



DVS SCIENCES INC

Cell Analysis Products

CyTOF[®]

MANUALS



DVS SCIENCES INC
Cell Analysis Products

CyTOF[®]

Mass Cytometer

SAFETY MANUAL

CELL ANALYSIS PRODUCTS

CyTOF Mass Cytometer Safety Manual

This is a Class A device and is for use in a commercial, industrial or business environment.

Warning: This is a Class A product. In a domestic environment this product may cause radio interference, in which case the user may be required to take adequate measures.

CyTOF and MAXPAR are registered trademarks of DVS Sciences Inc.
All products and company names mentioned herein may be the trademarks of their respective owners.

NOTE : this manual applies to CyTOF Model C5 instruments.

Revision 3, 2011.

© DVS Sciences Inc. 2011

70 Esna Park Drive, Unit 12, Markham, Ontario, L3R 6E7, Canada
telephone: 905-513-1704; fax 905-513-1704
www.dvssciences.com

CyTOF[®] Series Mass Cytometer Safety Manual

This document contains information proprietary and confidential to DVS Sciences Inc. and is for customer use in the operation and maintenance of CyTOF equipment or is for vendor use in the specification, fabrication and manufacture of DVS designed component parts. Any other use, disclosure or reproduction of the information contained herein is strictly forbidden, except as DVS Sciences may authorize in writing.

Equipment described in this document may be protected under one or more patents filed in the United States, Canada and other countries. Additional patents are pending. Software described in this document may be furnished under a licence agreement. It is against the law to copy the software on any medium, except as specifically allowed in the licence agreement.

Portions of this document may make reference to other manufacturers' products, which may contain parts that are patented and may contain parts whose names are trademarked. Any such usage is intended only to designate those manufacturers' products as supplied by DVS for incorporation into its equipment.

DVS Sciences Inc. assume no responsibility or contingent liability for any use to which the purchaser may subject the equipment described herein, or for any adverse circumstances arising therefrom.

Safety Practices

This document describes general practices designed to aid you in safely operating the Mass Cytometer and accessories.

This advice is intended to supplement, not supersede, the normal safety codes in your country. The information provided does not cover every safety procedure that should be practiced. Ultimately, maintenance of a safe laboratory environment is the responsibility of the operator and the operator's organization.

Please consult all manuals supplied with the Cytometer and accessories before you start working with the instrument. Carefully read the safety information in this chapter and in the other manuals supplied. When setting up the instrument or performing analyses or maintenance procedures, strictly follow the instructions provided.

Note: Maintenance procedures described in this document are to be performed only by a DVS-trained service engineer.

Laboratory Guidelines

This section describes some general laboratory safety guidelines. For additional information, we recommend *The CRC Handbook of Laboratory Safety* (Ref. 1) and *Prudent Practices for Handling Hazardous Chemicals in Laboratories* (Ref. 2).

General

The instrument operates with Inductively Coupled Plasma (ICP) ion source. This is a bright source of ultraviolet radiation, and the instrument is designed to contain the ion source without being assessable by a user during operation. Always wear safety glasses when using the instrument. Safety glasses with side shields will provide an extra margin of safety as well as mechanical protection for your eyes.

For safety reasons and to avoid contaminating samples, be sure that the instrument and work area are kept scrupulously clean. This is especially important when working with toxic elements or when measuring trace amounts of any element. Please note that some of the reporting elements are abundant in the environment, so care must be taken not to contaminate samples and other working solutions. Clean up spilled chemicals immediately and dispose of them properly.

Do not allow smoking in the work area. Smoking is a source of significant contamination as well as a potential route for ingesting harmful chemicals. Food should not be stored, handled, or consumed in the work area.



Figure 2-1. Warning Symbol

Environmental Conditions

The instrument should be used indoors in a laboratory having the environmental conditions recommended in the “Preparing Your Laboratory for the DVS Sciences Inc. CyTOF Mass Cytometer” guide.

Handling of the Instrument

Contact a DVS Sciences service engineer for assistance in installing or moving the system. The total weight of the instrument is 295 kg (650 lbs).

Laboratory Ventilation

Toxic combustion products, metal vapor, and ozone can be generated by the CyTOF system, depending upon the type of analysis. Therefore, an efficient ventilation system must be provided for your instrument. Detailed specifications for a recommended system are described in the “Preparing Your Laboratory for the DVS Sciences Inc. CyTOF Mass Cytometer” guide.



Warning! The use of CyTOF instruments without adequate ventilation to outside air may constitute a health hazard. Extreme care should be taken to vent exhaust gases properly.



Warning! CyTOF instrument is designed for analysis of fixed/permealized, non-live cells only. Under normal operation, cells are completely combusted in the ICP. High levels of UV radiation inside the torch box are significantly above the lethal levels for most of single airborne cells. However, in the event of plasma shutdown, the undigested portion of a sample can enter the torch box exhaust gases. Extreme care should be taken to vent exhaust gases properly.

General Safety Precautions

Adherence to the following safety precautions should be maintained at all times when setting up, operating, and maintaining the CyTOF system:

- Never view the ICP torch directly without protective eyewear. Potentially hazardous ultraviolet radiation may be emitted.
- ICP-based instruments generate high levels of radio frequency energy within the RF power supply and the torch box. The RF energy is potentially hazardous if allowed to escape. Safety devices and safety interlocks should not be bypassed or disconnected.
- The power supplies of the CyTOF instrument are capable of generating potentially lethal voltages. No maintenance should be performed by anyone other than a DVS Sciences Service Specialist or by the customer's own DVS-trained and appropriately certified maintenance personnel.
- Water lines should be located away from electrical connections. Condensation and potential leaks may create an unsafe environment in the proximity of electrical connections.

Electrical Safety

The CyTOF series products have been designed to protect the operator from potential electrical hazards. This section describes recommended electrical safety practices.



Warning! If this equipment is used in a manner not specified by DVS Sciences, the protection provided by the equipment may be compromised.

Electric Shock Hazard



Warning! Lethal voltages are present at certain areas within the instrument. Installation and internal maintenance of the instrument should be performed only by a DVS Sciences service engineer or similarly authorized and trained by DVS personal.

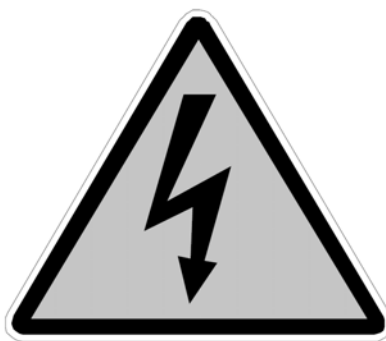


Figure 2-2 Electric shock hazard

- When the instrument is connected to line power, opening instrument covers is likely to expose live parts.
- High voltages can still be present even when the power switch is in the off position.
- Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.
- The instrument must be correctly connected to a suitable electrical supply.
- For 50 Hz installations, a means of electrically grounding the instrument must be available.

Earth/Ground Integrity

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 instrument.



Warning! Any interruption of the protective conductor (earth-ground) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous. Intentional interruption is prohibited.



Figure 2-3 Earth-ground symbol

Electrical Safety

- Connect the instrument to a correctly installed line power outlet that has a protective conductor connection (earth-ground).
- Do not operate the instrument with any covers or internal parts removed.
- Do not attempt to make internal adjustments or replacements except as directed in the manuals.
- Disconnect the instrument from all voltage sources before opening it for any adjustment, replacement, maintenance, or repair.
- Use only fuses with the required current rating and of the specified type for replacement. Do not use makeshift fuses or short-circuit the fuse holders.
- Whenever it is possible that the instrument is no longer electrically safe for use, make the instrument inoperative and secure it, with a lockout, against any unauthorized or unintentional operation. The electrical safety of the instrument is likely to be compromised if, for example, the instrument shows visible damage; has been subjected to prolonged storage under unfavorable conditions; or has been subjected to severe stress during transportation.



Warning! The Radio Frequency (RF) power supply driving the plasma torch provides up to 1.6 kW. The resulting voltages may cause extensive burns - even death. Under no circumstances should you attempt any physical adjustments of the plasma torch when it is operating. The instrument must be operated with the RF generator in the locked position at all times.

Safety Interlocks

The instrument has safety interlocks to protect the operator from ultraviolet radiation and radio frequency radiation, and to prevent access to high voltage areas. All interlocks must be engaged before you can ignite the plasma.



Warning! Do not attempt to defeat the safety interlocks. This would place the operator's safety at risk.

Other Hazards

Protection from Ultraviolet Radiation



Warning! The plasma generates high intensity ultraviolet radiation. A safety interlock is used to automatically shut off the plasma if the chamber and interface are not fully coupled. Do not defeat the interlock. Do not remove the shield which protects the sample introduction system: the shield is designed to block any residual amounts of the ultraviolet radiation.

Protection from Radio Frequency radiation



Warning! The instrument generates high levels of Radio Frequency (RF) energy, which is potentially hazardous if allowed to escape. The instrument is designed to contain the RF energy within the shielded enclosures of the torch compartment and the RF power supply. Safety interlocks prevent you from operating the system without all covers, doors, and shields in place.



Figure 2-4 Radio Frequency radiation symbol

Hot Surface Temperatures



Warning! The torch components, the 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.



Figure 2-5 Hot surface symbol

Hot Exhaust Gases

When the plasma is on, hot gases are vented through two exhaust vents located at the back of the instrument. Detailed information on exhaust vents are described in the appropriate “Preparing Your Laboratory for the DVS Sciences Inc. CyTOF Mass Cytometer” guide.

Safe Use of the Drain Vessel

A drain vessel is supplied with the CyTOF system. The vessel is made of HDPE and is used to gather the effluent from the Flow Injection Valve of the sample introduction system. For safe operation of your system, you should properly install and maintain the drain vessel and drain tubing.



Warning! Never place the vessel in an enclosed cabinet. Doing so could result in a build-up of hazardous gases.



Warning! Never use a glass drain vessel. A glass drain vessel may break and spill toxic or corrosive liquids.

Recommended safety practices for drain systems are as follows:

- Always place the drain vessel in an area that is visible to the operator, who can observe the level of collected effluent and empty the vessel when necessary.
- Check the condition of the drain tubing regularly to monitor deterioration. Organic solvents deteriorate the tubing more quickly than aqueous solutions. When the tubing becomes brittle or cracked, replace it.
- Empty the drain bottle regularly. Disposal of waste must be in accordance with all national, state/provincial and local health and safety regulations and laws.

Safe Use of Chemicals

Safe Use of Chemical Reagents

In this section, we have provided some general safety practices that you should observe when working with any chemicals.



Warning! Some chemicals used with this instrument may be hazardous or may become hazardous after completion of an analysis.

The responsible body (e.g., Lab Manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Material Safety Data Sheets (MSDS) or OSHA, ACGIH or COSHH documents. For more information, see Reference 5.



Warning! Venting for fumes and disposal of waste must be in accordance with all national, state/provincial and local health and safety regulations and laws.



Figure 2-6 Poison Hazard

Protective Equipment

Wear appropriate eye protection at all times while handling chemicals. Use safety glasses (with side shields), goggles, or full-face shields, according to the types of chemicals you will be handling.



Warning! Wear suitable protective clothing, including gloves specifically resistant to the chemicals being handled.

Use, Storage, and Disposal



Figure 2-7 Caustic Chemical Hazard



Warning! Wear protective clothing and gloves. Some reagents are readily absorbed through the skin.

When handling any chemical the following safe-handling guidelines should be strictly observed:

- Use, store, and dispose of chemicals in accordance with the manufacturer's recommendations and regulations applicable to the locality, state/ province, and/or country.
- When preparing chemical solutions, always work in a fume hood that is suitable for the chemicals you are using.
- Conduct sample preparation away from the instrument to minimize corrosion and contamination.
- Clean up spills immediately using the appropriate equipment and supplies, such as spill cleanup kits.

- Do not put open containers of solvent near the instrument.
- Store solvents in an approved cabinet (with the appropriate ventilation) away from the instrument.

Safe Handling of Gas Cylinders

Ar gas used with CyTOF systems is normally stored in liquid argon tanks or pressurized bottles.

The following hazards are associated with pressurized bottles of argon:

- Muscle strain
- Physical injury (i.e., from a bottle falling)
- Suffocation



Figure 2-8 Compressed Gas Hazard

The major hazard associated with argon is suffocation. This can occur if the gas is allowed to escape in an enclosed area and displace the oxygen in air. Argon is neither explosive nor combustible.



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

Carefully use, store, and handle compressed gases in cylinders. Gas cylinders can be hazardous if they are mishandled.



Warning! If liquid argon 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.

Following are some general safety practices for the proper identification, storage, and handling of gas cylinders.

Gas Cylinder Identification

Legibly mark cylinders to identify their contents. Use the chemical name or commercially accepted name for the gas. In North America, as in most countries, all chemical or gas storage containers must be identified by means of approved labels (i.e., WHMIS labels).

Storing Cylinders

- Store cylinders in accordance with the regulations and standards applicable of your locality, state/province, and country.
- When cylinders are stored indoors in storage rooms, the storage room should be well ventilated and dry. Ensure that the ventilation is adequate to prevent the formation of dangerous accumulations of gas. This is particularly important in small or confined areas.
- Do not store cylinders near elevators, gangways, or in locations where heavy moving objects may strike or fall against them.
- Use and store cylinders away from exits and exit routes.
- Locate cylinders away from heat sources, including heat lamps. Compressed gas cylinders should not be subjected to temperatures above 52^o C (126^o F).
- Do not allow ignition sources in the storage area and keep cylinders away from readily ignitable substances such as gasoline or waste, or combustibles in bulk, including oil.
- Store cylinders standing upright, fastened securely to an immovable bulkhead or permanent wall.
- When storing cylinders outdoors, they should be stored above ground on a suitable floor and protected against temperature extremes (including the direct rays of the sun).

