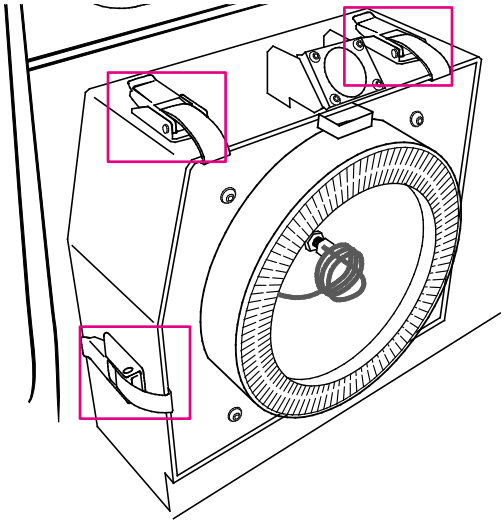
If the load coil (105398) is damaged or misshapen, it must be replaced. Inspect the load coil regularly to ensure that it is in good condition.



WARNING HOT SURFACE HAZARD. The torch components, the vacuum interface, and the sample introduction system components remain hot for some time after the plasma has been shut off. Allow sufficient time for these items to cool to room temperature before you handle them.

1. Disconnect the coupling tube and move the Hyperion or Hyperion+ Tissue Imager out of the way to access the injector.
2. Gently twist and pull the injector from the torch assembly.
3. Remove the torch assembly from the front face of the instrument.

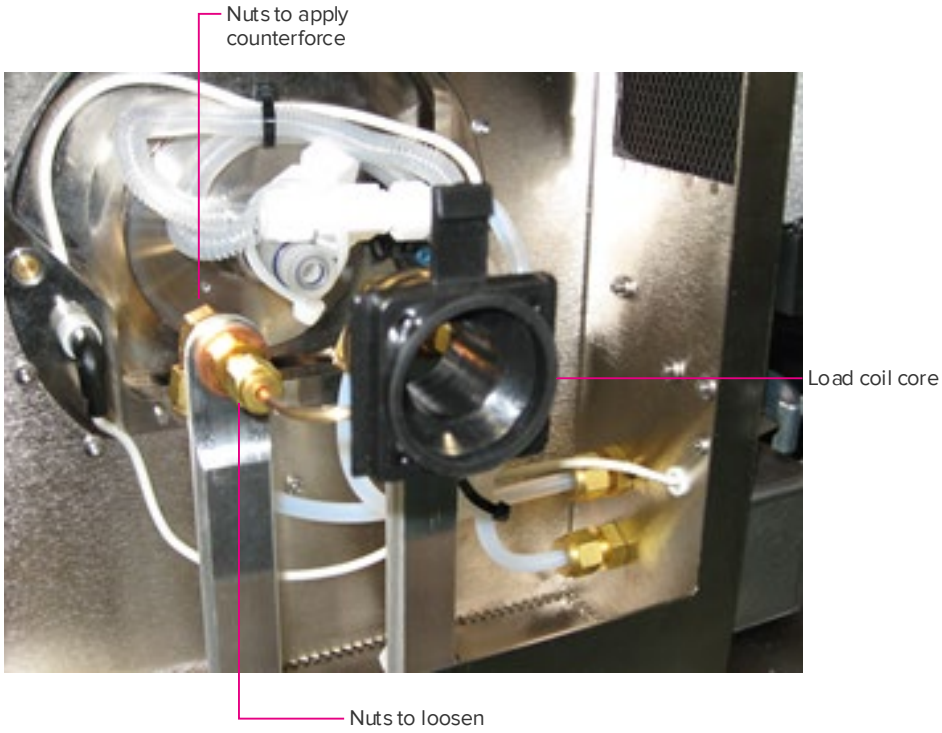
4 Open the front access door. Undo the 4 clips on the top and both sides of the front shield and lift off (magenta boxes below).



5 Using a 7/16 wrench loosen the nut holding the load coil. Use a 9/16 wrench simultaneously to apply counterforce on the larger nut. Repeat on the opposite side. Remove the old load coil.

6 Carefully remove the zip ties on the new load coil using a wire cutter. Remove the base of the load coil holder but keep the load coil core in place.

7 Install the new load coil using the 7/16 wrench to tighten the nuts and washers while applying counterforce on the larger nuts with the 9/16 wrench.



