FLUIDIGM®

PN 400250 A7

Helios, a CyTOF System

User Guide

SW Version 6.7

For Research Use Only. Not for use in diagnostic procedures.

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Contents

About This Guide

This document provides important safety information pertaining to the operation of Fluidigm mass cytometry systems. For detailed information about using a system, see the appropriate user 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.

For complete safety information, see Appendix B.

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 will result 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.

Indicator	Description
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: Introduction to Helios

Helios Instrument Overview

Helios™, a CyTOF® system, comprises the most recent advances in mass cytometry and is designed to provide a new and improved tool for bioanalytical single-cell detection and analysis. This high-performance mass cytometer, from the CyTOF family of instruments, enables the analysis of more than 40 markers and uniquely allows their quantitative determination with negligible spectral overlap, a result of exquisite resolution between mass detection channels. Helios provides users with 135 detection channels that can simultaneously resolve multiple elemental probes at high acquisition rates, thereby maximizing the per-cell information obtained from a single sample. The expanded mass range of 75–209 amu and superior mass resolution provide researchers with the ability to differentiate adjacent peaks. The Helios system provides fast instrument startup, an easy-to-use pneumatic sample loading system, an improved cell detection rate, and enhanced data storage capabilities. These attributes provide researchers with an unparalleled ability to generate high-resolution phenotypic and functional profiles of cells from normal and diseased states.

Figure 1. The Helios instrument front view

The Helios system is designed to provide an accessible and expandable platform for systems-level biology with single-cell resolution in a streamlined instrument. Helios consists of the Sample Loader, the main instrument, a chiller, and a system computer with workstation. The AS-5 Autosampler is designed to rest on a separate system cart. The system computer may be placed on a bench or a separate computer table. The instrument combines 4 vacuum pumps—the interface pump, the backing pump, the 3-inlet turbopump,

and the separate TOF turbopump—that work in concert to maintain a vacuum within the vacuum interface, the ion optics, and the time-of-flight (TOF) regions of the Helios instrument.

The CyTOF software system is designed to provide real-time information on instrument status.

IMPORTANT

- Do not make unauthorized modifications to your Helios system or accompanying computer system. The computer system has been configured for use only with the Helios system.
- It is recommended that no modifications or updates be made to the operating system and drivers. Keep installation of nonessential software to a minimum.

Instrument Specifications

Instrument Dimensions

Dimensions of major Helios components are shown below.

Table 1. Dimensions of Helios, Sample Loader, and chiller

Component	Width (cm/in)	Height (cm/in)	Depth (cm/in)	Weight (kg/lb)
Helios instrument	107 (42)	132 (52)	142 (56)	320 (705)
Sample Loader	18 (7)	32 (13)	22 (8.7)	5 (11)
Chiller	38 (15)	64 (25)	67 (27)	81 (178)

Operating Parameters

Table 2. Helios operating parameters

Parameters	
Operating temperature*	15–30 °C
Operating system	Windows® 7 Pro 64 bit
Sample introduction	Pneumatic round-bottom tubes
Instrument response	600,000 counts/^{159}Tb

*For optimal performance the temperature should be 22 °C ±2.

Power Consumption

Two dedicated electrical branch circuits (single-phase, 30 A single-phase 220–240 V AC, 50–60 Hz) are used to provide power to the Helios instrument.

Table 3. Power consumption specifications for the Helios instrument

Power Consumption	
Maximum volt-amperes (two circuits)	2 x 4,500 VA
Maximum continuous current per circuit	20 A
Operating voltage	200/208/220/230/240 V AC

Gas Requirements

Table 4. Argon gas requirements for the Helios instrument

Gas Requirements	
Ultra High Purity argon (≥99.996 purity)	80 ±1 psi
Flow rate	20 L/min

Standard Equipment

The following is a list of standard equipment* included with the shipment of the Helios instrument.

Table 5. Standard parts included in the Helios system shipment

Part Number	Standard Equipment
107002	Helios instrument
104042	4K LED computer monitor USB keyboard USB optical mouse Computer tower
101058/101859 (North America/Europe)	Chiller
106218	Sample Loader

* Standard equipment is subject to change at sole discretion of Fluidigm.

Consumables, Spare Parts, and Reagents

Table 6. Consumables and spare parts available for the Helios system

Part Number	Description	Unit
101508	Luer Adapter	1 count
101509	Union Body, Sample Capillary Kit	1 count
105922	Pre-assembled Sample Capillary Kit	1 count
101533	Union Elbow, Torch Assembly	1 count
101792	Torch Body	1 count
101802	Skimmer-Reducer Cone Assembly	1 count
101810	Vacuum Oil, 3.78 L	1 count
101813	O-Ring, Interface Pump Oil Port	5 pack
101815	O-Ring, Reducer	5 pack
101934	Blue Sleeves, Sample Capillary Kit	3 pack
105197	Sampler Cone	1 count
105350	Injector Sealer Cap	1 count
105398	Load Coil	1 count
105592	Air Filter	1 count
105641	O-Ring Kit, Torch Body	5 pack
105704	O-Ring, Sampler	5 pack
105652	Sample Capillary Tubing	1 count
105654	Coolant Solution, 1 L	1 count
106393	Sample Loader-Sample Holder–Large	1 count
107018	HT Injector	1 count
107028	Sample Loader-Sample Holder–Small	1 count
107033	Sample Loader-Sample Probe Line	1 count
107144	Nebulizer and Nebulizer Gas Line	1 count
107142	Nebulizer Gas Line	1 count
107085	Argon Gas Input, Sample Loader	1 count
107086	Sample Line, Sample Loader	1 count
105910	Nebulizer Cleaning Kit	1 count
107301	O-Ring, Pressure Chamber, Sample Loader	5 pack
107302	O-Ring, Spray Chamber, Body	5 pack

Part Number	Description	Unit
107313	O-Ring, Spray Chamber, Nebulizer Adaptor	5 pack
107304	O-Ring, Spray Chamber, Inner Cap	5 pack
107950	WB Injector	1 count

Table 7. Recommended reagents for use with Helios

Part Number	Reagents	Unit
201065	Fix I Buffer	50 mL
201066	Perm-S Buffer	250 mL
201067	Fix and Perm Buffer	100 mL
201068	Cell Staining Buffer	500 mL
201069	Maxpar® Water	500 mL
201070	CyTOF Washing Solution	1 L
201071	CyTOF Washing Solution	250 mL
201072	CyTOF Tuning Solution	250 mL
201078	Calibration Beads, EQ™ Four Element	100 mL
201237	Maxpar™ Cell Acquisition Solution	6 x 100 mL

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/contacts.

Chapter 2: Helios and Mass Cytometry

Helios Instrument Overview

Helios™ is a mass cytometer comprising the latest in CyTOF® technology (based on cytometry by time-of-flight), providing users a new and improved tool for bioanalytical single-cell detection. The Helios system analyzes individual cells labeled with stable heavy metal isotopes using state-of-the-art inductively coupled plasma time-of-flight mass spectrometry (ICP-TOF-MS) technology. With 135 detection channels, Helios has the exquisite ability to simultaneously resolve multiple elemental probes per cell at high acquisition rate without the need for compensation, thereby maximizing the per-cell information obtained from a single sample. These attributes provide researchers with an unparalleled ability to generate high-resolution phenotypic and functional profiles of cells from normal and diseased states.

Figure 2. The Helios instrument

Introduction to Mass Cytometry

Mass cytometry is a tool for accurate cell profiling. It employs elemental tags with a higher molecular weight than elements that are naturally abundant in biological systems. Helios has been designed to detect metals at very low concentrations with minimal background noise from signal overlap. The sample is ionized with inductively coupled plasma, then the ions are separated by mass and quantified.

Cells stained with metal-conjugated probes in a suspension are delivered to the nebulizer from the Sample Loader. The samples are aerosolized in the nebulizer and directed through the spray chamber to the ICP torch. The cells are vaporized, atomized, and ionized in the plasma. The cells undergo a multistep process within the instrument, resulting in generation of a file that records the identity and amount of each probe for each cell (Figure 3).

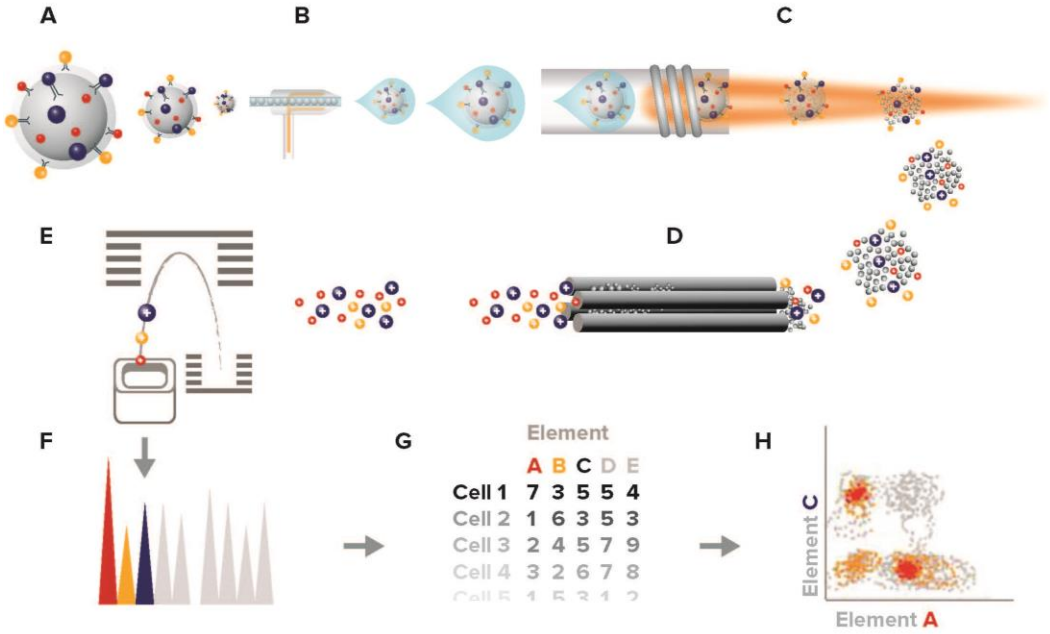


Figure 3. Mass cytometry workflow. Cells labeled with metal-conjugated antibodies in solution (A) are injected into the nebulizer (B). They are aerosolized and reduced to single cell-containing droplets. The cells are directed to the ICP torch, where they are vaporized, atomized, and ionized in the plasma (C). The high pass optic removes the low-mass ions (D), resulting in an ion cloud that enters the TOF mass analyzer. The ions are separated based on their mass and are accelerated to the detector (E). The detector measures the quantity of each isotope for each individual cell in the sample; data is generated in an FCS format (G) and analyzed (H).

Mass Cytometry Workflow

Mass cytometry can be divided into five major processes: sample introduction and ionization, ion transport through vacuum interface and high-pass ion optics, ion separation in the time-of-flight (TOF) mass analyzer, data acquisition and processing. Figure 4 is a schematic of the Helios system divided by color to indicate the major processes.

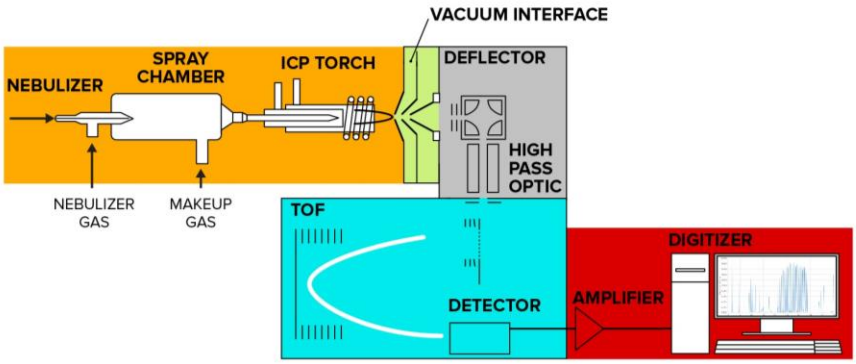


Figure 4. The Helios system. The mass cytometry workflow is divided into sample introduction and ionization (orange), ion transport through vacuum interface (green) high-pass ion optics (gray), ion separation in the time-of-flight (TOF) mass analyzer (blue), and data acquisition and processing (red).

Each of the components of these steps are described in detail in the following section.

Components of the Helios System

Sample Delivery

The Helios instrument is equipped with the Sample Loader that serves to deliver the sample into the inductively coupled plasma via the nebulizer. The Sample Loader is seated on the front bench cover of the Helios instrument (completed during installation). There are two inputs to the Sample Loader: the argon from the instrument, and the USB cable from the computer. The sample is loaded into a 5 mL round-bottom tube or 1.5 or 2 mL Eppendorf tubes and inserted into the holder. The Sample Loader is equipped with LED status lights that serve to notify users of the operational status of the Sample Loader (see Figure 5 below). The unit is equipped with a proximity safety mechanism that depressurizes the unit when the Sample Loader handle is moved to the open position.

Argon is forced into the pressure chamber providing the pressure required to drive the sample out of the tube and up into the sample line, where it is carried to the nebulizer for injection into the system. The Helios instrument control software provides the ability to

adjust and optimize the pressure of the argon in order to control the sample injection rate into the instrument. The Sample Loader, including its on/off control, is operated using the CyTOF software.

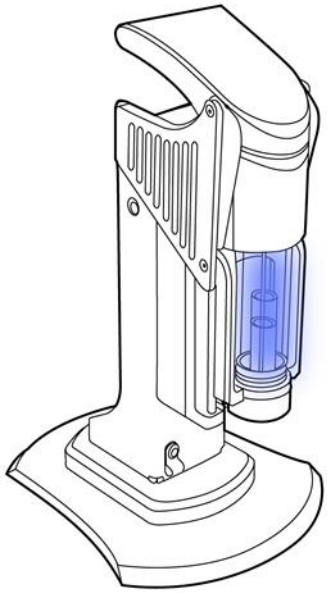


Figure 5. Sample Loader

Autosampler

If the Helios system is connected to the AS-5 Autosampler (Figure 6), samples loaded into 96-well plates are automatically introduced into the system, allowing unattended instrument operation and sample data acquisition. The AS-5 Autosampler contains a separate dedicated liquid sampling automation system. The AS-5 is designed to rest on a separate cart (Recommended vendor and cart: Anthro MiniCart, PN GT23BK).

Figure 6. The AS-5 Autosampler

Sample Introduction

The sample introduction system aerosolizes the liquid sample suspension and introduces cells one at a time into the ICP source for ionization (Figure 7). The liquid sample is introduced using the Sample Loader or the AS-5 Autosampler into a nebulizer inserted into a heated spray chamber, where the sample aerosolized. Within the spray chamber, the high temperature partially vaporizes the aerosol, and argon gas directs the aerosolized cells to the ICP source (see Figure 7).

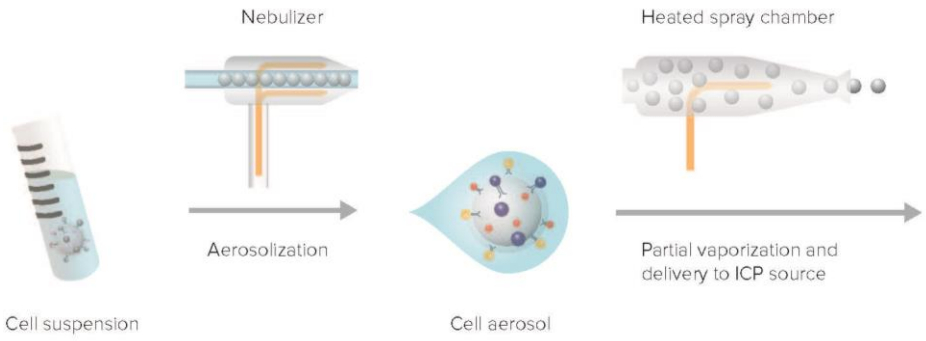


Figure 7. Sample introduction. The liquid sample suspension is directed to the nebulizer via the Sample Loader or the AS-5 Autosampler, then aerosolized by the nebulizer into the spray chamber, which partially vaporizes the aerosol and delivers it to the plasma.

Nebulizer

For liquid sample analysis, it is critical to remove as much water as possible from the sample so that it can be efficiently ionized in the plasma. This is achieved first by aerosolizing the sample in the nebulizer followed by delivery to the plasma through the heated spray chamber.

The Helios instrument employs a glass concentric nebulizer consisting of an inner capillary that carries the liquid sample and an outer chamber that carries argon gas (called nebulizer gas). The nebulizer gas flows at a rate of approximately 0.15–0.35 L/min. Both liquid and gas flows are directed towards the spray chamber through the tapered end of the nebulizer. The sample within the capillary travels at atmospheric pressure, and as the sample exits the tip, shear force exerted by the exiting nebulizer gas onto the liquid breaks it up into fine aerosol droplets.

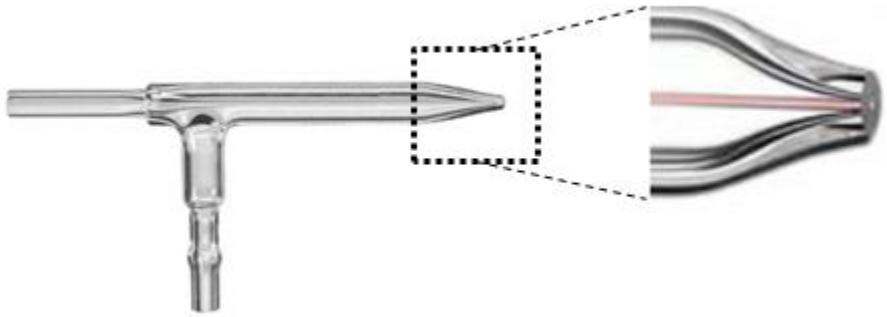


Figure 8. The concentric nebulizer. Liquid sample enters from the sample inlet (left), and argon (nebulizer gas) enters from the side arm (bottom). The sample chamber narrows into a capillary, pulling liquid rapidly to the tip (enlarged, at right, with liquid sample indicated in red), where shear forces exerted by accelerated nebulizer gas break the liquid into aerosol droplets.

Spray Chamber

The aerosolized sample exits the nebulizer directly into the heated spray chamber, which is housed within a heater block. Makeup gas (argon gas) is pumped into the designed polyetheretherketone (PEEK) spray chamber, and this high flow of heated gas partially evaporates the droplets, to reduce their size, as it directs the aerosol to the plasma torch.

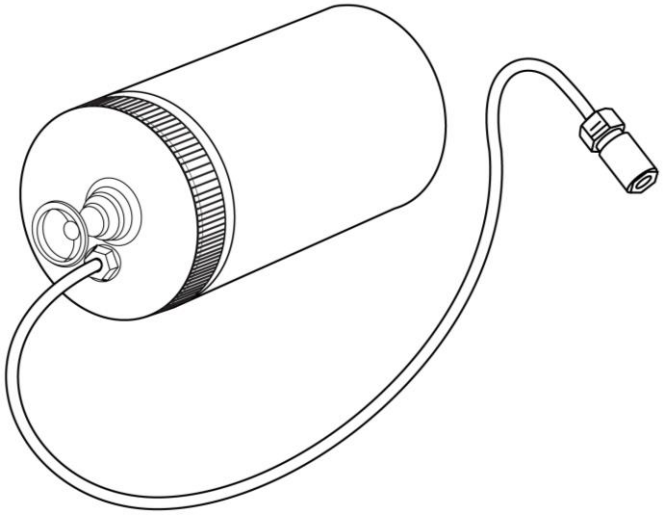


Figure 9. Schematic drawing of the spray chamber with the makeup gas connection

Sample Heater Assembly

The spray chamber is housed within the sample heater assembly, which heats the sample to 200 ˚C as it passes through the spray chamber. The nebulizer tip is inserted into the nebulizer adaptor port on the front face of the heater box. The heater box is covered by a heat shield, which protects the user from contacting instrument parts that may be at high temperatures.

WARNING The heat shield should not be removed during operation of the instrument. The heat shield provides protection from the residual ultraviolet (UV) energy from the plasma, torch flange (outer surface), and heater assembly, which are heated to high temperatures when Helios is in operation.

A nebulizer rest that holds a 50 mL tube allows users to remove the nebulizer from the heater assembly and rest it in deionized water (DIW) when the instrument is not in use.

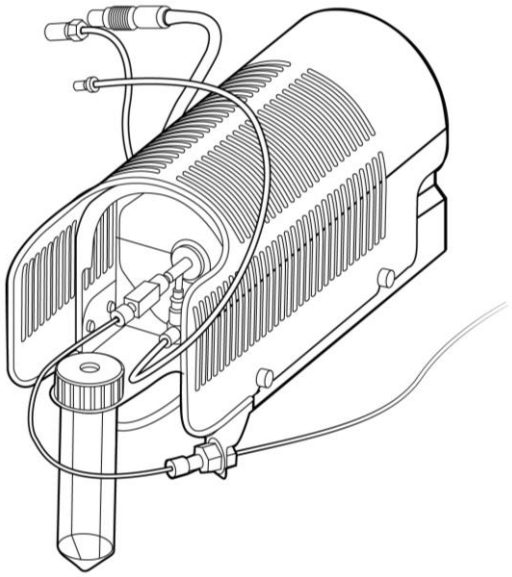


Figure 10. Sample heater assembly

CAUTION Ensure that the thumb screws on the heat shield (two on each side) are tight before proceeding with the operation of the instrument.

Ionization

The single-cell aerosol droplets that exit the spray chamber are transmitted to the ICP source where they are vaporized, atomized, and ionized in the plasma for subsequent mass analysis. This results in the formation of an ion cloud containing the ionized metal tags.

Plasma Torch

The plasma torch consists of three concentric chambers: the torch body–a fused assembly of two concentric quartz tubes–and a quartz sample injector tube that is inserted inside the torch body. The torch assembly on Helios enables shorter single-cell event duration, which yields higher cell detection rates.

The outermost chamber (between the torch body tubes) contains argon plasma gas flowing at 18 L/min that is ignited to form the plasma. The central chamber (between the inner torch body tube and the sample injector) contains argon auxiliary gas flowing at ~1 L/min that is used to change the position of the base of the plasma relative to the sample injector. The innermost chamber inside the sample injector transmits the argon stream and sample aerosol from the spray chamber directly into the center of the plasma.

Figure 11 The plasma torch

The torch assembly is mounted inside an induction load coil that is supplied with current that is generated by radio-frequency (RF) power. This current generates the electromagnetic field that sustains the plasma.

Sample Ionization

Plasma, consisting of charged particles, is formed by ionization of argon gas within the electromagnetic field generated in the load coil. First, argon plasma gas flows tangentially from the outer chamber of the torch body. RF power supplied to the load coil creates a strong electromagnetic field as the plasma gas exits the outer chamber of the torch.

A high-voltage spark strips away free electrons from the exiting argon atoms. These free electrons accelerate in the electromagnetic field and collide with sufficient energy to ionize the argon gas. Temperatures within the plasma typically range from 5,000 to 10,000 K. When the aerosolized sample is introduced through the injector into the plasma, the water droplets are rapidly vaporized. The desolvated individual cells are then vaporized forming a cloud of atoms which are then ionized. Thus the plasma contains bursts of ion clouds corresponding to individual cells that were introduced into the torch in aerosol form (Figure 12).

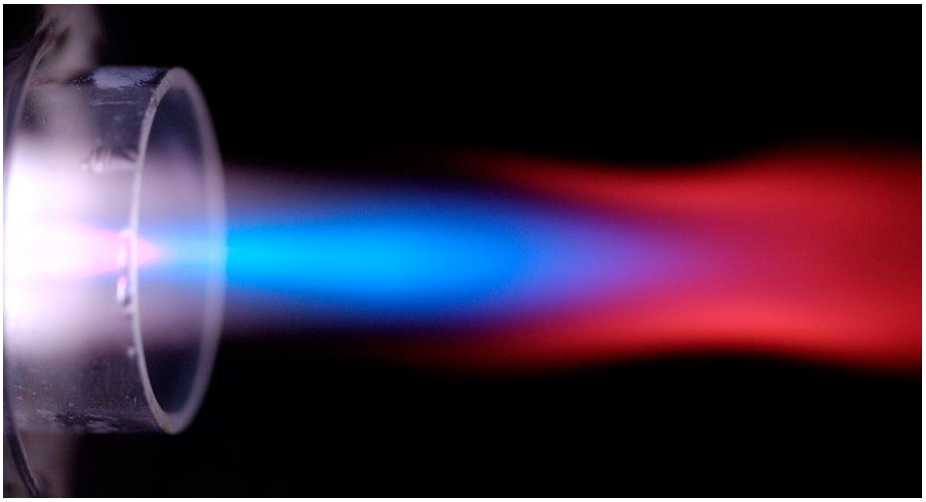


Figure 12. Plasma production with the plasma torch. The energized ions exit the plasma torch. Photo credit: Aeschliman, D.B. et al., *Journal of Analytical Atomic Spectrometry* 18 (2003).

Vacuum Interface

The plasma which is generated at atmospheric pressure (760 Torr) passes through the vacuum interface. (Figure 13).

The purpose of the vacuum interface is to efficiently transport ions from the plasma at atmospheric pressure to the chambers that house the ion optics at less than 10^{-3} Torr. Helios uses a 3-cone interface to transport the ion beam into a low-pressure vacuum: sampler (1.1 mm diameter orifice), skimmer (1 mm) and reducer (1.2 mm). All 3 cones are made of nickel, and the interface housing is water-cooled to dissipate the significant heat generated by the plasma. The plasma containing the cell-induced ion clouds enters through the sampler cone orifice into the sampler-skimmer region, and then passes through the skimmer cone to the skimmer-reducer region, and finally, through the reducer cone, which reduces the pressure and foccuses the ions to the downstream ion optics. The ions that emerge from the reducer cone are accelerated and deflected by an electrostatic field of the deflector toward the time-of-flight region.

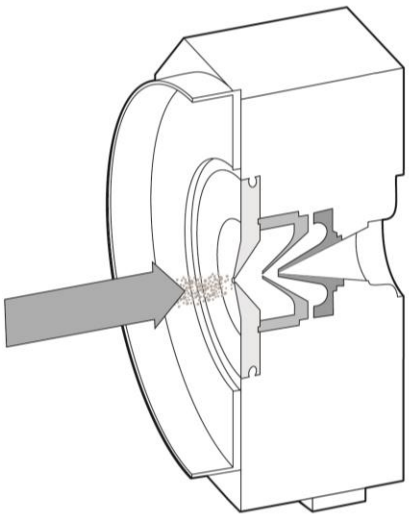


Figure 13. The vacuum interface includes 3 nickel interface cones: the sampler cone (light gray), and the 2 cones in the skimmer-reducer assembly (dark gray).

The ion beam propagating through the cones contains some non-ionized material and photons in addition to ions. If not filtered, these neutrals can attach to instrument components, resulting in signal drift. Photons that reach the detector are registered erroneously as ions. To eliminate these problems, the ions in the beam are deflected perpendicularly through a high pass optic. This turns positively charged ions toward the downstream ion optics, while neutrals and photons follow a pathway to the turbo molecular pump.

High Pass Ion Optics

The ion cloud leaving the vacuum interface is dominated by low-mass ions that are not of analytical interest and that are of such high abundance that they may quickly damage the detector. To remove these ions, the beam is focused and directed into the high-pass ion optic (Figure 14). High-mass ions are guided through this pathway and as a result, a stream of ions (corresponding to individual cells) that contains only the high mass isotopic probes exits the high-pass ion optic.

Figure 14. The high-pass ion optic removes unwanted low mass argon and other ions from the ion beam, transmitting clouds that contain isotopic probe ions (>75 amu) to the TOF.

Time-of-Flight Mass Analyzer

The ion clouds that exit the high-pass ion optic consist of a mixture of high molecular weight probes in a randomly distributed array. These ions are sent to the time-of-flight (TOF) mass analyzer, which separates the ions on the basis of the mass-to-charge ratio (Figure 15).

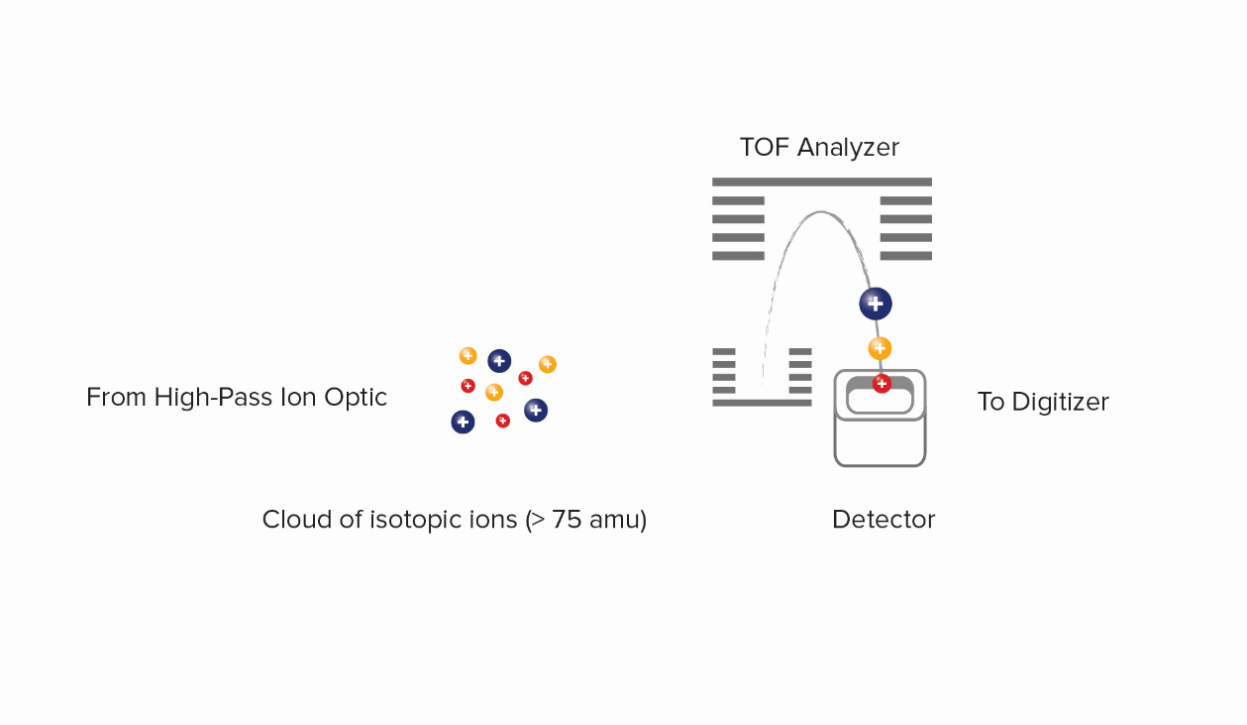


Figure 15. Separation of ions in the TOF. Ion clouds are subjected to an electrostatic force, which orthogonally accelerates the incoming ions toward the detector. As a result, the ions separate based on their mass-to-charge ratio, with lighter elements reaching the detector first.

The beam exiting the high-pass ion optic enters through the entrance slit into the accelerator chamber of the TOF analyzer. At 13 µs intervals (frequency of 76.8 kHz), a pulse of several hundred volts is applied to the push-out plate, accelerating the accumulated packet of ions orthogonally toward the reflector, which redirects the ions toward the detector. The electric fields in the accelerator and reflector are configured to focus ions into tight time-resolved bands regardless of initial position or energy. The relationship between time of ion flight to the detector and their mass-to-charge ratio (m/z) is:

$$t = t_0 + A\sqrt{\frac{m}{z}},$$

in which t_0 and A are derived from the mass calibration procedure. ICP ionizes metal atoms predominantly into a single positive charge state. Each packet of ions resolves into a series of bands, with the lightest probes reaching the detector first and each successively heavier mass reaching the detector at a later time. Each time resolved band of ions of mass M is separated from its M+/- 1 neighbor by 20–25 ns.

After the first packet of ions is pushed out and detected, a second pulse pushes out the next packet of ions for detection and the cycle repeats until data acquisition is complete.

Detector

The ions separated in the TOF chamber are detected using a discrete dynode electron multiplier. When an ion strikes the first dynode of the detector, several secondary electrons are liberated. These electrons strike the next dynode where they generate more electrons. This process is repeated at each dynode, resulting in an electron pulse that is captured by the anode of the detector. The output analog signal is amplified and converted by a dual 8-bit digitizer to digital values at 1 ns sampling intervals. The digitizer trigger delay dictates the first mass channel (the lowest mass registered) to be recorded per push, while the segment length dictates the mass range to be recorded per push. Instruments are set to collect data from at least 135 mass channels (each corresponding to 1 amu), typically starting at mass 75 for Helios.

Data Acquisition and Analysis

This section describes the process whereby the ions separated by mass in the TOF and generate signals which are converted into digital values and analyzed (Figure 16).

Dual Count Scale

Helios resolves multi-element samples using TOF, with ions from each isotope arriving at the detector centered in discrete 20–25 ns time windows (within each 13 µs push) depending on their mass-to-charge ratio. At very low ion concentrations, the probability of pulse signal overlap is negligible, and ion count is most precisely determined by simply counting the number of pulses (for example, the left-hand intensity/time curve in Figure 16). As ion concentration increases, ion pulses begin to arrive at the detector at the same time. In this situation, pulse count underestimates the true ion count, and integrated intensity becomes a more accurate measurement (Figure 16, right).

The range of data that Helios collects requires collection of dual data, which means that pulse count and intensity values are collected for every channel. CyTOF software plots the entire data range on a single dual signal scale, the units of which are actual counts of particles that hit the detector. To achieve this, two things are done. First, a dual count coefficient is applied, which converts analog intensity into actual counts according to the following formula:

Counts = intensity x dual count coefficient

Second, a dual switchover threshold is applied, below which pulse count is used and above which counts from coefficient-converted analog intensity is used. Using the dual count scale, Helios quantifies bound antibody labels per cell across a wide range of signal input.