Handling Cylinders

Move cylinders with a suitable hand truck after ensuring that the container cap is secured and the cylinder properly fastened to the hand truck.

- Never roll or drag a compressed gas cylinder. Use a wheel cart.
- Always use a stand or safety strap while using or storing a cylinder.
- Replace the protective cap on the valve when the cylinder is not in use.
- Use only regulators, tubing, and hose connectors specifically approved by an appropriate regulatory agency to be used with the gas in the cylinder.

- Never lubricate regulators or fittings.
- Do not force caps off with tools. If stuck, contact the supplier.
- Arrange gas hoses where they will not be damaged or stepped on, and where objects will not be dropped on them.
- Do not refill gas cylinders.
- Check the condition of pipes, hoses, and connectors regularly. Perform gas leak tests at all joints and seals of the gas system regularly, using an approved gas leak detection solution.
- Close all gas cylinder valves tightly at the cylinder when the equipment is turned off.

Sample Handling

Sample preparation for Mass Cytometry may require the handling of organic or corrosive solutions.

MAXPAR[®] reagents that are used with CyTOF instruments are supplied in a solution form.

Please refer to the information supplied with MAXPAR reagent Material Safety Data Sheets for safe handling of the reagents.

Please refer to appropriate safety practices documents when handling antibodies, cell cultures and samples. This manual does not deal with safety procedures related to handling biologically active materials. Reference 11 contains general information on biological hazards and safety.



Warning! Always observe manufacturer/ supplier 's recommendations on safe handling, storage and disposal of biologically active materials.

Reference 6 contains general information on sample preparation procedures as well as an appendix on laboratory safety. Reference 7 contains information on laboratory safety and sample preparation methods prescribed by the United States Environmental Protection Agency (EPA).



Warning! Always wear appropriate eye protection while preparing samples. Use safety glasses with side shields, goggles, or full-face shields, depending on the chemicals you are handling.

Acid Digestions

Acid digestions, either at atmospheric pressure or at increased pressure, require special care. Spattering and foaming of the sample/acid mixture may expose you to a hazard, as

well as compromise the sample integrity. A digestive sample containing concentrated acid will react violently with water. Perchloric acid and hydrofluoric acid are particularly hazardous to work with. Use hoods, ducts, and other devices for removing vapors specifically designed to accommodate this kind of fume. There is a severe explosion hazard if a normal hood is used, or if the hood is not properly used and maintained. Use goggles and face shields. Wear protective clothing and polyvinyl chloride gloves, not rubber gloves. Additional hazards and precautions are given in References 1, 2, 8, and 9.

Hydrofluoric Acid

Trace amounts (< 0.1 % w/v) of hydrofluoric acid (HF) may be present in the wash-out solution. Hydrofluoric acid is toxic and extremely corrosive. It will readily burn skin and lung tissue (if the fumes are inhaled). Burns may not be immediately painful or visible. Contact with eyes could result in blindness.



Warning! Before using hydrofluoric acid, you should be thoroughly familiar with its hazards and safe handling practices. Observe the manufacturer's recommendations for use, storage, and disposal.

When using hydrofluoric acid:

- Always wear suitable protective equipment, including goggles, a face shield, acid-resistant gloves, and protective clothing.
- Do not breathe HF vapors.
- Always work in a fume hood when using hydrofluoric acid.
- Hydrofluoric acid attacks glass. Do not use a glass beaker.
- Observe the additional hazards and precautions outlined in References 1, 2, 8, and 9.

Performing Sample Preparations

When carrying out sample preparation:

- Always work in a hood suitable for the type(s) of chemical(s) and biomaterials you are using.



Warning! Always wear powder-free gloves and observe necessary precautions to prevent foreign materials from contaminating the samples or reagents. Elemental contamination will jeopardize the lifetime of the detector.



Warning! Always use laboratory reagents and supplies that are fresh, are not expired and have not been exposed to contamination sources. For better control of contamination, dedicate laboratory reagents and consumables to use with CyTOF instrument and MAXPAR reagent only.

Waste Disposal Procedures

Drain vessels may contain flammable, acidic, caustic, or organic solutions, cells debris and small amounts of the elements analyzed. The collected effluent may have to be disposed of as hazardous waste. Carefully monitor the collection of effluent in the drain vessel and empty the drain vessel frequently.



Warning! It is necessary to follow appropriate waste segregation guidelines in order to prevent effluents from reacting in the drain vessel.



Warning! Dispose of waste in accordance with the regulations applicable to your locality, state/province, and/or country.

References

1. Furr, K., ed., *CRC Handbook of Laboratory Safety*, 3rd ed., The Chemical Rubber Co. Press, Florida, USA, 1990.
2. National Research Council, *Prudent Practices for Handling Hazardous Chemicals in Laboratories*, National Academy Press, Washington, D.C., USA, 1981.
3. Compressed Gas Association (USA), "Safe Handling of Compressed Gases in Containers," pamphlet no. P-1, 1984.
4. Compressed Gas Association (USA), "The Inert Gases - Argon, Nitrogen and Helium," pamphlet no. P-9, 1992.
5. Material Safety Data Sheets (MSDS), USA; DIN-Sicherheitsdatenblaetter (genormte Formular DIN-Nr 52900), FRG; Product Information Sheets, UK.
6. Other sources of information include: OSHA: Occupational Safety and Health Administration (United States) ACGIH: American Conference of Governmental Industrial Hygienists (United States) COSHH: Control of Substances Hazardous to Health (United Kingdom).
7. Helrich, K., ed., *Official Methods of Analysis*, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, VA, USA, 1990.
8. Bretherick, L., *Bretherick's Handbook of Reactive Chemical Hazards*, 4th ed., Butterworth & Co., Ltd., London, UK, 1990.
9. Sax, N., ed., *Dangerous Properties of Industrial Materials*, 7th ed., Van Nostrand Reinhold, New York, USA, 1989.
10. Bretherick, L., ed., *Hazards in the Chemical Laboratory*, 3rd ed., Royal Society of Chemistry, London, UK, 1981.
11. Wald P. H. and Stave G. M., eds. *Physical and Biological Hazards of the Workplace*, 2nd ed., Wiley, 2001.



DVS SCIENCES INC
Cell Analysis Products

CyTOF[®]

Mass Cytometer Guide

CELL ANALYSIS PRODUCTS

CyTOF[®] Mass Cytometer Guide

This is a Class A device and is for use in a commercial, industrial or business environment.

Warning: This is a Class A product. In a domestic environment this product may cause radio interference, in which case the user may be required to take adequate measures.

CyTOF and MAXPAR are registered trademarks of DVS Sciences Inc.
All products and company names mentioned herein may be the trademarks of their respective owners.

NOTE : this manual describes CyTOF[®] Model C5 instruments.

Revision 15, November 2012

© DVS Sciences Inc. 2012

70 Esna Park Drive, Unit 12, Markham, Ontario, L3R 6E7, Canada
telephone: 905-513-1704; fax 905-513-1704
www.dvssciences.com

Table of Contents

CHAPTER 1 CyTOF® MASS CYTOMETER OPERATION

Instrument Overview	1
Cell Introduction System	3
Description of the ICP Ion Source and the Time-of-flight Mass Analyzer	4
Preparing the Instrument for Plasma Start	8
Description of the Instrument Status Panel	8
Recommended Reagents and Running solutions	9
Activating the Heater	9
Preparing Syringe Pump and Flow Injection Valve	11
Connecting the Nebulizer	11
Starting Plasma and Detecting Ions	14
Plasma Start-up Sequence	14
PDA Board Calibration	17
Acquisition Parameters	18
Observing Real Time Snapshots of Detected Ions	20
Verifying Mass Calibration	21
Selecting Analytes	22
Detector Gain Optimization	27
Daily Performance Check	29
Measurements in Masses per Reading Mode	29
Pulse Counting, Intensity and Dual Modes of Data Processing	32

Calibrating detector for dual counts	33
Tuning instrument sensitivity	34
Auto-optimization of a parameter	35
Analyzing cells	38
Turning off the plasma	41

CHAPTER 2 CELL DATA ACQUISITION AND ANALYSIS

Acquisition Window	43
Input and output	43
Description	44
Action Controls	45
Acquisition Parameters	45
Analysis Parameters	47
Dual Count Calibration	50
Threshold Filtering	51
Processing Messages	52
FCS Conversion Window	53
Overview	53
Description	55
FCS Conversion Parameters	55
Action Controls	57
Analysis Window	58
Input and Output	58

Description	58
“Cell Analysis Parameters” Group	60
“Dual Count Calibration” Parameter Group	62
“Cell Analysis Data Source” Parameter Group	63
Files Parameter Group	64
IMD Files Conversion Window	65
FCS Fix Window	67

CHAPTER 3 MAINTENANCE

Spray Chamber	69
ICP Torch & Injector	71
Sampler Cone and Skimmer-reducer Assembly	75
Nebulizer	79
Rotary Pumps	83
Instrument Inputs and Outputs	84

CyTOF[®] Mass Cytometer Operation

Before attempting to operate the CyTOF[®] instrument, please read the CyTOF Safety Manual and this Guide carefully. Always observe all the recommendations provided in the CyTOF Safety Manual.

The DVS Sciences Inc. CyTOF[®] Mass Cytometer System is a self-contained instrument system which analyzes single cells via detection of elemental tags by atomic spectrometry. The instrument combines the efficient ion generation characteristics of the inductively coupled plasma (ICP) with the multi-element nature and excellent detection powers of mass spectrometry (MS).

The system consists of a cell introduction system, an ICP-based ion source, a time-of-flight mass spectrometer, an instrument control and data handling computer, and a coolant recirculator.

The cell introduction system aspirates cell-containing samples into a fine aerosol which is de-solvated at an elevated temperature before being transported to the ICP source. The sample flow is controlled by a syringe pump and the gas flows to the sample introduction system are controlled by mass flow controllers.

The inductively coupled plasma radio frequency (RF) power supply functions at a free-running frequency of approximately 40 megahertz (MHz). The maximum recommended operating power is 1300 watts (W). Free running RF circuitry ensures adequate delivery of RF power to the plasma during abrupt changes for example, when a single cell passes through it, providing complete vaporization, atomization and

ionization of elemental tags. The ICP operates at atmospheric pressure and temperature of ~ 5500 °K.

The time-of-flight mass spectrometer has a user-selectable mass range of at least 91 atomic mass units (amu) (typically set to 103 - 193 amu). The mass spectrometer and its detection circuitry handle positive ions. The time-of-flight mass analyzer is operated at 76,800 cycles per second spectrum generation rate which allows multiple point multi-element sampling of single cell-induced transient ion clouds. Fast data collection system operates at 1 GB/s peak data recording rate. The 1 Gs/s data digitizer system provides close to 5×10^4 dynamic range for detection of elemental tags in a single cell.

The power supplies used for the mass spectrometer operation are situated in a thermally controlled environment ensured by adequate air flows into and out of the instrument and by the coolant recirculator.

The mass analyzer operates at a pressure of $< 2 \times 10^{-6}$ Torr. An interface between the atmospheric pressure ICP ion source and the reduced pressure region of ion transfer optics is through a sampler orifice cone, skimmer orifice cone and a reducer orifice cone, all constructed of nickel. The interface and the RF generator designs minimize any electrical discharges that might form within the interface region. The turbomolecular high-capacity vacuum system achieves $< 5 \times 10^{-7}$ Torr base (stand-by) pressure or better within the time-of-flight analyser chamber. The system comprises two turbomolecular pumps and two rotary pumps.

Instrument control, data collection and processing are performed via CyTOF software which runs in a Windows XP environment.

Description of Cells Introduction System

The Cells Introduction System (Figure 1) consists of a Syringe Pump Module (SPM), a Heater Module (HM), a 6-port Flow Injection Valve (FIV), a Drain Vessel (DV), a Sample Loop and several liquid-transfer tubes. The Syringe Pump Module comprises the Syringe Pump (SP) and a Heater Control Unit (HCU) with digital LED display. It is connected to the Heater power outlet on the instrument cover, and is protected by a 1 A 250 VAC fuse.

WARNING: REPLACE THE SYRINGE PUMP MODULE FUSE ONLY WITH THE RECOMMENDED 1 A 250 VAC (SLOW BLOW) FUSE.

The syringe pump is controlled from the CyTOF[®] software. It typically operates at 0.06 mL/min flow rate. Carrier liquid is constantly run through the system, typically with use of a 3 mL syringe. In the LOAD position of the Flow Injection Valve lever, a sample can be loaded via the Sample Loading Port into the Sample Loop, which is typically done using 1 mL syringe. The sample syringe needs to be left in the Sample Loading Port during sample injection (INJECT position of FIV), in order to seal the tubing from the surrounding air. Recommended syringes are NormJect (Henke Sass Wolf), because of the absence of rubber wetted surfaces.