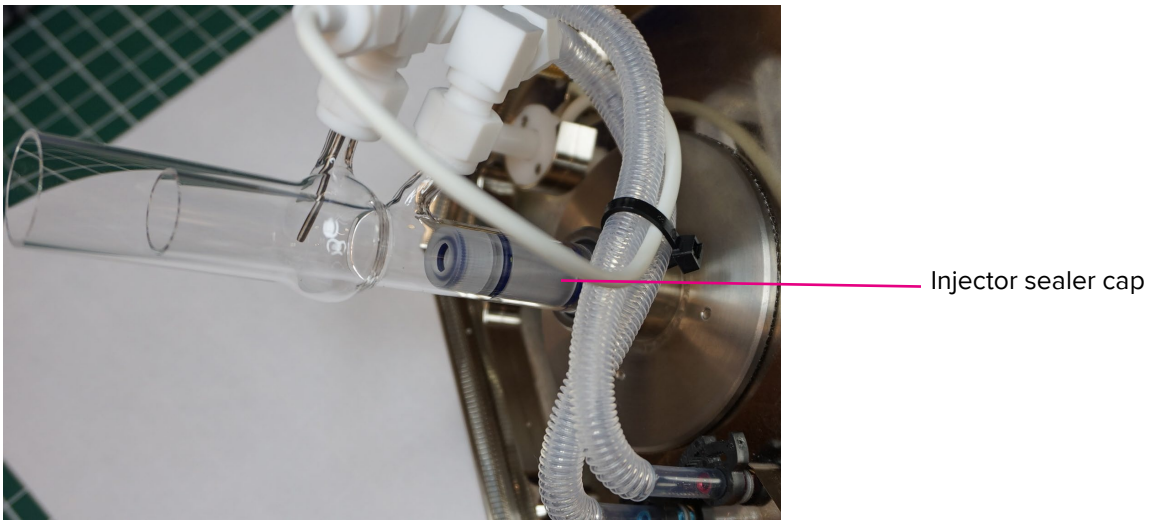
8 Remove the load coil core. Examine the new load coil and make sure that the coils are equally spaced. Also ensure that the arms of the coil are straight and aligned.

9 Replace the front shield and fasten the 4 clips.

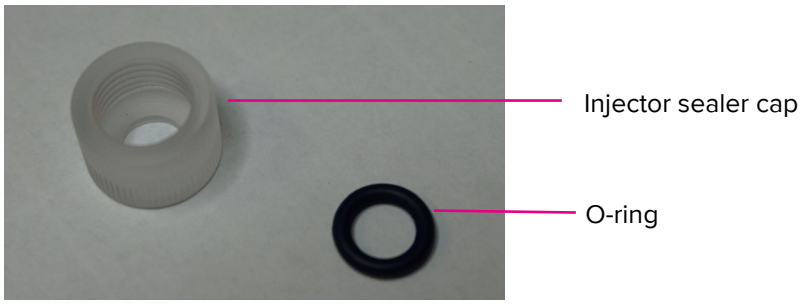
10 Reinstall the torch assembly. Check the alignment of the torch with the load coil (refer to the appropriate steps in the cleaning and maintenance section if necessary). See the Helios, a CyTOF System User Guide (400250) for the Z-alignment adjustment procedure.

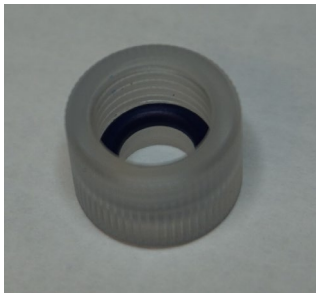
Replace the Injector Sealer Cap

Replace the Injector Sealer Cap if required (105350). You also need an O-ring, which is included in the O-Ring Kit, Torch Body (105641).



1 Insert the O-ring into the cap. Ensure that the O-ring is firmly seated in the inner cap and the center opening is unobstructed.





2 Loosely screw on the new injector sealer cap (with the O-ring) to the injector holder.

3 Carefully insert the injector into the injector holder. Pull the injector in and out to confirm that there is sufficient tension on the injector but that you are still able to insert and remove it.

WARNING Finger cut hazard. Broken glass may cause injury or cutting of fingers or hands.

4 Ensure that the cap is finger tight. Do not overtighten.

5 Remove the injector and place it aside.

Change the Interface Pump Oil

Before beginning the procedure have on hand:

- Funnel with extension tubing
- Flexible drain funnel
- Vacuum Pump Oil (101810)

1. Switch off the RF generator power using the RFG circuit breaker on the right side of the instrument.
2. Open the front access door using the door handle. Pull the spring pin and open the lower instrument door.
3. Open the lower right door of the instrument. The interface pump is on the right side of the instrument.