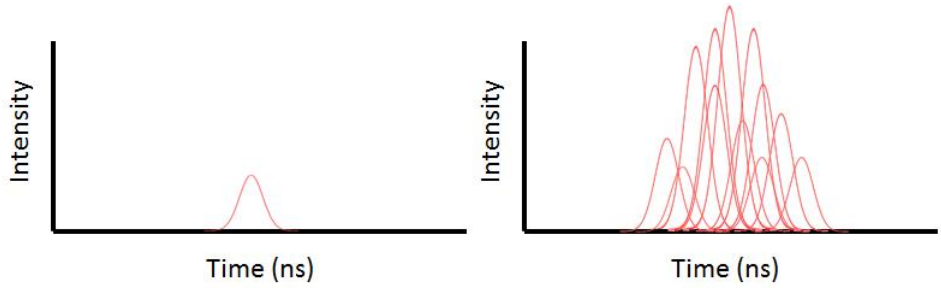


Figure 16. Impact of analyte concentration on signal measurement. At low analyte concentration (left), pulses do not overlap. Because each pulse delivers a different number of electrons to the anode and therefore a different intensity value, it is more precise to count pulses when ion concentration is very low. Here the pulse count is 1. At higher analyte concentrations (right), pulses overlap, and counting pulses will underestimate the true number of ions that hit the detector. Here the pulse count is 8 (if we count discernible peaks) even though 16 ions hit the detector. Thus, at high analyte concentration, it is more accurate to use integrated intensity, and convert this intensity value to counts using a calibration coefficient.

Cell Detection and Acquisition Data File format

Data for each 13 µs push is digitized sequentially and integrated to obtain ion counts for the channels selected for analysis. The resulting record is processed according to cell event selection criteria set by the user. These criteria include a minimum signal threshold and a range for event duration consistent with single-cell events. As a result, the data acquired contains the integrated number of total ion counts for each selected analyte on a per-cell basis. These data are saved as text (.txt) and flow cytometry standard (.fcs) 3.0 format for data analysis in compatible software programs.

Chapter 3: Instrument Components

Helios

This chapter contains annotated figures of the Helios™ system.

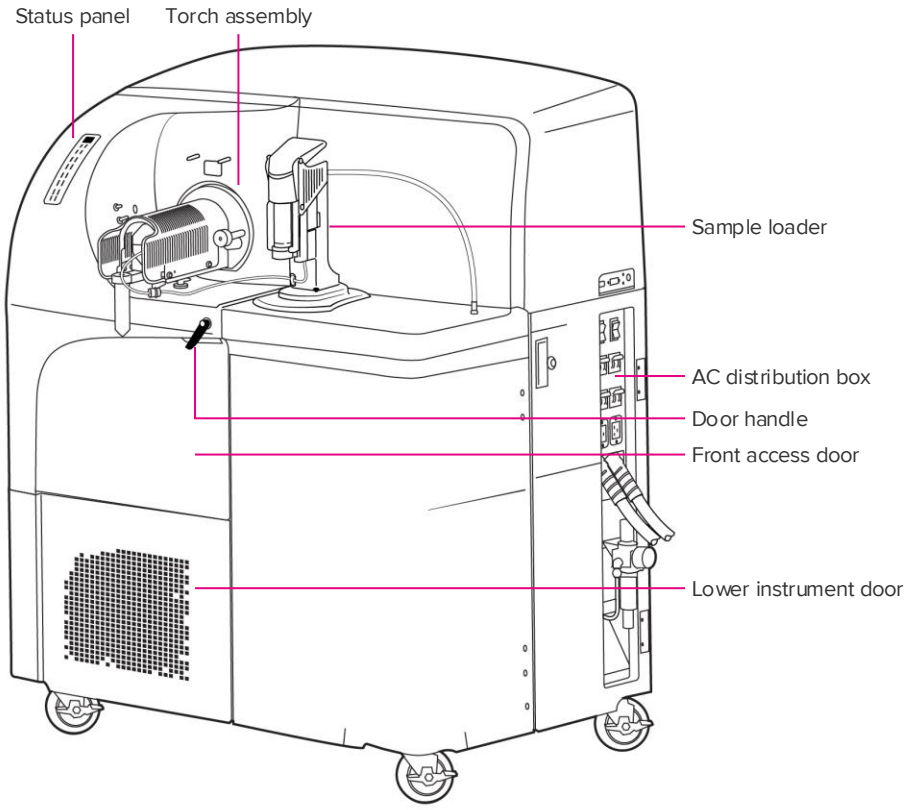


Figure 17. Helios front view

Sample Introduction

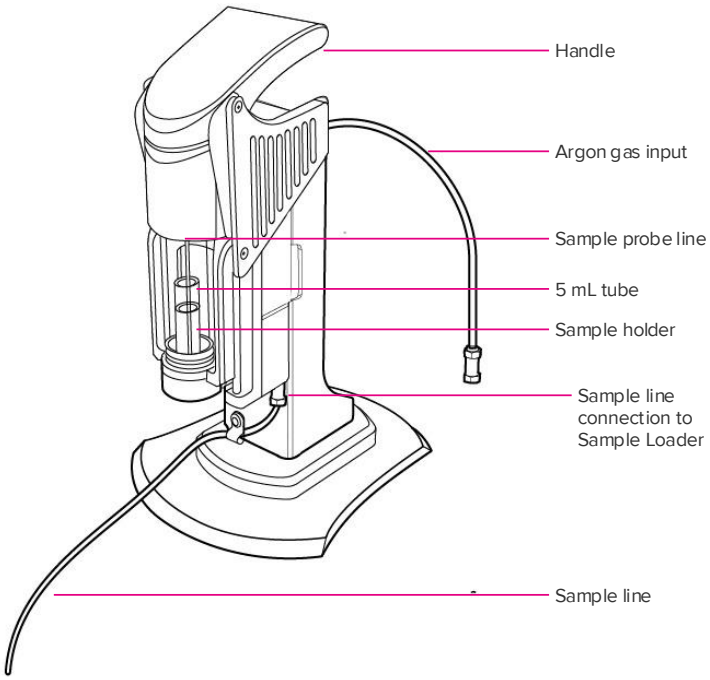


Figure 18. Sample Loader

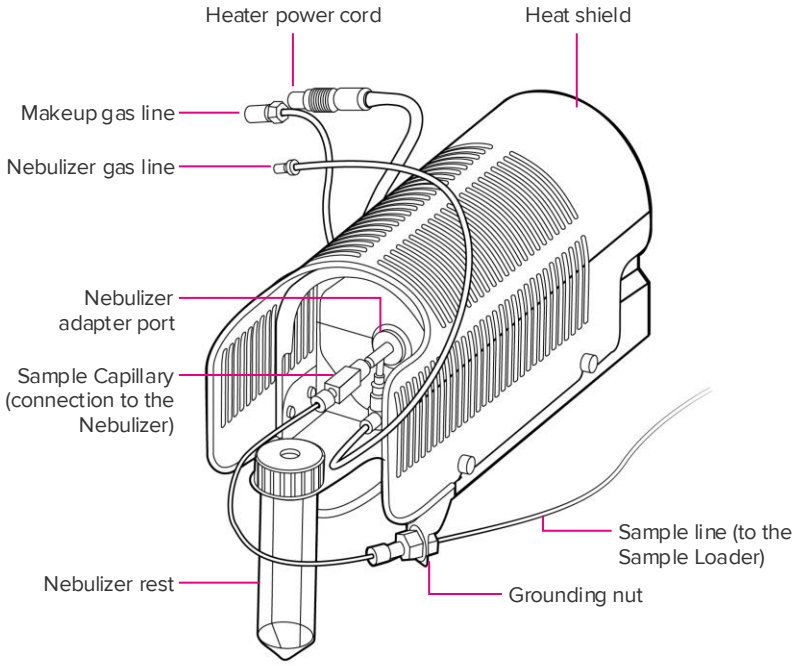


Figure 19. Heater assembly and connections to the Sample Loader

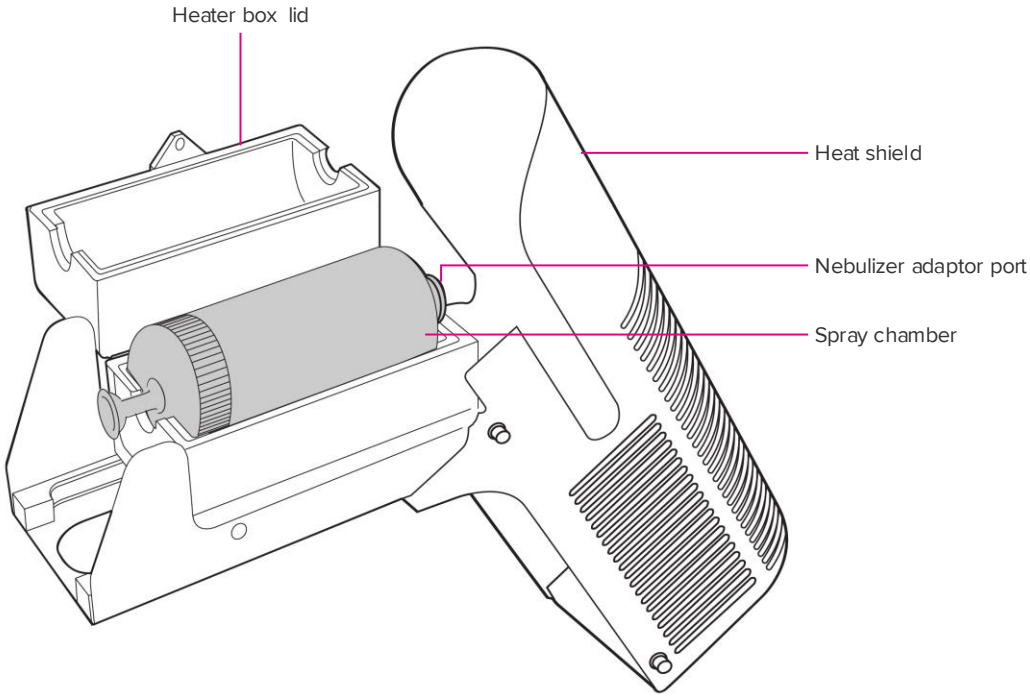


Figure 20. Spray chamber and connections

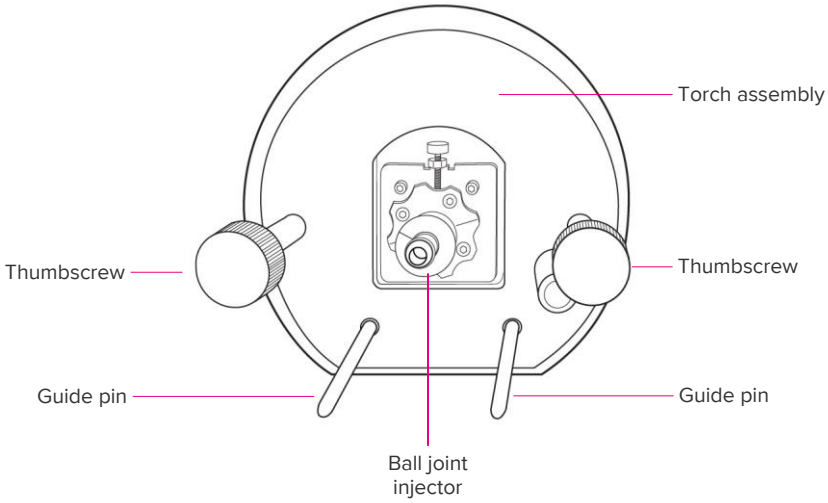


Figure 21. Front view of torch assembly

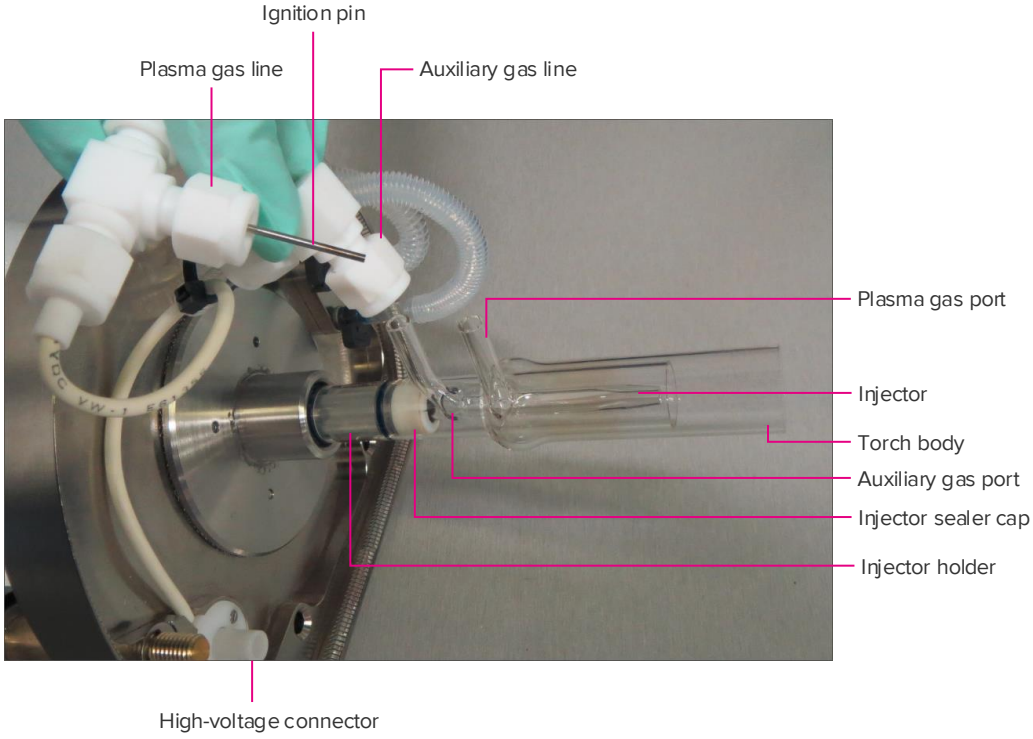


Figure 22. Rear view of torch assembly

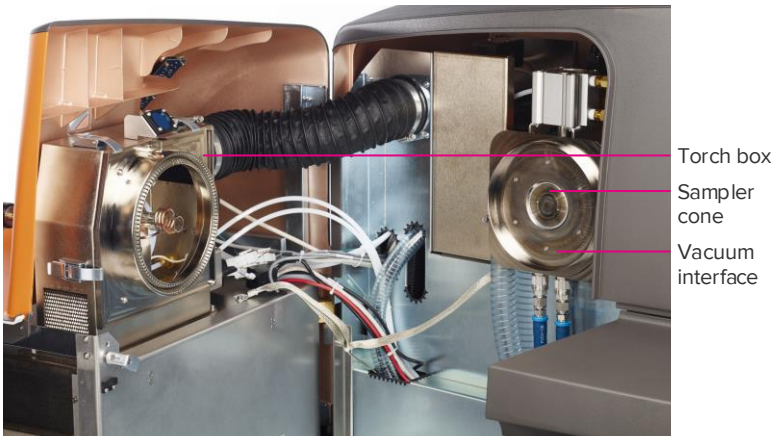


Figure 23. Interior view of Helios with front access door

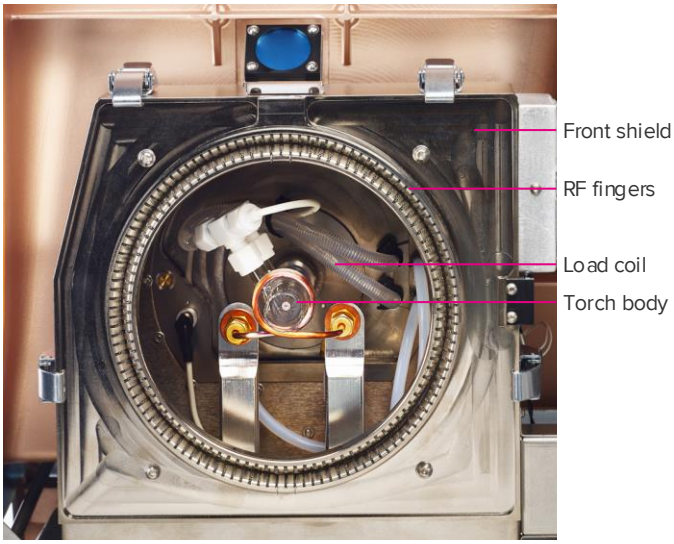
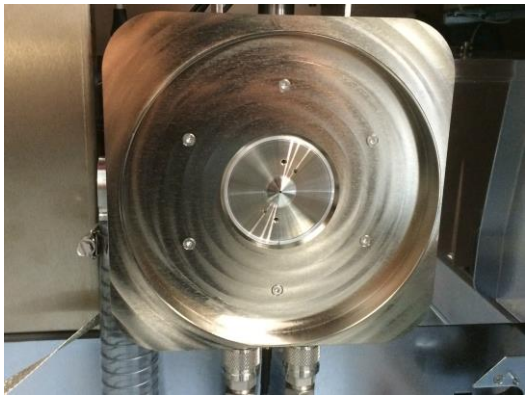


Figure 24. Torch box

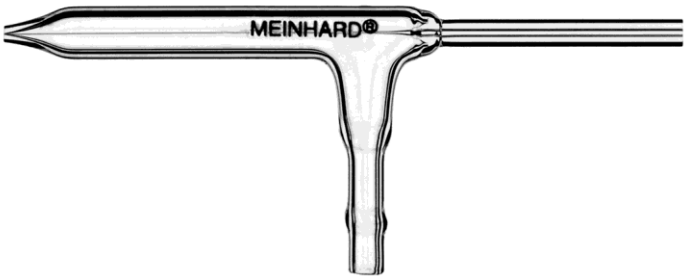
Other Components

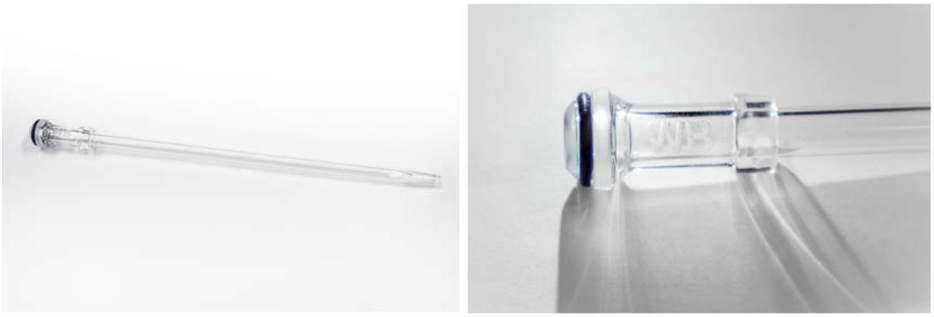
Table 8. Other Helios components

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

Cooling fans		Inside lower front door

Table 9. Helios sample introduction system glassware and spray chamber

Part	Image
Nebulizer	
Spray chamber	
HT injector	

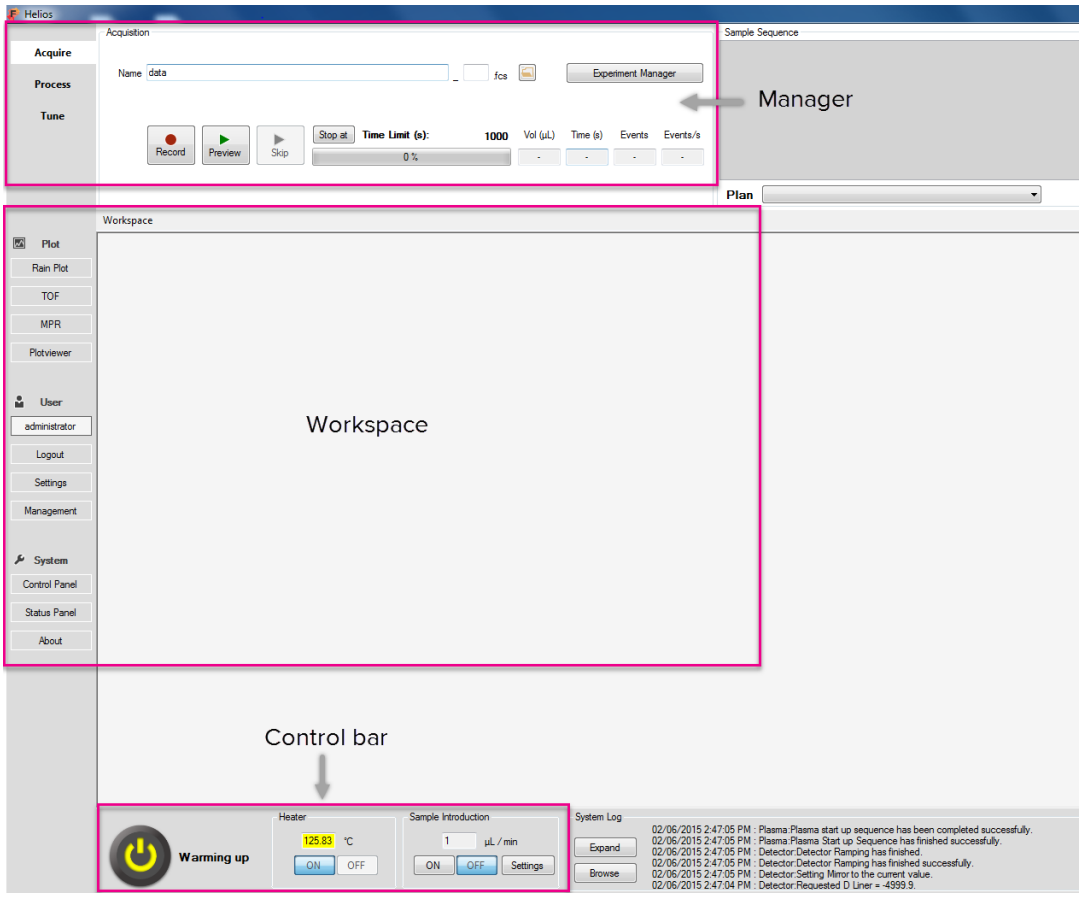
WB injector	
Torch body	

Chapter 4: Software Interface

CyTOF Software

The Helios™ system software is designed to be user-friendly and intuitive, and to provide continuous instrument feedback and diagnostic capabilities to users. This gives users the ability to troubleshoot quickly and effectively as well as to provide the highest quality of data output.

The software interface has three sections; Manager, Workspace, and Control bar.



The Manager section, at the top of the start page, allows users to control sample volumes, set up tuning protocols, set up experiments, preview samples, acquire samples, and record results.

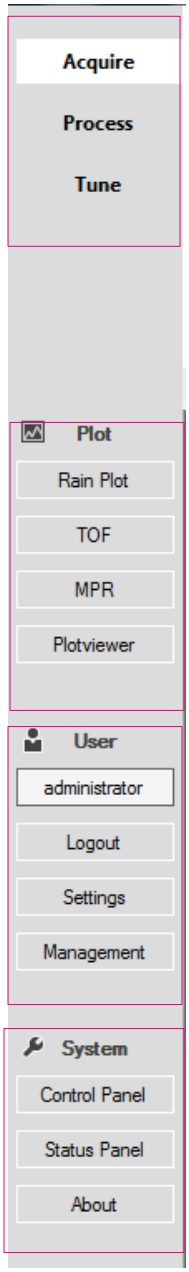
The Workspace contains all of the plots, including the rain plot, TOF plot, masses per reading (MPR) as well as the sample panels. Here users can visualize the tuning solution beads and samples that are run on the instrument.

The Control bar, on the bottom, allows users to start up and shutdown the instrument plasma, turn the heater on and off and monitor the temperature, turn on and off the Sample Loader, and view the system log to review the instrument status.

The software has 3 modes: user mode, administrator mode, and service mode. Administrator mode is available to Helios operators. User mode has simplified functionality for non-operators. Service mode is for Fluidigm service personnel only and should not be used to acquire samples by Helios operators.

Menu Panel

The menu panel on the left of the screen provides users with all the functions required for tuning the experiment, acquiring data, viewing the data (plot), monitoring the status of the instrument, and processing data (for example, performing debarcoding on your samples and normalizing data).



Acquire tab: Enables users to set up experiments (Experiment Manager), set acquisition limits and preview and record data.

Process tab: Enables users to debarcode data, normalize and concatenate data files, and export data to text or FCS files.

Tune tab: Allows you to select tuning protocols.

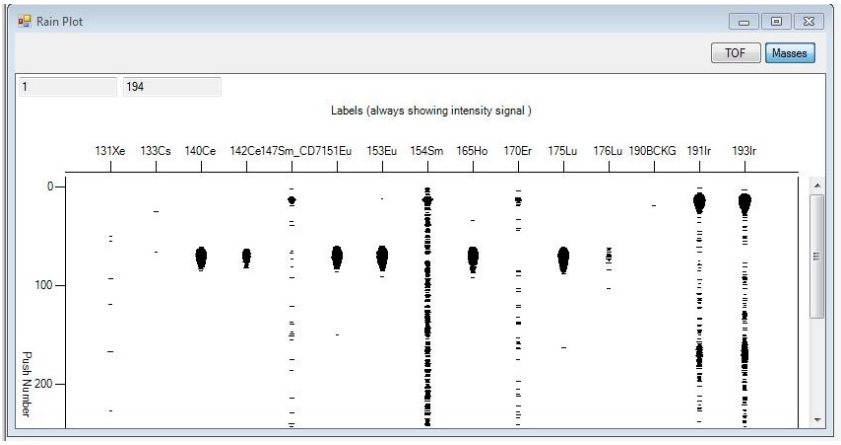
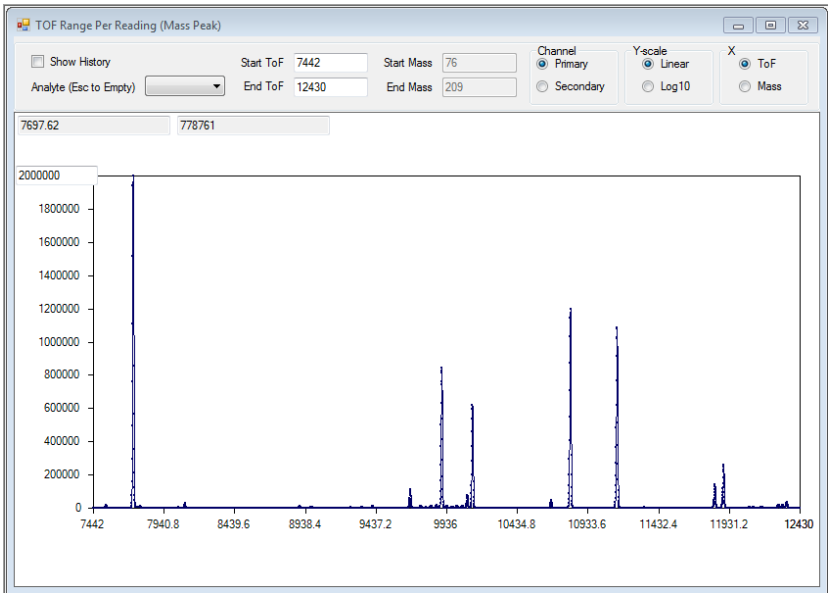
In the Plot section of the panel, users can select Rain Plot to view the live data, the TOF plot to view the mass range, the MPR (masses per reading) plot to view the intensities or dual counts of selected isotopes and the Plotviewer to cluster data and to view data in bivariate plots.

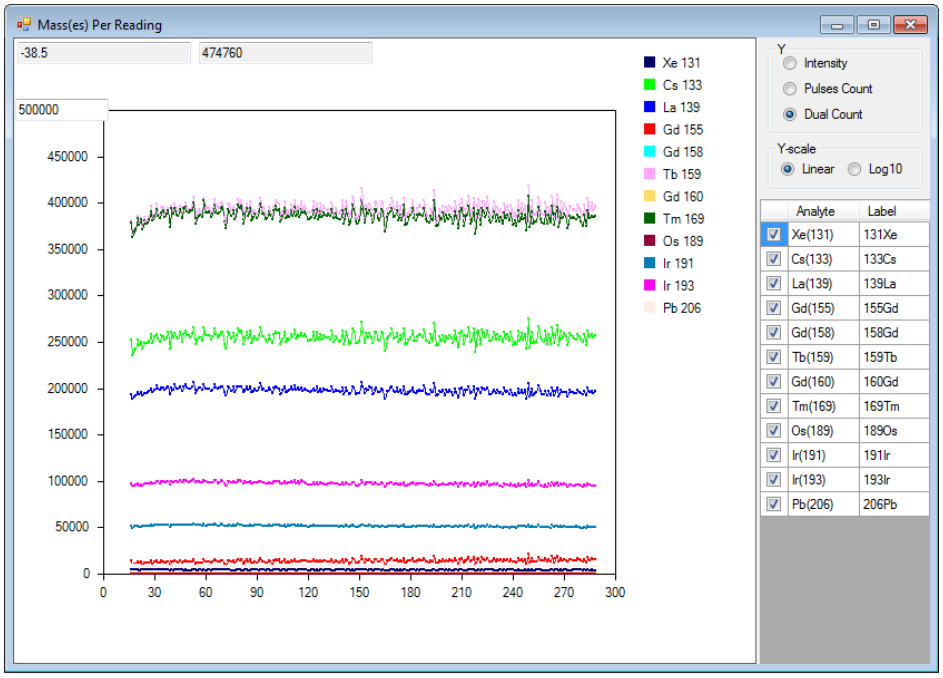
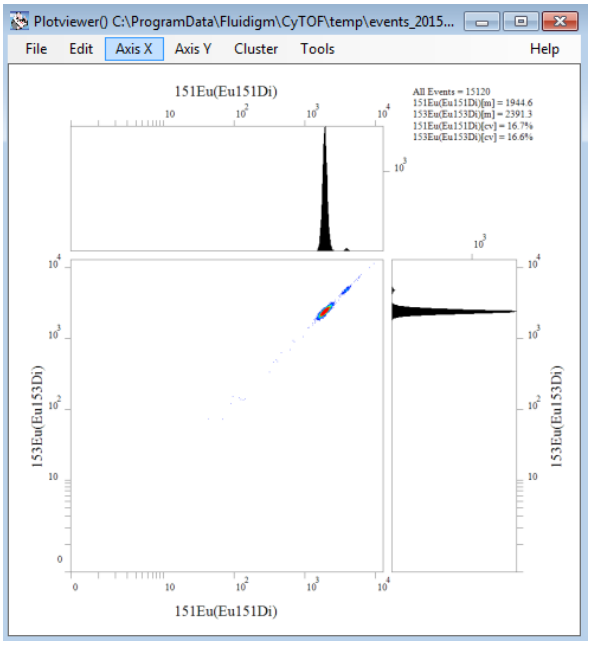
In the User section, users/administrators can manage system settings, log out, and view instrument usage time stamps.

In the System section, users can click Control Panel to view instrument status including plasma, nebulizer gas flow and makeup gas flow.

Plot

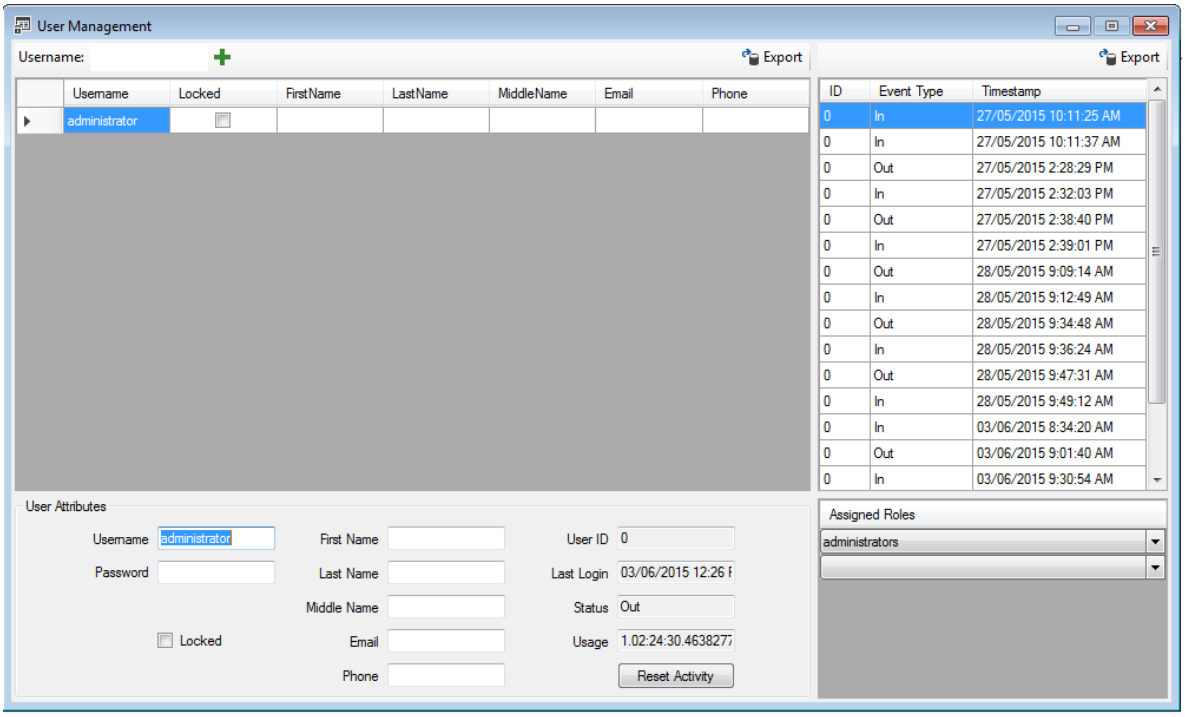
Table 10. The plot section of the menu bar allows users to visualize tuning solution, beads, or samples.