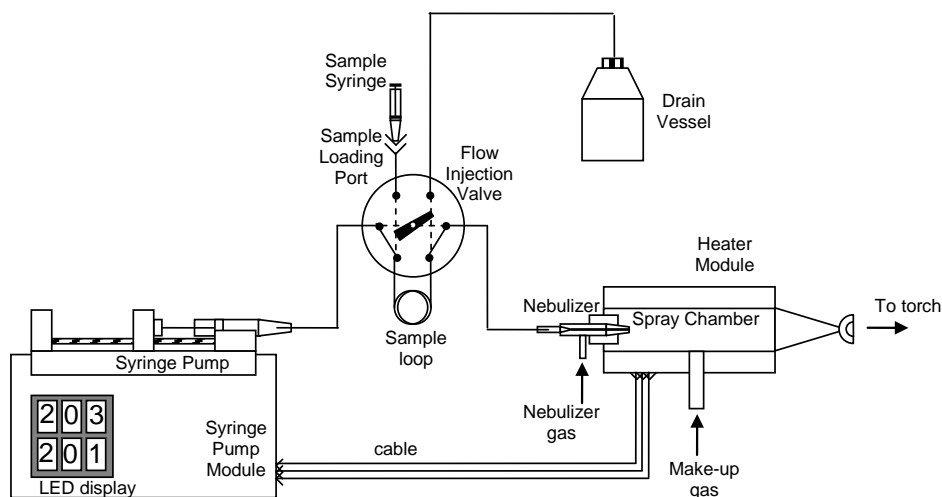


Figure 1. Schematic diagram of the Cells Introduction System

The Heater Module comprises a spray chamber which connects through 12 mm ball joint connector to the adapter of the ICP torch. The ball joint clamp secures coupling between the spray chamber and the adapter. The other end of the spray chamber has a Teflon adapter for holding a glass concentric nebulizer. The nebulizer is inserted only when the instrument is used for running samples. Prolonged exposure of the nebulizer to the high temperature inside the spray chamber without supplying nebulizer gas and without injecting a liquid can cause nebulizer clogging and should be avoided. The

nebulizer must be removed from the spray chamber immediately after turning the plasma off, so that it does not dry at elevated temperature while the spray chamber is cooling down. Keep the nebulizer submerged in deionised water for storage between operating sessions.

Description of the ICP Ion Source and the Time-of-flight Mass Analyzer

The schematic of the CyTOF[®] instrument is shown in Figure 2. Cells or other particles are introduced in the form of a liquid suspension by the syringe pump and then fine aerosol is produced by the nebulizer. The droplets are partially desolvated in the heated spray chamber, and the reduced in size cell- or particle-containing droplets are transferred by the gas flow into the plasma torch of the ICP ion source. The torch (Figure 3) consists of the torch body – a single-piece fused assembly of two concentric quartz “outer” and “inner” tubes, – and a demountable quartz injector tube, through which the sample aerosol is delivered into the ICP. Radio-frequency electromagnetic field of the induction coil placed around the torch accelerates electrons (initially produced by an ignition circuit) which transfer energy from the coil to the plasma in inelastic collisions with gas atoms. Typical gas kinetic and ionization temperatures of the analytical ICP are 5000 - 5500 K, and the ionization degree for argon is approximately 0.1 %. Elements with ionization energy smaller than 9 eV are practically completely ionized, which makes ICP an excellent ion source for multi-element and multi-isotope mass spectrometry analysis. The RF generator operates at ~ 40 MHz and is based on the free-running Collpitts oscillator with balanced load coil, which minimizes plasma offset potential and ensures low energy spread of ions.

The plasma is sampled through an interface which has 3 apertures: sampler (1.1 mm orifice diameter), skimmer (1 mm diameter) and reducer (1.2 mm diameter). The sampler – skimmer region is pumped by a 40 m³/h rotary pump SV40-BI and the skimmer-reducer region by the Holweck stage of a 3-inlet turbo-molecular pump TW400/300/25. The intermediate stage of the 3-inlet pump evacuates the portion of the vacuum chamber containing the ion deflection optics, and the high vacuum stage pumps the region containing the ion beam shaping optics. The TOF section is evacuated with a separate turbo-molecular pump TW250S. Typical pressures in the five stages of the vacuum system with the ICP operating under standard conditions are P1=2.3-2.5 Torr, P2 = 20 – 40 mTorr, P3= 300 – 500 μTorr, P4=2 – 5 μTorr and P5=0.3 – 1.5 μTorr, respectively.

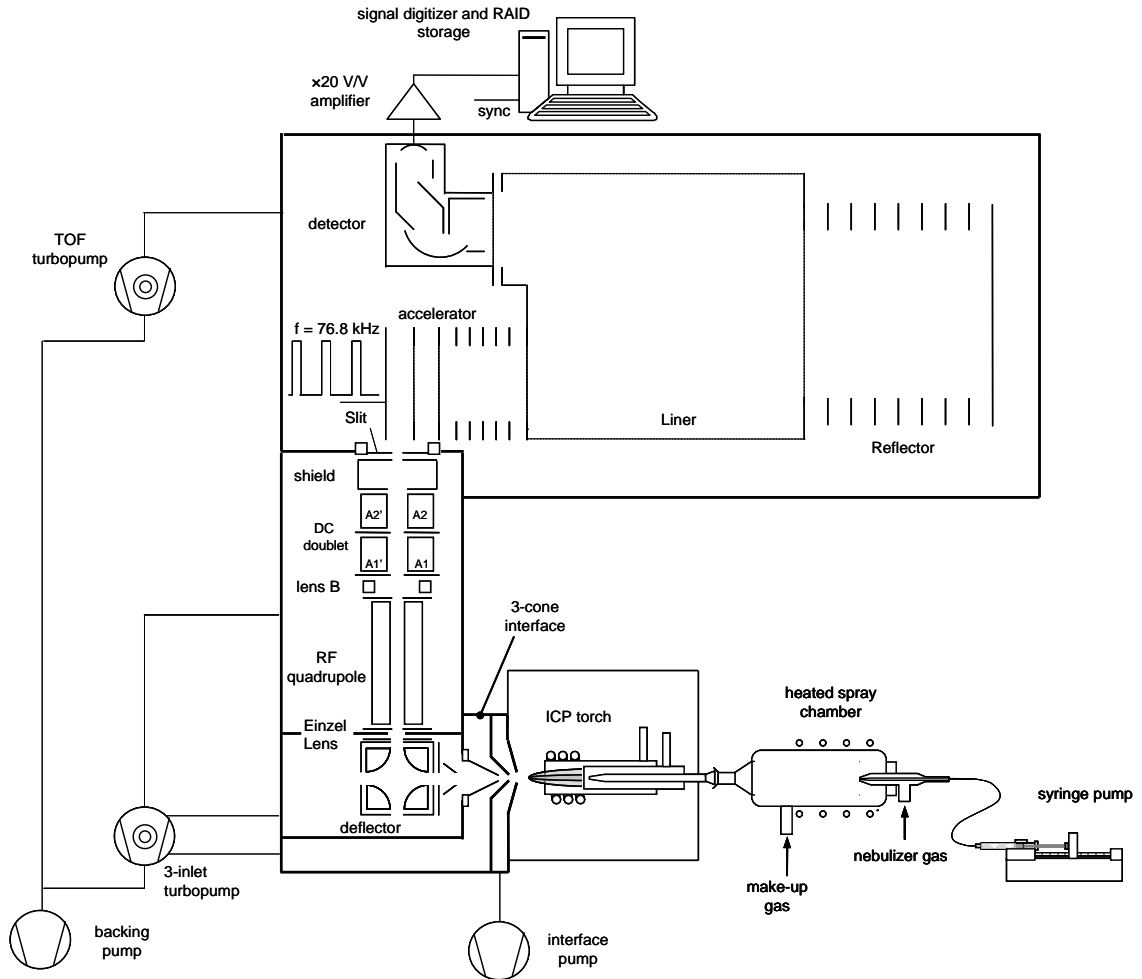


Figure 2. Schematics of the CyTOF[®] Mass Cytometer

The core of the supersonic plasma jet propagates through the reducer orifice, ions are accelerated and focused by an electrostatic field defined by the potentials of the reducer and a conical lens and then are deflected by the electrostatic quadrupole deflector, while un-ionized particles from the jet exit the deflector on a straight path to the turbo molecular pump.

An Einzel lens downstream of the deflector, consisting of the deflector exit aperture, ion guide entrance aperture, and an aperture between them, focuses the deflected ions into the RF-only quadrupole ion guide. The ion guide operates in the mode which allows only ions above certain mass-to-charge ratio (m/z) to be transmitted. The low mass cut-off of the quadrupole ion guide is set close to $m/z=80$, so that the low mass dominant ions (H^+ , C^+ , O^+ , N^+ , OH^+ , CO^+ , O_2^+ , Ar^+ , ArH^+ , ArO^+) are not transmitted.

The ions of $m/z>100$ are readily transmitted by the ion guide into the electrostatic quadrupole doublet.

ICP TORCH

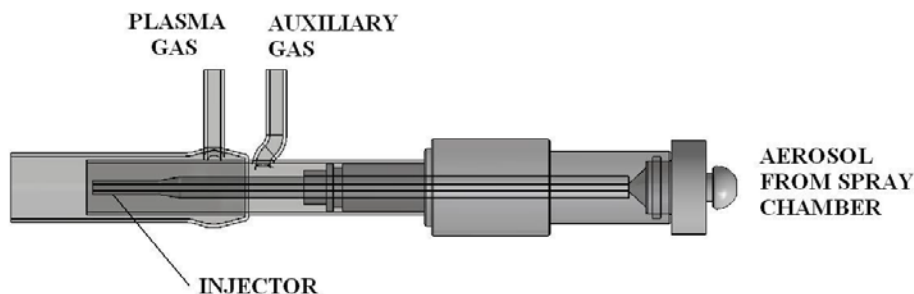


Figure 3. Plasma torch

The doublet shapes the ion beam exiting the round exit aperture of the ion guide into a beam of cross-section compatible with the rectangular entrance slit (3 mm x 12 mm) of the orthogonal-acceleration reflectron time-of-flight analyzer. The time-of-flight analyzer is a single stage mass-reflectron with two-stage orthogonal acceleration.

The ion beam shaped in the doublet enters the orthogonal accelerator gap formed by the push-out plate and the first grid, both kept at -120 V potential. A push-out pulse of several hundred volts is applied to the push-out plate, forcing all ions to leave the gap and to be accelerated in the second and third acceleration regions of the accelerator. The accelerated ions then travel through the field-free region into the ion mirror, wherein they are reflected toward the detector. The fields in the accelerator and the mirror and the length of the field-free regions are set in such a way that the ions of the same m/z but different initial position or energy arrive at the detector within a relatively narrow time interval distinct from that of the ions of other m/z . The relation between time of ion flight to the detector and their m/z is:

$$t = t_0 + A\sqrt{\frac{m}{z}}, \quad (1)$$

wherein constants t_0 and A are found from a mass calibration procedure based on the measured times of flight for two analytes of known m/z present in the calibration solution, typically in the beginning and at the end of the mass range of interest (e.g. ^{105}Rh at $m/z = 102.905$ and ^{193}Ir at $m/z = 192.963$).

After the ions are pushed-out of the first gap, the voltages on the push-out plate and the grid are restored to -120 V, so that the ion beam can re-fill the gap again. The total duration of the cycle is 13 μ s, and the push-outs are repeated at 76.8 kHz spectra generation frequency.

A fast discrete dynode TOF ion detector is used for ion detection. The output signal of the detector is amplified by a pre-amplifier and is transmitted through a coaxial cable to the data station, wherein the signal digitizer digitizes the signal at 1 ns sampling interval with 8 bit intensity resolution. Another coaxial cable carries the trigger pulse, which synchronizes the digitizer scan with the push-out pulses.

A trigger delay and the recording segment length of the digitizer are set to allow digitization of the segment of the signal that corresponds to $m/z = 103 - 193$. The data stream and the instrument parameters are controlled by the CyTOF software.

Two distinct modes of instrument control are realized: instrument tuning mode and cell data collection mode.

Instrument tuning is done with on-line integration of user-selected analytical mass channels, and for which the results (usually integrated ion counts per 1 s reading) are displayed in real-time and can be saved as text files. The software routines allow automated optimization of the make-up and the nebulizer gas flows, all ion optics electric potentials and detector voltages. In the tuning mode, each 1 s integration measurement requires extra ~ 0.5 s for two-way data transfer, data processing, storage and display.

The cell data collection mode is done in real time, with no data loss. Data for each push-out cycle are sequentially digitized by the digitizer, transferred at approximately 250 MB/s and in parallel compressed by integration of mass peaks for the analytes selected by the user. The resulting record of sequential single push-out spectra integrated for each mass is processed according to cell-event selection criteria. For example, the appearance of two isotopes of Ir, which is part of an iridium-DNA-intercalator, above a pre-selected threshold for a pre-selected number of sequential single push-out spectra indicates the presence of a cell-induced ion cloud and can be selected by the processing algorithm for further processing. The end result of the cell data acquisition is the record which contains integrated number, per each cell event, of total ion counts for each of the selected analytes. These results are recorded in a text file and FCS 3.0 file for each sample analysis.

Since the transmission of the instrument for each analyte is known from measurements of the known concentration calibration solution, the total per cell counts for each analyte can be converted into the total number of atoms present in each cell-induced cloud. The number of metal label atoms conjugated to each antibody is known from independent calibration assays of the MAXPAR[®] reagents. Thus, the number of antibodies bound to each cell can be quantified.

Preparing the Instrument for Plasma Start

WARNING: Wearing powder-free gloves is required when working with samples on the Cells Introduction System. This protects the operator and prevents contamination of the samples by the elements abundant in the environment.

Description of the Instrument Status Panel

The Instrument Status Panel on the front of the instrument cover (see Figure 4) shows the status of the interlocks and the instrument systems, namely

- Backing Pump (BP);
- Turbomolecular pumps (TP1 and TP2);
- Vacuum levels (VG1 and VG2);
- Gate Valve (GATE);
- Interface (Temperature and Cooling);
- Torch Box (Torch, Temperature);
- Argon pressure (ARGON);
- Instrument Exhaust (EXHAUST);
- RF generator mode (RFG, TEST).

The following Light Emitting Diodes (LEDs) on the Status Panel (Figure 4) should be lit when the instrument is in Standby Mode : VG1, VG2, TP1, TP2, BP, CLOSE, INTRF, TORCH, ARGON, EXHAUST.