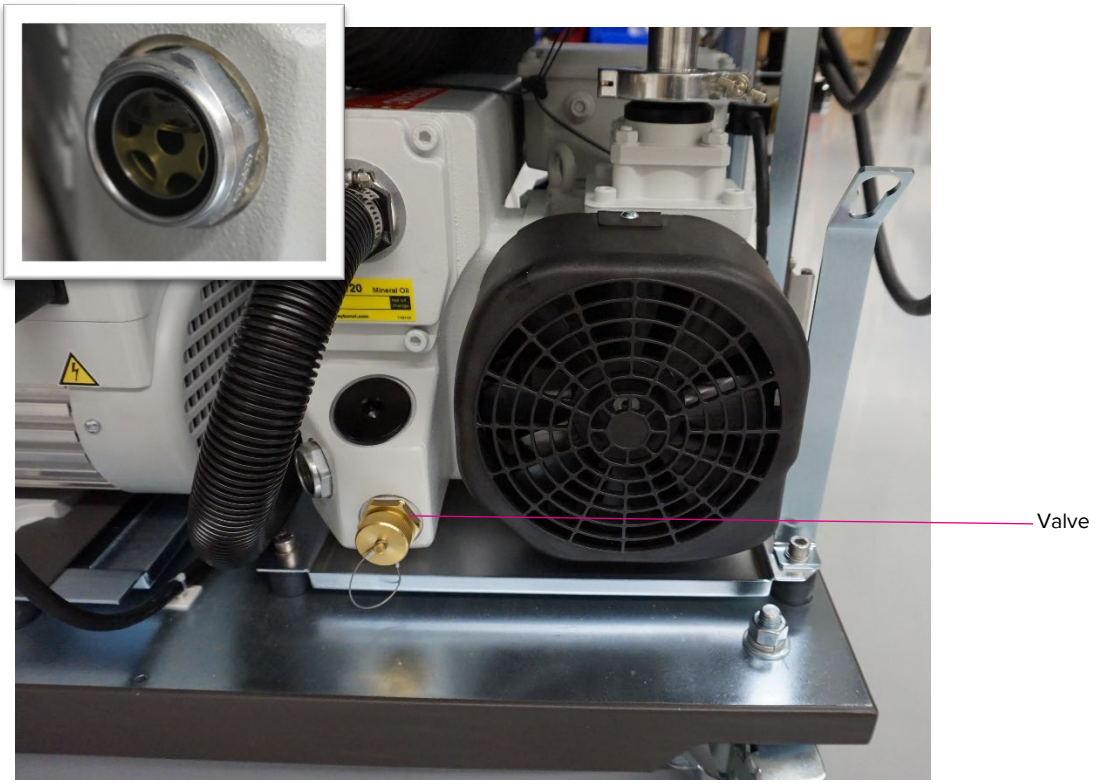


Figure 10. The interface pump in the lower right compartment of the Helios system. The visual inspection window is on the side of the interface pump (inset).

4. The oil level in the interface pump should be approximately ¾ full according to the Min and Max lines on the visual inspection window.

5 Verify the condition of the oil using the oil inspection chart. The oil should be below Level 4 as indicated in the pump oil condition chart.

IMPORTANT The interface oil condition should be checked weekly.

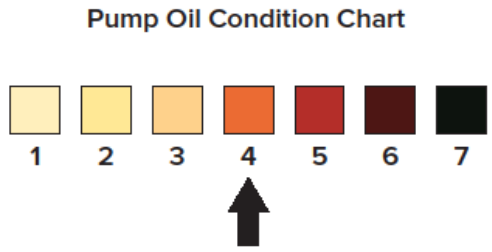
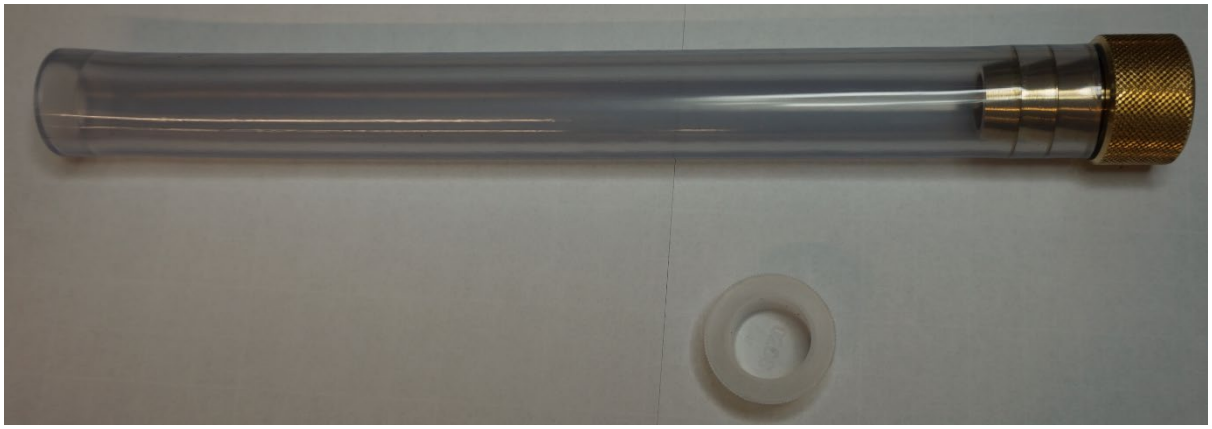
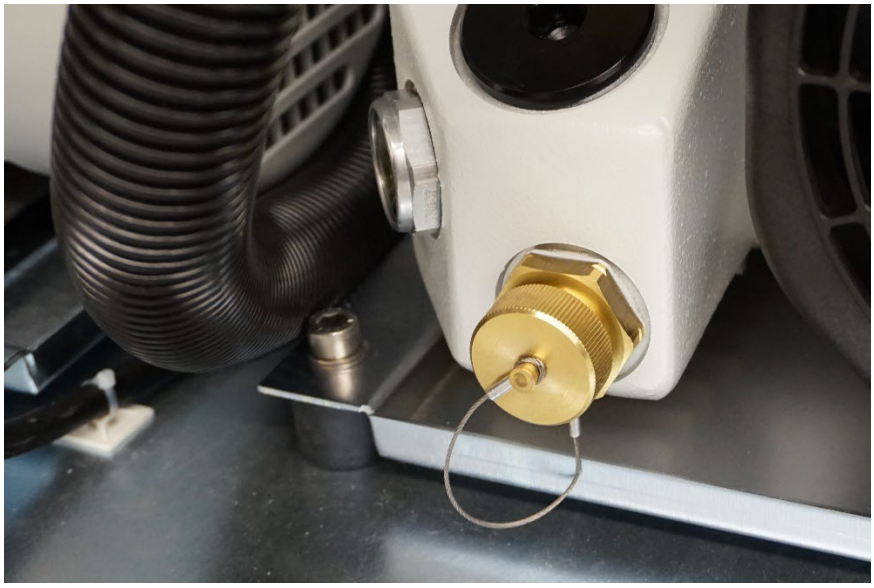