Plot Type	Example
Rain plot	
TOF plot	

Plot Type	Example
Masses Per Reading (MPR)	
Plotviewer	

User Management

In the User Management window, administrators can view and update user attributes and access information on instrument usage and timestamps.

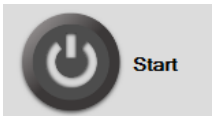


Control Bar

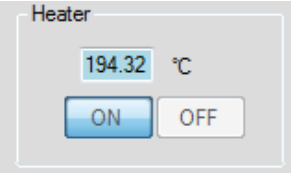
The Start/Ready button on the Control bar functions to start up the system and indicates when the system is ready for tuning or acquisition.

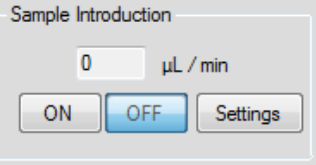
The Start button will turn yellow when the system is in warmup mode or shutdown mode.

The Start button will be remain grey when the system is off.



The Heater display indicates whether the heater is on or off. When the temperature box turns blue that means that the heater has reached optimal temperature. When it is yellow this indicates that the heater is warming up or cooling down.

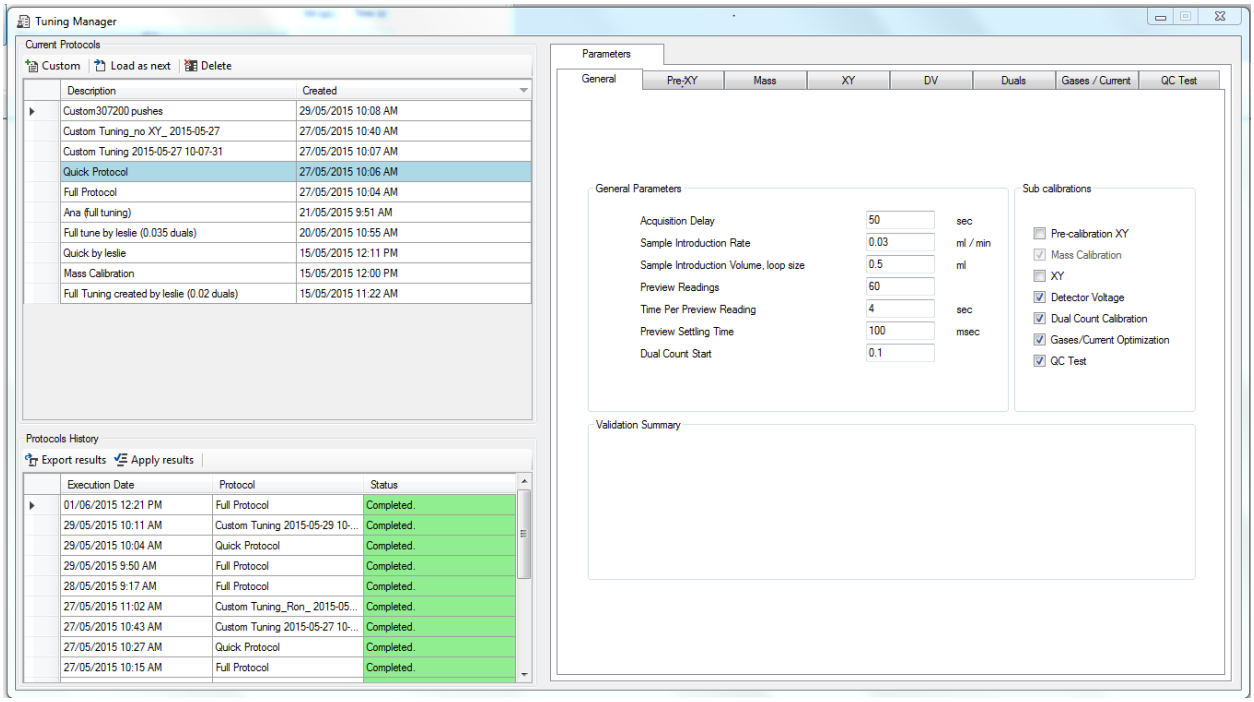




The Sample Introduction display indicates when the Sample Loader is on or off and shows the sample introduction flow rate. Users can click the **Settings** button to change the sample volumes.

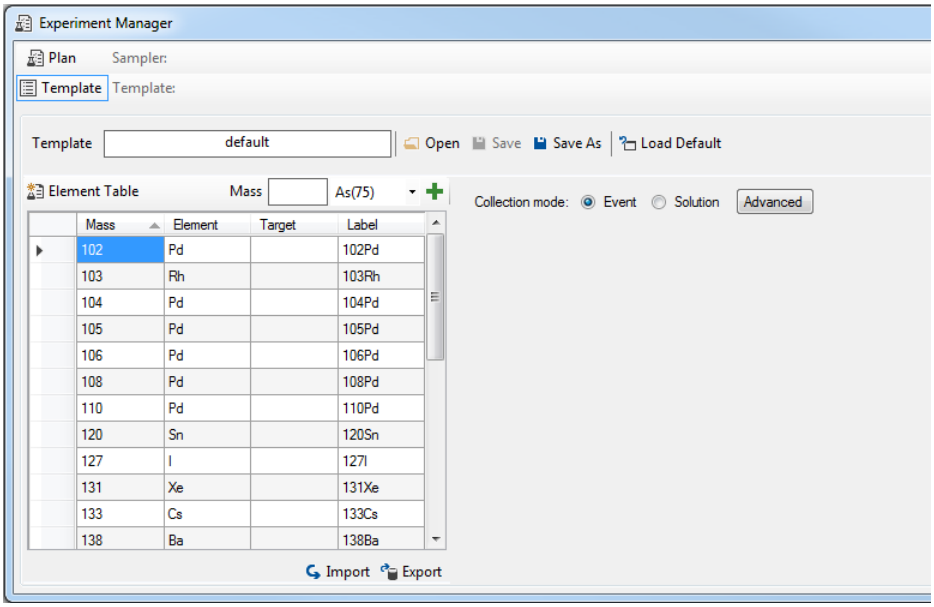
Tune

In the Tuning window users may click **Tuning Manager** to open the Tuning Manager window. There users can select a tuning protocol and tuning parameters. (See Chapter 5: Operation for details.) The Protocols History at the bottom of the window gives users a log of the tuning protocols that were performed on the instrument, including the time and date stamp.



Acquire

In the Acquire tab, users can click **Experiment Manager** to open the Experiment Manager window. There users can select collection mode for data acquisition, event mode for beads or samples, or solution mode for tuning solution. Use the element table to create a template for your experiment by selecting the isotopes or masses used for each target, or select the default template to obtain a template with preselected isotopes (masses).



Chapter 5: Operation

Introduction

This chapter describes daily operation of the Helios™ system, including:

- Preparation and startup
- Overview of the Sample Loader
- Quick and full protocols for tuning the system
- Instrument tuning
- Sample acquisition
- Normalization of data with EQ™ Four Element Calibration Beads
- Daily cleaning
- Instrument shutdown

Preparation and Startup

WARNING 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.

IMPORTANT Ensure that the thumbscrews on the heat shield (two thumbscrews on each side) are tight before proceeding to the next step.

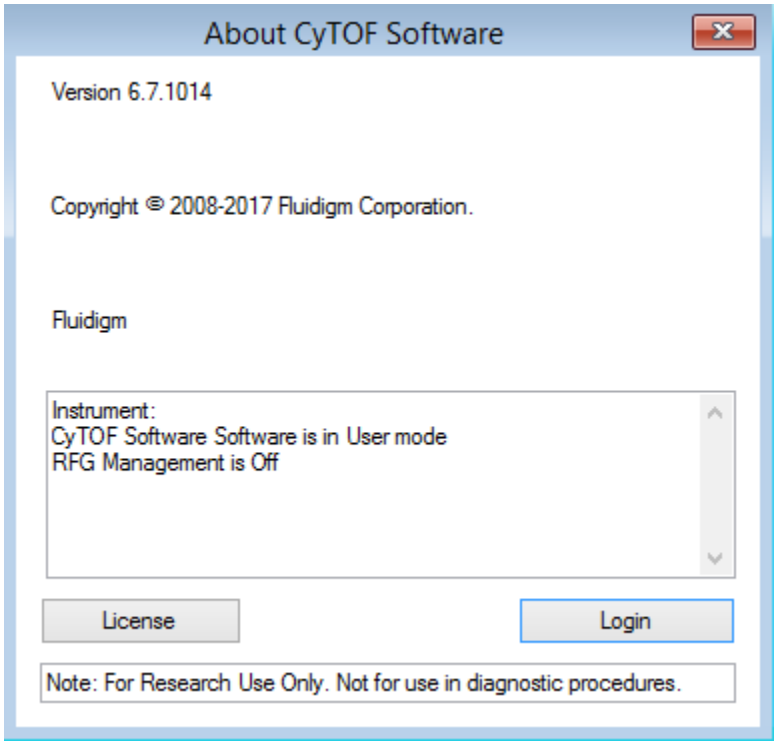
1 Check the Status Panel on the front cover of the instrument. The LED indicator lights should be lit. The ARGON and AIR lights should be green. If not check the argon supply pressure regulator and check that the exhaust air levels are correct.

   NOTE You may have to contact your local facilities manager to check the exhaust levels in your laboratory.

2 Open the CyTOF software.

3 Log in to the administrator mode of the software. To log in for the first time, enter “administrator” for username and leave the password box empty.

The CyTOF software interface opens.



4 Click **License** for the license agreement when opening the software for the first time.

5 Change personal settings and manager other users. (See

6 User Management section for instructions.)

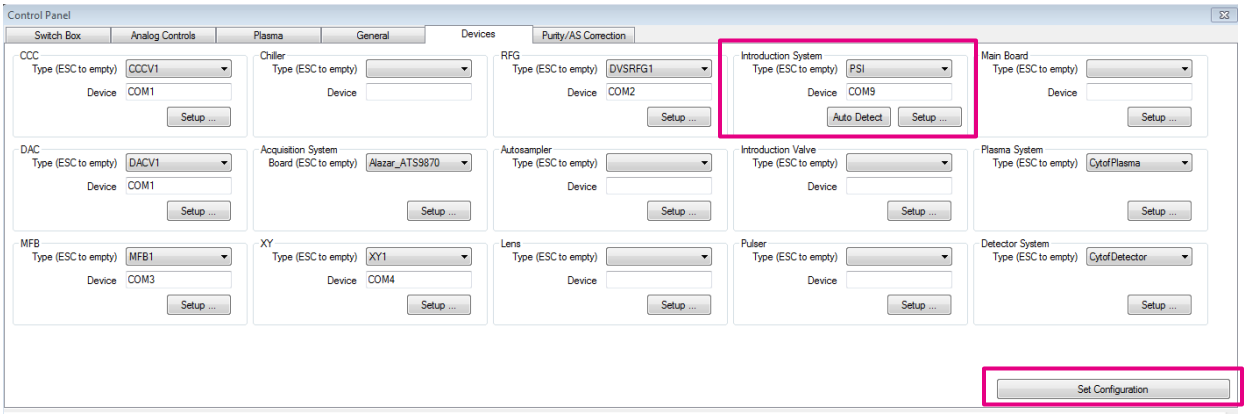
Check Sample Loader

Before beginning any procedures on the system, confirm that the USB cable for the Sample Loader is connected to the computer.

1 In the Control Panel window select the **Devices** tab.

2 In the Introduction System panel, click **Auto Detect** in order to identify the COM port, and then click **Set Configuration** at the bottom right corner of the Control Panel window.

3 A dialog box confirms that “Your device is ready to use.”

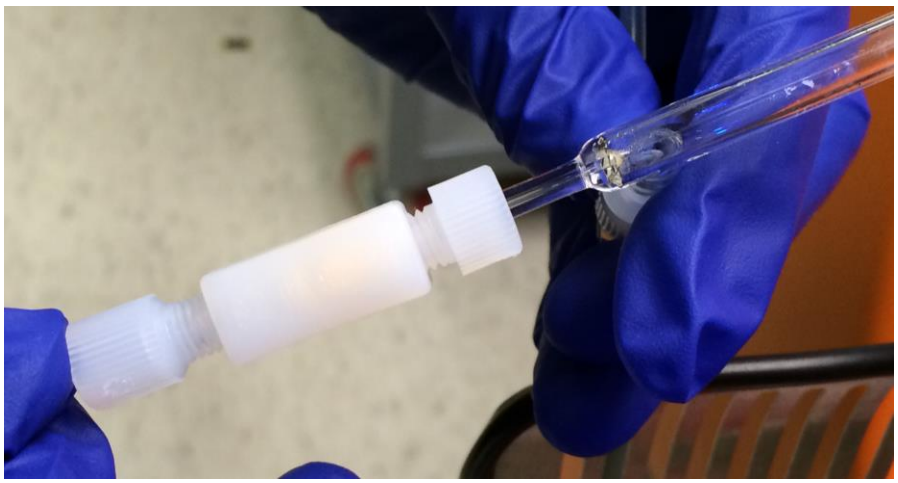


Connecting the Nebulizer

1 Before plasma start up, connect the nebulizer and insert it in the nebulizer adaptor port.

2 Remove the clean nebulizer from the nebulizer rest and dry thoroughly, being careful not to touch the nebulizer tip.

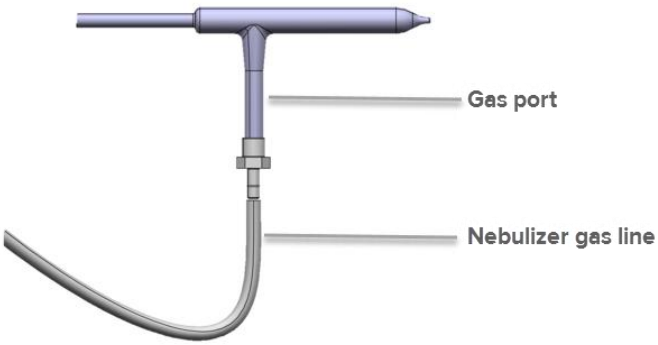
3 Insert the sample capillary (PN 105922) into the sample inlet end of the nebulizer (if required).



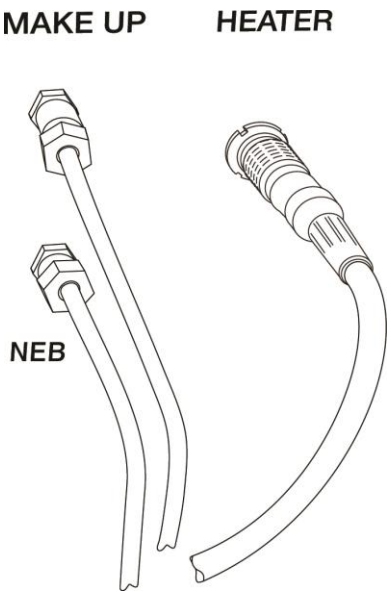
4 Push the sample capillary in gently until it reaches the tapered portion of the nebulizer.

5 Tighten the 1/8” nut.

6 Connect the nebulizer gas line to the gas port of the nebulizer.



NOTE Check that the nebulizer gas line, the makeup gas line, and the heater quick-connects on the front of the instrument tightly fit.



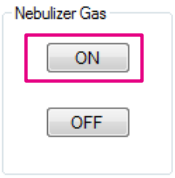
7 Fill a 5 mL round-bottom tube with 1 mL of Type 1 ultrapure (18.2 ΩM) water (DIW). Lift the handle of the Sample Loader and insert the tube into the holder. Close the handle. The LED indicator should turn blue.

Check the Nebulizer Spray

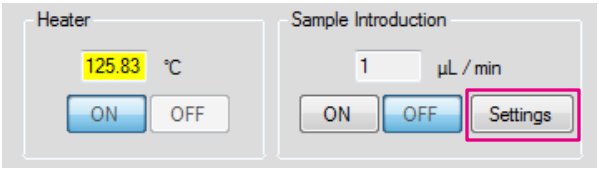
Before starting plasma, verify fluid flow by checking the nebulizer spray.

NOTE The nebulizer is held in your hand during this test.

1 Go to **Control Panel>Switch Box** and click Nebulizer Gas **ON**.

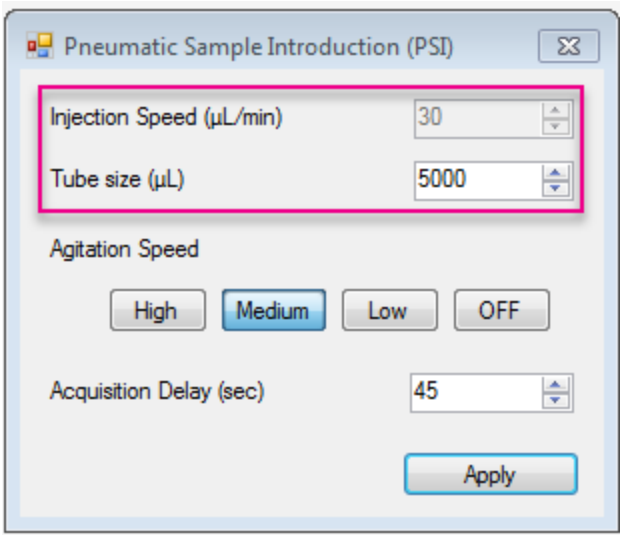


2 Load a 5 mL tube containing deionized water (DIW) into the Sample Loader.



3 Click **Settings**.

4 The Sample Introduction Settings window will open. The default injection speed is 30 µL/min and the Tube size is 5,000 µL.



5 Click Sample Introduction **ON** to start DIW flow through the nebulizer.

6 Observe the spray from the nebulizer using a flashlight. It should appear as a fine aerosol that leaves the nebulizer in an even, symmetrical pattern. If not, clean (see End-of-Day Cleaning) or replace the nebulizer.

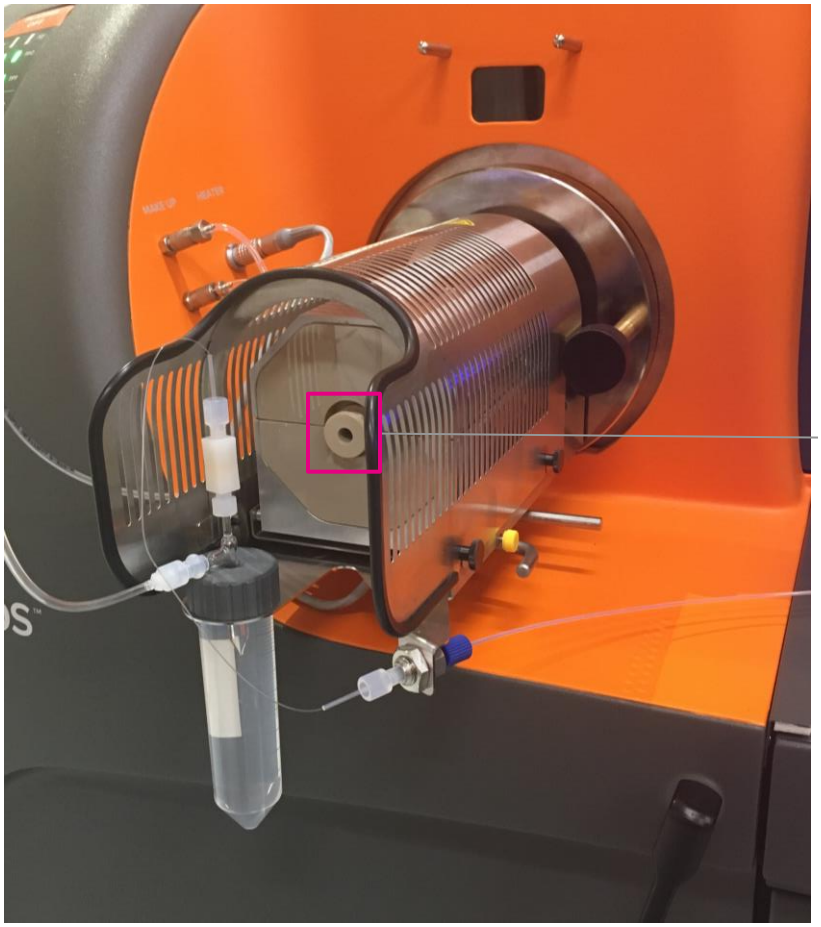
7 Go to **Status Panel>Sample Introduction**, verify that the PSI pressure does not exceed 14 psi.

NOTE If the PSI pressure is higher than 14 psi, clean (see see End-of-Day Cleaning).

8 Click Sample Introduction **OFF.**

9 Go to **Control Panel> Switch Box** and click Nebulizer Gas **OFF**.

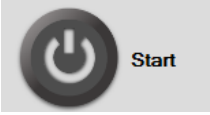
10 Loosen the nebulizer adaptor port a half-turn counter-clockwise, insert the nebulizer into the nebulizer adaptor port until it reaches a hard stop, and tighten clockwise a quarter-turn to secure the nebulizer into the adapter.



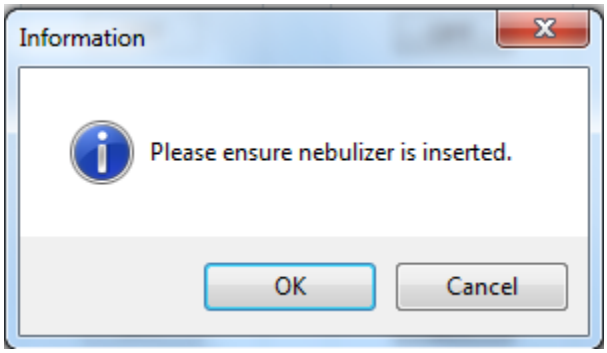
WARNING HOT SURFACE HAZARD. A safety interlock on the CyTOF 2 and Helios systems automatically shuts off the plasma if the chamber and interface are not fully coupled. Do not defeat the interlock. Do not remove the shield that protects the sample introduction system. The heat shield is designed to protect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

Plasma Start Up

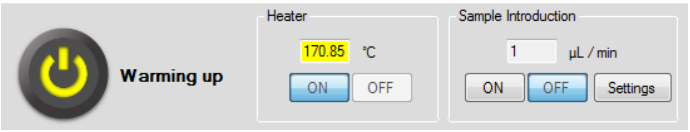
1 Click the **Start** button in the bottom panel of the CyTOF software.



2 When the insert nebulizer dialog box appears, click **OK**.



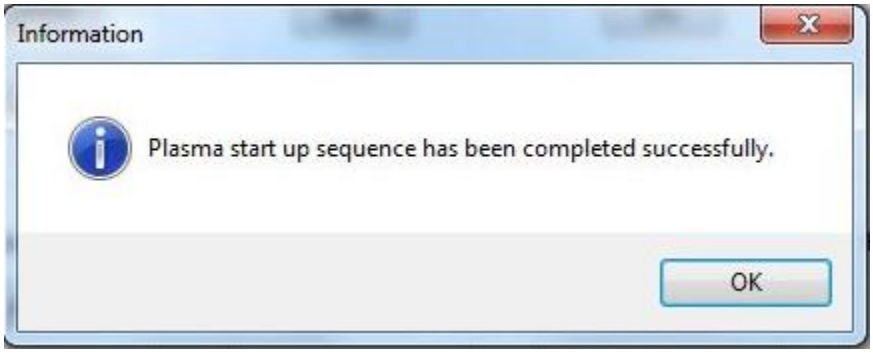
This will turn on the heater and begin to heat up as well as turning on the plasma in the system. The **Start** button will turn yellow after a few seconds.



NOTE The plasma startup sequence will begin. The plasma, heater, and interface pump take approximately 15–30 minutes to warm up.

IMPORTANT Please ensure that the instrument is not left unattended during the plasma startup sequence. User intervention may be required to address instrumentation issues.

3 When the plasma startup information box appears, click **OK**.



4 When the heater reaches approximately 200 °C the Startup button will turn green, Ready will appear, and the temperature field will turn blue when it has reached 200 °C.

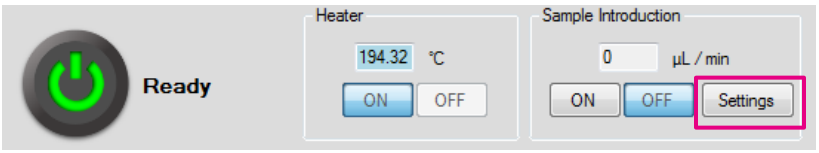
NOTE It is safe to run DIW. Let the instrument warm up for 15–30 minutes after which point you may begin tuning procedures.

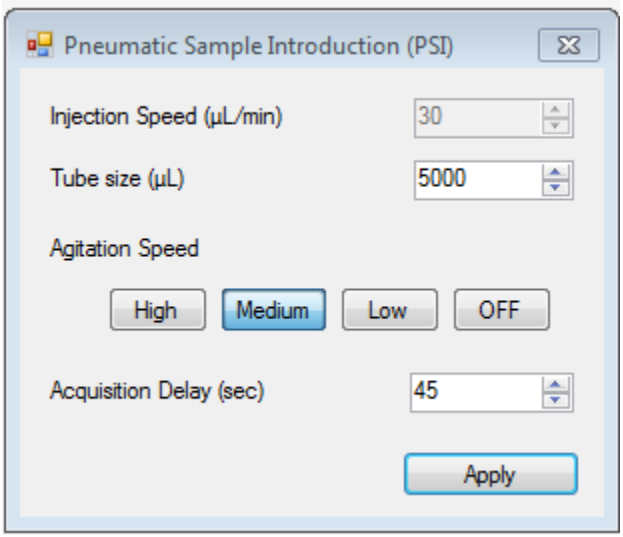
Sample Loader

Sample Agitation

When a filtered 5 mL round-bottom tube is placed in the tube holder of the Sample Loader (uncapped), the sample is agitated prior to injection into the nebulizer. The default setting for agitation is 8 seconds in the clockwise direction and 8 seconds in the counterclockwise direction. (There is a 1 minute delay between agitations.) The sample is then directed to the nebulizer.

NOTE The agitation frequency, speed, and time can be adjusted in administrator mode. In the control bar at the bottom of the screen click the **Settings** button. There are four settings available for agitation: Off, Low, Medium, and High.





The sample is carried through the sample capillary to the nebulizer, which is inserted into the nebulizer port of the heater box. It then travels through the sample introduction system towards the plasma.

Figure 25. Sample Loader and sample heater assembly

Sample Loader Configurations

The Sample Loader has been designed to simplify sample loading.

IMPORTANT

- Take care to remove the cap from the 5 mL round-bottom tube before closing the holder of the Sample Loader.
- Take care not to damage the silica sample probe line during sample loading and sample run. Load the sample tube in the upright position.

Sample Loader LED Indicator Colors

The Sample Loader has three LED colors with flashing function to indicate the operational status of the module. When the LED indicator is white, the handle is in the open position and the Sample Loader is ready for sample loading.

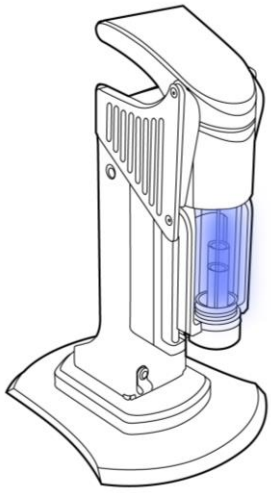


Figure 26. Sample Loader LED indicator

When a sample of cells, beads, or tuning solution has been loaded into the 5 mL tube and the Sample Loader handle is pushed down and closed, the LED indicator turns to blue.

Go to **Sample Introduction** and click **ON** in the CyTOF software and the module begins to pressurize until the LED indicators begins to glow blue. This means that the system is pressurized and ready to begin tuning the system or to preview or to record your data in the CyTOF software. The 5 mL round-bottom tube containing the sample will be agitated and sample will be taken up into the nebulizer.

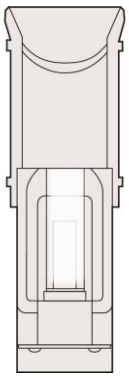
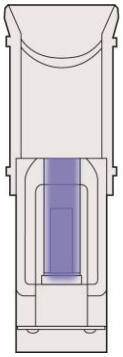
NOTE To check the pressure in the Sample Loader, open **Status Panel > Sample Introduction >** Pressure. The pressure should read up to approximately 14 psi.

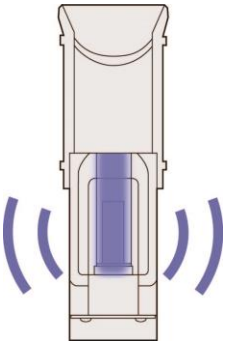
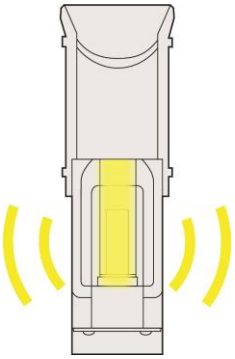
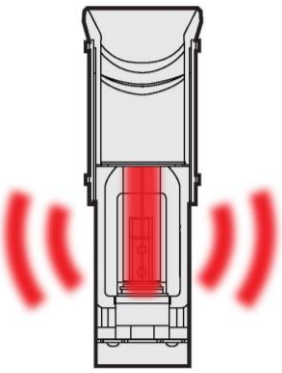
NOTE If there is no more sample left in the tube, the LED indicator will turn from blue to flashing yellow.

When the module begins to turn a flashing yellow, the module is depressurizing and it is not safe to open the unit. Wait for the unit to completely depressurize and turn solid yellow before unlocking the handle.

IMPORTANT Do not open the handle of the sample delivery module when the LED indicator is blue and pressurized. This may cause the sample to become aerosolized. In this case the LED indicator will quickly flash yellow to indicate an error and the system will immediately begin depressurizing.

Table 11. Summary of LED indicator colors on the Sample Loader

	LED Indicator Color	Description
	White	Handle is open. Ready for sample loading
	Blue	The sample is loaded; the handle is closed and the Sample Loader is ready to pressurize.

	LED Indicator Color	Description
	Glowing blue	The system is pressurized and sample is being delivered to the nebulizer. ⚠ **CAUTION** Do not open the Sample Loader.
	Flashing yellow	The system is depressurizing. ⚠ **CAUTION** Do not open the Sample Loader.
	Flashing Red	The Sample Loader agitator has a fault detected. See Helios Troubleshooting for more details.

Daily Quality Control

Daily quality control (QC) of Helios involves checking the instrument performance, monitoring background, and tuning the system.

Before beginning tuning, ensure that all the parts have been cleaned and correctly assembled on the instrument.

- The nebulizer has been cleaned correctly.
- The connections of the nebulizer sample capillary line are tight.
- The nebulizer has been inserted correctly into the nebulizer adaptor port.

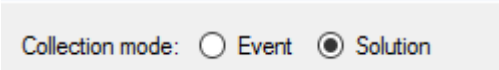
- The external sample introduction line is correctly connected to the heater block/assembly.
- The makeup gas line is connected.

Monitoring Background Signal

Due to the high sensitivity of the system, it is important to create a background template for all metals that have been run on the Helios system which can be used to monitor metal contamination. This should be done daily before tuning procedures and in between samples.

1. On the Menu Panel click the **Acquire** tab.
2. Click Experiment Manger, and the **Default** Template should appear.
3. To add additional metals to be monitored open the Elements table and select the masses or enter the mass in the Mass Field and click +.

4 In Collection mode, click Solution.



5 Save the template as “Background_Solution.tem”, and close the Experiment Manager Window.

6 Enter a name for the background sample and set the **Stop At** limit to 60 readings, 1 second per reading.

7 Load 1 mL of DIW onto the Sample Loader, and click 

to collect data.

The Masses Per Reading window opens.

8 Select **Dual Count** for the Y-axis.

9 Change the y-axis range upper limit to 10,000. The selected elements should fall below 1000 and there should be no signal from the Ir 193 channel. Keep washing until the contamination disappears.

10 Click **Stop**.

11 When the preview dialog box appears, click **OK**.

12 Use this template to compare subsequent samples on the instrument and to compare the background.

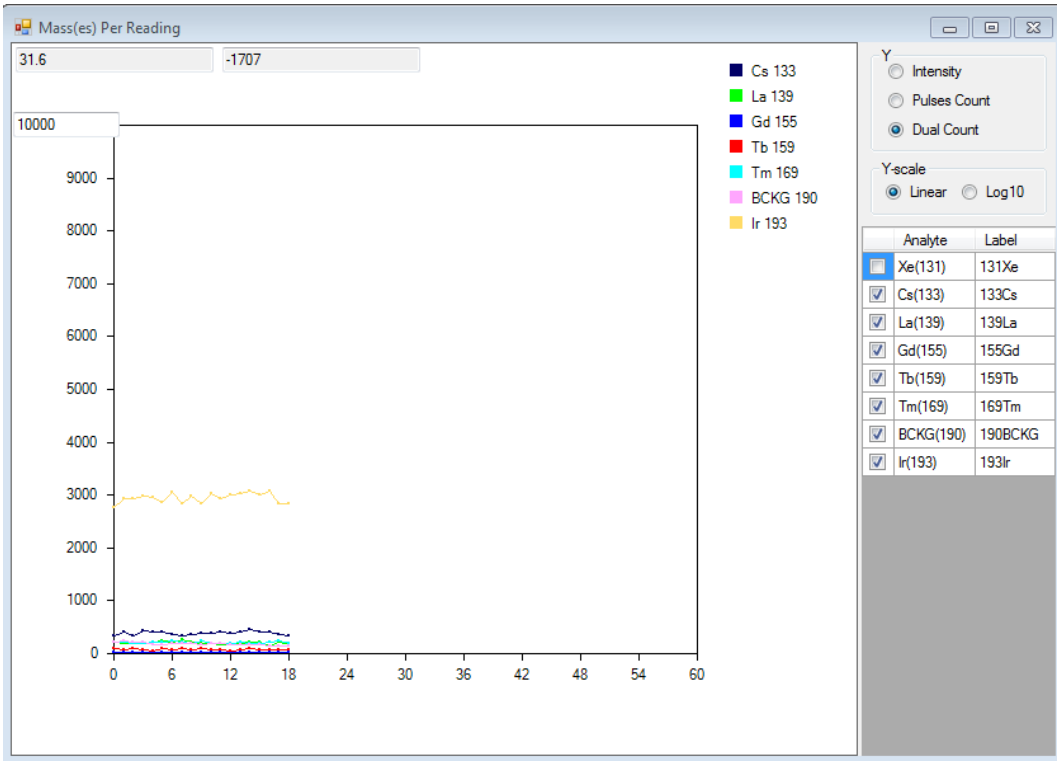


Figure 1. Masses per reading window. In this example, the 193Ir signal is very high which suggests the need for instrument maintenance (cleaning).