Note: Do not attempt to start the plasma if the above mentioned LEDs are not on, or if any other LED is lit. Record the LEDs status and contact DVS Sciences Inc. Technical Support to report the problem.

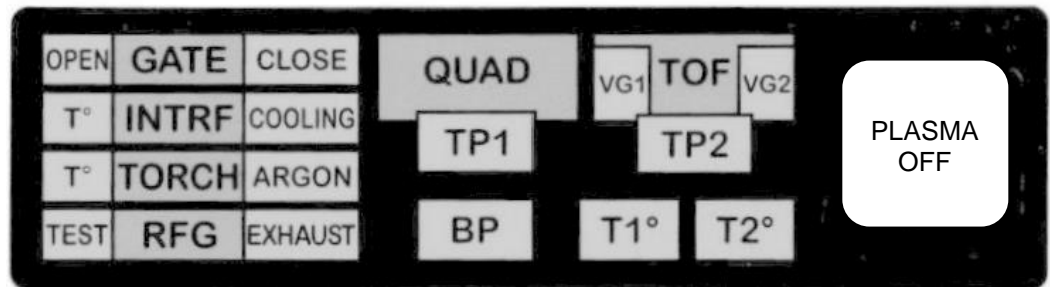


Figure 4. CyTOF[®] Instrument Status Panel in Standby Mode

After initial instrument power up, if power to the System was disconnected, TEST LED may become lit. This does not indicate a fault. Press and release the RFG Test button on the side of the instrument to turn the LED off.

The recirculator (chiller) is activated by the instrument control software during execution of the Plasma Start Sequence. When the recirculator is running, the

COOLING LED is turned ON. When Plasma is On, the Gate Valve is open and the GATE OPEN LED remains lit.

Recommended Reagents and Running Solutions

The highest analytical grade reagents must be used in sample preparation, in order to ensure lowest contamination of samples. The CyTOF[®] instrument is a very sensitive elemental detector, with the capability to detect sub-particle-per-trillion ultra-trace impurities. Contamination of samples with, for example, barium (Ba) or lead (Pb) can jeopardize instrument response during analysis and also shorten the ion detector lifetime. DVS Sciences supplies the following ultra pure solutions needed for operating the instrument and sample preparation:

CyTOF[®] Washing Solution, E-Pure (Part # 201071)

CyTOF[®] Tuning Solution, E-Pure (Part # 201072)

CyTOF[®] Calibration Beads, E-Pure (Part # 201073)

Deionised water (DIW) of highest grade. For example, gradient grade, Milli-Q, (Millipore) must be used in sample preparation.

Note: Gravimetric dilutions of the stock solutions, without use of dispensers or pipettes, is the best way to avoid elemental contamination.

Please refer to the Material Safety Data Sheets (MSDS) on the DVS Sciences website (<http://www.dvssciences.com/msds.php>) for storage and handling instructions regarding the aforementioned reagents and solutions.

Activating the Heater



Instrument Setup -> Card Cage -> Heater ON

When the heater is turned ON, the display on the Syringe Pump Module shows the current temperature setting and the actual temperature of the Heater Module. The default set-up of the heater temperature is 200 °C and should appear in the top part of the display. The lower part of

the display shows actual temperature and may blink during ramp-up of the instrument. The system is ready for plasma start when the lower display shows temperature within 10 degrees of 200 °C (typically within 30 min. after turning the heater on). Attempts to aspirate solutions into the plasma prior to reaching operating temperature of the heater box will result in plasma overloading with large droplets and can interfere with reliable instrument operation.

Preparing Syringe Pump and Flow Injection Valve

Move the lever of the Flow Injection Valve into LOAD position. Prepare Sample Carrier Solution in a 3 mL syringe, making sure there is no air in the syringe (tap and move the plunger several times while holding the syringe facing upwards to remove air). Install the syringe into the syringe pump, adjust the pump so that the liquid meniscus is formed on the syringe output, and connect the female luer fitting of the delivery tube to the syringe.

WARNING: When changing carrier syringes during operation, never leave the delivery tube open to air for prolonged periods. The nebulizer develops negative pressure at the tip during aspiration, and large amounts of liquid stored in the delivery tube can be abruptly transferred to the plasma, which may overload it. Always have the replacement syringe ready with liquid first, remove the old syringe from the pump with the tubing attached to it, secure the new syringe in the pump and only then quickly move the luer connection from the old syringe to the new one.

Samples are usually introduced into the Sample Loading Port using a 1 mL syringe. The supplied sample loop volume is 0.45 mL. Prepare the sample syringe, make sure there are no air bubbles in it, insert it into the Sample Loading Port of the Flow Injection Valve, load the sample into the Sample Loop and move the valve lever to the Inject position.

Note: It takes about 40 s for the sample to reach the plasma, and then about 40 s more for the aspiration rate to stabilize.

Connecting the Nebulizer

WARNING: The nebulizer is fragile and will be damaged if dropped. Its tip can be easily contaminated, and its capillary clogged. Do not touch the tip of the nebulizer. Always wear gloves when handling the nebulizer. Store the nebulizer in deionized water between operating sessions.

Connecting the nebulizer gas line using the High Pressure Gas Fitting

The High Pressure Fitting is a Nylon Swagelok 1/4" to 3/16" reducing union. The 1/4" end connects to the Nebulizer Gas line. The other end holds the nebulizer sidearm. A small o-ring is used instead of a back ferrule in the 3/16" end of the union. The high pressure fitting is more than adequate for the gas pressure used in the instrument (< 100 psi).

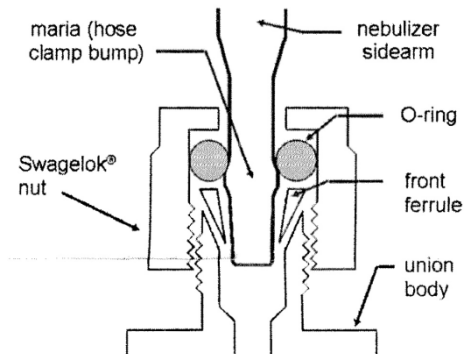


Figure 5. High Pressure reducing union connection to the nebulizer sidearm

To connect the nebulizer, remove the 5/16" Swagelok nut from the union and insert the nebulizer sidearm through it. Then push O-ring over the maria on the sidearm (see Figure 5). A plastic nebulizer tip protecting cup (supplied with the nebulizer) can be used to push the O-ring. Next put the front conical ferrule on the sidearm with the base facing the O-ring and the cone pointed toward the union body. Screw the union body and nut together firmly.

The other, 1/4" end of the union, is secured to the nebulizer gas line and should not be disconnected during removal of the nebulizer.

If there is residual water inside the annular gas channel of the nebulizer, dry it with argon flow by turning on the Nebulizer Gas Flow at 0.3 (L/min) using the instrument software

Instrument Setup -> DAC Channels Setup ->Nebulizer Gas ->
->Actual Current Value = 0.3 ->Set Actual Current Value

Connecting sample capillary tube to the nebulizer

WARNING: Do not install sample capillary connector kit while nebulizer is installed in the spray chamber.

Unscrew the two flangeless nuts on the connector/adaptor approximately one-half turn or until they are loose (Figure 6). They do not need to be removed.

Insert the liquid input end of the nebulizer into the 1/8" short nut until it rests against the web of the connector/adaptor. Tighten the nut until the nebulizer's liquid input end is held in place. The nebulizer should be able to withstand gentle tugging and the nebulizer should remain against the connector/adaptor's web.

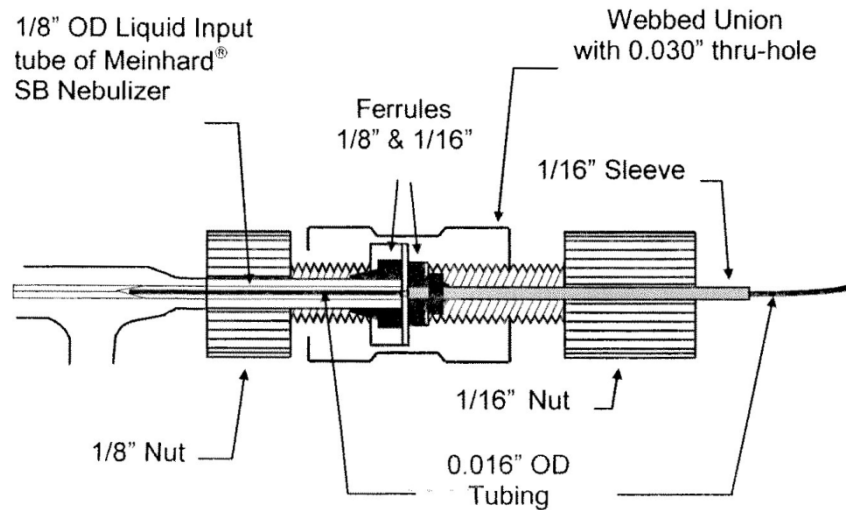


Figure 6. Sample Capillary Assembly connection to the liquid input of the nebulizer

WARNING: Do not over tighten the nut.

Insert the end of the 0.016" OD Teflon Tubing of the Sample Capillary assembly into the nebulizer liquid input end. Adjust the length of the 0.016" tubing so it stops against the tapered portion of the glass capillary inside the nebulizer. While applying gentle pressure to the tubing to maintain the contact with the glass capillary, tighten the 1/16 " nut so that the 1/16" sleeve holds the 0.016" OD tubing securely.

Insert the other end of the Sample Capillary Assembly into the union on the Flow Injection Valve.

Insert the nebulizer into the Teflon adapter of the spray chamber, aligning the nebulizer so that the tip is prevented from touching the adapter internal channel and O-rings inside the adapter. The sidearm of the nebulizer should touch the adapter.

Starting Plasma and Detecting Ions

Plasma Start-up Sequence

The lifetime of the detector depends on its exposure to ion currents. Between data acquisitions, the ion beam is prevented from reaching the detector.

WARNING: Never switch syringes, samples, or do any adjustments to the sample introduction system while acquisition is activated. The spikes of ion current during the changes and adjustments will reach the detector and can shorten its lifetime.

The ion beam is deflected from the detector due to Turn B voltage set to -200 V between acquisitions.

WARNING: Do not override it by changing the current Turn B value (typically -50 V).

During acquisition, detector protection from high ion currents of dominant plasma species is achieved by low-mass cut-off operation of the quadrupole ion guide.

WARNING: DO NOT SET THE RF VOLTS ABSOLUTE VALUE TO BELOW 90 V. Typical operational value of RF Volts is -130 V. Setting it to above -90 V will allow dominant plasma ions to reach and damage the detector.

Note: The recommended plasma power is 1300 W.

The Syringe Pump should be in Idle Mode, not supplying any liquid, during the plasma start-up. Syringe pump controls (Pause, Run, Stop) can be found in the top right corner of the CyTOF[®] software window. Settings of the syringe Diameter, total injection Volume and injection Speed are shown, as well as the Sampling Progress SP (Injected / Total volumes) (see below).



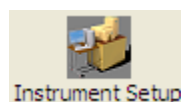
Default carrier syringe settings are as shown above: Diameter 9.3 mm, injection Volume 3 mL, injection Speed 0.060 mL/min. The syringe settings can be changed by opening the Syringe Pump window



WARNING: Setting the injection speed above 0.060 mL/min, either directly or by setting syringe diameter to a wrong value can result in plasma overload and shutdown.

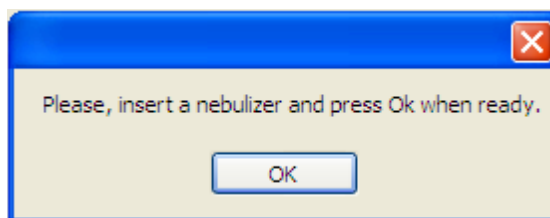
The recommended syringes are NormJect[®] (Henke Sass Wolf) 1 mL and 3 mL (nominal 2 mL) with 4.7 mm and 9.3 mm diameters, respectively. The default sample injection parameters for the recommended syringes are saved in the Syringe Pump settings.

The plasma startup sequence is executed by CyTOF[®] software by pressing the “Start Plasma” button.



Instrument Setup -> RFG Controller->Start Plasma

A reminder about the necessity to insert the nebulizer into the spray chamber will be issued.



Make sure that the nebulizer is inserted, and that the temperature is within 10 degrees of the set heater temperature (typically 200 °C), then press OK.

The plasma startup sequence takes approximately 4 min.

During the plasma start sequence the Log part of the RFG window (see Figure 7) will reflect the sequence steps, as will the status of RFG change.