Figure 11. Pump oil condition chart. When the oil color is above Level 4 (as indicated by the black arrow), the oil should be replaced in the interface pump.

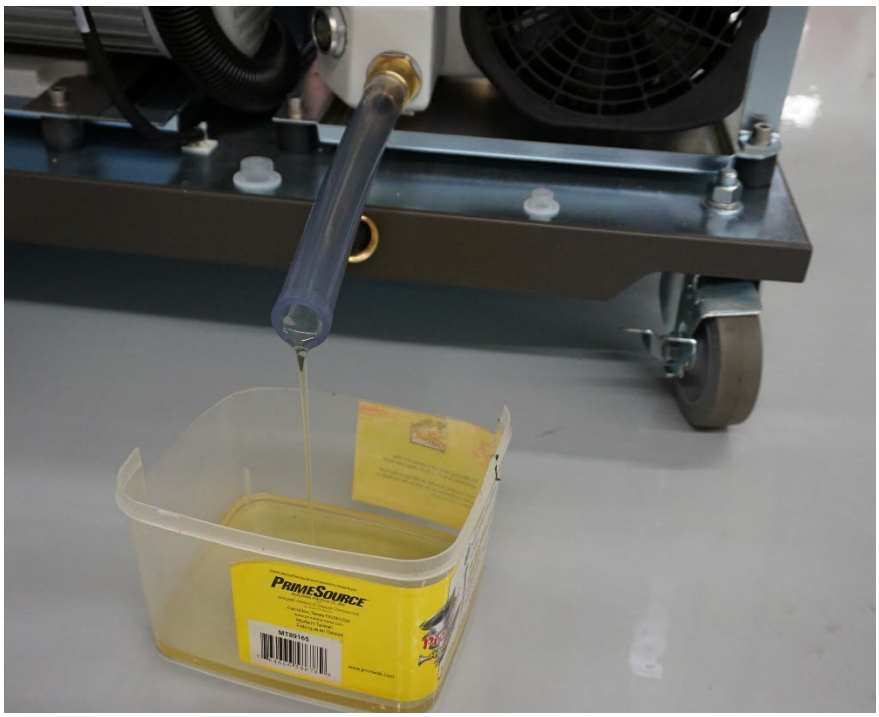
6 Remove the 2 white caps on each end of the Drain Kit (107125).



7 Unscrew the gold valve on the interface pump, and connect the Drain Kit provided.



8 Drain the oil into a tray or plastic container.



9 Remove the drainage tube and reconnect the gold valve.

10 Unscrew the top cap, using the Allen key provided.

11 If the oil is very dirty, add 100 mL of fresh Vacuum Pump Oil (101810) to allow the oil to drain freely, and then drain using the drainage tubing before proceeding to the next step.

12 Using the funnel and drain tube provided, fill the oil slowly until it reaches ¾ full by viewing the oil inspection window.