Tuning

The Helios system has fully automated instrument tuning and calibration. The tuning protocol requires the addition of Tuning Solution (PN 201072). The software will automatically select the following isotopes for tuning: ^{133}Cs, ^{139}La, ^{155}Gd, ^{159}Tb, ^{169}Tm, and ^{193}Ir.

NOTE Lanthanum oxide (^{155}LaO) is monitored and used for gases and current optimization (see section below). During tuning the ^{155}Gd channel is used to monitor LaO.

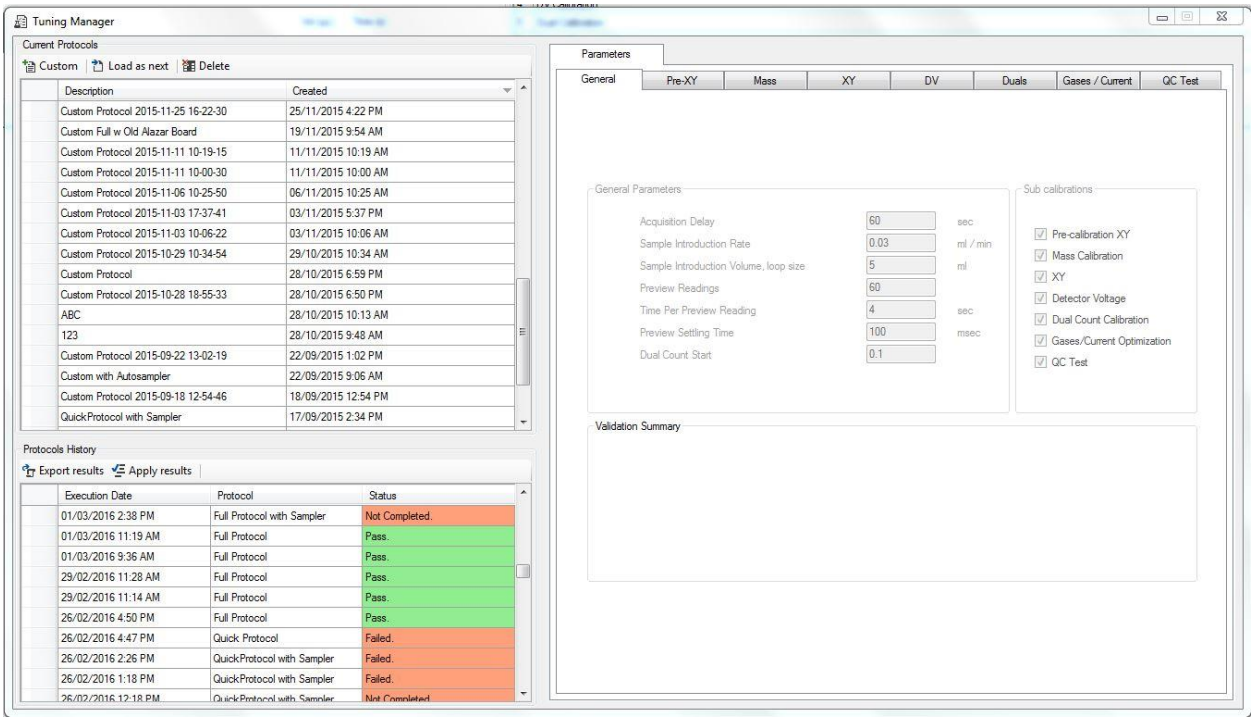
Tuning Manager

In the Tuning Manager window users can select the appropriate tuning protocol.

- The Full Protocol includes mass resolution, mass calibration, pre-calibration XY, XY Optimization, Detector Voltage (DV) Calibration, dual calibration, gases/current calibration, and QC test. The Full Protocol is recommended at the start of each day, when the results from Quick Protocol tuning do not pass all the specifications, or each time maintenance procedures have been performed. It is also recommended when the instrument has not been in use for an extended period of time.
- The Quick Protocol includes mass resolution, mass calibration, dual count calibration and QC test. This protocol is recommended as a performance check at the beginning of each

operating session or experiment. Users are recommended to check that the tuning results meet specifications for performance before moving on to QC with beads and samples.

- Users and administrators also have the ability to create and run their own Custom Protocols; custom protocols that have already been created can be selected from the Custom dropdown menu. Alternatively, the Tuning Manager allows users to create a new custom protocol.
- The Load as next button allows users to select the protocol to be run. The protocol that is loaded as next will show up as the selected protocol from the dropdown menu.
- The Protocols History summarizes the results of the most recent tuning protocol and provides the time that it was executed. Users and administrators can select a protocol history and apply the results to update the settings of the instrument.



Tuning Procedure

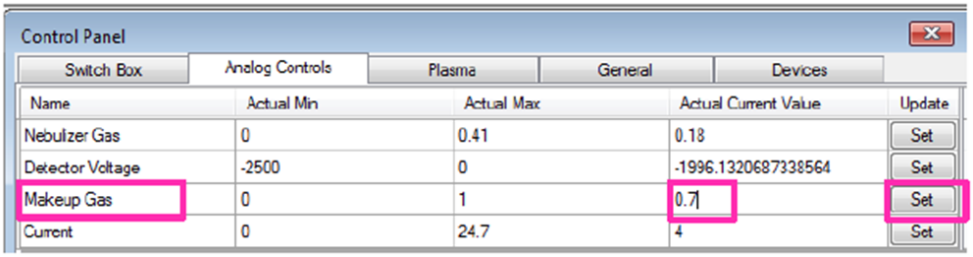
Adjusting the Makeup Gas with the WB Injector

In order to use the WB injector in your instrument you must change the Makeup Gas Flow Value from what is set for the HT injector.

IMPORTANT **Do not adjust the makeup gas value if you are using the HT injector on Helios**.

1 Click Control Panel in the menu panel of the CyTOF software, and then click Analog Controls.

2 Set the Actual Current Value for Makeup Gas to +0.2 L/min higher than current value, and then click Set. In the example below, the Actual Current Value was set to 0.5 L/min for the HT injector so the Actual Current Value for the WB injector is set to 0.7 L/min.



NOTE CyTOF Tuning Solution (PN 201072) is required for this procedure.

Helios should be tuned daily or between runs to ensure optimal data quality.

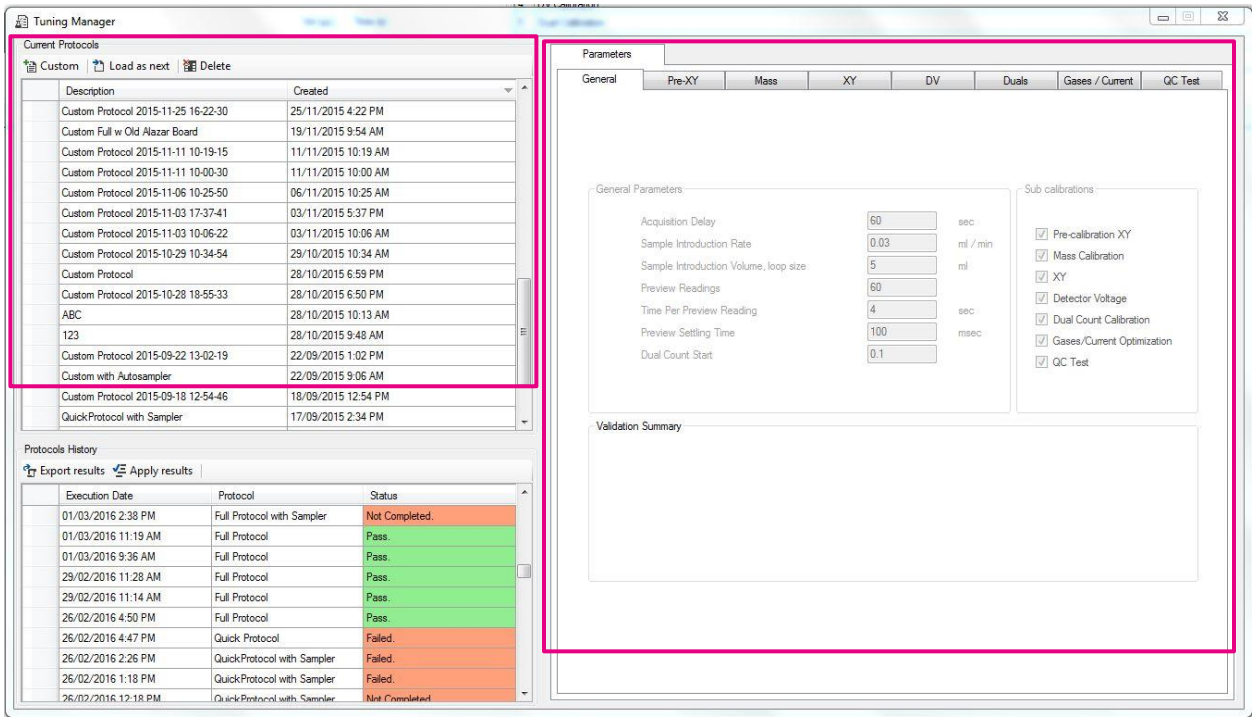
1 Click the **Tune** tab in the menu panel section of the software.

2 Select the tuning protocol to run from the dropdown menu.

NOTE The Full Protocol should be run at the start of each day.

3 Click **Load as next**.

Alternatively, click the **Tuning Manager** button and the Tuning Manager window will open for selecting and setting up the tuning protocol to run.



4 Once the tuning protocol has been selected, load 1 mL of tuning solution into a 5 mL round-bottom tube.

5 Open the Sample Loader and load the tube in the holder.

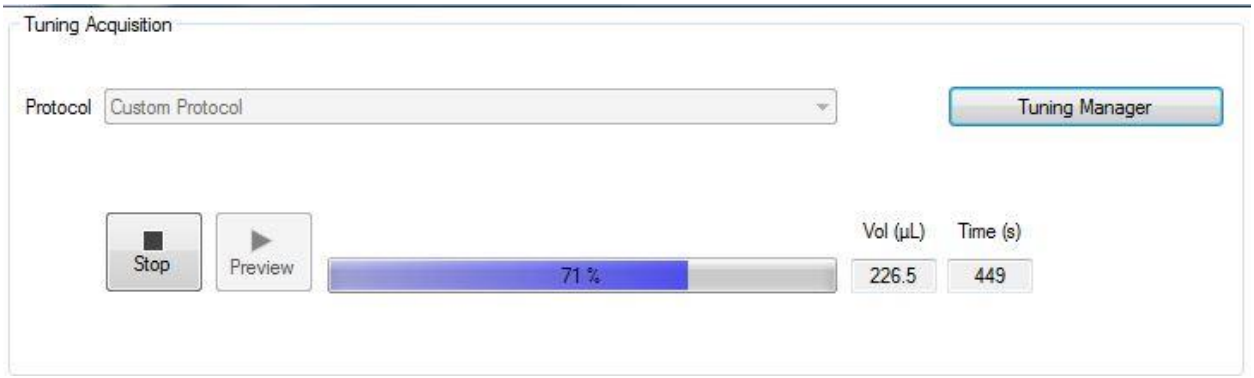
6 Close the Sample Loader.

7 Click 

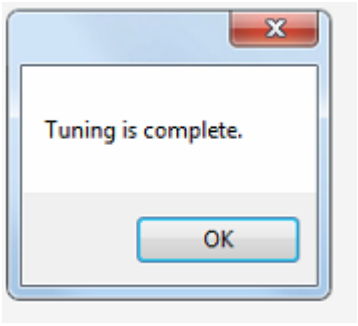
to start the tuning protocol with the pre-set acquisition delay. Users can also click 

in the **Tuning Acquisition** window to ensure that tuning solution is injected properly before starting the tuning protocol The MPR window will appear in the workspace with Preview. The default number of readings shown on the MPR graph is 60; after 60 readings Tuning solution will continue to be acquired without the protocol being displayed or run. Click **Record** anytime during Preview to start the tuning protocol and the acquisition delay will be skipped.

A progress bar appears to indicate the progress of tuning procedures.



8 Click **OK** in the dialog box that appears when the tuning procedure (calibration) is complete.



9 Click the **Results** button at the bottom of Tuning Sequence to access the Results tab in the Tuning Manager window to access the report generated at the end of the tuning procedure.

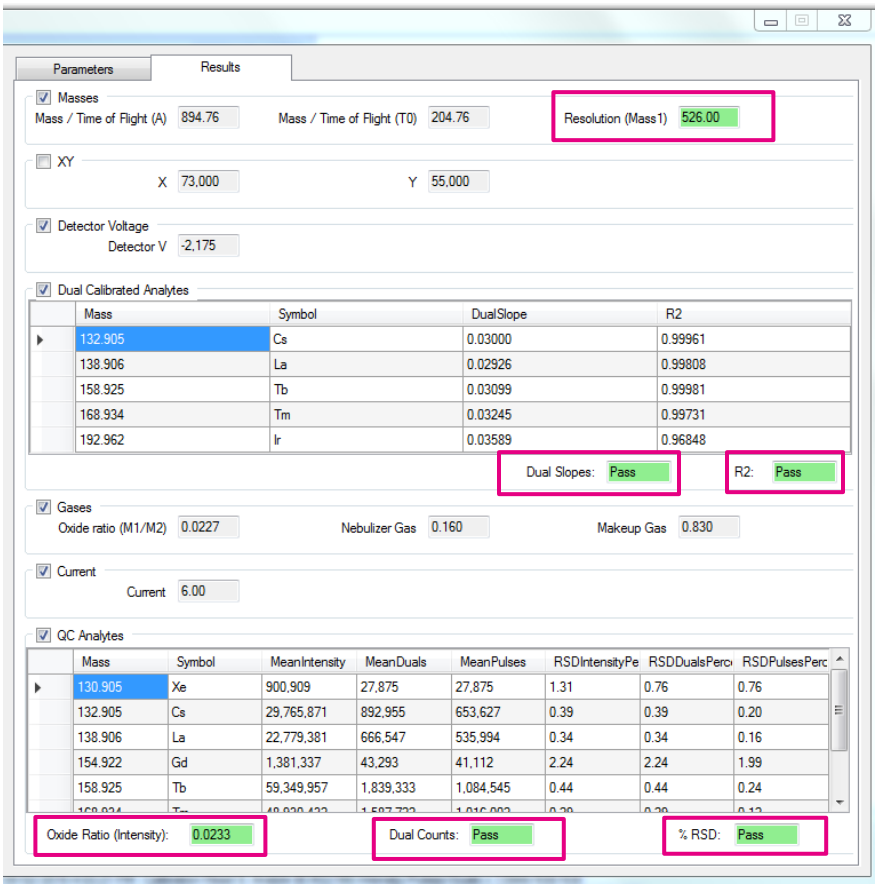
Tuning Sequence

	Tuning Protocol	Status	Date/Time Run
1	Pre-XY Optimization	Completed.	22/06/2015 3:08 PM
2	Mass Calibration	Completed.	22/06/2015 3:08 PM
3	XY Optimization	Completed.	22/06/2015 3:08 PM
4	DV Calibration	Completed.	22/06/2015 3:08 PM
5	Dual Calibration	Completed.	22/06/2015 3:08 PM
6	Gases/Current Calibration	Completed.	22/06/2015 3:08 PM
7	QC Report	Completed.	22/06/2015 3:08 PM

Results

Tuning Results

Once the tuning procedures are complete, select the **Results** tab in the Tuning manager. The Results tab indicates the subcalibrations that have been run in the protocol and contains the results of the tuning procedures. These results include the optimized settings of the subcalibrations selected for tuning Quick Protocol, Full Protocol, or Custom Protocol, as well as results of the QC test. For subcalibrations that have not been run in the protocol, the settings used for the protocol are displayed.



To verify that the tuning procedures were successful, verify the following have passed (indicated by a green Pass box):

- Resolution (Mass1) is >400

- The Mean Duals for ^{159}Tb is >600,000.
- The Dual Slopes are between 0.03 and ±0.003.
- The R2 is >0.8.
- If Gas/Current optimization was selected this Oxide ratio (M1/M2) is displayed in Gases. This should be lower than <0.03.
- The %RSD (relative standard deviation) values for Cs, La, Tb, Tm, and Ir should be <3%.

  NOTE Relative standard deviation (RSD) is equivalent to coefficient of variation (CV).

If all of the above criteria have been met, proceed to the bead sensitivity test.

Tuning Parameters

General Parameters

The General Parameters tab contains the selection of subcalibrations in auto-tuning that can be adjusted by users in a custom protocol. There are also general acquisition settings for the protocol to run. The acquisition delay is set at 50 seconds, by default. The detector stability delay is set to 5 seconds and the sample introduction rate is set to 0.03 mL/min for the tuning procedure. The settings for Preview can also be adjusted within this tab.

Mass Resolution

This calibration ensures that there is sufficient separation between ions of different mass. The mass resolution is determined from the Tb and is equal to the ratio of the time-of-flight (TOF) and twice the width of the Tb peak at half maximum. A mass resolution above 400 indicates a pass.

Mass Calibration

The auto-tuning checks the TOF values for ^{133}Cs and ^{193}Ir and then calculates the TOF values for the remaining isotopes. This also aligns the correct ions to the detection channel so that the entire signal for each ion is collected.

Dual Count Calibration

In dual count calibration, dual count coefficients (dual slopes) are determined to correlate pulse count and intensity. The dual count coefficient converts analog signal to ion count signal. This correlation is important when ion concentrations increase and pulses overlap, for example, during a cell event.

IMPORTANT Mass resolution, mass calibration, and dual count calibration should be done with each run.

Pre-XY and XY Optimization

The XY optimization is the process by which the optimal alignment of the torch with the vacuum interface is determined to provide the maximum Tb signal from the tuning solution during calibration. Optimizing the alignment of the system is important for maximum transmission of ions into the vacuum interface. Pre-calibration XY happens prior to mass calibration and provides a coarse alignment, while XY optimization fine-tunes for maximum signal after masses are aligned.

Detector Voltage

Detector voltage calibration uses the dual count calibration to determine the detector voltage that provides the best signal while ensuring the longevity of the detector. The optimum detector voltage is achieved when the dual count coefficient is 0.03 ± 0.003. The detector voltage should not be more positive than -1,100 V.

Gases/Current Calibration

This function optimizes the nebulizer gas flow and the makeup gas flow using the maximum ^{159}Tb signal that can be achieved by varying the makeup gas flow and nebulizer gas flow while controlling oxide formation. This will ensure that the plasma temperature is optimal in the system and minimal metal oxides are formed. The current that is applied at the vacuum interface is increased in increments to drive the transfer of the ion cloud through the interface. The value that provides the highest ^{159}Tb signal is selected in the system.

IMPORTANT Pre- XY and XY Optimization, Detector voltage, and gases/current calibration should be run when specifications are not met in the quick protocol tuning results.

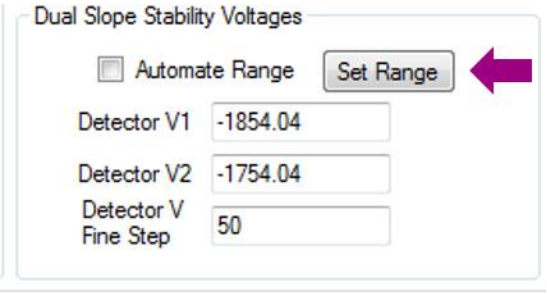
QC Test

At the end of the Quick or Full Protocol for tuning a QC report is generated to show the results of the different tuning procedures.

IMPORTANT See Tuning Results section for further details about how to interpret QC test results and recommendations on subsequent procedures.

Automatic Range Selection

By default, a Full Protocol tuning has the ranges for detector voltage, gases, and current optimization automated during tuning. When setting up a custom protocol, users can also automate these ranges by checking the **Automate Range** box within the respective tabs for the subcalibrations.



Deselect **Automate Range** to enter your own ranges, or click **Set Range** to have the instrument set the range for you prior to adjusting it.

Bead Sensitivity Test

NOTE EQ Four Element Calibration Beads (PN 201078) are used for the bead sensitivity test.

1. Flush the Sample Loader with washing solution for 2 minutes.
2. Flush with DIW for 5 minutes before beginning the test. Click Preview to observe the signals in the rain plot to ensure that the system has been cleaned.
3. Click the Acquire tab. Open the Experiment Manager and select the default template.
4. Vigorously shake or vortex the bottle with EQ Beads for at least 30 seconds to ensure thorough agitation.
5. Add approximately 500 µL of EQ Beads to a 5 mL round-bottom tube and load on Sample Loader.
6. In the Menu Bar click **Stop at.**
7. Click **Time** and set the acquisition time to 120 seconds.
8. Click 

to open the rain plot. When you see signal in the bead channels, proceed.
9. Click **Record** 

.

Analyze Bead Data

1. Once the acquisition is complete, open Premium Cytobank, create a new experiment, and upload your files.
2. Gate the singlet population. Calculate the event number of singlet events.

   The All Events count should be at least 6,000 events. If not, vortex the EQ beads again and rerun EQ Beads. If rerunning the EQ Beads results in lower than 6,000 events in total, tune the instrument again.
3. Check that the mean of singlet population for ^{151}Eu or ^{153}Eu is at least 1,000.
4. If the mean of singlet population for ^{151}Eu and ^{153}Eu is <1,000, tune the instrument again.

WB Injector Only

5 Following tuning and bead sensitivity, **condition** the system by running Maxpar Cell Acquisition Solution for 15 minutes prior to acquiring samples.

Data Acquisition

Sample Preparation

NOTE Go to fluidigm.com/support/Helios for sample preparation protocols.

To maximize data quality, filter samples to reduce clumps and dilute them to optimal concentration before loading them onto the Sample Loader.

1 Vigorously shake or vortex the bottle with EQ Beads. Then dilute the EQ Beads 1/10 based on the type of injector in use.

- For HT Injector: Dilute the EQ Beads 1/10 in DIW.
- For WB Injector: Dilute the EQ Beads 1/10 in Maxpar Cell Acquisition Solution.

2 Immediately before data acquisition, adjust cell concentration to 1.0 x 10^6 cells/mL or concentration appropriate for the sample type in the diluted EQ bead solution.

3 Filter cells into cell strainer cap tubes. The sample is now ready for acquisition.

IMPORTANT A higher concentration may result in a higher number of aggregates and lower singlet throughput.

4 Acquire sample data, leaving a minimum of 50 µL in the tube at the end of recording.

IMPORTANT This avoids collection of the end of the sample, which may contain a higher concentration of debris and/or aggregates that can contribute to clogging.

Use one of the following methods:

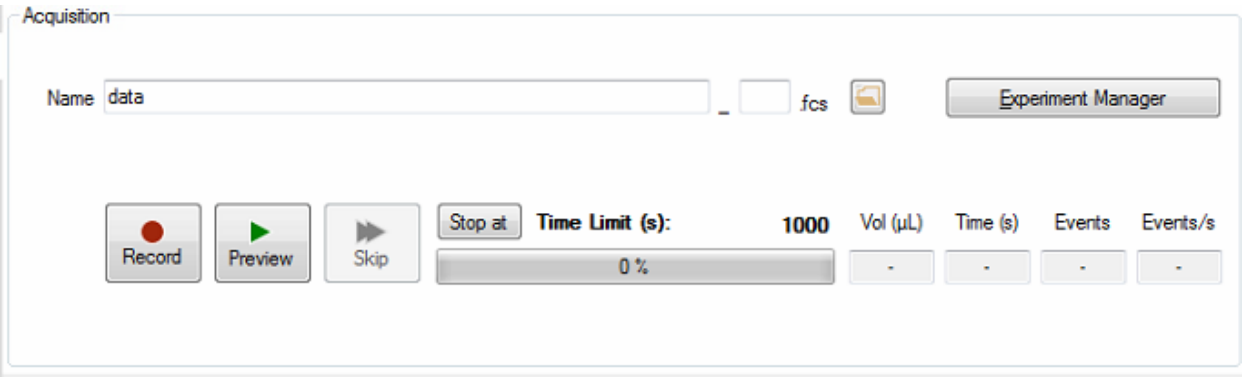
- In the Acquisition box, set the Stop at Time limit to the number of seconds that will leave at least 50 µL of sample remaining in the tube at the end of recording. Use the flow rate of 30 µL/min to calculate this time.
- In the Acquisition box, set the Stop at Event limit to the number of events that will leave at least 50 µL of sample remaining in the tube at the end of recording.

5 If the sample is precious and the last 50 µL is required, dilute it and collect data in a subsequent run.

IMPORTANT The sample introduction rate for Helios is set to 0.03 mL/min by default.

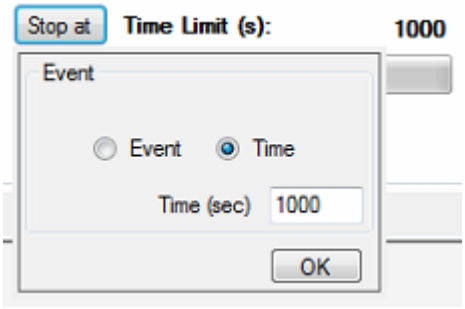
Acquisition Window

The Acquire tab has features similar to those of the Tune tab.

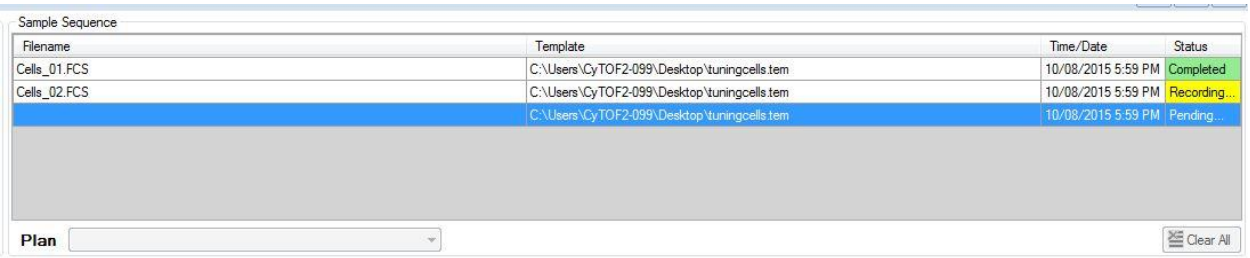


- Preview allows you to view data without an acquisition delay, but the data is not recorded.
- Record applies the acquisition delay and then begins to collect and display the data.

  The Stop at button allows users to manually stop the acquisition. Time specifies the acquisition period in seconds, and Event specifies the target number of events collected before the acquisition is stopped. If the event limit is not reached and the sample runs out, a popup window asks the user either to load more samples or stop the acquisition.



The Sample Sequence shows the samples being run.

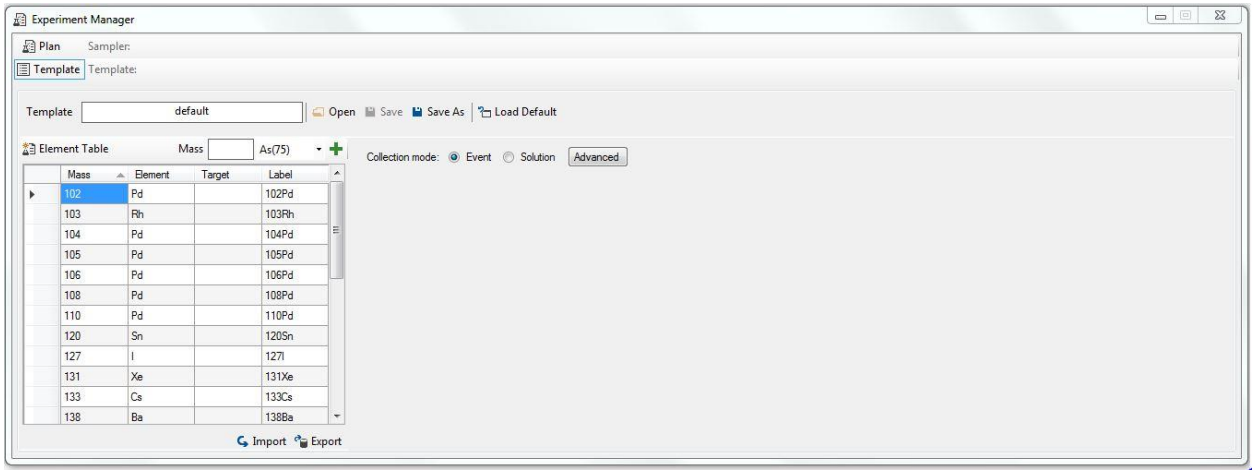


Experiment Manager

You can manage your experiments and set up new experiment plans in the Experiment Manager window.

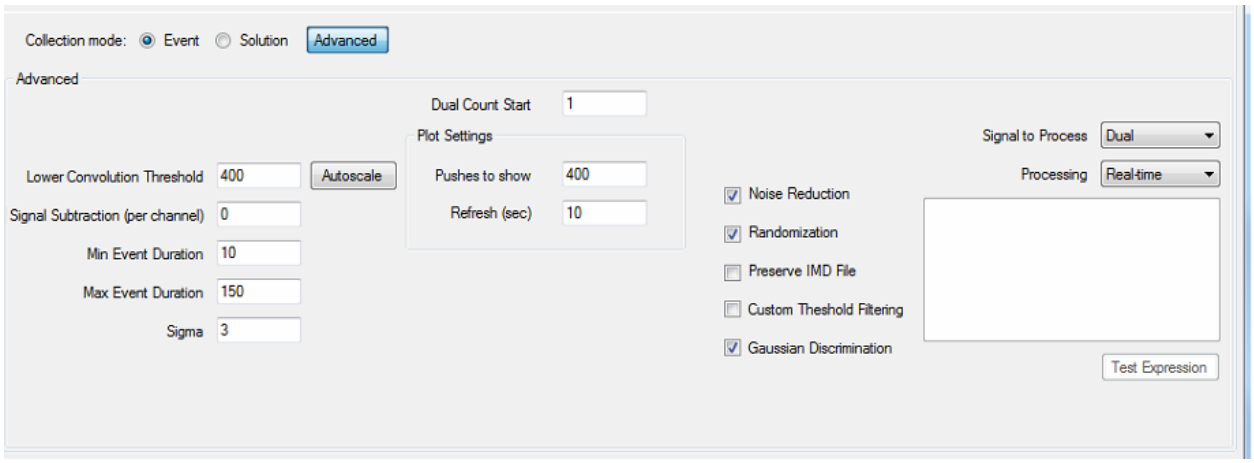
1 Click **Experiment Manager**. The Default template appears.

2 Add to the list of analytes by selecting masses from the Elements table or enter it into the Mass field and click +. A previously saved template may also be used.



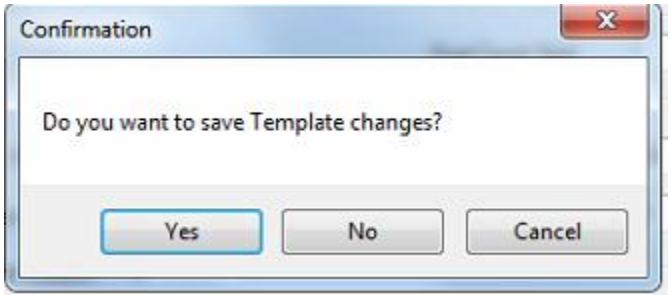
3 Users can also import analytes from the Maxpar® Panel Designer with the Import button. To save a modified template, click **Save as** and specify where it will be saved.

Administrators may adjust data processing settings by clicking on **Advanced** in the Template window.



- In the Advanced tab of the Experiment Manager**,** template users can adjust the lower convolution threshold.
- Administrators may also adjust the refresh rate of the Plotviewer (in seconds) in this window.

4 Close the Experiment Manager to prepare to run samples. A dialog box asks you to save the template changes. Click **Yes** for the changes to be applied in the following acquisition.

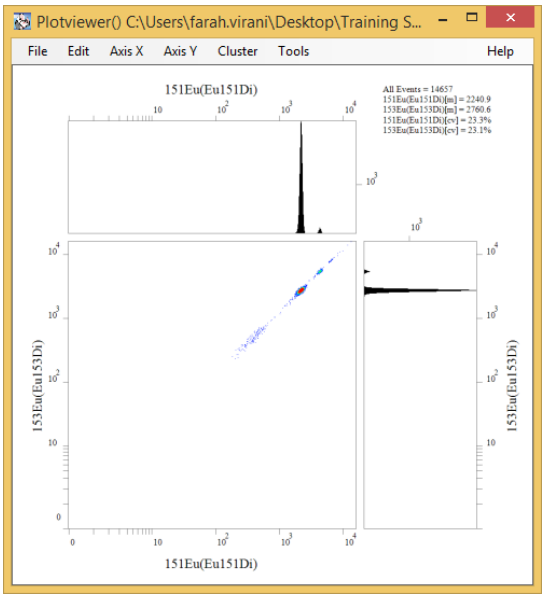


5 Load the sample onto the Sample Loader.

6 (Optional) Click **Preview** to check the quality of the sample.