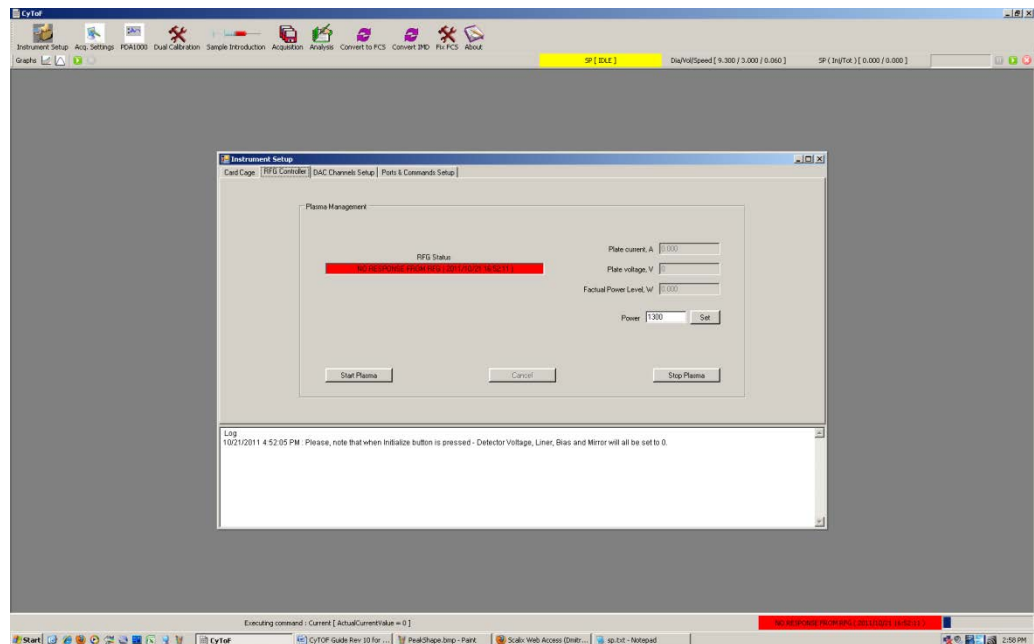


Figure 7. RFG Controller pane before first plasma start of the CyTOF[®] acquisition session

The following actions can be observed during a normal plasma start:

Re-circulating chiller activation and coolant flow starting (Cooling LED on the instrument front panel will become lit, refer to Fig. 4) ;

Change of RFG status from “No Response from RFG” to “Idle”, then “Warm Up”, then “Ready”, then “Ignite” and then “Plasma” modes ;

When the RFG is in “Ready” mode, the Interface rotary pump is started (additional sound of working interface pump will be heard);

When RFG is in “Ignite” Mode, plasma ignition, accompanied initially by slight “hissing” sound of the ignitor discharge, will produce a characteristic “popping” sound of quickly heated and expanding plasma gases forming audible shock waves in the torch.

When the RFG status changes to “Plasma”, values of the Plate Voltage, Plate Current and the Factual Power Level values will appear.

The Gate valve of the instrument will open and the “Open” LED will turn on in the instrument panel (see Fig.4).

The plasma start finishes with initialization of all of the instrument voltages and with ramping up of the Detector Voltages.

“Plasma Start Finished Successfully” message will appear.

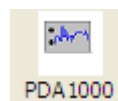
Once the plasma is successfully started, sample injection can be started by starting the Syringe Pump.

WARNING: Correct syringe pump and flow rate/ injection volume need to be selected from Syringe Pump menu. Maximum allowed injection flow rate is 0.06 mL/min.

Calibration of the PDA Board

All panels in the CyTOF software must be closed before the PDA Board calibration is attempted.

Click the PDA1000 button



The following panel opens, showing the parameters of the board.

PDA1000 Calibration

Trigger Mode: Segmented

Trigger Source: External

Trigger Level: 3000

Negative Slope Trigger

Trigger Delay: 9000

Segment Size: 3072

Sampling Frequency: 1000.000 MHz

Master Board

Master Board #: 1

Master Volt. Range: 1 V

Slave Board

Slave Board #: 0

Slave Volt. Range: 3 V

Apply to board(s) and Save

Figure 8. PDA1000 signal digitizer set up and calibration panel

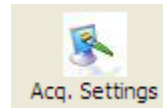
The parameters Board #, Frequency, Trigger Mode, Trigger Source, Trigger Level, Segment Size and Voltage Level are service only and are not accessible.

Trigger Delay defines the analytical mass range start of the instrument. After a change, please press “Apply to board(s) and Save” button.

The default settings are instrument dependent and set to cover the range $m/z = 103-193$. If operation with a different mass range is required, the Trigger Delay can be changed.

Acquisition Parameters

To open the “Acquisition Settings” window, please press “Acq. Settings” button in the top menu bar



->Data Acquisition Settings ->Parameter

Parameter

Parameter Min Value

Parameter Max Value

Parameter ▼

Start Value

End Value

Step Value

Settling Time(ms)

Pushes / Reading

Dual Count Start Point (in pulses per push)

Figure 9. Data acquisition Parameter set up window (tuning mode)

Parameters shown in this pane include:

Parameter - a user selectable parameter which will be ramped between “Start Value” and “End Value” with “Step Value” increments and “Settling Time” which represents a pause.

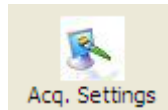
Pushes/Reading is the number of single-push-out spectra which will be integrated in a single reading. The default value is 76,800, corresponding to the number of single spectra generated in 1 s.

”Dual Count Start Point” is the number of ion counts per push starting from which the processing of the Tuning mode switches from counting to analog data, if Dual Counts are selected. The details of the modes of data processing are discussed on Chapter 2.

Observing Real Time Snapshots of Detected Ions

To quickly verify that all modules of the ion signal detection and processing are operational, snapshots of detected ions can be observed in real time, without summation of the readings.

Argon gas usually contains xenon as an impurity, which is ionized in the plasma. Even when ultrapure water is aspirated, or no sample is aspirated at all, ion detection events can be observed in the Mass Calibration window.



The vertical scale shows the push (single mass spectrum) number, and the horizontal scale represents the time-of-flight. Only the first 500 spectra of each reading are displayed. Black pixels indicate the presence of a signal of the level between PDA Max and PDA Min ($=0$), while white dots indicate absence of any signal.

When the tuning solution is aspirated, ions of the elements Tb and Tm present in the solution will appear in every single push spectrum, thus forming vertical lines in the snapshot window (as shown below). Ions of Cs and La will appear in approximately every second spectrum. The ions of Ir (which is at half the concentration and has two isotopes) appear in two vertical semi-populated lines. The width of the vertical lines indicates the full width of the mass peaks at the base, which depends on the selected Resolution Mode.

When transient events (e.g. ion clouds induced by cells), are detected, the vertical streaks of ions are not continuous, but last for 10-40 pushes, corresponding to 130 - 520 μ s duration of the cell-induced transients.

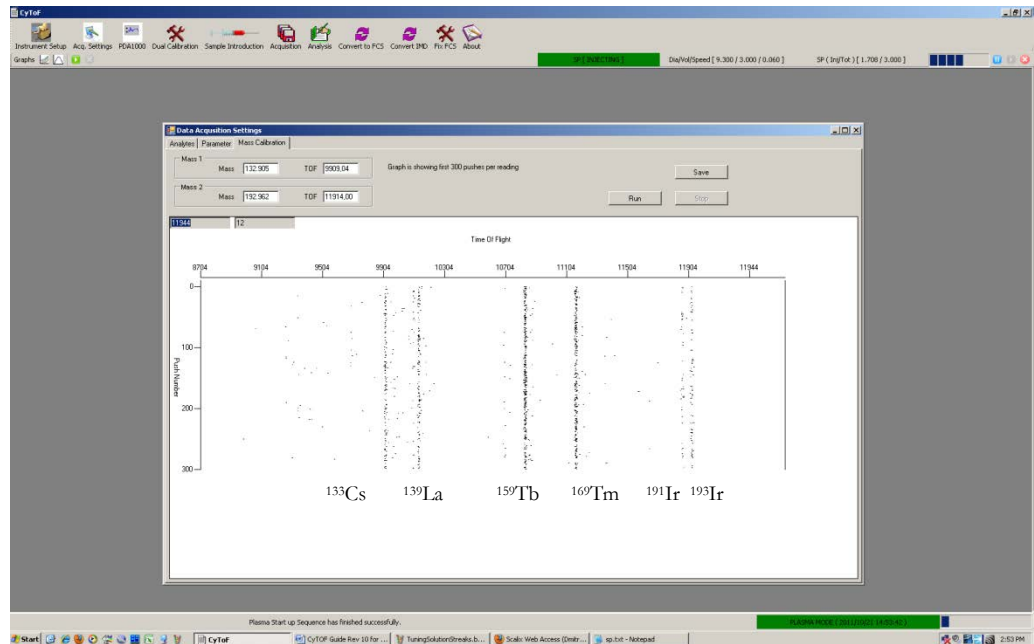


Figure 10. Typical snap-shot of ion signals in Data Acquisition Mass Calibration window for tuning solution aspirated at 0.06 mL/min rate

Verifying and Adjusting Mass Calibration

Mass calibration of the CyTOF[®] instrument is stable, and if none of the instrument voltages in Instrument Setup/DAC Channels Setup was changed deliberately, Mass Calibration does not need adjustment.

To verify Mass Calibration, Tuning Solution needs to be injected. Note it takes ~ 80 s for the sample aspiration to stabilize after a sample change.

Set the acquisition parameters as follows:



Start value = 0

End value = 100

Step value = 1

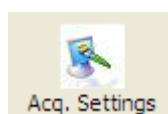
Settling time = 0

Pushes per reading 76800

Such settings mean that sequentially, 101 readings will be taken without settling time between the readings. Each reading will be a sum of 76,800 individual single TOF cycle (single push-out) spectra. The sum is taken separately for each digitizer time bin of 1 ns duration and can be presented as integral mass spectrum, or as a quantified (in counts or analog intensity) integral response for each selected analyte.

For verifying mass calibration, analytes ^{133}Cs and ^{193}Ir must be selected. This is done via pop-up periodic table.

Selecting analytes



Acquisition settings -> Analytes->Periodic Table

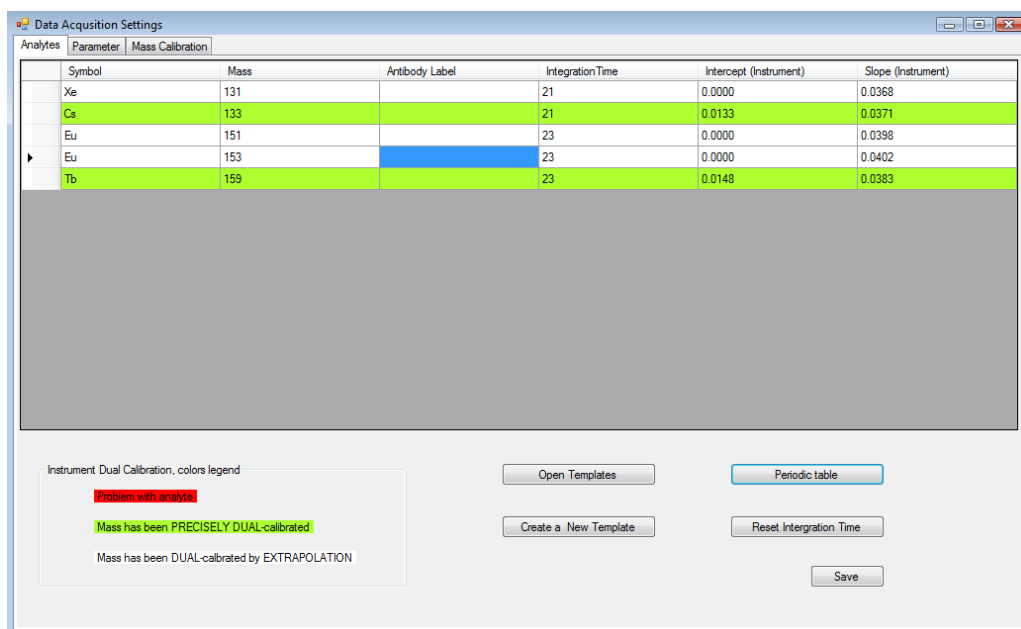


Figure 11.1 Selection of Analytes in Acquisition Settings Analytes window

Click on the “Periodic table” button. The Periodic Table Window will open (see Figure 11.2).

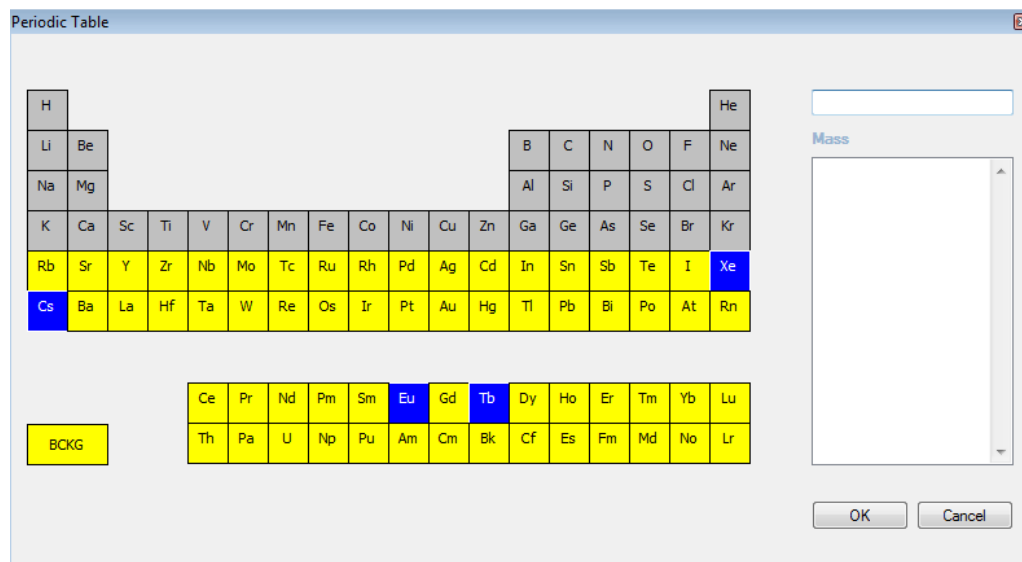


Figure 11.2 Periodic Table window

Click on the element on the Periodic Table and then click on the isotope of interest in the pop-up list of isotopes. The selected analytes appear in the analytes table. Click the “Save” button to save the analytes table.

You can also use templates when populating the analytes table with a preset list of analytes.

To create a template from the current set of analytes in the analytes table, “Create a New template” button. The “Acquisition Templates” window will open (Figure 11.3). To open the “Acquisition Templates” window without creating a new template, press the “Open templates” button.

In the “Acquisition Templates” you can manage and select templates to be loaded in the analytes table. To select a template and load it in the analytes tables, double click on a chosen template or navigate to a template and press the “Select Template” button.