13 Replace the top cap and tighten with the Allen key. Do not overtighten.

14 Close the lower left and right instrument doors and the front access door of the Helios system.

NOTE Dispose of the oil in accordance with applicable local, regional, and national regulations.

Change the Oil on the Backing Pump

NOTE The backing pump oil is changed annually by the Fluidigm Field Service Engineer (FSE) as part of the preventive maintenance of the instrument. Contact Fluidigm technical support if you believe the oil in the backing pump requires changing.ng

Appendix A: Troubleshooting

IMPORTANT If the symptoms below are not resolved using the information in the table, contact Fluidigm Support.

Table 18. Troubleshooting tips for the Hyperion™ and Hyperion+™ Imaging Systems

Symptom	Possible Cause	Recommended Solution
Instrument Initialization Errors		
Laser not turning on in software.	Instrument not connected to power source.	Click Start Laser in the software.
	Low helium or helium/hydrogen gas mixture supply pressure	Check helium or helium/hydrogen gas mixture pressure and change if helium or helium/hydrogen gas mixture tank if required.
High chamber pressure reading in the Status Panel window (>2 psi)	Blocked injector	Use the recommended brush to clean the injector. If pressure remains high contact Fluidigm Support.
Stage does not move or moves erratically.	XY start position not set correctly.	Click **Camera** > **Action** > **Home XY Stage**. If this does not resolve the issue you may need to power-cycle the instrument. Shut down the software and then turn off the power on the laser to re-establish correct communication.
Ablation quality poor, blurry image, no ions detected	Slide may be inserted incorrectly.	Ensure that the sample is facing up.

Symptom	Possible Cause	Recommended Solution
Coupling Tube Connection Errors		
Lu(175) mean duals <500 (during tuning)	Coupling tube not well-seated into the ablation chamber or into the injector.	Verify coupling tube connections to the injector and the ablation chamber.
	Coupling tube fitting and nuts are too tight.	Check that coupling tube is correctly connected. Nuts should be finger tight.
	Coupling tube not correctly connected.	Reinstall coupling tube.
	The injector/fitting may be pulled out slightly because the tube is under tension.	Check that the injector and fitting are correctly installed. The coupling tube should be as straight as possible.
No ions in the Masses per Reading window	Coupling tube not correctly connected.	Check that coupling tube is correctly connected. Nuts should be finger tight.
	Blocked coupling tube or blockage in the ablation chamber lid.	Flush argon through the system by adjusting the makeup gas flow to 1.0 L/min in the **Control Panel** > **HTI Setup** > **Chamber/Gases** > **Flushing Makeup gas flow.** Flush for 1 min.
	Helios™ XY has not been tuned correctly.	In the CyTOF® Software click AutoTune and click New. In the General tab, under Subcalibrations, check Coarse XY Optimization, Fine XY Optimization, QC Report, Enable HTI™ Current/Gases Calibration, and Transients Calibration.
	Detector voltage is incorrectly set.	Manually adjust detector voltage in the software. Refer to the CyTOF Software v7.0 Help.

Symptom	Possible Cause	Recommended Solution
Wide transient width.	O-ring seals on coupling tube connections not correctly made.	Reinstall coupling tube. In the HTI Status Panel check the chamber pressure and the helium or helium/hydrogen gas mixture flow.
Poor signal	The injector and fitting may be pulled out a bit because the tube is under tension.	Check that injector and fitting are correctly installed. The tube should be as straight as possible.
Laser Not Firing		
Laser not firing	Laser interlock tripped	Turn the laser off and then on in the software. Contact Fluidigm Support.
Tuning Errors		
No ion signals	Leak in chamber	Check for gas leaks. Tighten fittings and nuts on the coupling tube.
	Gases are set incorrectly.	Makeup gas should be approximately 0.8–1.0 L/min and helium or helium/hydrogen gas mixture should be approximately 0.15–0.3 L/min. These values vary depending on the specific Hyperion and Hyperion+ Imaging Systems. Contact Fluidigm Support.
	Ablation chamber door may be open.	Close the door. Flush the gases again.
	Ablation chamber cover not properly secured.	Contact Fluidigm Support.
Lu(175) mean dual counts <500 during tuning	Small fiber on 1 of the ends of or inside of the coupling tube, causing turbulent gas flow.	Disconnect coupling tube. Inspect with magnifying loupe and remove any fibers found.
	Injector not inserted correctly.	Check that the injector is firmly seated in the torch assembly, but also that the injector sealer cap is tightly secured.