7 Click **Record** to begin recording data.

NOTE During acquisition the **Plotviewer** window will appear in the Workspace to allow you to view the samples as the data is recorded. Select the appropriate X and Y axes for your purpose.



Cleaning the System Between Samples and Running Beads

It is important to run DIW between samples to maintain proper plasma conditions, remove residual sample within the tubing and nebulizer, minimize any potential cross-contamination, and prevent clogging.

IMPORTANT If using the WB injector with Maxpar Cell Acquisition Solution, replace DIW with CAS in steps 1–13 below.

1. Immediately after the end of an acquisition, replace the sample tube with a 5 mL tube containing DIW.
2. Click Sample Introduction **ON** to start DIW flow. Run DIW for 2 minutes.
3. Click **Preview** and observe signals in the rain plot.
4. Click **Stop**.
5. Repeat steps 2 to 4 until the rain plot is clear of residual cell signals.
6. Load the next sample immediately and click **Record** to begin acquisition.

   NOTE If you are not ready to load the next sample, load 5 mL of DIW into the Sample Loader. Click Sample Introduction **ON** and run DIW until the next sample is ready to be acquired. Go to step 6 above

7. For a more thorough wash:
8. Immediately after the end of an acquisition, replace the sample tube with a 5 mL tube containing washing solution.
9. Click Sample Introduction **ON** to start the flow of washing solution.
10. After 2 minutes, click Sample Introduction **OFF**.
11. Replace the washing solution with a 5 mL tube containing DIW.
12. Click Sample Introduction **ON** to start DIW flow for 5 minutes.
13. Go to step 4 in the procedure above.

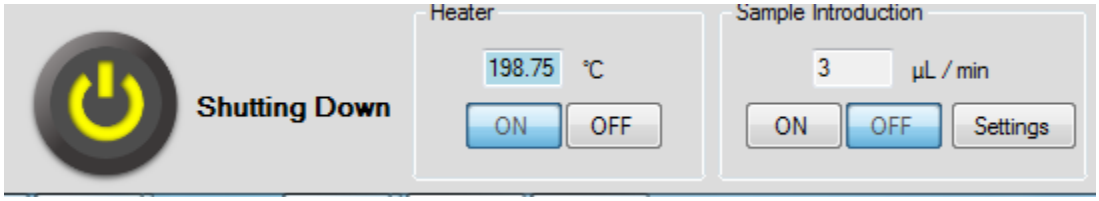
End-of-Day Cleaning

1. With plasma on, run washing solution for 2–3 minutes, then run DIW for 5–6 minutes.
2. Click **Preview** in the Acquisition tab. Check that the rain plot is clear and there are no residual cells or beads with minimal background.

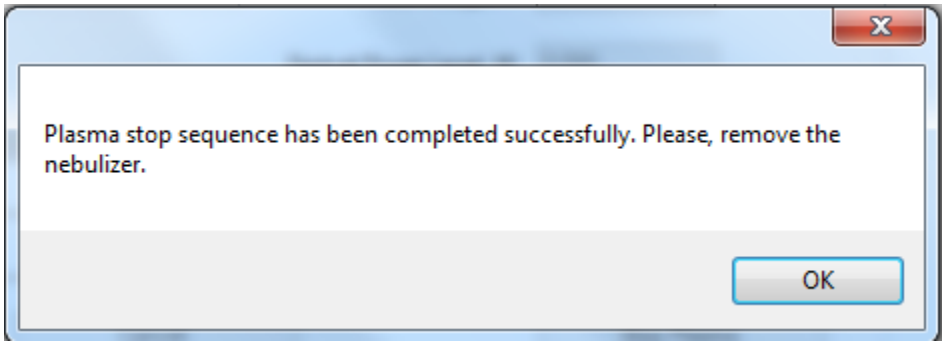
Shutdown: Turning Off Plasma

1 Click 

to shut down the system. The Ready button turns yellow and then gray when the plasma stop sequence is complete.



The plasma stop sequence dialog box appears. The sample delivery module, chiller and heater are automatically turned off when the plasma stop sequence is complete.



2 Loosen the nebulizer adaptor port half a turn, then remove the nebulizer from the nebulizer adaptor port.

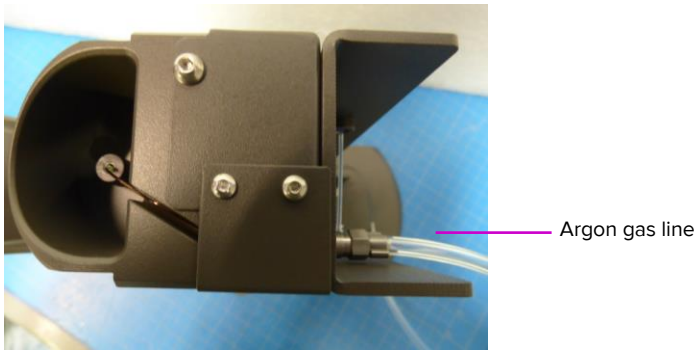
3 (Optional) Proceed to cleaning the nebulizer. See Chapter 6: Maintenance for a detailed nebulizer cleaning protocol.

4 Place the nebulizer back in the nebulizer rest containing DIW.

5 Fill at least 4 mL of DIW in a 5 mL tube.

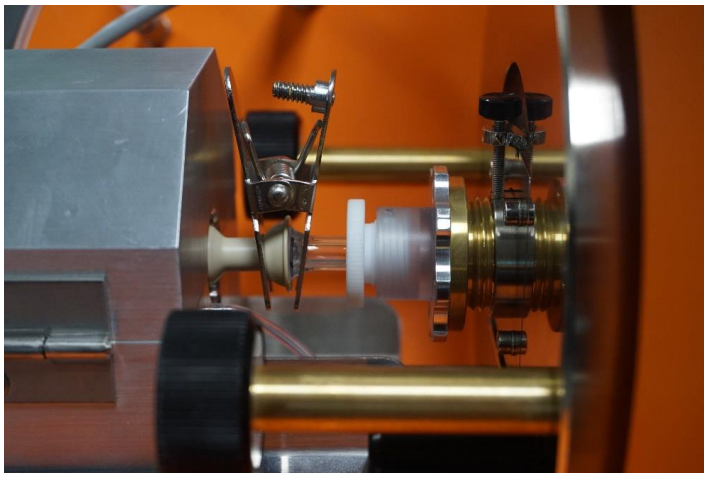
6 Load into the Sample Loader.

IMPORTANT Residual pressure remains in the system after plasma shutdown. This pressure is sufficient to allow a small amount of fluid to pass through the sample line and nebulizer. Please follow steps 5 and 6 upon plasma shutdown. For extended instrument inactivity (>2 days), disconnect the argon gas line from the back of the Sample Loader.



For WB Injector

1 Remove the ball joint clamp that secures the spray chamber to the WB injector.



2 Slide the heater off of the heater box pins and rest it on the upper support pins.

3 Remove the WB injector by gently pulling and turning until it comes loose from the torch assembly.

4 Rinse the WB injector in DIW and dry thoroughly.

Optional Cleaning Procedure without Plasma

To flush the Sample Loader before plasma start or after plasma has been turned off, use the following procedure.

1. Empty the 50 mL conical tube in the nebulizer rest.
2. Insert the nebulizer into the hole of the nebulizer rest cap.
3. Load the 5 mL tube containing washing solution into the Sample Loader.
4. Click Sample Introduction **ON** to start the flow of washing solution through the nebulizer.
5. After 5 minutes, click Sample Introduction **OFF**.
6. Repeat steps 5–7 with a 5 mL tube containing DIW for 5 minutes.
7. Remove the nebulizer from the nebulizer rest.
8. Replace the 50 mL tube of the nebulizer rest with a clean 50 mL tube filled with DIW.
9. Top up the 5 mL tube in the Sample Loader to contain at least 4 mL of DIW.
10. Place the nebulizer back in the nebulizer rest filled with DIW.

    IMPORTANT Residual pressure remains in the system after plasma shut down. This pressure is sufficient to allow a small amount of fluid to pass through the sample line and nebulizer. Please follow steps 9 and 10 upon plasma shutdown. For extended instrument inactivity (>2 days), disconnect the argon gas line from the back of the Sample Loader.

NOTE Disconnect the nebulizer gas line from the nebulizer if the nebulizer will remain in the nebulizer rest for more than two days to prevent DIW from backing up and entering the gas port.

IMPORTANT It is recommended that the software is completely shut down at the end of day to free up memory in the system.

Data Processing

The events are identified by an algorithm based on the minimum event duration of 10 and the Maximum event duration of 150. These parameters can be adjusted in the Advanced tab of the Experiment Manager window. Users can specify to preserve the integrated mass data (IMD) file which contains all data from cell events and non-events. This IMD can be used for processing after the sample has been acquired.

NOTE IMD files are very large. Remove these files from the computer regularly to ensure optimal performance and prevent the loss of data.

The Process tab of the CyTOF software provides post-acquisition processing options.

Data Normalization

CyTOF Software version 6.7 is pre-loaded with the bead passport EQ with the effective bead identification algorithm and an optimized Helios mass response curve for the EQ beads.

NOTE Remember to prepare freshly diluted 0.1 X EQ beads for each experiment to add to each sample.

1. In the Process tab, click **FCS Processing.**
2. In the FCS Processing window > Source file field, uncheck **Original Data** and uncheck **Concatenate**.
3. Click the browse button  and select the FCS files to be normalized.
4. Uncheck **Remove Beads** to provide an additional measure of instrumentation and data quality and allow the user to set gates to remove the beads after normalization.
5. Check **Time Interval Normalization** and set to 100 seconds (Default value).
6. Click **Start**.

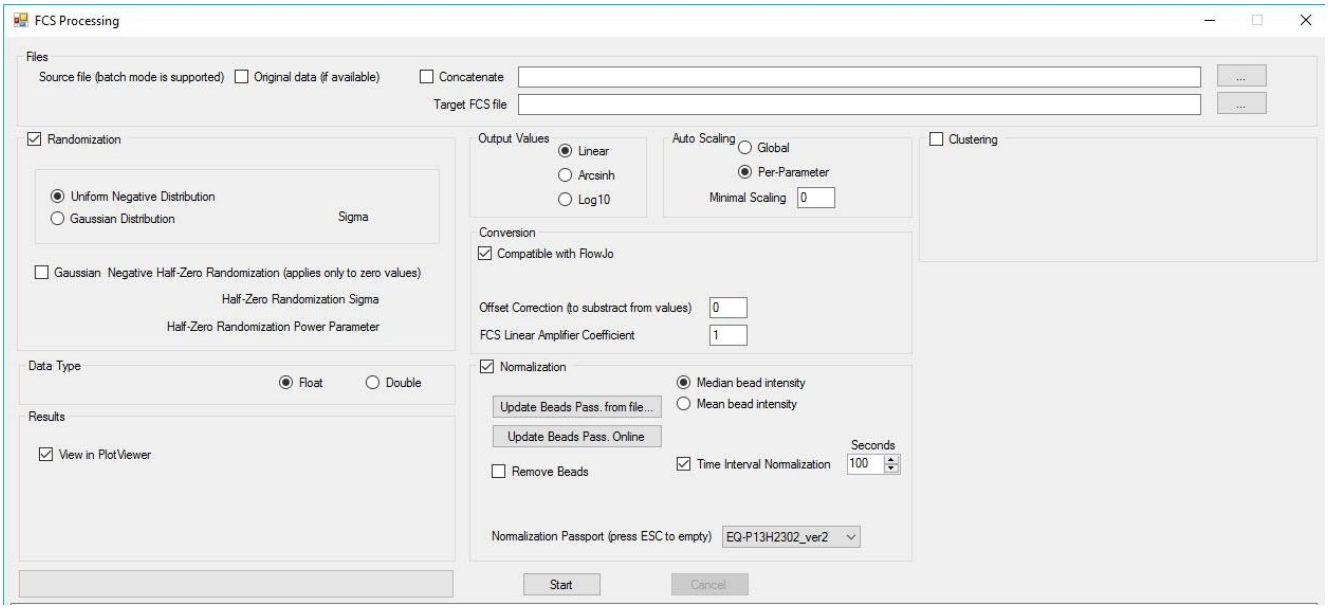
7 Each FCS file that is normalized will have a new file created in the folder that was selected. The normalized data can be opened in Premium Cytobank or any other FCS data analysis program.

FCS Processing

Once acquisition is finished, FCS 3.0 files are generated by the software which are compatible with third party cytometry data analysis platforms such as FlowJo and Premium Cytobank.

The FCS Processing window will process FCS or TXT files. The features in this window include randomization, concatenation, and normalization. Other data scaling settings can also be changed. The **Update Beads Pass from file** allows users to select and import a new Beads Passport for normalization. The **Update Beads Pass Online** provides a Beads Passport from the Fluidigm portal. Randomization is unchecked by default which allows you to only process the raw data.

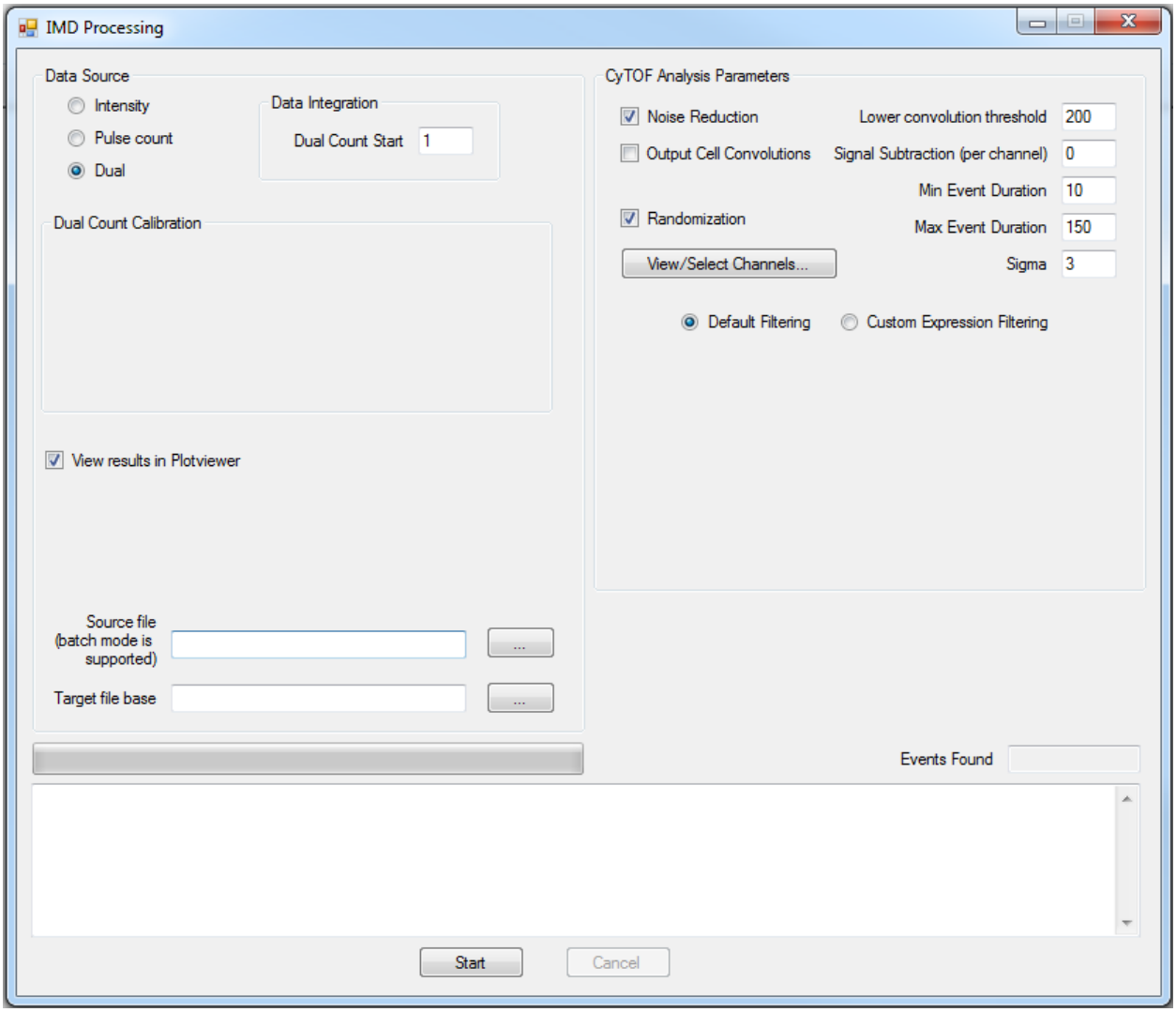
In order to concatenate multiple output files, first raw data is normalized. Check the Normalization box. Once this is complete, select the files to be concatenated or type the file names separated by commas. Remember to uncheck the Normalization box before concatenating files.



IMD Processing

Users can reprocess data based on different event definitions after sample acquisition by changing the Min Event Duration and Max Event Duration values in terms of the number of pushes. Channels with high background can be removed using the **View/Select** Channels button. The Source file feature at the bottom of the window allows you to select a file to process and then to change the output file. The Lower convolution threshold allows users to

differentiate the noise from the cell event, this represents the border or threshold of the noise and the cell event.



Expected Power Outages

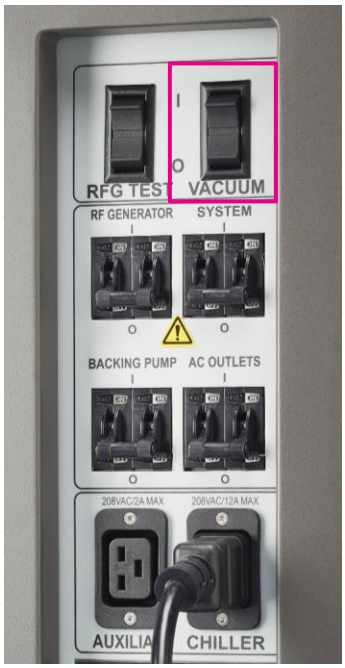
When a power outage is scheduled for the facility, the Helios instrument needs to be correctly shut down. Follow the steps below to shut down prior to the power outage and restart after power is restored.

NOTE The following procedures may also be used for shutting down and restarting the instrument for other purposes.

Helios Shutdown Procedure

1 Ensure that the system is connected to the argon supply for the vacuum chamber to be filled with argon when vented.

2 Turn off the **Vacuum** switch on the right side panel of the instrument above the circuit breakers.



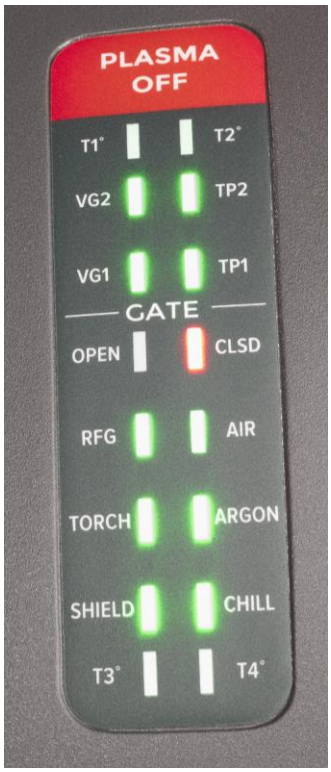
3 Wait 10 minutes for the turbopumps to gradually slow down. At this point, the venting valve opens and the chamber is slowly being filled with argon at a controlled pressure.

4 Shut off the instrument power by turning off the circuit breakers in the following order: AC OUTLETS, BACKING PUMP, RF GENERATOR, and SYSTEM.

IMPORTANT Leave the argon supply on during shutdown if you plan to restart the Helios instrument immediately.

Helios Startup Procedure

1. If the argon supply is on during the instrument shutdown, proceed to step 2. Otherwise, turn on argon gas supply for 2 hours prior to restarting Helios. This will allow the instrument to achieve the necessary vacuum levels.
2. Turn on the circuit breakers in the following sequence: SYSTEM, RF GENERATOR, BACKING PUMP, and AC OUTLETS.
3. Turn the **Vacuum** ON switch. In the Status Panel on the instrument cover, **VG1** will turn green, followed by **TP1** and **TP2** in approximately 6 minutes, and finally **VG2** will turn green in approximately 30 minutes.
4. Check in the monitor window to ensure the required vacuum levels are reached: VGauge1a below 1E-6 Torr and VGauge2a within the 1E-4 Torr range before plasma start.



Chapter 6: Maintenance

Clean the Sample Loader

Sample aggregation within the Sample Loader may result in clogs that impede sample flow and reduce throughput. The following procedures outline how to perform daily cleaning of the various parts of the Sample Loader.

Materials

Item
Luer Adapter (PN 101508)
Union Body (PN 101509)
Type 1 ultrapure (18.2 MΩ) water (DIW)
2 mL Norm-Ject® luer slip syringes (VWR PN 89174-492)
5 mL conical tubes

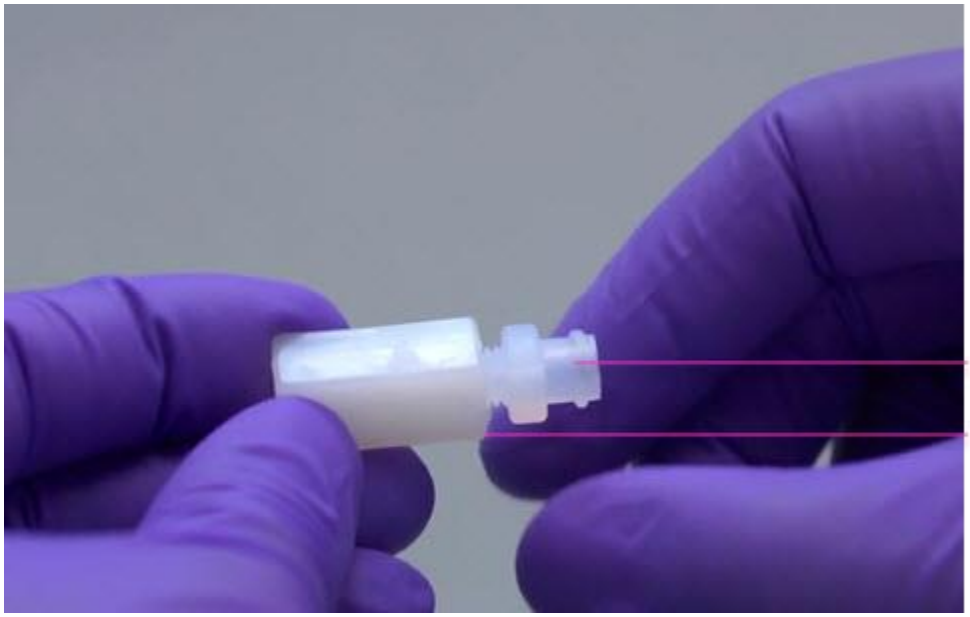
Nebulizer

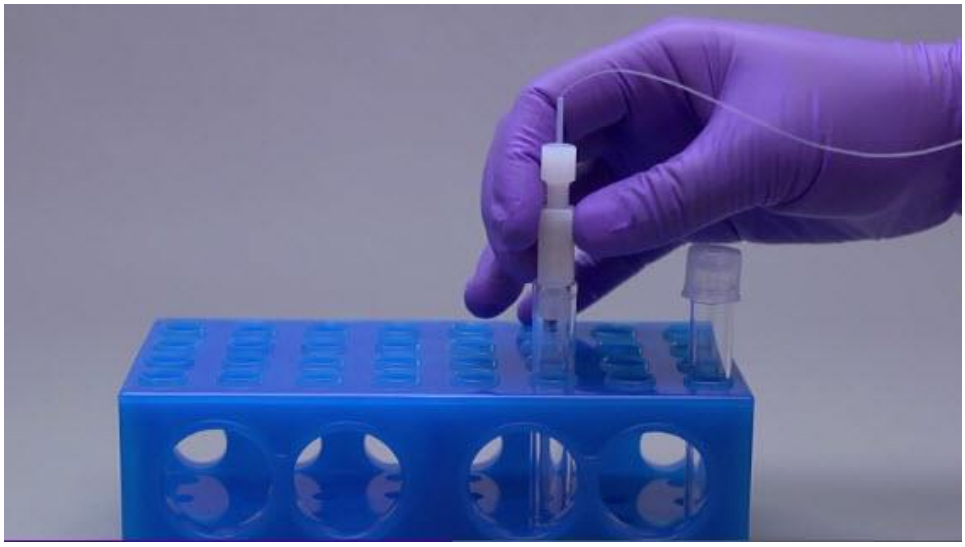
Either replace the clogged nebulizer with a new nebulizer, or:

1. Remove the nebulizer and disconnect the sample capillary.
2. Go to Nebulizer Removal and Cleaning below.

Sample Capillary

1. Remove the nebulizer from the Nebulizer Adaptor port and place in the nebulizer rest. Disconnect the Sample Capillary from the nebulizer and disconnect the sample line from the grounding nut.
2. Connect the luer adapter to the union body. Connect a syringe to the luer adapter. Place the nebulizer end of the sample capillary into a clean 5 mL tube on a tube rack to catch any residual sample and DIW.





3 Fill the 2 mL syringe with DIW and inject DIW into sample capillary. When the droplets fall consistently remove the syringe and place the sample capillary in a clean, dry area.

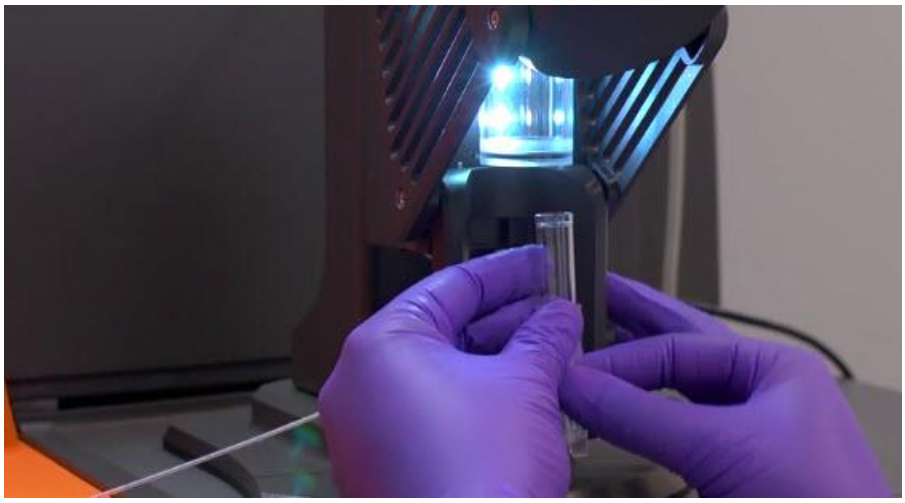
4 Reconnect all tubing to the original configuration.

Sample Line and Sample Probe Line

1 Disconnect the sample line from the grounding nut.

2 Connect the free end of the sample line to the other end of the union body with the luer adapter. Connect the syringe to the luer adapter.

3 Open the handle of the Sample Loader and place clean 5 mL tube into the sample holder. Close the handle.



4 Load the syringe with DIW. Using the syringe, carefully inject DIW through the luer adapter. Steady pressure will be required to create adequate flow into the tube. Gently inject DIW until a few droplets have come out of the sample probe line.

5 Remove and discard the 5 mL tube.

Grounding Nut

1 Remove the luer adapter from the union. Connect the luer adapter to the back side of the grounding nut.

2 Connect a syringe containing DIW to the luer adapter. Gently, inject DIW through the grounding nut. Place a 50 mL conical tube on the front side of the grounding nut to catch any debris that comes out of the grounding nut.

3 Remove and discard the 50 mL tube. Remove the luer adapter from the grounding nut.

4 Reconnect the sample line to the grounding nut.

5 Reconnect the sample capillary to the grounding nut. Reconnect the sample capillary to the sample inlet of the nebulizer. The capillary should reach the tapered portion of the sample inlet.

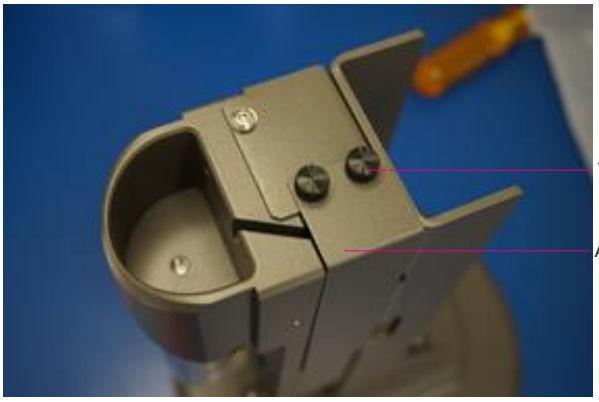
Replace the Sample Probe Line

If unclogging was unsuccessful or the Sample Probe Line (PN 107033) is damaged, replace the sample probe line using the following procedure.

1 Disconnect the sample line from the grounding nut and connect it to the side of the sample loader. Disconnect the USB cable to the computer and disconnect the argon line.

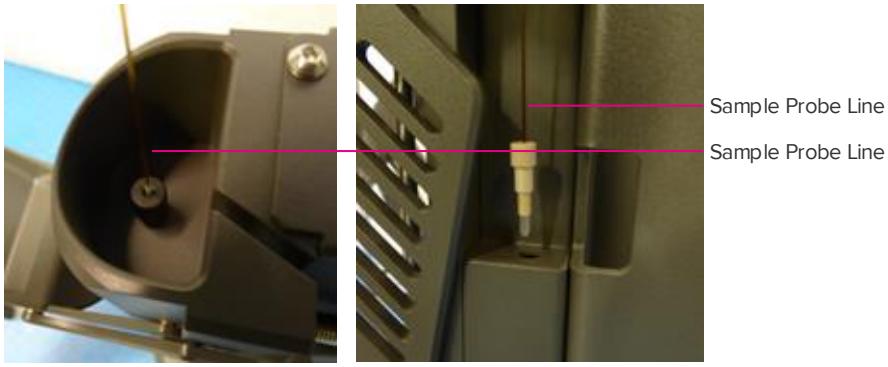
2 Gently lower the Sample Loader handle ensuring that it does not hit the platform.

3 Remove the two thrumbscrews that hold the access cover in place.

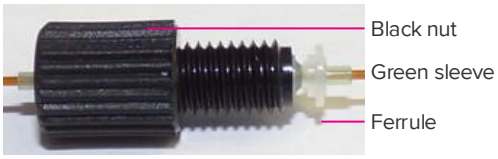


4 Remove the access cover.

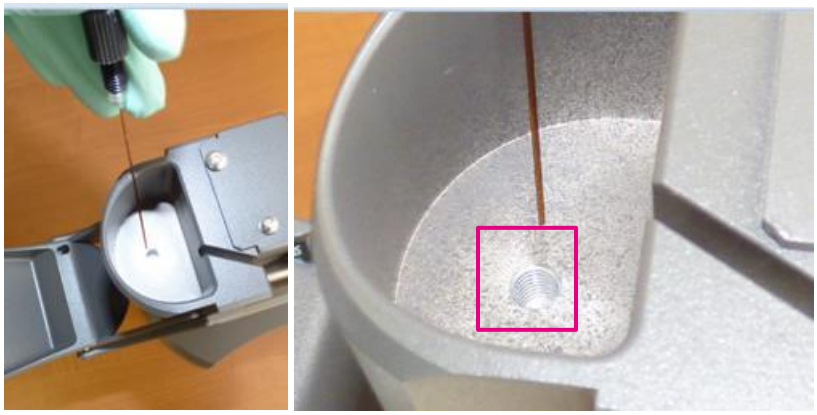
5 Remove the sample probe line by unscrewing the tan nut from the bottom right side of the Sample Loader. Unscrew the black nut at the top under the access cover and gently pull the line out from inside the pressure chamber.



Install the New Sample Probe Line



1 Carefully thread the sample probe line into the hole at the top of the Sample Loader, with the ferrule oriented closest to the hole, while securing the other end of the line with one hand.



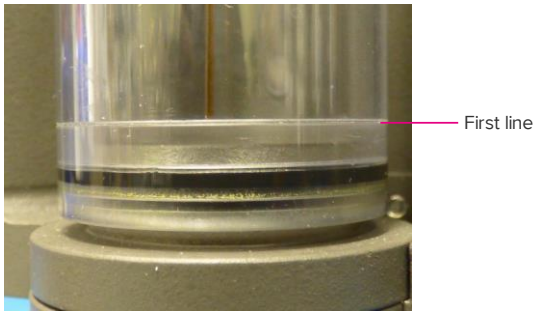
NOTE Handle this end of the sample probe line with care because it is used to acquire the sample.

2 Slide the assembly in until the black nut rests in the hole at the top of the Sample Loader. Push the sleeve through the black nut until the ferrule is firmly seated.

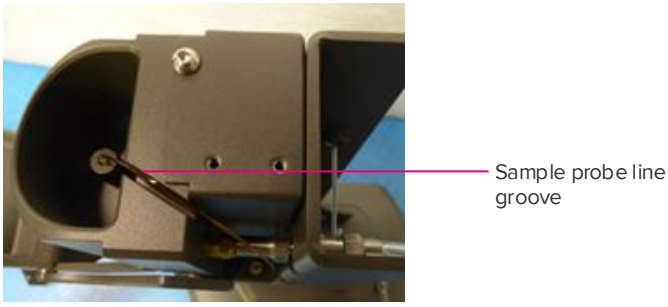
3 Tighten the black nut so that the green sleeve is partially visible.

NOTE After tightening the black nut, if the green sleeve is loose, the Sample Loader will not pressurize. Loosen the black nut, remove all sample probe line parts, and repeat the procedure from step 5 (above).