To open a menu with auxiliary features – navigate to a template and press right mouse button, a menu will appear providing the following items: “Add”, “Add a copy”, “Delete”, “Delete All” and “Select Template”.

To save templates changes – press the “Save” button.

Features “Export” and “Import” allow exporting and importing of templates for exchange and backup purposes.

To export – Select templates to be exported and press the “Export” button. A standard file dialog pops up, enter a new file name and press “Ok”. If no templates are selected, all templates are exported.

To import – Press the “Import” button, a standard file dialog appears, select a file to import and press “OK”.

If during import a “same name” existing template is discovered - the import procedure will skip the duplicate template from an import file and will preserve the existing template from being replaced.

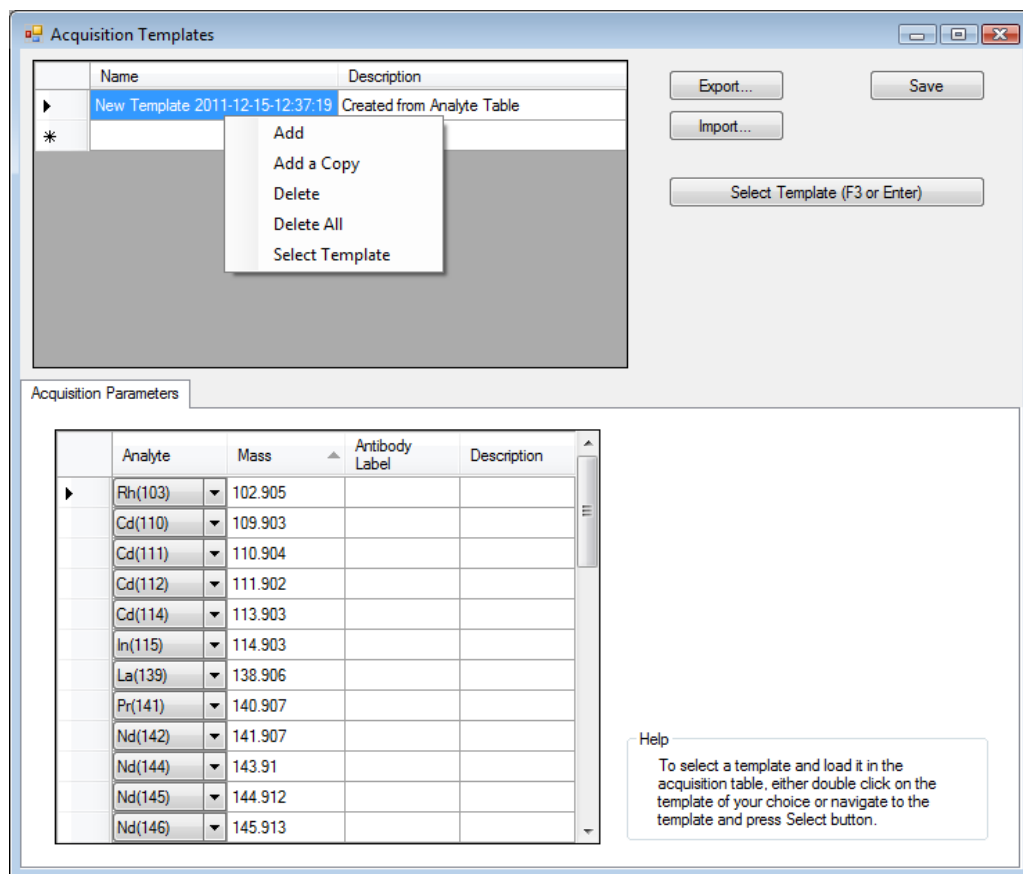
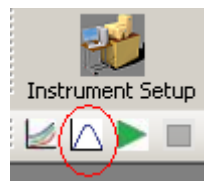


Figure 11.3 Acquisition Templates window

To observe mass peak shapes simultaneously for ^{133}Cs and ^{193}Ir , open the TOF Range per Reading pane twice:



->TOF Range Per Reading

Two panels will open. Drag them apart to allow simultaneous viewing.

Select Cs in Mass in one of the panels, and Ir in the other panel.

Start acquisition by pressing the green “Run” button.

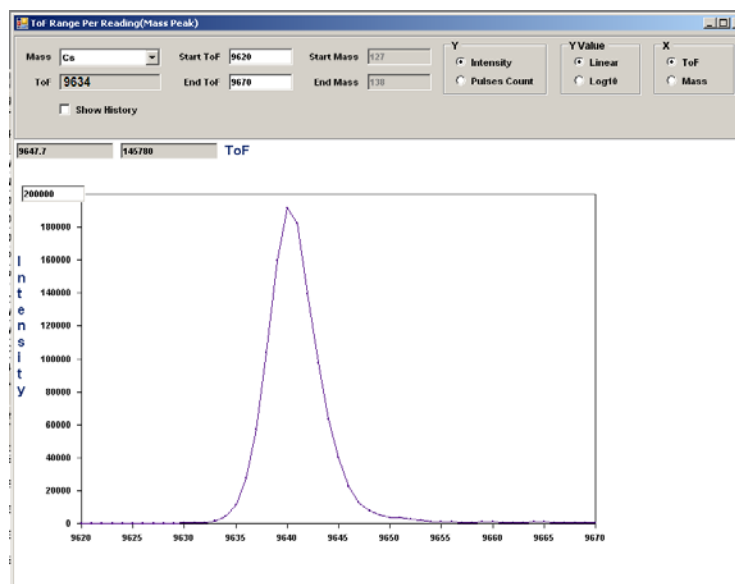


Figure 12. Typical shape of $^{133}\text{Cs}^+$ mass peak measured at 76,800 spectra per reading (1 s per reading) for tuning solution aspirated at 0.06 mL/min

Mass peaks will appear in both panels. Verify that the ToF value (shown under the analyte symbol) corresponds to the beginning of the mass peak, at <3 % of its height (this value 9634 in the example given above). Similarly, verify the ToF value for the beginning (< 3% of peak height) of the ^{193}Ir peak.

Note also at what ToF the tail end of the peak is crossing 3 % of the peak height, to calculate peak width at 3 % peak height. Typically, integration of the peak area is done with the default width of 20 points (20 ns).

If both Cs and ^{193}Ir ToF values correspond to the 1-3 % of the peak height, mass calibration does not need to be changed.

If not, record the new ToF values of the position of the leading edges of the Cs and ^{193}Ir peaks at ~ 3 % of peak height and enter the new values in the Mass Calibration panel.

->Acquisition Settings ->Mass Calibration

Make sure that the Mass 1 and the Mass 2 specified masses correspond to the examined analytes (132.905 for ^{133}Cs and 192.962 for ^{193}Ir in this example), and enter the new values of ToF into the TOF fields.

Note the format of TOF entry: Integer (TOF), 1.

Save the new Mass Calibration by clicking the “Save” button.

Starting TOFs for all other analyte peaks will be calculated by the software according to the $t(m/z)$ relation

$t = t_0 + A\sqrt{\frac{m}{z}}$, wherein t_0 and A are calculated from the masses and TOFs of the two reference peaks (^{133}Cs and ^{193}Ir).

Verifying mass resolution of the CyTOF[®] instrument is performed by measuring the full width of ^{159}Tb mass peak at half of its maximum. Open the TOF Range Per Reading pane for Tb and point the cursor at the points on the leading and trailing edges of the peak which correspond to the half of the peak maximum (the coordinates of the cursor are shown in grey boxes right above the plot). Mass resolution is calculated as

$$R = M/\Delta M = t/2\Delta t,$$

where t is the TOF which corresponds to the peak maximum, and Δt is peak width at half maximum.

Detector Gain Optimization

The detector is typically operated at an average gain of $\sim 3 \times 10^5$ electron/ion. At such gain, the detector outputs a pulse of approximately 1 mV and 2.5 ns width (full width at a half maximum) into a 50 Ohm load. The preamplifier amplifies it further to ensure that each single ion pulse is easily distinguishable from the background. At an adequately set detector gain and ion detection threshold, more than 85 % of single ions are detected by pseudo-counting algorithm. Since the detector gain depends on the voltage applied to the detector much more than the background signal does, optimization of the detector gain is done at a fixed ion detection threshold (PDA Max).

The voltage applied to the detector is the difference between the Detector Bias (typically - 5000 V) and the Detector Voltage.

In order to optimize detector gain, tuning solution needs to be aspirated.

Select ^{191}Ir as the analyte (see the section on Selecting Analytes in Chapter 1). In order to lower the probability of reflected waves being counted as ions, set Integration Time to 6. Save the new analytes setting.

Set the acquisition parameters as follows:



Start value = -2500

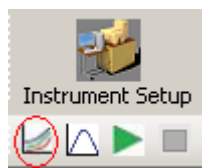
End value = -1900

Step value = 50

Settling time = 1000

Pushes per reading = 76800

Set the measurement mode to Masses per Reading:



Select "Mass" -> ^{191}Ir , select Y axis to show Pulses Count, in Linear scale

Pressing the “Run” button (green arrow) will ramp Detector Voltage from -2500 V to -1900 V, effectively ramping the voltage applied to the detector from 2500 to 3100 V. A typical graph showing signal of ^{191}Ir in pulse counting mode as a function of Detector Voltage is shown below.

When the number of ions counted changes by less than 15 % per step (per 50 V), the Detector Voltage is at an optimum value (highlighted by the square in the Figure 13).

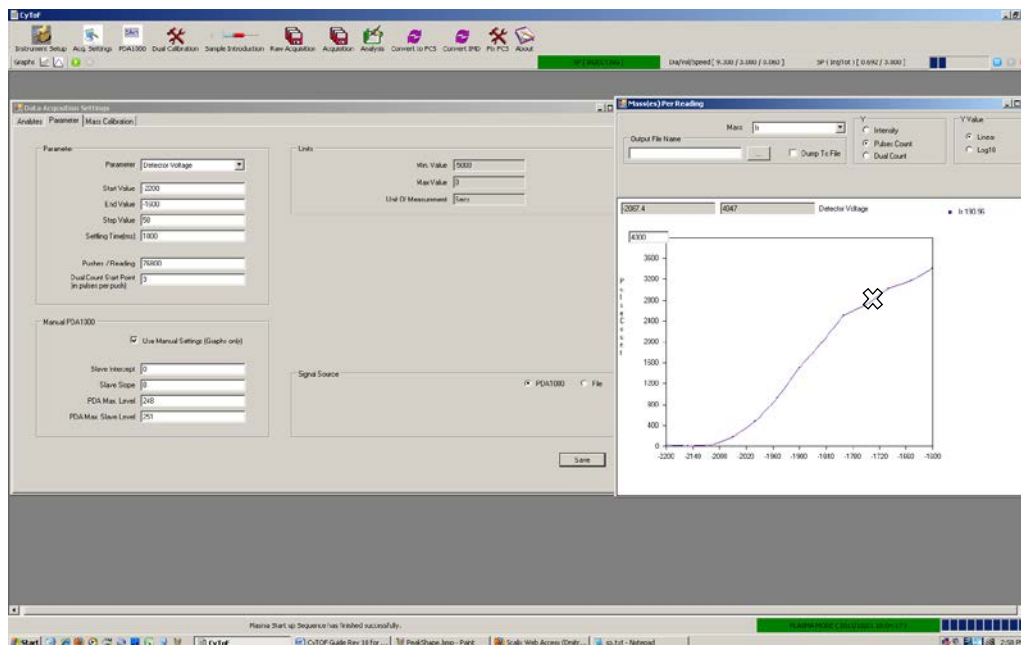


Figure 13. Typical Detector Optimization Curve

Note: The Detector Voltage optimization is done using an analyte of a relatively high m/z . For ions of the same energy, secondary electron emission efficiency decreases with m/z . In order to ensure efficient detection of ions for which the detector gain is the lowest, the detector is optimized for the heaviest ions of interest. Operating the detector at a voltage found in the prescribed procedure ensures efficient detection of ions of all m/z of interest, and provides sufficient dynamic range for multi-ion events. A check of adequacy of the detector voltage can be done by comparing the maximum intensity of the ^{159}Tb mass peak to the counting mode signal in counts per second. Typically, the analog peak intensity measured in the “TOF Range Per Reading” mode is 3 – 5 times higher than the counts per second (cps), e.g. for 100,000 cps in Mass Per Reading mode, peak height intensity of 300,000 -500,000 indicates the normal range of detector gain.

Daily Performance Check

In the analytes table () select ^{131}Xe , ^{133}Cs , ^{139}La , ^{155}Gd , ^{159}Tb , ^{169}Tm , ^{191}Ir and ^{193}Ir . Default integration time for ^{131}Xe is 21 ns. Set its integration Time in accordance with the observed mass-peak width during the Mass Calibration check (see the section on Selecting Analytes in Chapter 1). Reset Integration Time button will scale integration windows according to their m/z.

For the Daily Performance Check, it is advisable to inject the tuning solution directly from a 3 mL syringe run by the syringe pump. The Sample Loop can hold only 450 microliters of sample, which may not last long enough for reliable tuning and performance check.