Symptom	Possible Cause	Recommended Solution
	AutoDV optimization performed with abnormally high Xe131 (or alternate isotope) levels.	Identify sources of leak and resolve. Check the torch for correct seal. Check coupling tube for damage. If the issue persists contact Fluidigm Support.
Lower-than-expected ion signals	XY position on Helios is not optimized.	Perform Pre-Calibration XY Optimization and Fine XY Optimization (see Auto Tuning).
	Makeup gas setting not optimized.	Optimize the makeup gas settings for better sensitivity. In the Data Acquisition Settings window select **Makeup Gas** in the drop-down menu and perform transient analysis.
	Current setting not optimized.	Optimize the Gases/Current settings for better sensitivity. In the Data Acquisition Settings window select **Current** in the drop-down menu and perform transient analysis.
Transient width not within acceptable range.	Helium or helium/hydrogen gas mixture flow is not optimum.	Check that the helium or helium/hydrogen gas mixture gas line is connected correctly to the communication panel of the Hyperion or Hyperion+ Tissue Imager. Check that the gas cylinder is open and the gas line is connected correctly to the cylinder.
	Incorrect height of ablation stage.	Contact Fluidigm Support.
Low mass peak means and signals.	Sample not fully ablated.	Select another region to ablate and test the efficiency. If the ablation is still not clean, power cycle the instrument.
High CVs (>50%) during tuning procedures. Multiple peaks in Plotviewer.	High or low gain channel cables may be switched on the Helios preamplifier.	The gain channel cables are located on the right side of Helios on the AC panel. The Low Out 1 and the High Out 2 cables may be switched.
Acquition Errors		
Ablation is occurring, but there is no or low ion signal.	Gas lines not connected.	Check helium or helium/hydrogen gas mixture and argon gas line connections.

Symptom	Possible Cause	Recommended Solution
	Coupling tube not connected or leaking.	Check coupling tube, and check that the connections are tight.
	Incorrect Ablation Power settings.	Determine the most efficient ablation energy for the sample. Change the Ablation Power settings.
No ablation on the slide.	Slide may have been inserted upside down.	Check that the slide has been inserted correctly. Contact Fluidigm Support.
No ablation on the slide.	Ablation energy has been set too low.	Increase the Ablation energy in the Acquisition window.
Slow/irregular stage movement during ablation.		Check that the slide size is compatible with the Hyperion and Hyperion+ Imaging Systems. Power-cycle the instrument and try ablating a new area on the slide.
Other		
Streaking in TIFF images	Vibrations in the system	Check that the Hyperion or Hyperion+ Tissue Imager has been fully lowered to the ground. Check that the Helios instrument castors have been locked to minimize vibrations. Contact Fluidigm Support.
Uneven spacing of marks on the glass slide	Vibrations in the system	Check that the Hyperion or Hyperion+ Tissue Imager has been fully lowered to the ground. Check that the Helios instrument castors have been locked to minimize the vibrations. If this continues to occur, contact Fluidigm Support.
Laser produces a thin/wavy ablation pattern.	Laser has not sufficiently warmed up.	Allow 10 min for laser to warm up.

Appendix B: Decontamination

Decontamination of the Hyperion and Hyperion+ Imaging Systems

Biological Agents

Using a soft cloth, apply 5% bleach solution to all accessible surfaces of the Hyperion™ or Hyperion+™ Imaging System.

- Keep surfaces wet for at least 5 min, then wipe dry.
- Repeat Steps 1 and 2 once.
- Clean all decontaminated surfaces with a wet cloth to remove residual alcohol and wipe dry.

Hazardous Chemicals

Using a soft cloth, apply 70% ethyl alcohol or 70% isopropyl alcohol to all accessible surfaces.

IMPORTANT Before use, ensure that alcohol is compatible with the chemicals used.

- Keep surfaces wet for at least 5 min, then wipe dry.
- Repeat Steps 1 and 2 once.
- Clean all decontaminated surfaces with a wet cloth to remove residual alcohol and wipe dry.

Radioactive Materials

Using a soft cloth, apply an industry standard radioactivity decontaminant to all accessible surfaces.

- Wipe the surfaces as directed by the decontaminant manufacturer.
- Survey the instrument with an appropriate radioactivity measuring device.
- Ensure that the survey results are at or below background level.

Disposal of Products

Used slides and reagents should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

Do not dispose of this product in unsorted municipal waste. This equipment may contain hazardous substances that could affect health and the environment. Use appropriate take-back systems when disposing of materials and equipment.

Learn more at fluidigm.com/compliance.

Appendix C: Related Documents

Table 19. Go to fluidigm.com to download these related documents.

Title	Document Number
Helios, a CyTOF System User Guide	400250
CyTOF System Site Requirements Guide	400252

Appendix D: Revision History

Table 20. Table History

Revision	Date	Description of change
[06]	03/2022	**1** Include the procedure for Hyperion+ Imaging System setup and use. **2** Include the procedure describing how to use the Wheel Retraction Switch Access Tool.

Appendix E: Safety

IMPORTANT For translations of the system safety information, see Safety Information for Mass Cytometry Systems (400319).