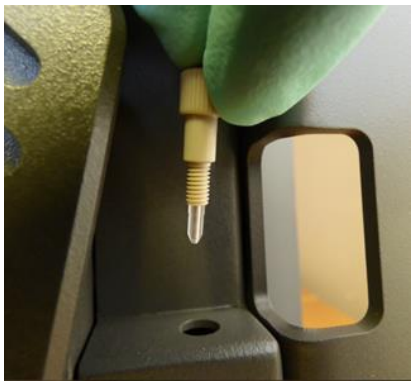
4 Push the sample probe line until it reaches the first line of the pressure chamber when viewed at eye level.



5 Tuck the sample probe line into the probe line groove.

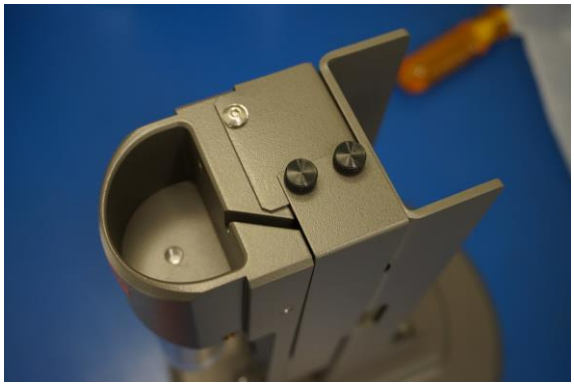


6 Insert the tan nut of the sample probe line into its position on the right side of the Sample Loader.



7 Push the sample probe line through the tan nut until it stops and tighten the nut.

8 Reattach the access cover using the two thumbscrews.



9 Reconnect the sample line to the grounding nut. Reconnect the USB cable form the computer to the Sample Loader. Reconnect the argon line from the instrument to the back of the Sample Loader.

The installation is now complete. Follow the procedure the section entitled Check the Nebulizer Spray to verify fluid flow and nebulizer spray.

Helios Cleaning

Several sources of metal contamination may occur in the Helios™ system. It is important to clean the lines, glassware, spray chamber, cones, and load coil regularly to ensure optimal instrument performance. Table 12 summarizes the cleaning frequency and the agents required. Table 13 summarizes required equipment.

IMPORTANT Use only Type 1 ultrapure (18.2 MΩ) DIW to rinse Helios system parts.

Table 12. The cleaning frequency and reagents required for correct maintenance and cleaning of the Helios system

Parts	Frequency	Performed by	Agents/Equipment	Company and Part Number
Sample line, sample probe line, grounding nut, sample capillary	Daily	Operator	Type 1 ultrapure (18.2 MΩ) water (DIW)	
Nebulizer	Weekly	Operator	10% Contrad® 100 in DIW	Decon Labs
Nebulizer rest	Weekly	Operator	Type 1 ultrapure (18.2 MΩ) water (DIW)	
Spray chamber	Weekly	Operator	Isopropanol	
Torch and HT injector	Weekly	Operator	10% Contrad 100 in DIW, glassware brushes	Decon Labs
WB injector	Daily Weekly	Operator Operator	DIW 10% Contrad 100 in DIW, glassware brushes	 Decon Labs
Sampler and skimmer-reducer cones	Weekly (approximately 40 hours of sample acquisition)	Operator	10% Citranox®	Alconox Z273236
Load coil	Weekly	Operator	Isopropanol Scotch-Brite™ Ultra Fine Hand Pad 7448	3M PN 19-047-254
Interface pump oil inspection	Weekly	Operator	Oil Condition Chart	
Interface pump oil replacement	As required	Operator	Vacuum Pump Oil	Fluidigm PN 101810
Air filters	Annually	Fluidigm field service engineer	Air Filter	Fluidigm PN 105592

Table 13. The equipment required for correct maintenance and cleaning of the Helios system

Parts	Equipment	Product Name	Part Number
Torch	Glassware brushes	Nylon Tube Brushes and Pipe Cleaner	Restek® PN 20108

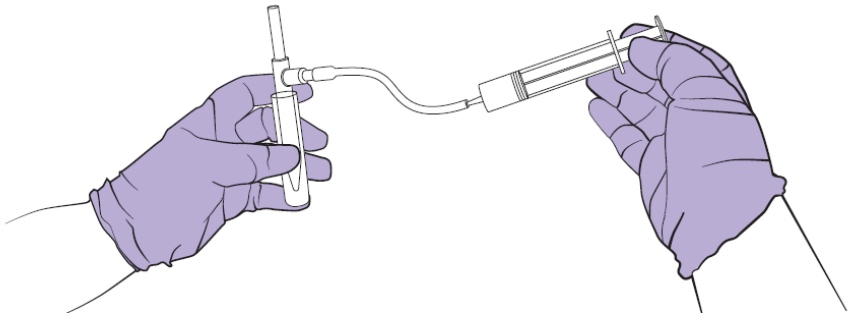
Parts	Equipment	Product Name	Part Number
Injector	Glassware brush	Nylon 0.030 Miniature Single-Spiral brushes with a stainless steel stem	Gordon Brush PN 01023
Sampler and skimmer-reducer cones	Sonicator	Branson® M1800 Ultrasonic Bath or sonicator	VWR PN 89375-450
Load coil	Ultrafine hand pad	Scotch-Brite Ultra Fine Hand Pad 7448	3M PN 19-047-254

NOTE The following procedure should be done when the instrument plasma is off and the nebulizer is resting in DIW after use.

Remove and Clean the Nebulizer

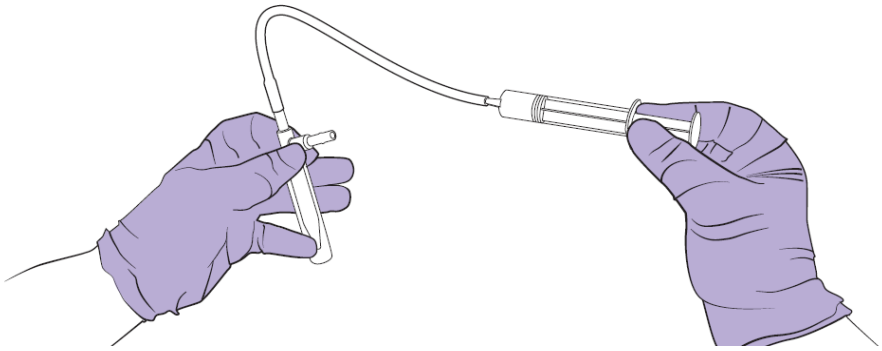
The nebulizer is exposed to considerable cellular material and therefore should be thoroughly cleaned daily. Two methods for daily maintenance and/or unclogging of the nebulizer are provided below.

1. Loosen the nut on the sample inlet and remove the sample capillary line from the nebulizer. Disconnect the nebulizer gas line from the Helios and then remove the gas line from the nebulizer side arm.
2. Label a 5 mL round-bottom tube and fill it with approximately 3 mL of 10% Contrad.
3. Lubricate one end of Tubing 1 from the Nebulizer Cleaning Kit (PN 107210).
4. Connect the side arm of the nebulizer to the syringe, provided in the kit, with Tubing 1.
5. With the nebulizer tip submerged in detergent, pull slowly on the syringe plunger, filling the nebulizer body with detergent. Continue until the syringe body is filled with detergent.



6. Detach the tubing from the side arm and depress the syringe plunger to remove all the liquid.

7 Attach Tubing 2 from the nebulizer cleaning kit to the sample inlet and fill the sample capillary with detergent.



8 Discard the detergent from the syringe and tubing by depressing the syringe plunger. Remove the syringe and tubing from the sample inlet.

NOTE If the nebulizer is clogged, soak it for an hour in a detergent bath. If the clog still remains, the nebulizer can be soaked overnight.

IMPORTANT Do not sonicate the nebulizer.

9 Remove the nebulizer from the tubing-syringe assembly and pull DIW into each tubing several times, and expel, to rinse detergent from the tubing pieces.

10 Repeat steps 3–7 several times with Tubing 1 followed by Tubing 2, using DIW to rinse the nebulizer.

11 Disconnect the sample line from the connector to the nebulizer line and remove the sample line and the nebulizer line.

Use the Hot DIW Method to Clean the Nebulizer

Materials

Item
Heat-resistant glove or mitt
Beaker
Nebulizer-to-aspirator tubing: 3/16 inch OD, 1/8 inch ID 1/8 inch OD, 1/16 inch ID
Deionized water—Type I ultrapure, >18.2 MΩ (DIW)
Light microscope (optional)

Procedure

1 Detach the nebulizer gas line and sample capillary from the nebulizer.

NOTE You might be able to identify the location of the clog by examining the nebulizer with a light microscope.

2 Fill a small beaker halfway with DIW.

3 Boil the DIW.

4 Using a heat-resistant glove or mitt, carefully transfer the beaker to a lab bench.

5 Connect a vacuum aspirator (suitable for liquid waste disposal) to the nebulizer using the appropriate tubing.

- If the clog is near the nebulizer tip, connect the tip to the aspirator using 3/16 inch outside diameter (OD), 1/8 inch inside diameter (ID) tubing.
- If the clog is near the sample inlet, connect the sample inlet to the aspirator using 1/8 inch OD x 1/16 inch ID tubing.

6 Submerge the open end of the nebulizer (tip or sample inlet) and side arm fitting (nebulizer gas inlet) of the nebulizer in the hot DIW.

7 Draw DIW through the nebulizer until the clog is removed.

WARNING The nebulizer may be very hot. Carefully remove the nebulizer from the beaker using a heat-resistant glove or mitt.

8 Visually inspect the nebulizer using a light microscope to verify that the clog has been removed.

9 Verify the nebulizer spray.

a Connect the nebulizer gas line and connect the sample capillary to the nebulizer (see Chapter 5: Operation).

b Observe the spray from the nebulizer using a flashlight. It should appear as a fine aerosol that leaves the nebulizer in an even, symmetrical pattern.

10 Reconnect the sample capillary and nebulizer gas line to the nebulizer for use, or store the nebulizer in the nebulizer rest.

NOTE If the nebulizer remains clogged, refer to the detergent-based nebulizer cleaning kit method.

Remove the Spray Chamber for Cleaning

WARNING Hot Surface. Ensure that the heater has sufficiently cooled before performing any maintenance procedures.

1 Remove the heat shield by loosening the four screws, two on each side of the shield. Lift the shield off and place it in a clean, dry area.

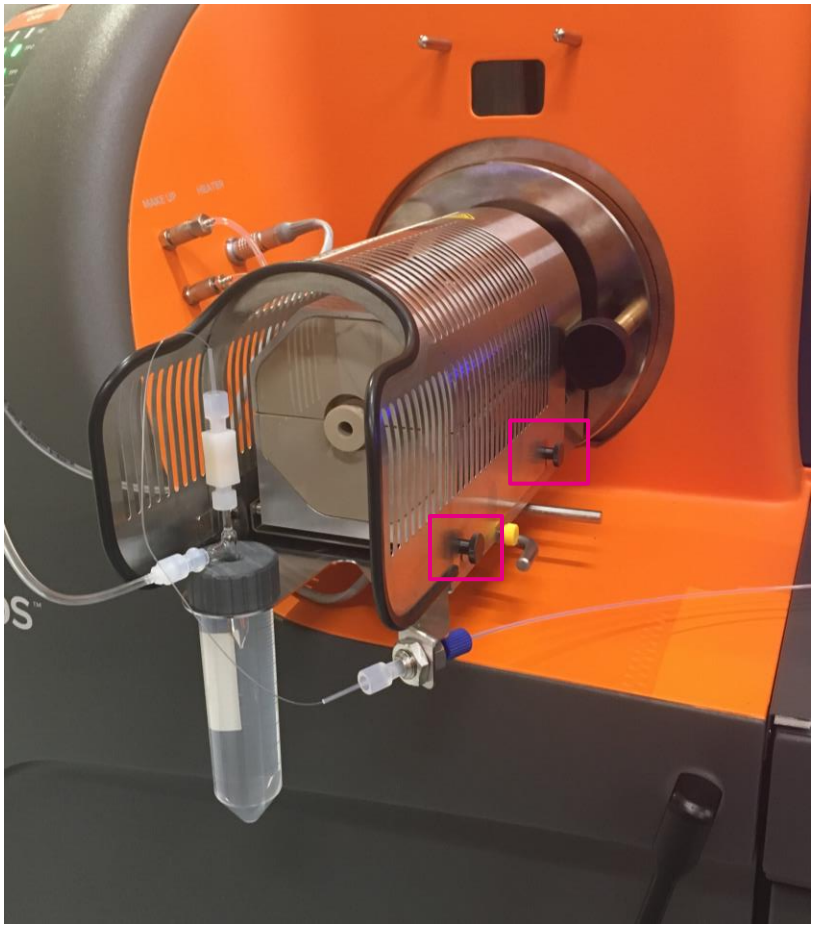


Figure 27. The heat shield on the Helios heater box. There are four screws, two on each side of the shield, that must be loosened before removing the shield.

2 Remove the ball joint clamp that secures the spray chamber to the injector.

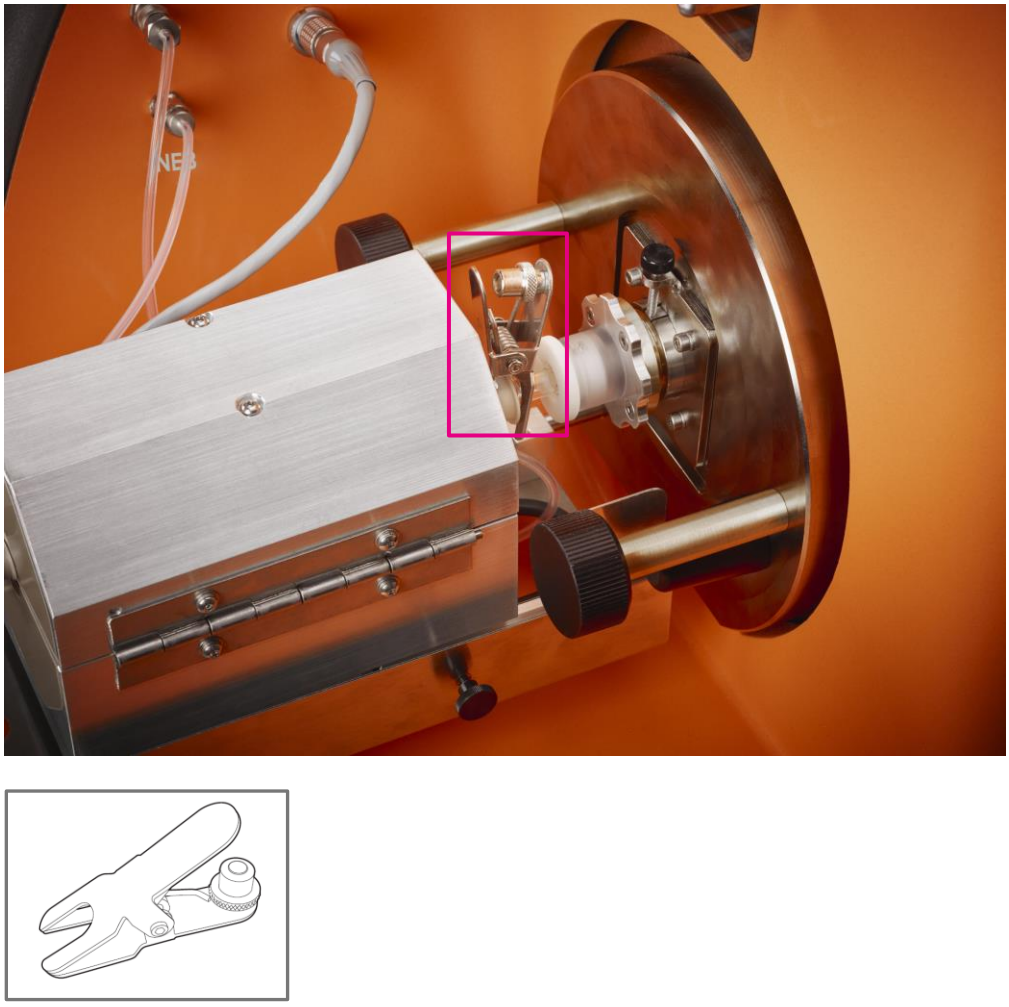


Figure 28. The spray chamber within the heater assembly. The injector ball joint clamp (bottom) connects the spray chamber to the injector. The magenta box indicates where the clamp is inserted.

3 Lift the clip on the side of the heater and open the heater box.

4 Remove the spray chamber from the heater.

5 Disconnect the makeup gas line from the front of the instrument.

6 Unscrew the outer cap of the spray chamber, and then remove the inner cap. Inspect the inside of the body of the spray chamber. If there is visible residue on the cap or the interior, clean with isopropanol and Kimwipes®. If the O-rings are damaged, replace the O-rings.

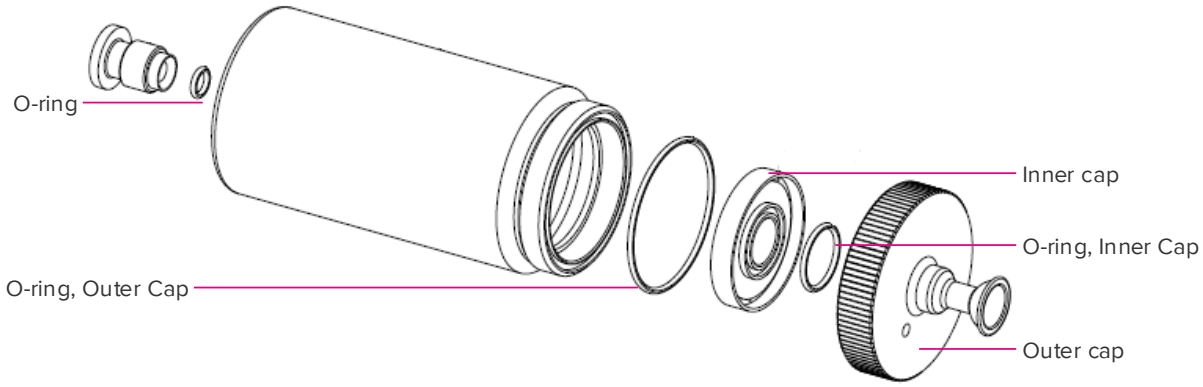


Figure 29. Spray chamber with outer cap and inner cap removed

7 Slide the heater off of the heater box pins and rest it on the upper support pins.

Remove the Torch Assembly to Clean and Reassemble the Injector and Torch

DANGER

- Before opening the front access door and disengaging the torch box from the vacuum chamber, switch off the RF generator power using the breaker located on the right-side panel of the Helios system.
- Wait a minimum of 15 minutes after turning off the RF generator power before opening the Helios access door to torch/cone area.

WARNING Hot Surface. Ensure that the ICP torch and the load coil have sufficiently cooled before performing any maintenance procedures.

1 Switch off the RF generator power using the RFG circuit breaker on the right side of Helios.

Figure 30. The RF generator circuit breaker on the Helios circuit breaker panel

2 Remove the ball joint injector by gently pulling and turning until it comes loose from the torch assembly.

3 In unison, loosen the two thumbscrews on the front of the torch assembly.

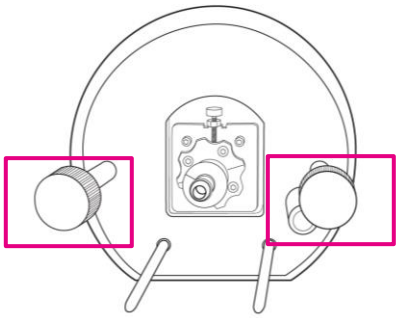
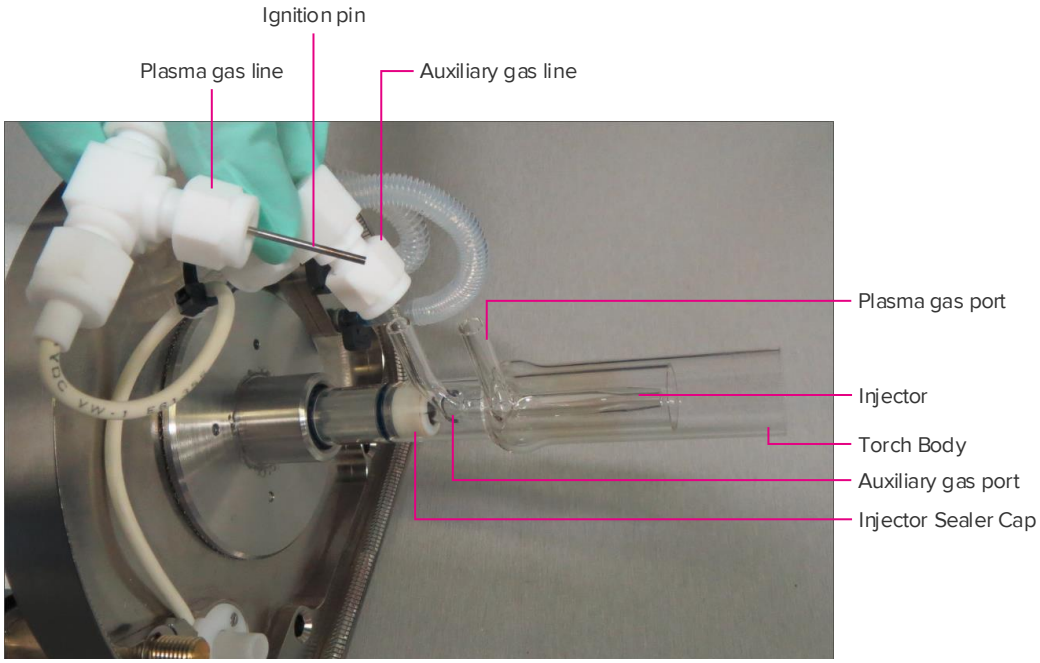


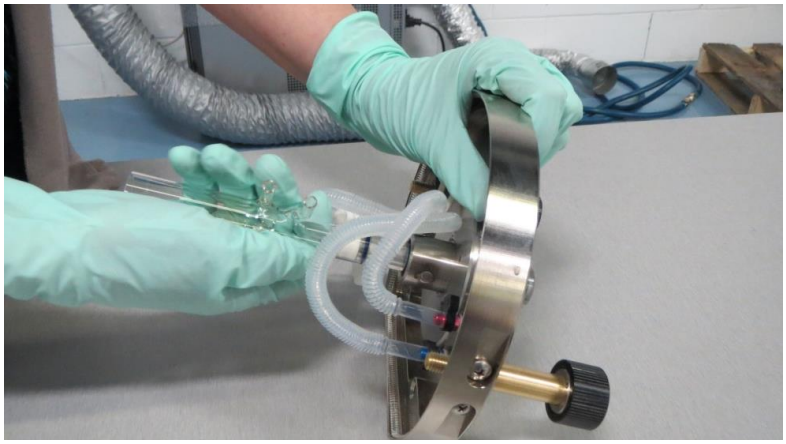
Figure 31. Torch assembly with thumbscrews (magenta boxes)

4 Slide the torch assembly off the heater box pins and set it down on a clean, dry workspace.

5 Loosen the nut on the plasma gas line (approximately ½ turn), and remove the plasma gas line with ignition pin from the torch body. Loosen the nut on the auxiliary gas line (approximately ½ turn) and remove the auxiliary gas line from the torch body.



6 Firmly hold the torch assembly with one hand. With the other hand gently twist and pull the torch off of the injector holder from the base of the torch assembly.



Clean the Torch and Injector

1 Soak in 10% Contrad for up to 1 hr.

2 Scrub the parts with the recommended glassware brushes.

3 Rinse thoroughly with DIW.

4 Spray isopropanol on the torch and injector and air dry.

IMPORTANT Do not spray isopropanol on the injector O-ring as this may cause damage.
IMPORTANT Inspect the O-ring (PN 107212) for damage. Replace if necessary (refer to section Replace the Injector O-Ring).

5 Alternatively, completely dry the glassware with a blow dryer or heat gun.

NOTE Ensure that the parts are completely dry before reinstalling.

IMPORTANT The injector must be cleaned weekly to prevent clogging. If using the WB injector in the system, remove the WB injector and rinse daily in DIW.

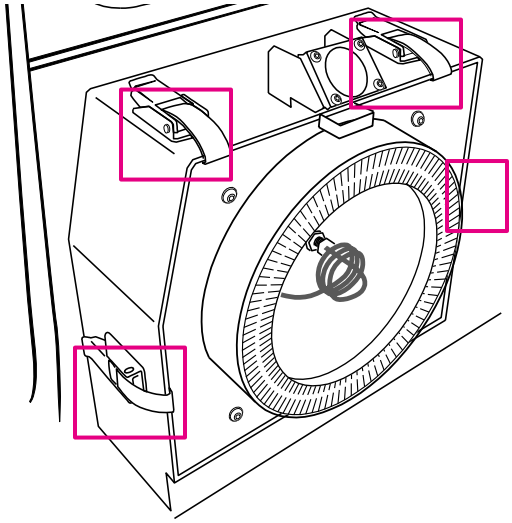
Clean the Load Coil

NOTE The torch body should be removed before beginning this procedure.

1 Disconnect the sample line of the Sample Loader from the grounding nut.

2 Open the instrument front access door.

3 Undo the clips on four sides of the front shield and lift off (magenta boxes below).



4 Install the load coil core.

5 Using a Scotch-Brite Ultra Fine Hand Pad moistened with isopropanol, gently rub the surfaces of the load coil to remove any deposits. Be careful not to bend the coils.

6 Remove the load coil core.

7 Visually inspect the coil to look for deposits and/or damage to the coil.

8 Gently clean in between the coils with the hand pad and isopropanol being careful not to bend the coils.

9 Wipe the coil with a kimwipe moistened with isopropanol to remove any residue.

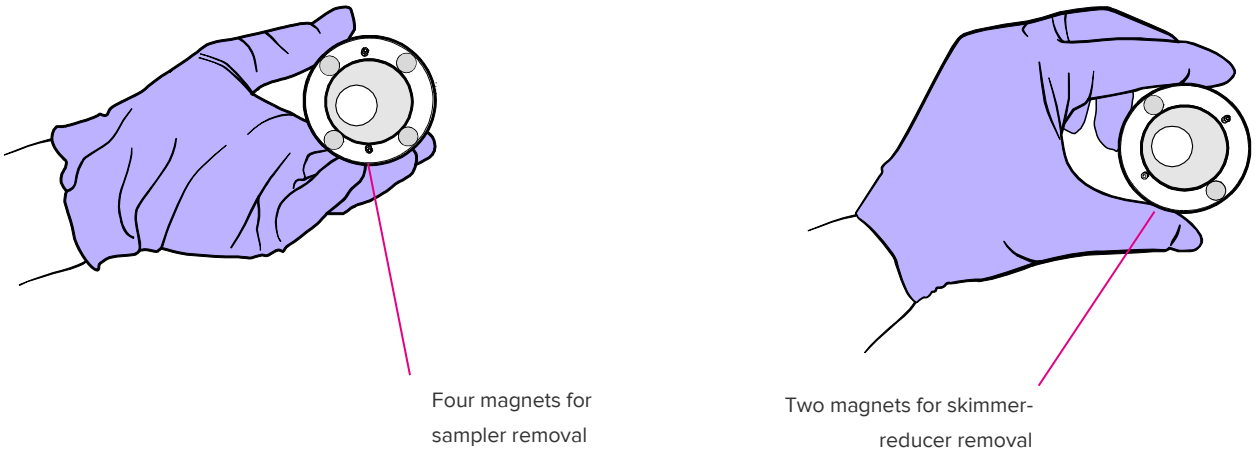
10 Reinstall the front shield.

Remove, Clean, and Reinstall the Vacuum Interface Cones

1 Remove the torch assembly prior to beginning this procedure.

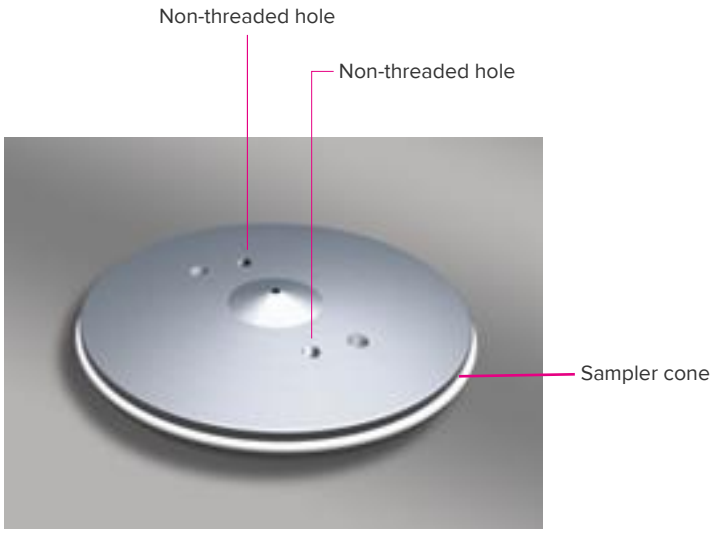
2 Open the front access door of the instrument.

NOTE The cone removal tool has two ends, one to remove the sampler cone, and the other to remove the skimmer-reducer.



3 The sampler cone has four holes on the face. Insert the cone removal tool (with four magnets) into the two non-threaded holes. Rotate the cone removal tool and pull towards you to release the sampler cone from the vacuum interface.

NOTE You may need to use isopropanol to lubricate the sampler O-ring .



4 Remove the sampler cone from the cone removal tool. Be careful not to touch the sampler orifice. Place the sampler cone face down on the bottom adapter.

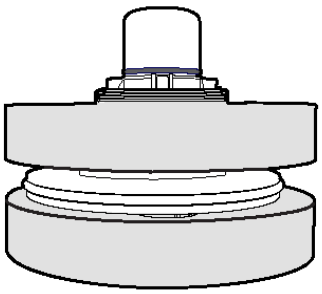
5 Insert the other end of the cone removal tool (with two magnets) into the two holes on the skimmer-reducer assembly. Turn the tool counterclockwise to remove the skimmer-reducer assembly from the vacuum interface.

6 Remove the skimmer-reducer assembly from the cone removal tool and place face down on the top adapter.

7 Inspect the sampler O-ring for damage or discoloration and replace if needed.

8 Stack the cones as follows in the cone cleaning container: First stack the bottom adapter. Then place the sampler cone face down on the bottom adapter. Follow with the top adapter. Finally place the skimmer-reducer assembly on the top adapter with the screws facing up.

IMPORTANT Add 10% Citranox as each part is placed in the container to ensure that all parts come in contact with the cleaning solution.

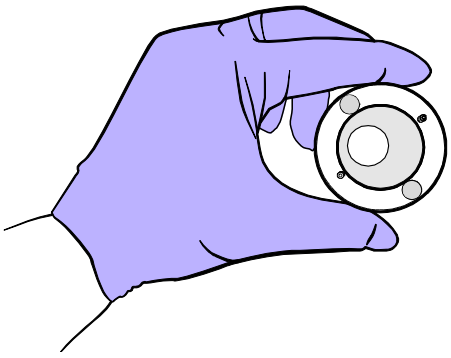


9 Sonicate cones in 10% Citranox for no more than 15 minutes.

10 Pour off the Citranox and rinse the container with DIW. Stack the cones and adapter again as described above adding DIW as each piece is added to the container. Sonicate for an additional 15 minutes. Repeat the DIW rinse step two times.

11 Air dry the cones thoroughly before reinstalling.

12 Place the skimmer-reducer assembly on the side of the cone removal tool with two magnets.



13 Coat the threads of the skimmer-reducer assembly with graphite using a No. 2 pencil.

14 Place the skimmer-reducer assembly flush into the interface and begin to turn clockwise. After a few clockwise turns, turn back counterclockwise by a quarter turn. If this occurs smoothly, the skimmer-reducer assembly is being threaded properly; if it turns back with difficulty, completely remove the skimmer-reducer assembly and start again. Continue to turn clockwise until the skimmer-reducer assembly is firmly seated in the interface. Detach the cone removal tool.

15 Place the other side of the cone removal tool (with four magnets) in the non-threaded holes of the sampler cone. Seat the sampler cone flush with the interface. Turn the

sampler cone clockwise while applying gentle forward pressure. You may need to lubricate the sampler O-rings with a small amount of DIW. Remove the cone removal tool.

16 Press the edges of the sampler cone to make sure it is firmly seated taking care not to touch the orifice.

Reassemble the Torch Assembly

1 Inspect the injector sealer cap and ensure that it has not been damaged or cracked.

2 Gripping the torch body at its base, push and turn the torch body to install it over the two O-rings of the injector holder. Sprinkle isopropanol as needed to lubricate the O-rings.

3 Turn the torch body so that the gas ports are oriented on top.

4 Connect the auxiliary gas line to the port closest to the injector holder. This port is slightly angled. Tighten the nut.

5 Connect the plasma gas line (with the ignition pin) to the second port. This port is straight. Tighten the nut.

6 Check that both of the gas connections are tight.

7 Install the ball joint injector by pushing and turning until it is fully inserted.

8 Confirm that the injector is 1.5–2 mm from the end of the inner portion of the torch.

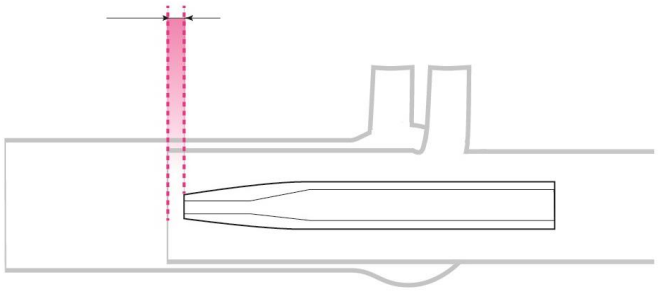


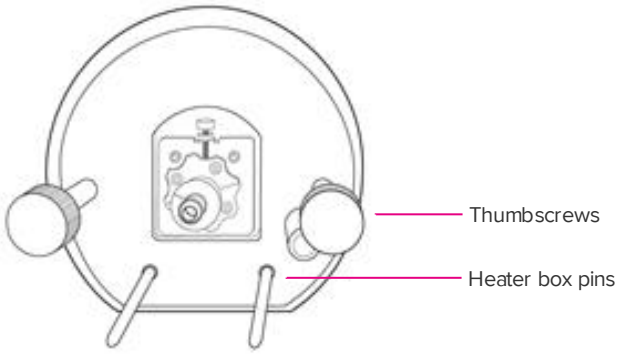
Figure 29. The injector should be positioned 1.5–2 mm from the end of the inner portion of the torch (as indicated by the pink dashed lines).