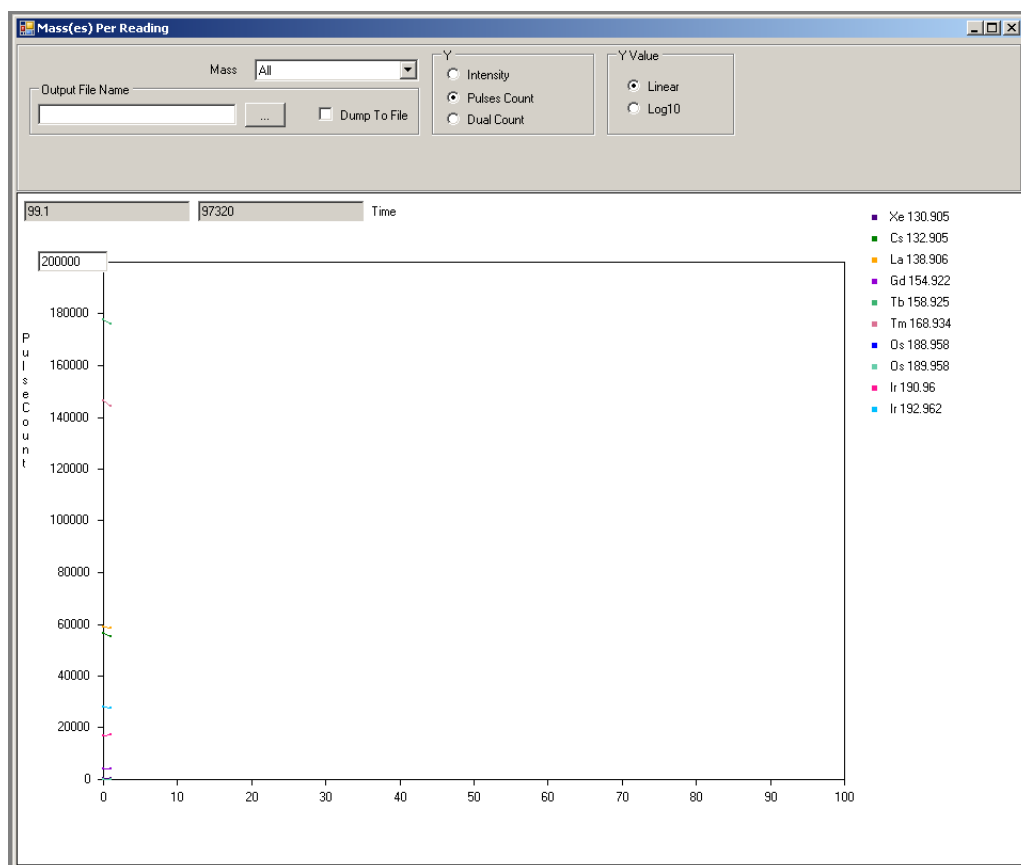
Keep the Flow Injection Valve lever in Load position, so that the Sample Loop is bypassed.

Set number of readings to 10 () , and click on the “Save” button.

After 80 s of injecting the Tuning solution, run Mass Calibration sampling and observe pseudo-continuous vertical lines indicating presence of sample (as shown in Fig.10). Then run Masses per Reading acquisition.

Measurements in Masses per Reading Mode

In the Masses Per Reading mode, the signal from the ion detector is integrated after digitization, both on the integration time for each analyte (selected in Acq. Settings ->Analytes) , and on the number of push-outs per reading (selected in Acq. Settings ->Parameter). The default number of pushes per reading is 76800, thus the integral data correspond to ion signal obtained in 1 s. The results for each analyte are presented on the Masses per Reading graph and can be saved in the text file. The data can be processed, presented and saved in three formats: Ion Counts per reading, Intensity per reading and Dual Counts per reading. Ion Counting counts exact number of detected ions, but is valid only for low strength ion signals, when single ion pulses do not overlap. Analog “Intensity” data processing is used for higher signals. The analog data is converted to ion counting data using calibration coefficients derived via calibration procedures.



Since Tuning and Daily Performance Check are done using relatively low concentration solutions (0.5 ng/mL or less), the resulting average ion signals are below 2 ion counts per mass channel per single push mass spectrum (e.g. integrated ion signal is less than 153,600 cps), at which point the Ion Counting mode of processing is valid. Thus tuning and Daily Performance Check can be done using just Ion Counting Mode (Pulse Counting).

In the panel shown above, select Pulses Count and All for masses (this selects all analytes from the acquisition analytes table to be displayed), adjust the vertical scale (usually to 200,000 counts) and linear or Log10 to display the integrated per reading data.

To record the results of Masses per Reading measurements in a text file, select file name and check the “Dump to File” checkbox.

Note: Results will be recorded for all three processing modes: Intensity, Pulse Counts and Dual Counts.

To start data collection, press green button in the left top corner

Typical results of the Daily Performance Check done with use of the 0.5 ppb tuning solution of are shown in Table 1.1 below

Table 1.1. Typical results of the Daily Performance Check

Analyte	Ion signal / cps
^{133}Cs	70300
^{139}La	59500
^{155}Gd	2750
^{159}Tb	115800
^{169}Tm	97900
^{191}Ir	11360
^{193}Ir	19410
Oxide ratio ($^{155}\text{Gd}/^{159}\text{Tb}$)	2.4%

The results of measurements should satisfy sensitivity specifications: pulse counting signal for ^{159}Tb should be above 100,000 cps (which corresponds to 200 Kcps/ppb). The ratio of $^{155}\text{Gd}/^{159}\text{Tb}$ pulse counting signals should be below 0.03. M/z=155 ion signal for Tuning Solution (which does not contain Gd) is that of $^{139}\text{La}^{16}\text{O}$ ion, and its ratio to the atomic ^{159}Tb ion signal indicates the robustness of the plasma for successful vaporization, atomization and ionization of samples.

If Sensitivity Specification is not met, perform instrument tuning by sequential auto-optimization of the Make-Up Gas Flow at set Nebulizer Flows, and then perform Current Optimization. These routines are discussed in more detail later (see the section on Tuning Instrument Sensitivity).

WARNING: Daily Performance Checks must be performed and results recorded before and after each use of CyTOF[®] instrument. This will ensure the instrument's performance to specifications and reproducibility of results.

Pulse Counting, Intensity and Dual Counts Modes of Data Processing

In Ion Counting Mode, distinct detector signal pulses produced by single ions are detected separately in each single push-out spectrum. A simple peak detection algorithm, based on comparison of each sampling point to two adjacent sampling points, is used. The detected peaks are assigned to a particular mass channel using Mass Calibration (see page 22). The typical width of a mass channel is ~ 20 -25 data points (sampled at 1 ns interval), while the typical width of a single ion pulse is 3 data points. For ion signals with < 2 single ion pulses per mass channel, the probability of the two ion pulses overlapping within the mass channel window in a single push-out spectrum is very low.

In Intensity Mode, all signals above the selected threshold (in the section on Acquisition Parameters in Chapter 1) are summed for each mass channel.

The ion detector single ion pulse signal strength has relatively large spread, resulting in a high uncertainty of the Intensity Mode data with < 2 ions per single push-out spectrum (e.g. $< \sim 30$ -60 ions per characteristic to a cell event transient of 15 – 30 push-outs long). For signals of an intermediate strength, both modes are valid which allows Intensity-to-Pulse conversion coefficients to be established. For analyte signals with an average strength of more than two ion counts per single push-out spectrum, intensity data is converted to “Dual Counts”.

Calibrating the Detector for Dual Counts Coefficients

The coefficients to convert analog Intensity signals into ion counts are derived from data obtained via the Pulse Dual Calibration routine. During execution of the routine, ion transmission of the instrument is varied by changing the potential on one of the electrodes of the Quadrupole Deflector (Turn B) from -100 V to 0 V. Settling time of 100 ms is set to ensure complete transition to the next data point. Analog and pulse data are collected for each point. Processing of the data is done only for the points for which the results fall within the Pre-reading Pulse Regression limits. In the example below the limit is 1000 – 20000 ion counts per reading, each reading comprising 76,800 single spectra, e.g. 1 s. The Intensity-to-Pulse linear fits are derived for each 10 consecutive points. Out of several derived fits, the fit with correlation closest to 1 is selected. The intercept and slope are then recorded. The coefficients are mass dependent.

Open the Pulse Dual Calibration panel

Symbol	Mass	Integration Time	Cor
Cs	133	21	0
La	139	21	0
Tb	159	23	0
Tm	169	24	0
Ir	193	25	0

The analytes for which the analog-to-pulse coefficients are derived are selected in the table above by the operator (recommended are at least four analytes: ^{139}La , ^{159}Tb , ^{169}Tm , ^{193}Ir), as are the start and end values (-100 to 0 V), the step (1 V), the number of pushes per reading (76,800), settling time (100 ms), range of pulse counting (1000 to 20,000 counts per reading) and the number of points in regression ($n=10$).

The slopes and intercepts for the analytes of which calibration was not directly performed, are derived by linear extrapolation of the coefficients for the closest two

adjacent analytes. In the Acq. Settings -> Analytes table, the analytes for which dual detector coefficients were derived directly appear in green. Other analytes are shown in yellow and red. Analytes indicated in yellow have their coefficients extrapolated from the coefficients of two adjacent analytes with m/z below and above the analyte. Coefficients for analytes shown in red are derived from “one-sided” extrapolation from two analytes with m/z of both either above or below the analyte. These coefficients are the least accurate. It is advisable to perform Pulse Dual Calibration on the highest and lowest m/z analytes of the complete mass range of interest. For example, if measurement range of m/z is from 115 to 208, then selecting ^{115}In and ^{208}Pb (plus as many other analytes of intermediate m/z as practical) is advisable.

The decision of which ion signals are processed by converting the analog Intensity data to dual counts is made based on the user selectable parameter. The upper counting mode limit for a single push-out for Masses per Reading and TOF Range per Reading is set in

Acquisition Settings ->Parameter->Dual Count Start Point.

In addition to the “Instrument” dual mode calibration, the dual mode coefficients can be determined during particles/cells data acquisition, from the actual collected data for the cells. In this case, the data is sorted according to the selected range of the pulse signals (default 0-2 per push), and the average of 30 analog intensities is calculated for each pulse counting value. Such “Data” calibration is preferable during cell measurements, since it mitigates changes of the ion detector gain which may occur due to excessive ion currents during cell detection.

Tuning Instrument Sensitivity

Instrument sensitivity is defined by the efficiency of a) sample nebulization, b) vaporization, atomization and ionization in plasma, c) transmission of the ion optics and d) efficiency of ion detection.

The main parameters that define a) and b) are the Nebulizer Gas Flow and Make-up Gas Flow. The temperature of the spray chamber heater, which also affects a) and b), should be kept at 200 °C.

Transmission of the ion optics is defined by the mode of operation (High Sensitivity or High Resolution) and the optics parameter “Current”.

To perform instrument tuning, begin by mapping the Sensitivity - Make-up- Nebulizer gas flows phase-space. The following ranges are recommended: Nebulizer Gas Flow 0.2 – 0.45 L/min, Make-up Gas Flow 0.55- 0.95 L/min.

Set Nebulizer Gas Flow to 0.3 L/min and Make-up Gas Flow to 0.7 L/min in

Instrument Setup -> DAC Channels Setup

by entering the corresponding values in Actual Current Value cells and pressing “Set Actual Current Value”.

Card Cage	RFG Controller	DAC Channels Setup	Ports & Commands Setup				
Parameter Name	Actual Min	Actual Max	Actual Current Value	Update	F1	F2	
Tun A	-413	0	-300	Set Actual Current Value			
Tun B	-210	0	-48	Set Actual Current Value			
Exit Lens	-210	0	-25	Set Actual Current Value			
Thick Lens	-210	0	0	Set Actual Current Value			
RF Entrance	-410	0	-200	Set Actual Current Value			
Quad Bias	-210	0	-140	Set Actual Current Value			
RF Exit	-410	0	-50	Set Actual Current Value			
shield	-210	0	-150	Set Actual Current Value			
B1andA2	-211	0	-196	Set Actual Current Value			
DCommon	-210	0	-70	Set Actual Current Value			
Grid1	-209	0	-119	Set Actual Current Value			
Lens B	-210	0	0	Set Actual Current Value			
B2andA1	-209	0	-200	Set Actual Current Value			
RFVolts	-267.5	0	-138	Set Actual Current Value			
Middle Point	-146.4	0	-120	Set Actual Current Value			
▶ Nebulizer Gas	0	1.2	0.3	Set Actual Current Value			
Detector Bias	-5000	0	-5000	Set Actual Current Value			
Detector Voltage	-5000	0	-2150	Set Actual Current Value			
DO2	0	40	0	Set Actual Current Value			
PulserP	0	713	430	Set Actual Current Value			
PulserN	-703	0	-702	Set Actual Current Value			
Liner	-5000	0	-4999.9	Set Actual Current Value			
Mirror	0	1000	920	Set Actual Current Value			
Makeup Gas	0	2	0.7	Set Actual Current Value			
Current	0	25	5	Set Actual Current Value			
400V1	-410	0	-80	Set Actual Current Value			
Slit	-209	0	-140	Set Actual Current Value			
400V2	-410	0	0	Set Actual Current Value			

Next, auto-optimize Make-up Gas Flow by following the instructions below.