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Laser instruments generate potentially hazardous UV radiation. Do not remove the top cover of the Hyperion™ or Hyperion+™ Tissue Imager. Only a Fluidigm Field Service Engineer should remove the top cover and perform maintenance.
- Inductively coupled plasma-based systems generate high levels of radio frequency (RF) energy within the RF power supply and the torch box. RF energy is potentially hazardous if allowed to escape. Do not bypass or disconnect safety devices and safety interlocks.
- The system power supplies are capable of generating potentially lethal voltages and currents. Store the removable system handle separately from the system. Maintenance should be performed only by a Fluidigm Field Service Engineer or by maintenance personnel, employed by the customer, who have been trained by Fluidigm and are appropriately certified.
- Do not remove the side panel on the electrical box of the Hyperion or Hyperion+ Tissue Imager. Only a Fluidigm Field Service Engineer should remove the side panel and maintain the electrical box.
- Use the appropriate personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves, according to your laboratory safety practices.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Laser Safety for the Hyperion and Hyperion+ Tissue Imagers

The Hyperion and Hyperion+ Tissue Imagers are solid-state pulsed lasers classified as Class 1 laser systems. They comply with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007.

They comply with 21 CFR 1040.10 and 1040.11 except for conformance with IEC 60825-1 Ed. 3 and IEC 60601-2-22 Ed. 3.1, as described in Laser Notice No. 56, dated May 8, 2019.

Instrument Safety

The system should be serviced by authorized personnel only.

WARNING Do not modify this instrument or system. Unauthorized modifications may create a safety hazard.

WARNING BIOHAZARD. If you are putting biohazardous material on the instrument or system, use appropriate personal protective equipment and adhere to Biosafety in Microbiological and Biomedical Laboratories (BMBL), a publication from the Centers for Disease Control and Prevention, and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines online at cdc.gov/biosafety/publications/index.htm.

WARNING TIPPING HAZARD. The Hyperion and Hyperion+ Tissue Imagers have a high center of gravity, and therefore there is risk of tipping when moving the instrument or system.

WARNING HOT SURFACE HAZARD. A safety interlock on the CyTOF® 2 and Helios™ systems automatically shuts off the plasma if the chamber and interface are not fully coupled. Do not defeat the interlock. Do not remove the shield that protects the sample introduction system. The heat shield is designed to protect users from burns from the heater.

WARNING HOT SURFACE HAZARD. The torch components, the vacuum interface, and the sample introduction system components remain hot for some time after the plasma has been shut off. Allow sufficient time for these items to cool to room temperature before you handle them.

WARNING PHYSICAL INJURY HAZARD. When installing or moving the instrument or system, contact a Fluidigm Field Service Engineer for assistance. See the user guide for the weight of the boxed or crated instrument or system.

Symbols on the Instrument

The following table describes the hazard symbols that may be used in this document or on labels on the system.

Symbol	Description
	Hazard. Consult the user guide for further information.
	Hot surface hazard. Do not touch; potential for personal injury.
	Biohazard
	Electricity hazard. Indicates high electricity levels and a threat of electric shock from machines and/or equipment in the vicinity. You may suffer severe injuries or death.
	Pinch hazard. Indicates where pinch hazards exist. Exercise caution when operating around these areas.
	Lifting hazard
	Indicates rotating blades can crush or cut fingers or hands. Keep hands clear.
	Laser hazard. Indicates the presence of a laser.
	Finger cut hazard. Broken glass may cause injury or cutting of fingers or hands. Caution when loading and unloading the sample slides.
	Non-ionizing radiation hazard. Exposure to high-frequency radio waves and radio frequency radiation can result in injuries.
	Tipping hazard. Movement or impact with the instrument or system may cause tipping.
	Trip hazard. Watch your step to avoid falling over objects.
	Indicates specific chemical harm
	Indicates hazardous, toxic, or very toxic materials that are very hazardous to health or potentially fatal when inhaled, swallowed, or in contact with the skin
	Indicates caustic and acid materials that can destroy the skin and eat through metals

Symbol	Description
	Indicates the presence of material contained under pressure, including compressed gas, dissolved gas, or gas liquefied by compression or refrigeration
	A compressed gas cylinder can become a projectile when ruptured, with the potential to cause significant damage.
	Indicates a health hazard
	Power and standby symbol
	Power switch is in the Off position.
	Power switch is in the On position.
	Protective conductor terminal (main ground). It must be connected to earth ground before any other electrical connections are made to the instrument or system.
	To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision. Contact customer service for information about responsible disposal options.

Electrical Safety

WARNING ELECTRICAL HAZARD. DO NOT REMOVE THE COVERS. Electrical shock can result if the system is operated without its protective covers. No internal components under the covers are serviceable by the user.