9 With the Helios front access door closed, slide the torch assembly onto the heater box pins and push flush, making sure to line up the high-voltage connector with its port.

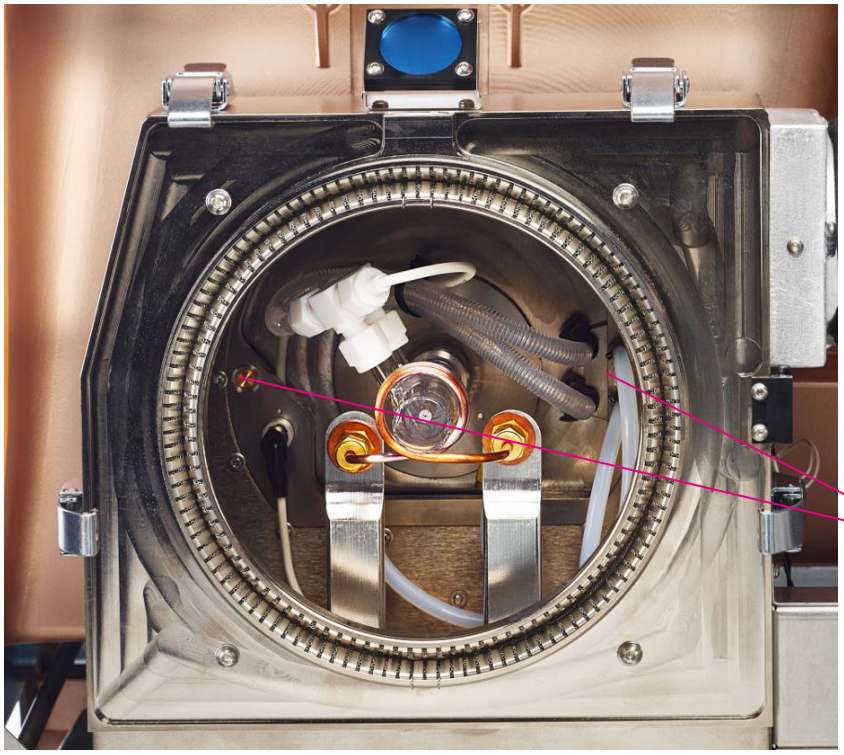
10 Turn the thumbscrews in unison. Continue to tighten the thumbscrews until an audible click is heard on each side. This will ensure that the torch assembly is installed correctly.

NOTE The thumbscrews have an internal ratcheting system on the black knobs. Over a small range, these knobs are free to rotate without the brass screw being turned. Therefore, when installing/removing the torch assembly, always be sure that the knobs are moving in the same direction as the screw.

NOTE If one of the thumbscrews becomes significantly more difficult to turn, loosen the thumbscrews completely and repeat step 10 (above).



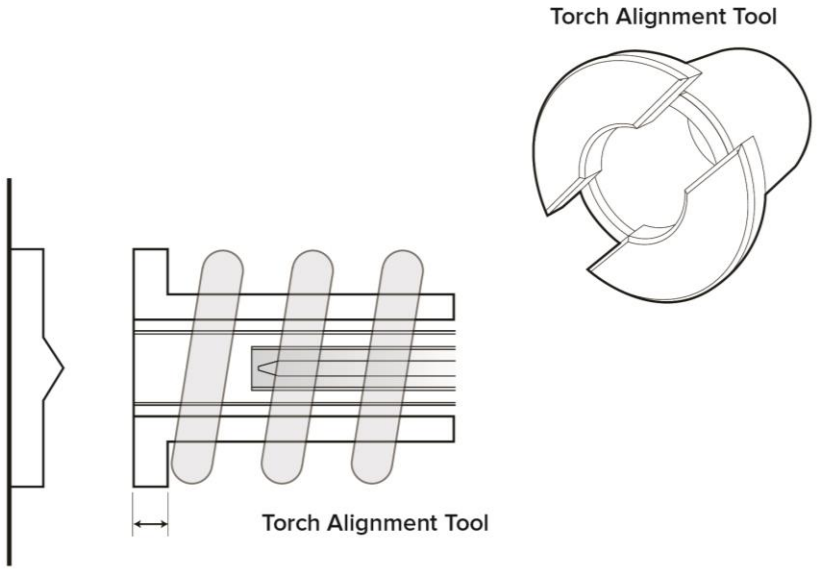
11 Open the front access door of the Helios instrument and view the positions of the thumbscrews with respect to one another. Ensure that they have an equivalent amount of thread engagement. Loosen or tighten the thumbscrews on the front to equalize the thread engagement.



Check the Alignment of the Torch with the Load Coil

1 Install the torch alignment tool into the end of the torch. Check that it spins freely. If it does not, then the torch is not aligned properly with the load coil. Remove the torch assembly and reinstall being particularly careful to turn the thumb screws in unison.

2 Gently push the torch alignment tool in as far as it will go.



3 If the outer edge of the torch alignment tool is not flush with the edge of the torch, remove the torch assembly and check that the torch is installed over both O-rings.

IMPORTANT If the tool is not flush with the torch (±1 mm), inspect the coils to ensure that they are not distorted or damaged.

NOTE If a new torch has been installed, see Z alignment Adjustment in Periodic Maintenance.

4 If the torch is aligned properly in the load coil, remove the torch alignment tool.

5 Close the front access door.

Reassemble the Spray Chamber and Heater

1 Place the inner cap of the spray chamber back into the outer cap and screw it back onto the body of the spray chamber.

IMPORTANT Check that the spray chamber inner and outer cap have been tightened prior to re-installing.

NOTE If a sqeaking noise is noticed, remove the outer cap and wipe the threads of the cap and the spray chamber with a Kimwipe moistened with isopropanol.

2 Gently place the spray chamber in the heater box making sure that the outer cap and makeup gas line are sitting snugly in the groove of the heater box.

3 Close the heater lid.

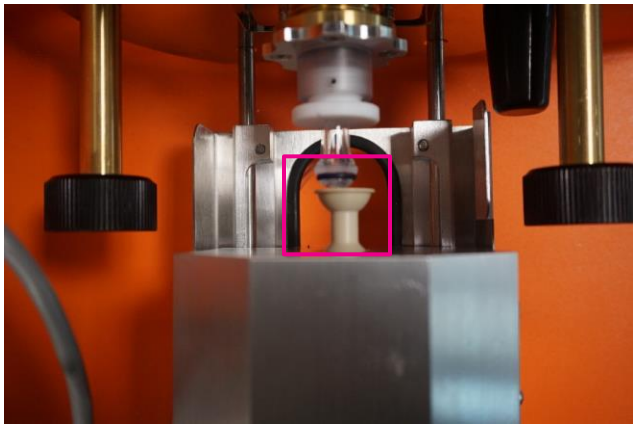
4 Slide the heater box off of the heater guide pins and slide back onto the support pins towards the torch assembly so that the spray chamber ball joint connection meets the injector.

Adjust the Position of the Heater Tray Assembly and Check the Spray Chamber Alignment with the Injector

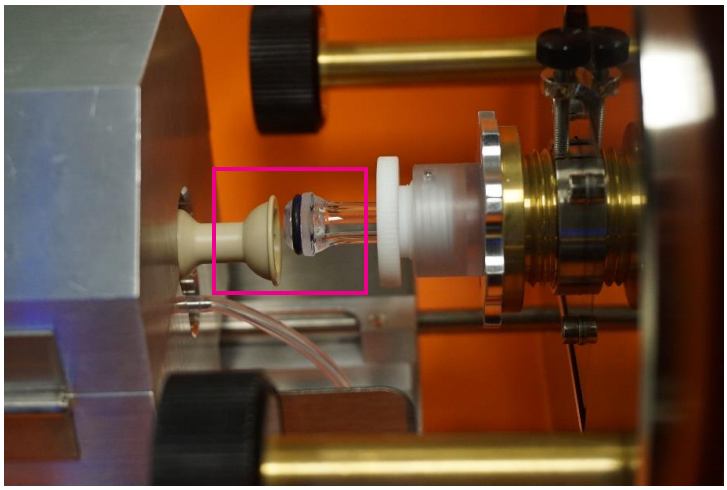
The new Helios heater tray design enables easier alignment to the injector. The new design features:

- An adjustment lever to align in the horizontal direction
- An adjuster wheel to align the tray in the vertical direction
- A bent shaft lever to align the heater/spray chamber front-end in the vertical direction

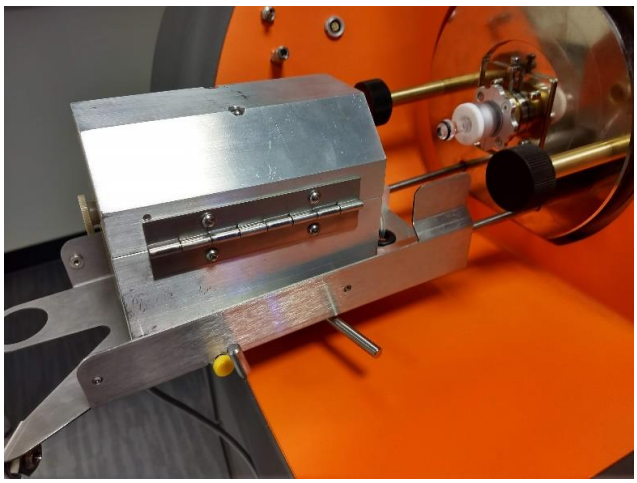
1 View the spray chamber and injector from above to observe the horizontal alignment of the spray chamber port and the ball joint of the injector.

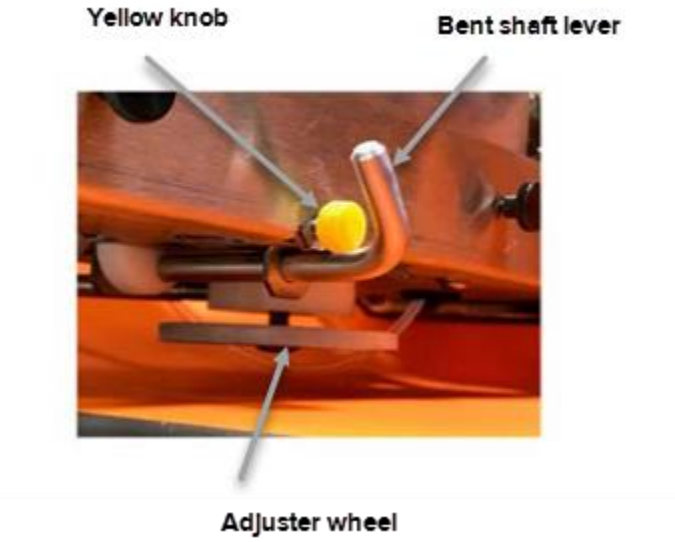


2 View the two parts from the side to observe the vertical alignment.



3 The vertical and horizontal adjustment for the heater is on the lower right-hand side.





4 Use the adjuster wheel to move the vertical alignment of the back end of the heater.

a Slide your hand under the heater being careful not to move the heater. Use your index finger to turn the knob.

b Turn it in the counter-clockwise direction to raise the heater, or clockwise to lower the heater. View the injector and spray chamber from the side to ensure that it is aligned vertically.

5 Use the adjuster lever to adjust the horizontal alignment of the heater box (left or right) to ensure that the heater is sitting perfectly straight on its axis.

a Push the lever back (toward the instrument) to move the heater to the left or pull the lever forward (toward the nebulizer) to move the heater to the right.

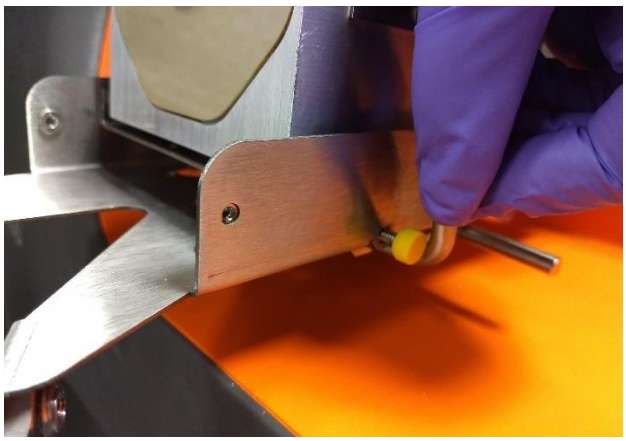
NOTE Care should be taken when peforming the horizontal adjustment because the lever cannot be locked into place.

6 Use the bent shaft lever to adjust the vertical alignment of the front part of the heater.

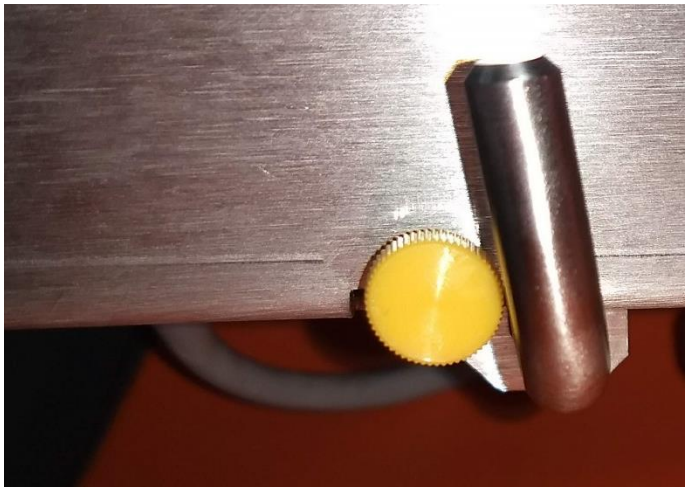
a Loosen the yellow knob to free the bent shaft.

NOTE Do not unscrew the knob too much as it will interfere with the movement of the bent shaft lever.

b Pull up on the bent shaft lever up to raise the anterior part of the heater or push it down to lower the anterior part of the heater.



c While holding the bent shaft lever, tighten the yellow knob to secure the position.

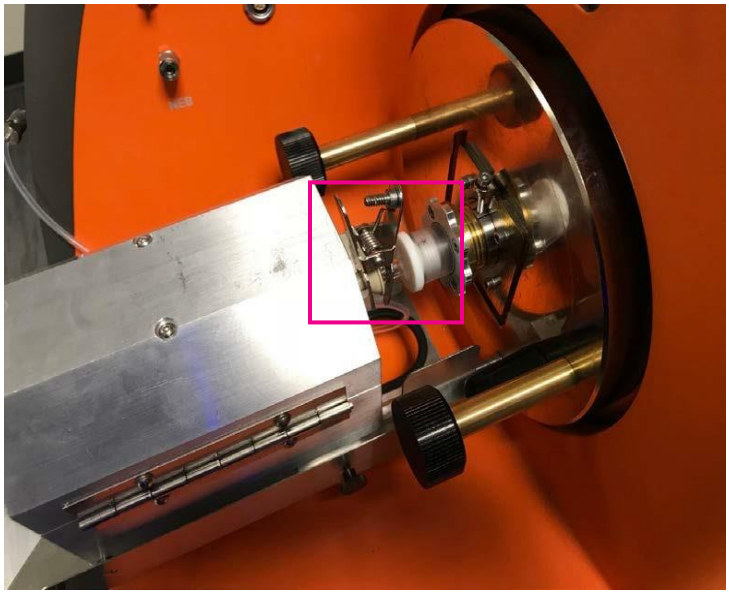


7 View the connection of the spray chamber and the injector from above and from the side to ensure that it is aligned.

NOTE Adjusting the front vertical alignment may affect the back vertical alignment. If needed, re-adjust the back vertical alignment using the adjuster wheel.

8 Carefully slide the heater so that the injector ball joint connects with the cup in the spray chamber cap.

9 Secure the ball joint clamp and ensure that it is securely seated on the ball joint injector and spray chamber.



10 Place the heat shield back onto the heater and tighten the four screws, two on each side of the shield.

11 Reconnect the makeup gas line.

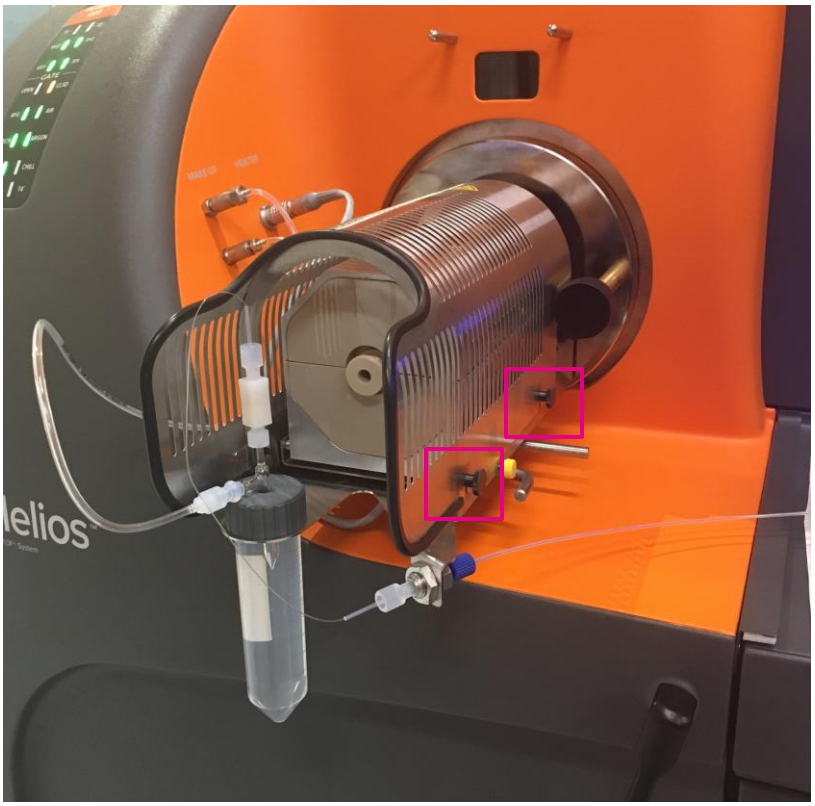


Figure 31. The heat shield on the Helios heater box. There are four screws, two on each side of the shield, that must be loosened before removing the shield.

Check the Condition of the Interface Pump Oil

1 Open the front access door using the door handle. Pull the spring pin to the left and open the lower instrument door.

2 Open the lower right door of the instrument. The interface pump is on the right side of the instrument.

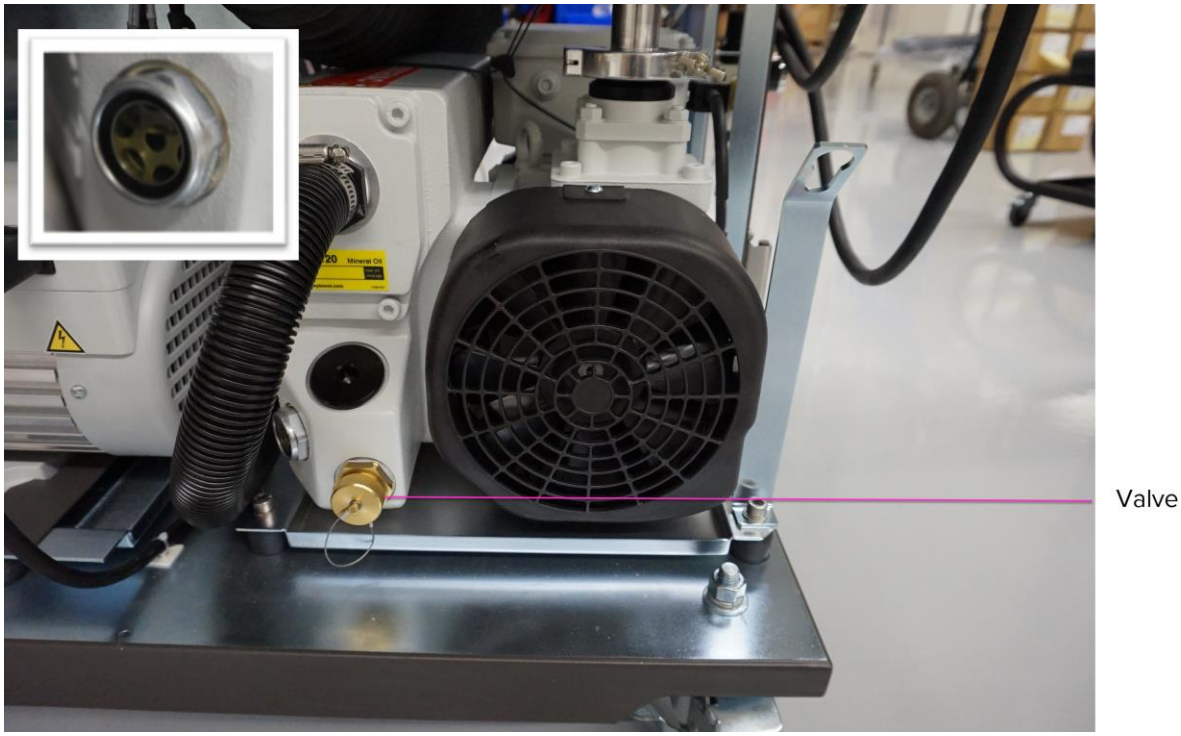


Figure 32. The interface pump in the lower right compartment of the Helios system. The visual inspection window is on the side of the interface pump (inset).

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

4 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. If the oil condition is above 4 the oil needs to be replaced.

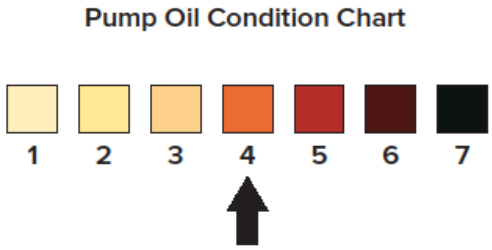


Figure 32. Pump oil condition chart. When the oil is above Level 4 (as indicated by the black arrow) the oil should be replaced in the interface pump.

Cleaning Summary

Table 14. A summary of the cleaning procedures for the Helios system glassware and parts

Part	Frequency and Cleaning Agent	Procedure
Sample capillary Sample line Sample probe line	Daily Type 1 ultrapure (18.2 MΩ) water (DIW)	**1** Use a 2 mL luer syringe and luer adapter. **2** Place one end the line into a 5 mL tube. **3** Push DIW through the line.
Grounding nut	Daily Type 1 ultrapure (18.2 MΩ) water (DIW)	**1** Use a 2 mL luer syringe and luer adapter. **2** Push DIW through the grounding nut.
Spray chamber	Weekly Isopropanol and Kimwipes	**1** If there is visible residue on the cap or the interior, clean with isopropanol and Kimwipes. **2** Air-dry. **3** Dry thoroughly before reinstalling on instrument.
HT Injector	Weekly Contrad 100/Decon 90 (dilute to 10% in DIW)	**1** Soak in 10% Contrad/Decon for 1 hr. **2** Scrub with brush. **3** Rinse with DIW. **4** Dry thoroughly before reinstalling on instrument.
WB Injector	Daily Weekly	**1** Rinse in DIW. **2** Dry thoroughly. **1** Soak in 10% Contrad/Decon for 1 hr. **2** Scrub with brush. **3** Rinse with DIW. **4** Dry thoroughly before reinstalling on instrument.
Torch body	Weekly Contrad 100/Decon 90 (dilute to 10% in DIW)	**1** Soak in 10% Contrad/Decon for 1 hr, or overnight if needed. **2** Scrub with brush. **3** Rinse with DIW. **4** Dry thoroughly before reinstalling on instrument.
Nebulizer	Daily Contrad 100/Decon 90 (dilute to 10% in DIW)	• For storage, soak in DIW between operating sessions. • For washing: **1** Draw 10% Contrad/Decon through side arm and sample inlet. **2** Soak in 10% Contrad for 15 min. **3** Repeat with DIW to rinse. **IMPORTANT** • Do not sonicate. • Do not clean with acidic detergent (for example, Citranox).
Nebulizer rest	Weekly	Discard the old DIW and add 50 mL of DIW to the conical tube.

Part	Frequency and Cleaning Agent	Procedure
Sampler cone Skimmer-reducer assembly	Weekly (approximately 40 hours of sample acquisition) Citranox (dilute to 10% in DIW)	**1** Place cones with adapters into the cleaning container. **2** Soak and sonicate in 10% Citranox (15 min maximum). **3** Rinse with DIW. **4** Repeat soak and sonication with DIW three times. **5** Dry thoroughly before reinstalling.
Load coil	Weekly Isopropanol	Scrub gently with isopropanol and a Scotch-Brite Ultra Fine Hand Pad (3M 7448).
Interface pump	Weekly Vacuum Pump Oil	**1** Inspect the visual inspection window and verify the condition of the oil using the oil inspection chart. **2** Verify the level of the oil is approximately ¾ full. **3** Drain out the oil and fill new oil if necessary.

Maintenance Checklist

The following are some checks to ensure that the system is clean and has been put back together correctly.

Nebulizer

- ☐ Nebulizer gas and sample line connections are tight.
- ☐ Sample capillary is correctly inserted into the nebulizer.
- ☐ The nebulizer is free of damage and clogs.

Spray Chamber

- ☐ Makeup gas line connection is tight.
- ☐ Injector ball joint clamp is in place.
- ☐ Nebulizer is fully inserted into adapter port.
- ☐ The spray chamber is aligned with the injector and tightly fitted with the ball joint clamp to the injector.

Torch Body and Injector

- ☐ Injector is pushed fully into injector holder.
- ☐ Plasma and auxiliary gas connectors are tight and correctly connected.
- ☐ Torch is aligned properly relative to the load coil.

Cones

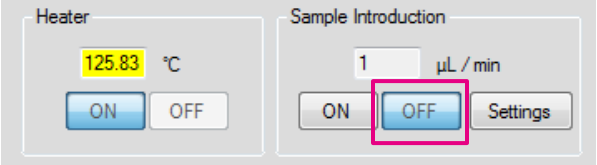
- ☐ Cones are clean and properly installed.

Periodic Maintenance

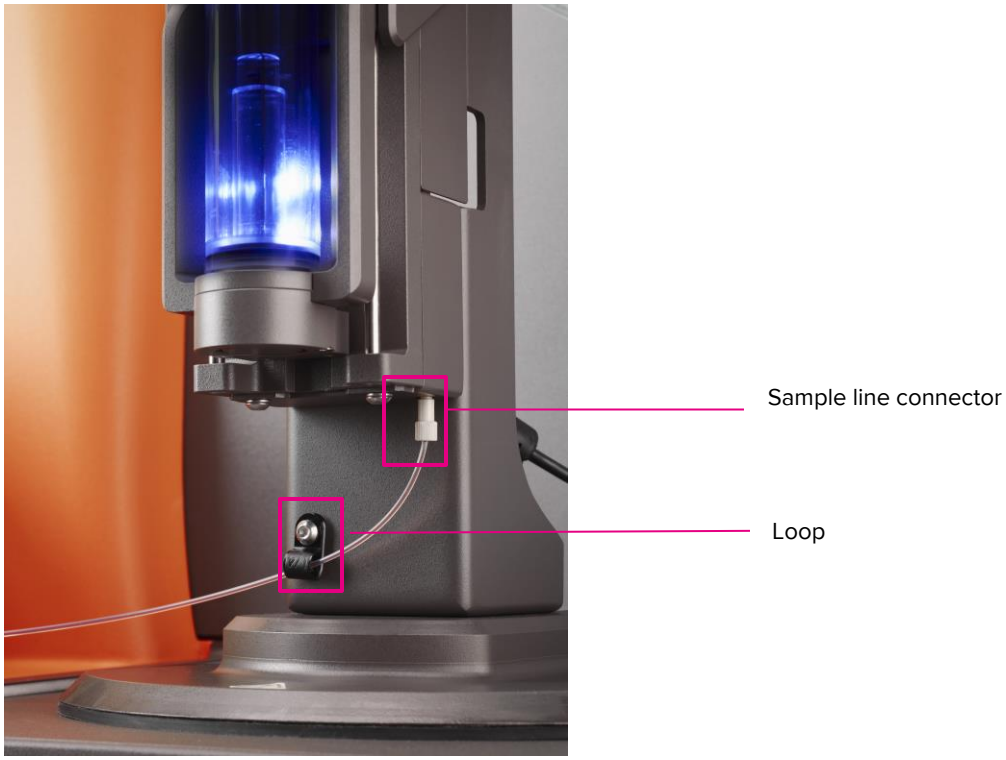
Replace the Sample Line on the Sample Loader

If the Sample Loader does not pressurize or the pressure is quites high when it is on in the software, there may be a clog in the sample line. Clean the sample line and if this does not fix the issue the sample line will need to be replaced.

1 Go to Sample Introduction and click **OFF** to fully depressurize the Sample Loader. Wait until the LED on the Sample Loader is white.



2 Unscrew the sample line connector underneath the Sample Loader.



3 Unscrew the blue connector on the grounding nut of the heater assembly and slide the sample line out of the loop. Set aside.

4 Obtain a new sample line.

5 Separate the tan connector from the new sample line; thread sample line through the loop.

6 Slide the tan sample line connector over the sample line.

7 Pull approximately 1.5 mm of the brown line out of the clear sleeve.

8 Holding brown line of the sample line, partially screw the tan sample line connector into the opening underneath to the Sample Loader.

9 Push the brown line into the Sample Loader until it stops.

10 Screw the tan sample line connector until it is finger-tight.

11 Push the clear sleeve towards the tan sample line connector until it stops. The clear sleeve should now fully cover the brown line.

12 At the other end of the sample line, ensure that the brown line is flush with the clear sleeve and the clear ferrule with blue sample line connector.

13 Screw in the blue sample line connector into the grounding nut of the heater assembly until finger-tight.

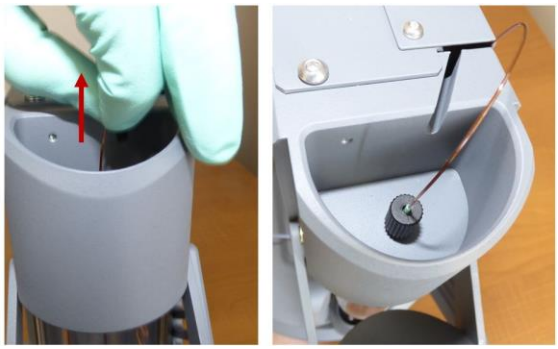
Replace the O-Ring of the Sample Loader Pressure Chamber

When the Sample Loader cannot pressurize, check if the O-ring is misaligned or damaged. Replace the O-ring if it is damaged.

1 Move the sample probe line to avoid damage or contamination to the sample inlet.

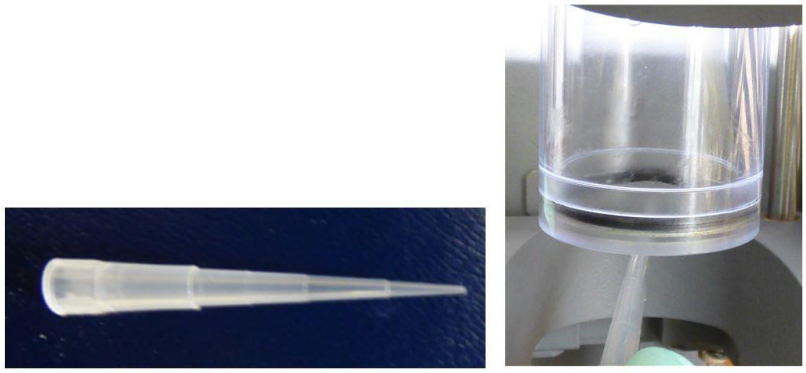
   a Lower the handle of the Sample Loader to open up the pressure chamber and remove the test tube and holder.

b Gently pull up the sample probe line from the top of the Sample Loader toward the corner of the access cover until the line is halfway up the pressure chamber.



2 Remove the O-ring from inside the pressure chamber.

a Using a standard 200 µL plastic pipette tip, gently lift the O-ring out of the groove in the pressure chamber.



b Using the pipette tip, detach the O-ring from the groove and remove it from the pressure chamber.

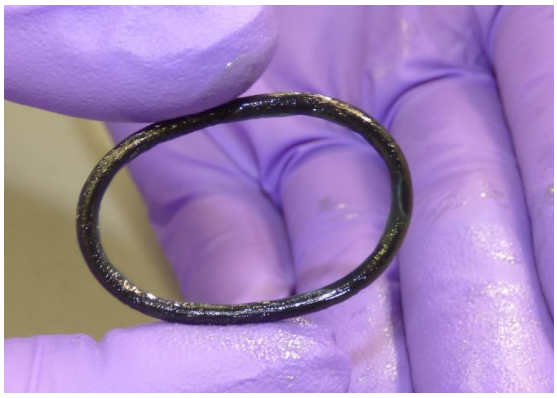
c Using Kimwipes, wipe any excess O-ring grease from the inside of the pressure chamber.

3 Obtain a new O-ring (PN 107301) and a tube of silicone grease (Magnalube, Cat. No. MG75).

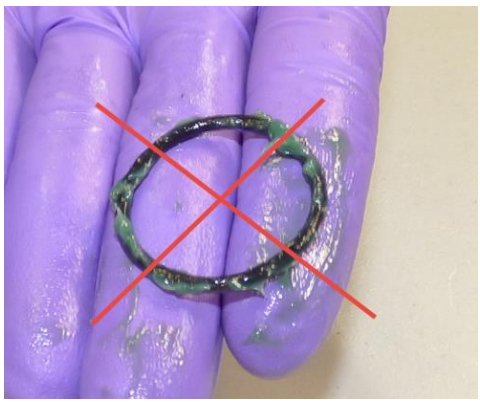
4 Apply a 1 inch length of Magnalube-G on a gloved finger.

5 With the clean hand, apply the Magnalube-G to the O-ring while moving it in a circular manner.

6 Ensure the entire O-ring is covered with the Magnalube-G as shown.

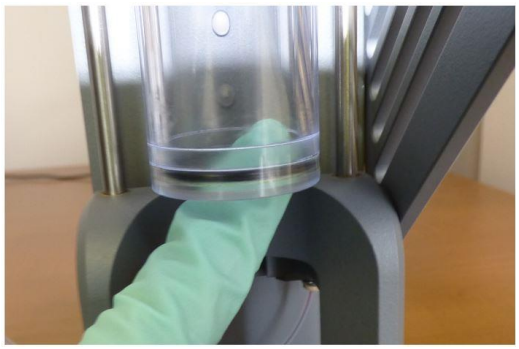


NOTE Avoid applying too much or too little Magnalube-G, as shown below.



7 Change gloves before proceeding to the next step.

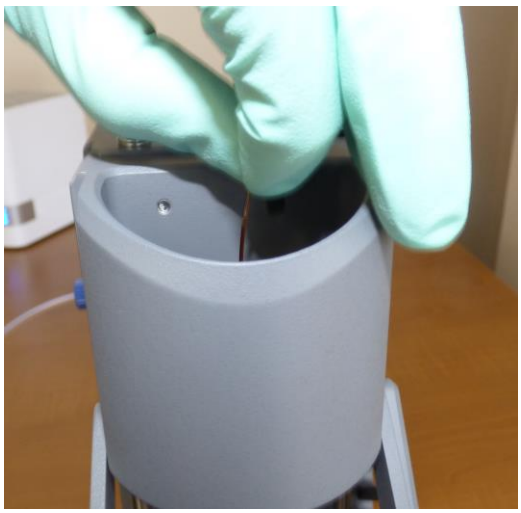
8 Carefully place the new O-ring covered with Magnalube-G into the groove underneath the pressure chamber.



9 Slide your finger along the edge of the O-ring to ensure a consistent seal within the groove.

NOTE After replacing the O-ring, use a Kimwipes (as shown in step 3) to wipe any excess O-ring grease from inside the pressure chamber.

10 Slowly push the sample probe line back into the top of the Sample Loader until it reaches the first line of the pressure chamber when viewed at eye level.

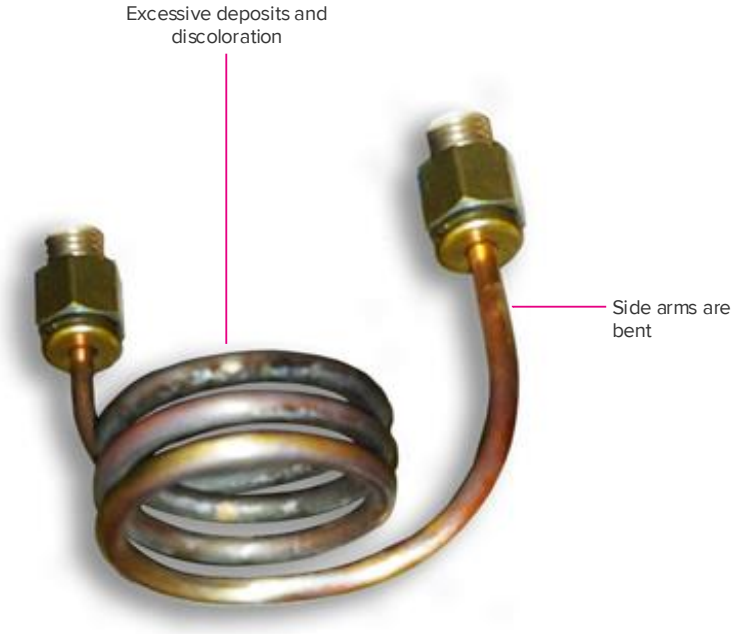


11 Close the handle of the Sample Loader. Observe whether a consistent seal is maintained by the pressure chamber O-ring.

12 Open and close the handle four more times to ensure that the O-ring remains secured within the groove.

Replace the Load Coil

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

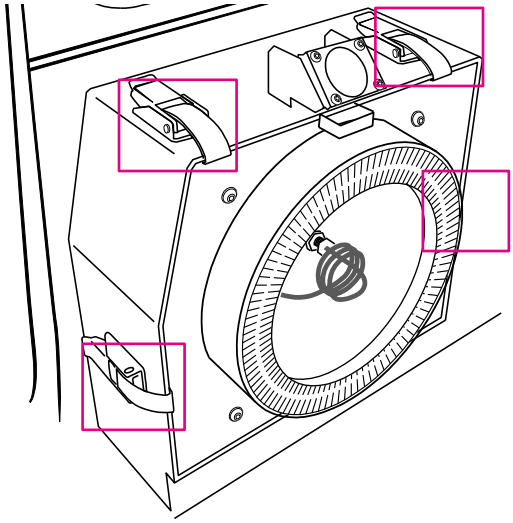


WARNING Before performing maintenance on the cones or torch, switch off the radio frequency generator power using the circuit breaker at the right rear of the system. Wait at least 5 minutes for residual electrical charge to dissipate. Additional time is required to allow the inductively coupled plasma torch, cones, and load coil to reach room temperature.

1 Remove the nebulizer gas line. Disconnect the sample line from the grounding nut and connect to the Sample Loader.

2 Remove the injector ball joint clamp. Slide the heater (with heat shield) off of the heater guide pins and rest it on the support pins. Remove the torch assembly from the front face of the instrument.

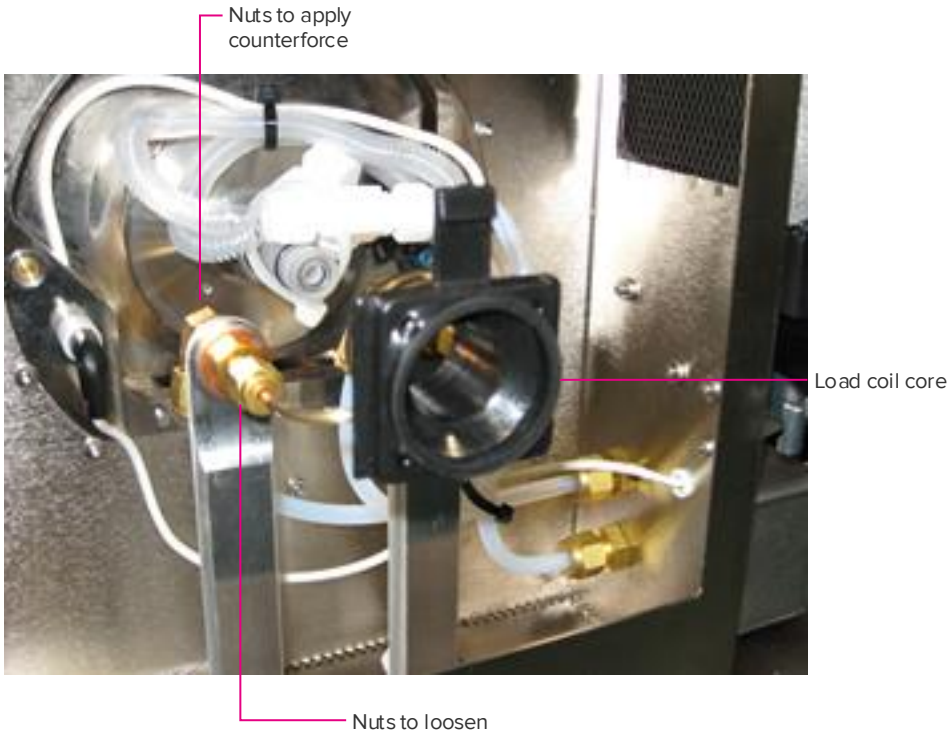
3 Open the front access door. Undo the clips on four sides of the front shield and lift off (magenta boxes below).



4 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.

5 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.

6 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.



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

8 Replace the front shield and fasten the four clips.

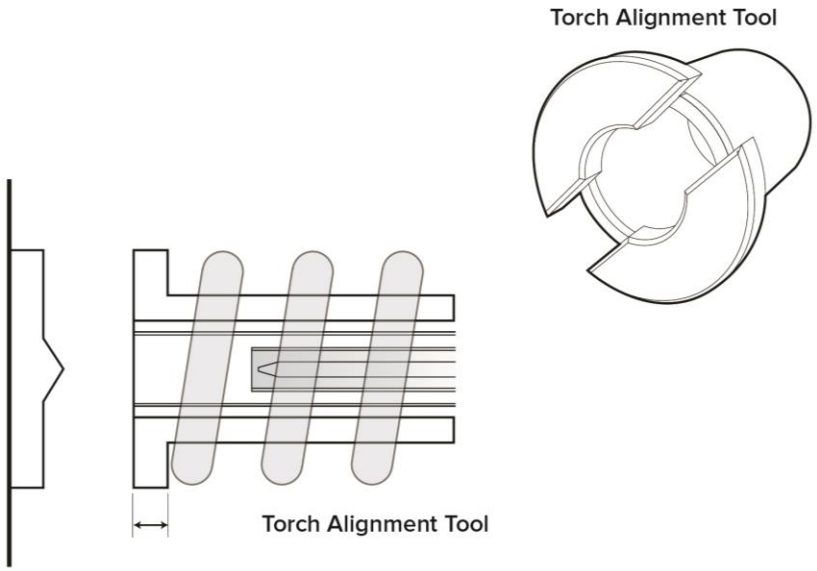
9 Reinstall the torch assembly. Check the alignment of torch with the load coil (refer to the appropriate steps in the cleaning and maintenance section if necessary).

Z-alignment Check

NOTE When a new torch has been installed it is necessary to check the Z-alignment.

1 Install the torch alignment tool into the end of the torch.

2 Gently push the torch alignment tool in as far as it will go.



3 The outer edge of the torch alignment tool should be flush with the edge of the torch.

IMPORTANT If the torch is not flush with the torch alignment tool follow the procedure in the Adjust the Z-alignment section below.

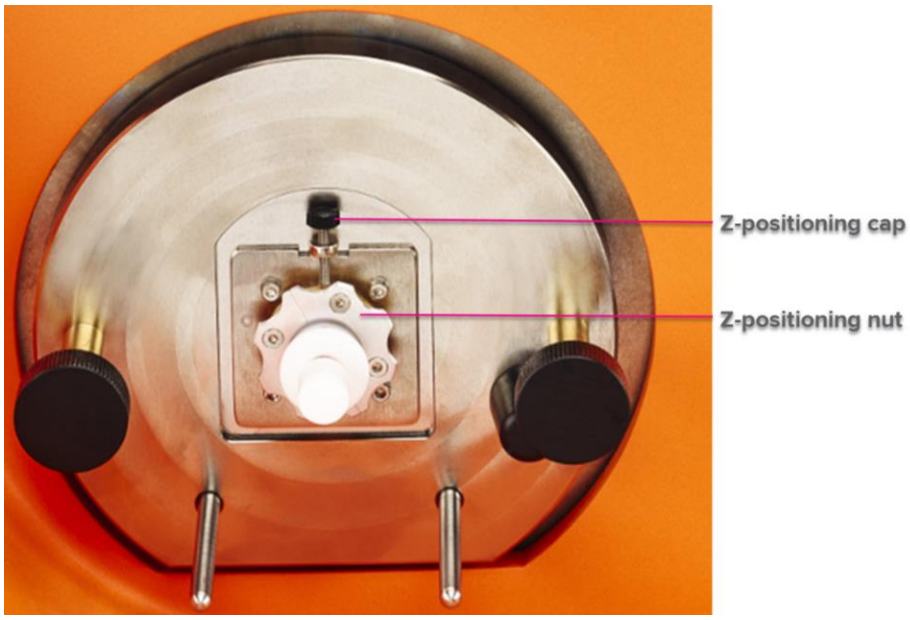
4 Remove the torch alignment tool.

5 Close the front access door.

Adjust the Z-alignment

IMPORTANT Before beginning the procedure, check that the load coil is straight and not bent or damaged.

1 Loosen the black z-positioning cap on the front of the torch assembly.



2 Spray isopropanol on the z-positioning nut. Turn the z-positioning nut until the edge of the torch is flush with the outer edge of the torch alignment tool.

3 Retighten the Z-positioning cap.

4 Remove the torch alignment tool.

5 Close the front access door.

Replace the Injector O-Ring

1 If the Injector O-Ring is damaged or worn you will need to replace it. Inspect the O-Ring (PN 107212) when you remove the injector for cleaning (refer to section Clean the Torch and Injector).

2 Using a gloved hand remove the damaged injector O-ring.

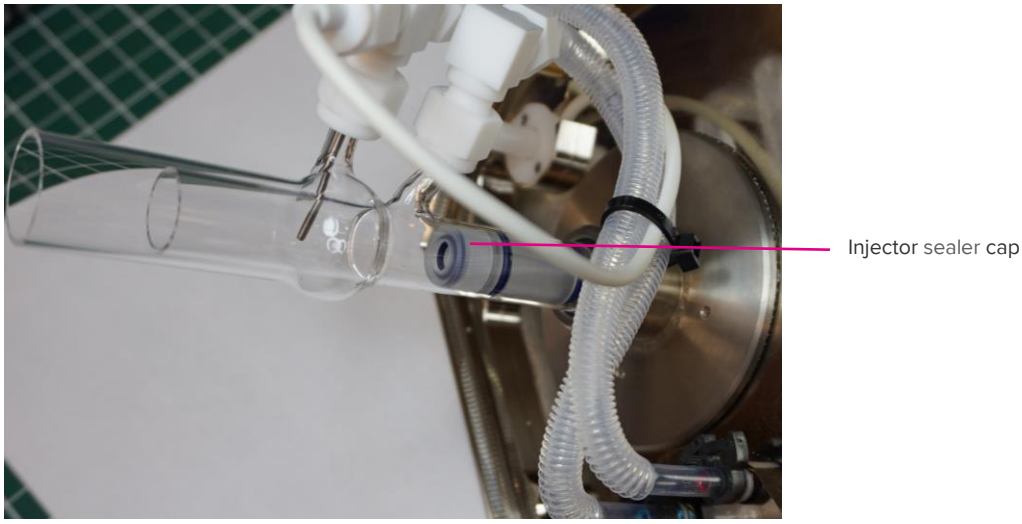
NOTE If the O-ring is difficult to remove, use a plastic pipette tip to cut out the O-ring. Do not damage the groove on the injector.

3 Carefully slide the new O-ring over the groove on the ball joint of the injector.

Replace the Injector Sealer Cap

IMPORTANT The torch assembly must be removed from the instrument prior to performing this procedure.

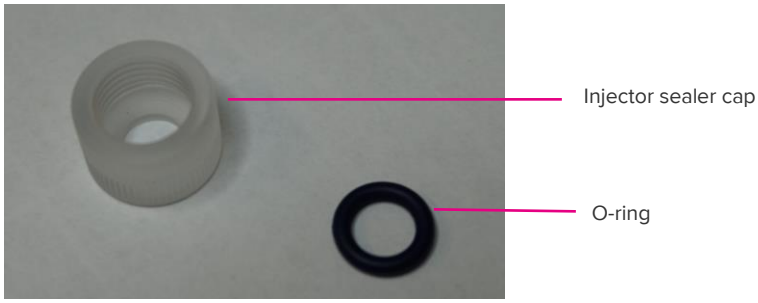
1 Replace the injector sealer cap if required (PN 105350). You will also need an O-ring, which is included in the O-Ring Kit, Torch Body (PN 105641).

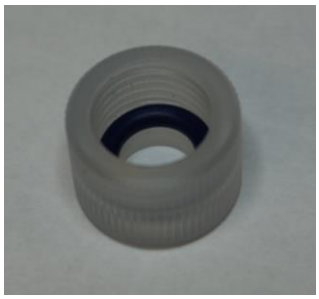


2 Disconnect the plasma gas line and the auxiliary gas line. Grip the torch body at the base and gently turn and pull the torch body over the two O-rings and place on the lab bench.

3 Carefully remove the old injector sealer cap and discard.

4 Insert the O-ring into the new injector sealer cap. Ensure that the O-ring is firmly seated in the inner cap and the center opening is unobstructed.





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

6 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.

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

IMPORTANT The injector may break if excessive pressure is used to insert the injector into the injector holder.

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

Change the Interface Pump Oil

Before beginning the procedure have on hand:

- Funnel with extension tubing
- Vacuum Pump Oil (PN 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 to the left 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.

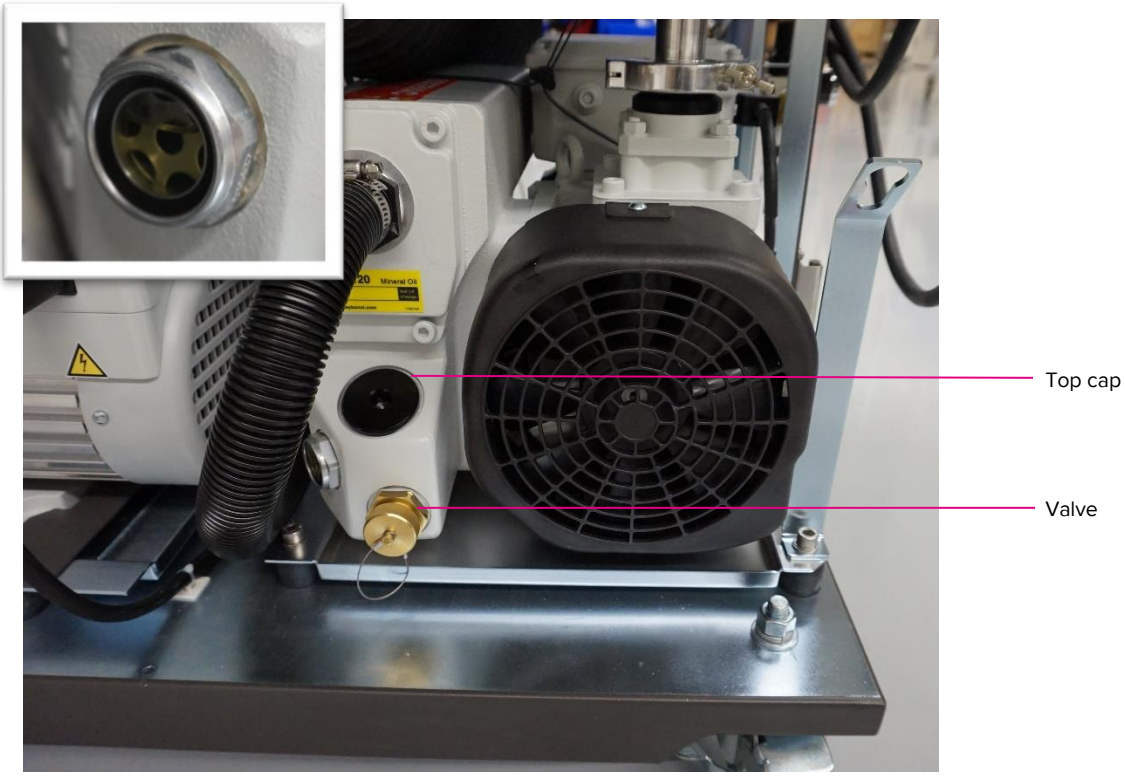


Figure 33. The interface pump in the lower right compartment of the Helios system. The visual inspection window is on the side of the interface pump (inset).

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.

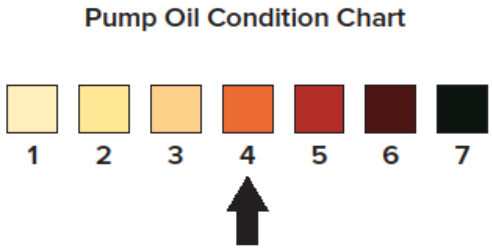
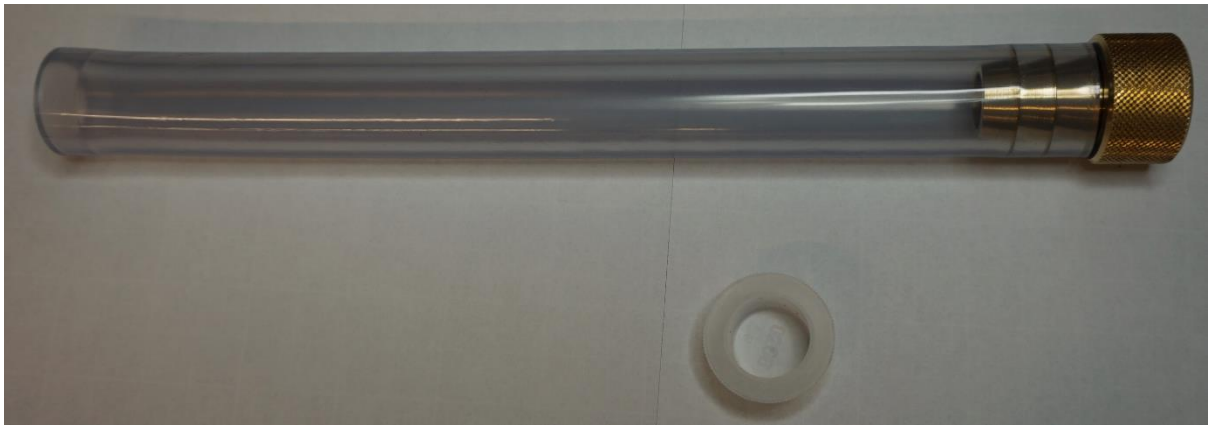


Figure 34. Pump oil condition chart. When the oil is above Level 4 (as indicated by the black arrow) the oil should be replaced in the interface pump.

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



7 Unscrew the gold valve on the interface pump, and connect the drain kit provided.

8 Use the Allen key provided to remove the top cap from the pump.

NOTE This allows for more effective draining of the oil.

9 Drain the oil into a tray or plastic container.

10 If the oil is very dirty, add 100 mL of fresh pump oil to allow the oil to drain freely, and then drain using the drainage tubing before proceeding to the next step.

11 Remove the drainage tube and reconnect the gold valve.

12 Using a 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 regional, national and local regulations.

Change the Oil on the Backing Pump

NOTE The backing pump oil is changed on an annual basis by the Fluidigm Field Service Engineer (FSE) as part of the Preventative Maintenance of the instrument. Contact Fluidigm technical support if you believe the oil in the backing pump requires changing.

Chapter 7: Troubleshooting

Helios Troubleshooting

Table 15. Recommended solutions for symptoms you may encounter while operating the Helios™ instrument

Observation	Possible Cause	Recommended Action
Plasma does not ignite	RFG circuit breaker is switched off.	Switch the circuit breaker on.
	Vacuum levels are not achieved.	Check the Status Panel on the front of the instrument and check that the vacuum gauges VG1 and VG2 LEDs are green. If these are not OK, shut down and restart Helios. Refer to Chapter 6: Maintenance.
	Argon pressure is incorrectly set/ there is not enough argon.	Verify and adjust argon pressure on tank to around 100 psi and Helios regulator pressure behind the instrument to around 50 psi. Also check level of argon in the tank and replace tank if necessary.
	Exhaust is out.	If the EXHAUST LED light is off, it indicates that there may be a problem with the exhaust fan within the building.
	Chiller is not turned on.	The chiller should turn on automatically within 20 seconds after user confirms plasma start. If CHILL LED light is off, it indicates that the chiller has not been turned on by the software. Manually switch on the chiller in the Control Panel and ensure the CHILL LED light comes on in the instrument status panel.
	There is moisture in glassware.	Inspect glassware for moisture that may be present and interfering with plasma ignition. Completely dry glassware and spray chamber.
	Connections of gas line are incorrect.	Ensure tight and correct gas line connection on nebulizer, spray chamber, and torch.
	Torch has melted	Check for leaks in torch assembly and gas lines near the interface area. Check argon pressure. Check load coil for deposits. Replace torch and, if necessary, load coil.
	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer.

Observation	Possible Cause	Recommended Action
	The load coil is not clean/has punctures/deposits.	Clean the load coil so that the surface is smooth and free of debris. If necessary, such as when there are small punctures present, replace the load coil.
	The O-rings on the vacuum cones are damaged or worn out.	Check that the O-rings of the sampler and skimmer/reducer cones are not worn out or damaged and replace if necessary.
	Vacuum interface cones are misaligned.	Check that the sampler cone is flush with the vacuum interface.
	Glassware and spray chamber are not installed correctly after cleaning.	Reassemble and realign the glassware and spray chamber to ensure that they have been installed correctly.
	Ignitor pin is not correctly inserted or it is dirty.	Clean the ignitor pin, reverse it, and put it back into place to improve connection to the wire.
No signal is detected during tuning	One of the above causes.	Follow the corresponding recommended solution for the cause.
	Sample capillary is clogged.	Replace the capillary.
	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer.
	Nebulizer is damaged or clogged.	Go to Sample Introduction and click **ON** in the software. Carefully remove the nebulizer from the nebulizer adaptor port (with all other connections intact) and check the spray with a flashlight. If the spray is absent or intermittent, clean or replace the nebulizer.
	Masses are incorrectly calibrated.	Select Quick Protocol in the Tuning Manager and perform the tuning. See Chapter 3: Operation.
	The analytes are not selected correctly.	Check your elements template in the Experiment Manager and make sure the analytes of interest are selected.
Unstable signals	One of the above causes.	Follow the corresponding recommended solution for the cause.
	Nebulizer is not connected properly or needs replacement.	Check nebulizer gas quick connection on the front of the instrument and reconnect if necessary. Check nebulizer flow. Refer to Chapter 5: Operation.

Observation	Possible Cause	Recommended Action
Low tuning solution signals (Tb signals <600,000 dual counts per picogram)	One of the above causes.	Follow the corresponding recommended solution for the cause.
	Heater is not on and has not reached the correct temperature.	Ensure heater temperature is at or near 200 ˚C. If not at or near 200 ˚C, check for moisture in the glassware and if necessary remove the nebulizer and spray chamber to dry after shutting off plasma.
	Argon pressure is not maintained.	Ensure steady argon supply and proper argon pressure is maintained (~100 psi on tank and ~50 psi on regulator).
	Plasma is unstable	See above for recommended solutions for plasma ignition/stability issues.
	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer
	Masses are not correctly calibrated.	Perform mass calibration. Refer to Chapter 5: Operation.
	Nebulizer and makeup gas flows are not optimal.	Perform gas optimization. Refer to Chapter 5: Operation.
	Current is not optimal.	Perform current optimization. Refer to Chapter 5: Operation.
	Detector voltage is not optimal.	Perform detector voltage optimization. Refer to Chapter 5: Operation.
	One or more hardware parts are not aligned properly.	Refer to Chapter 6: Maintenance for proper alignment of parts and refer to Chapter 5: Operation for optimizing signals.
	The glassware is not clean.	Remove the glassware according to the instructions in Chapter 6: Maintenance.
	The interface cones are not clean.	Remove the cones according to the instructions in Chapter 6: Maintenance.
	One or more hardware parts of the instrument need to be replaced.	Inspect all accessible hardware parts including the spray chamber, Nebulizer, torch, injector, and cones. If there are any signs (such as damage, clogging, and irremovable stains) that suggest the part is no longer functioning optimally, replace with a new one.
	The O-rings on the sampler and skimmer-reducer are damaged.	The O-rings may have been damaged during cleaning procedures or may be worn out and require replacing.
	The sampler cone is not inserted correctly.	The sampler cone is not flush with the interface orifice.

Observation	Possible Cause	Recommended Action
	The O-rings on the injector holder are worn out.	Check the two O-rings on the injector holder to ensure that they are not worn out and that the torch body covers both O-rings.
	The injector is not fitted correctly.	Check that the injector is correctly fitted into the injector holder and ensure that it is 1.5–2.0 mm from the inner tube of the torch.
	The alignment of the torch and load coil is incorrect.	Check the alignment of the torch and the load coil using the torch alignment tool. Refer to Chapter 6: Maintenance.
Oxides are >3%	Nebulizer and makeup gas flows are too high.	In the tuning tab set up a custom protocol and select gas optimization and perform gas optimization. Refer to Chapter 5: Operation.
Unstable Signal or Oscillations from Tuning Solution	Proper exhaust level is not reached/maintained in the exhaust hoses.	Ensure proper and consistent exhaust by checking with your laboratory facilities manager.
	Nebulizer is damaged or clogged.	Carefully remove the nebulizer from the nebulizer adaptor port (with all other connections intact) and check the spray using a flashlight. If the spray is absent or intermittent, clean or replace the nebulizer.
	Nebulizer gas line is not connected properly.	Check nebulizer gas connection and reconnect if necessary.
	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer. Refer to Chapter 5: Operation.
No signal from sample	Sample has not been delivered to the nebulizer.	Ensure the argon pressure in the Sample Loader is OK. Check the connection on the external sample line.
		The sample line is clogged and must be replaced. Refer to Chapter 6: Maintenance.

Observation	Possible Cause	Recommended Action
	Sample is not present.	It is highly recommended that users add 0.1X EQ Beads with the sample as an internal standard. Refer to Product Insert for usage instructions. If the beads are present but the cells are not, it indicates the absence of cells in the sample itself. If both the beads and the cells are not visible on Helios, there could be problems with one or more parts of the instrument that need to be addressed before continuing to record.
	One or more parts of the instrument are causing the problem.	Refer to “No Signal detected during performance check” for possible causes and recommended solutions.
	The injector is clogged/has not been cleaned efficiently.	The injector must be removed and cleaned according to the instructions in Chapter 6: Maintenance.
Sample is leaking from sample capillary	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer. With leakage, the capillary is often too far in and has bent. Trim capillary or replace if damaged. Refer to Chapter 5: Operation.
Sample Loader LED indicator is flashing red	There is a fault detected with the sample agitator.	Remove the sample holder and sample tube. Visually inspect the base of the holder to ensure that there is no liquid in the base of the pressure chamber. User a KimWipe to dry the base and let it air dry. Power cycle the Sample Loader. If the flashing red light continues contact Fluidigm support.
Cells are indistinct from each other (streaky signals)	Cell concentration is likely too high.	To prevent detector damage, immediately stop the acquisition when there are more than three continuous refreshes of streaky signals. Look for the antibodies that produce this continuous streak of signals.
	Too many cells are introduced	Dilute the sample with Type I ultrapure (18.2 MΩ) (DIW). Concentration of cells introduced should be 1,000,000 cells/mL, at 30–45 µL/min introduction rate. Lower cell concentrations improve signal resolution.

Observation	Possible Cause	Recommended Action
	The concentration of intercalator is too high.	Before recording, wash the sample once more with DIW. If the signals are still too strong, wash once again with DIW.
	The source of streaky signals is one of markers used.	Make sure the antibodies are titrated prior to the experiment, ideally with the cell type of interest.
The Operating System is not turning on properly and the “System Recovery” prompt appears	The Windows® files may have been corrupted.	Do not attempt to recover the operating system. Contact Fluidigm support for instructions on repairing.
Sample Loader is not pressurizing.	The sample line is blocked or damaged.	The sample line needs to be replaced.
	The argon gas connection is not connected.	Check the quick connect behind the Sample Loader and ensure that it is connected.

Appendix A: Helios Specifications

Helios® Specifications

Description		Specification
Channels		135
Mass range		75–209 amu
Abundance sensitivity		0.3% for ^{159}Tb
Instrument response		600,000 counts/pg ^{159}Tb
Detection limit		350 antibodies/cell
Dynamic range		4.5 orders of magnitude
Calibration		Automated
Operating system		Windows® 7 Pro 64-bit
Data storage		7.2 TB RAID (mirrored)
Sample introduction		Pneumatic single-tube loader with agitation, up to 5 mL volume
Average event rate		500 events/sec
Peak throughput		2,000 events/sec
Flow rate		30 µL/min
Replicate sample CV (normalized)		<3%
Dimensions	Width	103 cm (41 in)
	Height	132 cm (52 in)
	Depth	87 cm 35 in
Weight		320 kg 750 lb

Workstation Specifications

Description		Specification
Operating system		Windows 7 Pro 64-bit
CPU		Intel® Core™ i7
RAM		16 GB
Hard drive		223 GB SSD, RAID 1
Data storage		7.2 TB HDD, RAID 10
Video		NVIDIA Quadro® 410
Power supply		650 W
Serial adapter board		8 Port Native PCI Express RS232
Monitor		24 in (61 cm) LED
Keyboard/mouse		Wired
DVD/Blu-ray		SATA 16X
Dimensions	Width	20 cm (8 in)
	Height	46 cm (18 in)
	Depth	58 cm (23 in)
Weight		35 kg (77 lb)

Data File Size

File Type	Size
IMD	0.3 MB/sec/channel
FCS	12.4 bytes/event/channel
TXT	2.4 bytes/event/channel

Chiller Specifications

Description		Specification
Dimensions	Width	38 cm (15 in)
	Height	64 cm (25 in)
	Depth	67 cm (27 in)
Weight		81 kg (178 lb)

Autosampler

Description		Specification
Sample volume		50–900 µL
Sample format		3 x 96-well plate, up to 2 mL deep well
Sample resuspension		Probe pipet motion
Reagent reservoirs		
	Carrier (DIW)	250 mL
	Wash solution	250 mL
	Rinse	250 mL
Dimensions	Width	39 cm (16 in)
	Height	24 cm (10 in)
	Depth	36 cm (14 in)
Weight		20 kg (44 lb)

Appendix B: Safety

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

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 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.

Symbol	Description
	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.
	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.

Symbol	Description
	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 minutes 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 two 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.

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 is used, the gas cylinder must be fitted with an overpressure regulator, which will vent 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
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