WARNING ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

WARNING Lethal voltages are present at certain areas within the system. Only a Fluidigm Field Service Engineer or those similarly authorized and trained by Fluidigm personnel should install or repair the system.

WARNING The interface and backing pumps in the system are in close proximity to areas where high voltages are present. User access to the pumps is not advised. Only Helios operators trained by Fluidigm may access the pump compartment and change the interface pump oil. Disengage the radio frequency generator circuit breaker on the right side of the system before accessing this area.

WARNING Do not touch electrical wires, contacts, transformers, or transformer components during the oil inspection procedure (see the user guide). A metal shield located in the system compartment above the interface pump contains the transformers and electrical wires. There is no need to access this section when servicing the pumps. When the system is connected to line power, opening system covers is likely to expose live electrical parts. High voltage can still be present even when the radio frequency generator power switch is off. Water lines should

be located away from electrical connections. Condensation and potential leaks may create an unsafe environment in the proximity of electrical connections.

WARNING Before performing maintenance on the cones or torch, switch off the radio frequency generator power using the circuit breaker at the right rear of the system. Wait at least 5 min for residual electrical charge to dissipate. Additional time is required to allow the inductively coupled plasma torch, cones, and load coil to reach room temperature. Capacitors inside the system may still be charged even if the system has been disconnected from all voltage sources. The system must be correctly connected to a suitable electrical supply (see the site requirements guide for further details). The power supply must have a correctly installed protective conductor (earth ground) and must be installed or checked by a qualified electrician before connecting the system.

WARNING Any interruption of the protective conductor (earth ground) inside or outside the system or disconnection of the protective conductor terminal is likely to make the system dangerous. Do not operate the system with any covers or internal parts removed. Do not attempt to perform internal adjustments or replacements except as directed in this user guide.

Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that system operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier. When handling any chemical, the following safe-handling guidelines should be strictly observed:

- Do not inhale fumes from chemicals. Use adequate ventilation and return caps to bottles immediately after use.
- Use, store, and dispose of chemicals according to manufacturer recommendations and to regulations applicable to the locality, state, province, and/or country.
- When preparing chemical solutions, always work in a fume hood that is suitable for those chemicals.
- Conduct sample preparation away from the system to minimize corrosion and contamination.
- Store solvents in an approved cabinet (with the appropriate ventilation) away from the system.

Laboratory Ventilation Safety

Toxic combustion products, metal vapor, and ozone can be generated by the system, depending on the type of analysis. An efficient ventilation system must be provided for your system. When the plasma is on, hot gases are vented through 2 exhaust vents at the back of

the system. Detailed information on exhaust vents is included in the site requirements guide.

WARNING Use of the instrument or system without adequate ventilation to outside air may constitute a health hazard. Take extreme care to vent exhaust gases properly.

WARNING The instrument or system is designed for analysis of fixed/permeabilized, non-live cells only. Under normal operation, cells are completely combusted in the inductively coupled plasma. High levels of UV radiation inside the torch box are significantly above lethal levels for most single airborne cells. However, in the event of plasma shutdown, the non-ionized portion of a sample can enter the torch box exhaust gases.

Pressurized Gas Safety

Safe Handling of Gas Cylinders

Argon gas used with the system is normally stored in liquid argon tanks or pressurized containers. Carefully use, store, and handle compressed gases in cylinders. Gas cylinders can be hazardous if they are mishandled. Argon is neither explosive nor combustible.

Helium gas is supplied in the non-liquefied or liquid form in a compressed gas cylinder for use with the Hyperion Imaging System.

Helium/hydrogen gas mixture is stored in a compressed gas cylinder for use with the Hyperion+ Imaging System.

Contact the gas supplier for a safety data sheet containing detailed information on the potential hazards associated with the gas.

IMPORTANT If liquid argon or liquid helium or helium/hydrogen gas mixture is used, the gas cylinder must be fitted with an overpressure regulator, which vents the cylinder as necessary to prevent it from becoming a safety hazard.

WARNING Do not use electronic pressure regulator and auto switching valves because doing so may affect the plasma stability and may result in frequent loss of plasma.

WARNING It is recommended to install an oxygen sensor in the room where the operator and gas storage are located.

Sample Handling and Preparation Safety

WARNING For better control of contamination, dedicate laboratory reagents and consumables to use with CyTOF instruments and Maxpar® reagents only.

Radio Frequency Radiation Safety

WARNING RADIO FREQUENCY RADIATION. The system generates high levels of RF energy, which is potentially hazardous if allowed to escape. The system is designed to contain the RF energy within the shielded enclosures of the torch compartment and the RF power supply. Safety interlocks prevent the system from operating without all covers, doors, and shields in place.

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For technical support visit
fluidigm.com/tech-support.