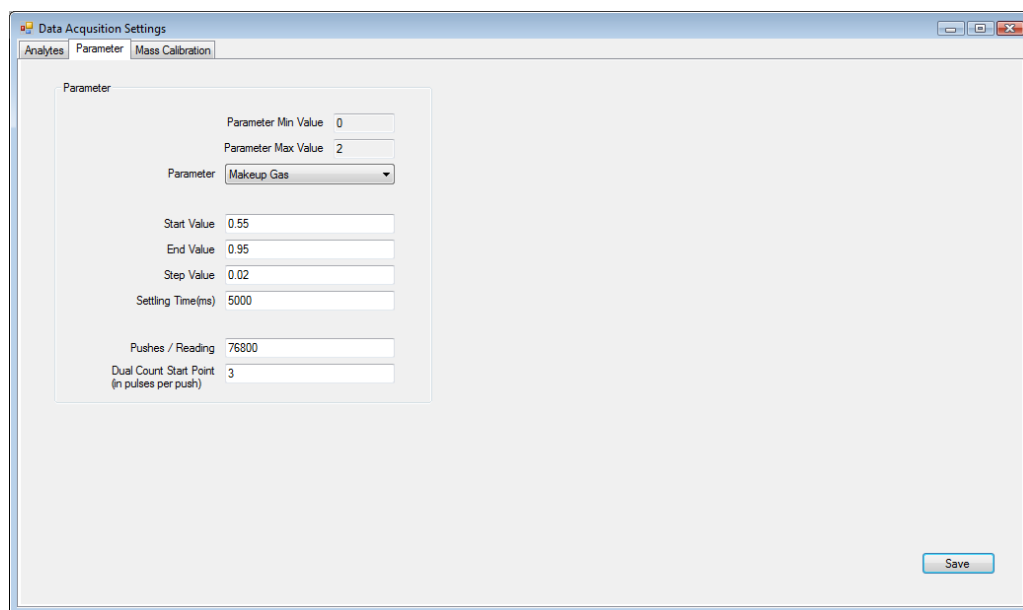
Auto-Optimization of a Parameter

Any DAC parameter is available for ramping in the Auto-Optimization routine. However, only three (Nebulizer Gas, Make-up Gas, Current) are part of routine tuning for sensitivity. Consult with DVS Sciences Inc. before attempting to optimize other parameters.

WARNING: Selection of incorrect ramping ranges for RF Volts, Detector Bias, Detector Voltage, or Liner may cause instrument malfunction. These are service parameters only, and will be inaccessible in future versions of the software. Do not attempt to ramp these using the Auto-Optimization routine except as specified in the section on Detector Gain Optimization in Chapter 1.

To optimize Makeup Gas, select it in Acquisition Settings:

->Acquisition Settings ->Parameter->Makeup Gas



Set the following values:

Start value =0.55
 End value =0.95
 Step value =0.02
 Settling time =5000
 Pushes per reading=76800

Note: The gases are controlled by mass flow controllers, which are thermal devices and require long settling time (5000 ms is recommended).

Save the above acquisition parameters and close the panel.

Now, set up the Masses per Reading Measurements (see the section on Measurements in Masses per Reading in Chapter 1).

Select All, Pulse Counts, vertical scale maximum at 200,000 and name the file in which to save the data. It is advisable to name files so that the parameters are mentioned in the name, e.g. "TuningMUGas055to095atNeb03_Curr4V").

Press the green "Run" button.

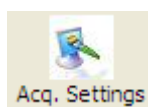
The name of the ramped parameter appears at the top of the graph.

Run the same measurement two more times, once at Nebulizer flow equal to 0.35 L/min and then at 0.25 L/min. Rename file for each measurement. Open the saved files in any spreadsheet program, calculate the $^{155}\text{Gd}/^{159}\text{Tb}$ pulse counting ratio for

each Make-up Gas Flow value, and select the largest value of ^{159}Tb pulse counting signal for which the ratio is below 0.03. Compare the obtained values of Tb signal for the measurements and select the largest. If the value is above the sensitivity specifications, further instrument optimization is optional.

If the specifications are not satisfied, repeat the measurement at Nebulizer Flow 0.2, 0.4, and 0.45.

In the case that conditions for meeting sensitivity specification are not found, select the best Nebulizer Flow-Makeup Flow combination, set their values in the Instrument Setup -> DAC Channels Setup, Save the DAC Channels Setup and perform Current Optimization in a manner similar to the Makeup Gas optimization.



->Acquisition Settings ->Parameter->Current

Start value = 0
End value = 10
Step value = 1
Settling time = 5000
Pushes per reading= 76800

If sensitivity specifications cannot be met after the above operation, cleaning of the sample introduction system, torch and/or cones may be required.

Once sensitivity specifications are met under robust plasma conditions, the instrument can be used for analysis of beads or cells.

Analysing Cells

WARNING: Thorough washout of the instrument sample introduction system after using Tuning Solution is required.

Use Washing Solution in the Carrier Syringe, load Washing Solution into the sample loop, and then run the instrument for 5 min. (without activating Data Acquisition).

Prepare and load Sample Carrier Solution into the Carrier Syringe, and Cell Buffer Solution into the Sample Loop.

Run for 5 minutes to equilibrate the tubing internal surfaces with the new reagents.

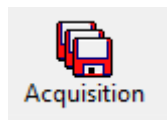
Edit the analytes table in

->Acquisition Settings ->Analytes

WARNING: In order to cover the mass range for analog-to-pulse calibration from the data, it is desirable to measure analytes at the start and the end of the mass range of interest. Include extra analytes which are usually present in the ion beam: ^{131}Xe and ^{206}Pb , for example.

In case of the Model Cells used during instrument installation (KG1A with ^{159}Tb , ^{169}Tm , and ^{166}Er labels), ^{131}Xe , ^{133}Cs (if running in 0.2 % NaCl buffer), ^{159}Tb , ^{169}Tm , ^{166}Er and ^{206}Pb are suggested as analytes.

Set the Acquisition parameters (see Chapter 2) in:

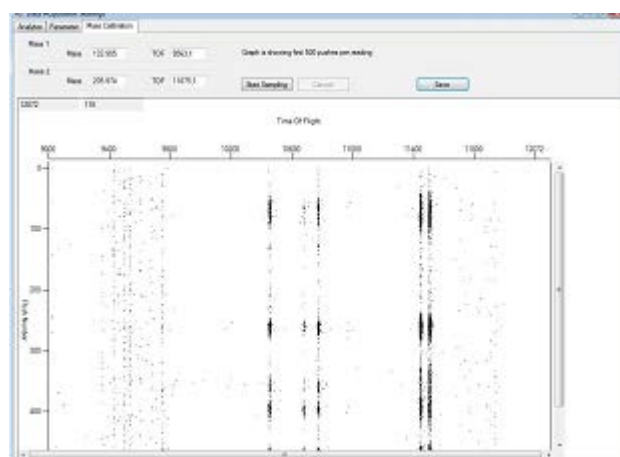


Load the sample in the syringe pump. Switch the Flow Injection Valve into the Inject position. After ~ 70 s, the sample is steadily delivered into the plasma. This delay can be set by choosing Detector Delay of 10 s and Acquisition Delay of 60 s, for example.

Presence of the cell-induced transient ion clouds can be verified by running acquisition snapshots in

->Acquisition Settings ->Mass Calibration

The example below shows such snapshots for the KG1A cells run in Deionised Water as buffer and sample carrier.



The pseudo-continuous signal lines on the left are Xe and Ba isotopes. The transient cell-induced ion clouds are evident, with the brightest two signals on the right being from DNA-Ir intercalator (^{191}Ir and ^{193}Ir isotopes), while the three signals in the middle of the screen correspond to ^{159}Tb , ^{166}Er and ^{169}Tm . Note that the between-the-cell signal streaks are pseudo-continuous lines indicating the presence of unbound elements in the sample. Nevertheless, the cell-induced transients are easily detectable by the cell detection algorithms. Figure 14 below shows FlowJo 2D graphs for the same cells used in the analysis above.

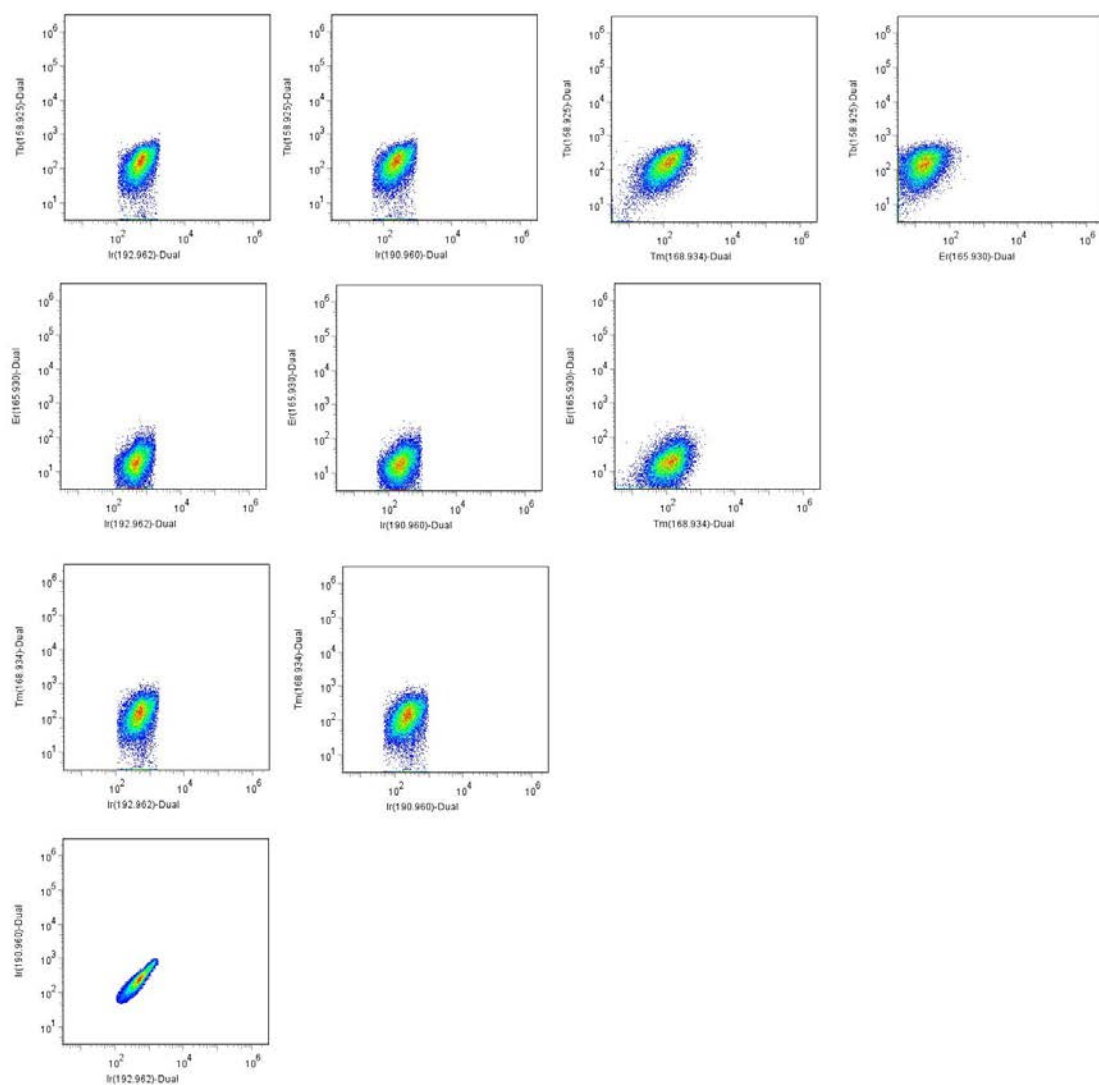


Figure 14. 2-D representation of Dual Counting ^{159}Tb , ^{166}Er , ^{169}Tm , ^{191}Ir and ^{193}Ir signals for KG1A cell

Turning Plasma Off

Instrument Setup->RFG Controller -> Plasma OFF

The plasma stop sequence is initiated.

The following events can be observed during the plasma shut-down:

Gate Valve closes, GATE Close LED lights up, GATE Open LED turns off;

Syringe Pump goes to “Idle”;

RFG goes to “Ready” then to “Idle”

Log records events of Detector voltages ramping down, Plasma Gases switching off, turning off of the Interface Pump, switching off the Chiller, turning off the Nebulizer Gas flow; and High Voltages switching off.

A message about successful plasma shutdown appears, with suggestion to remove the nebulizer.

TURN OFF THE HEATER



Instrument Setup -> Card Cage -> Heater OFF

WARNING: The nebulizer must be removed from the heated spray chamber after plasma is shut down. Exposing nebulizer to heat without Nebulizer Gas will deteriorate the nebulizer performance and shorten its lifetime. Clogging of the gas nozzle and sample capillary may occur.

Please remove the nebulizer from the spray chamber by gently pulling it out. Loosen the 3/16” nut of the high pressure gas fitting and remove the nebulizer side arm from the fitting (the o-ring, the front ferrule and the nut do not need to be removed). Loosen the 1/8” nut secured to the nebulizer liquid input, and remove the nut and the Sample Capillary Assembly.

Store the nebulizer submerged in DIW water.

USER NOTES :

Cell Data Acquisition and Analysis

Acquisition Window

The Acquisition window is intended for saving experimental data acquired from the Instrument. Data Analysis may be done during the acquisition process, or optionally after the acquisition is completed.

Input and Output

The acquisition window uses data acquired by a PDA Acquisition board.

The output data is represented by the following files, created through the process of acquisition and one of the Data Analysis options:

- “*.imd” – file containing integrated mass data
- “*_cells_found.txt” – found cells file
- “*_cells_found_long_cells.txt” – found long cells file
- “*_cells_found.conf” – dual calibration coefficients
- “*_cells_found_noise_statistics.txt” – noise statistics
- “*_cells_found_convolution.txt” – convoluted found cells values
- “*_cells_found_noise_convolution.txt” – convoluted noise data
- “*_cells_found.fcs” – found cells in the FCS3.0 format

- “*_cells_found.tcs” – found cells in the txt format, matching the FCS3.0 file values

Description

The acquisition window (shown on Figure 2.1) has several parameter groups specifying acquisition and analysis parameters, a graphical snapshot area to visualize sample analytes being introduced into the Instrument and action controls such as “Start” and “Cancel” buttons.

The acquisition process can run in one of two modes:

- Preview Mode, displaying acquired data on a graph only (for example, to monitor introduction of a sample)
- Normal mode, in which data is saved to disk while the acquisition process is visualised

Once it begins, the acquisition process runs until one of the criteria below is met:

- Acquisition time is over
- A Found Cells limit is reached
- An acquisition error has been discovered

At the end of an acquisition, a message information window opens indicating status of the acquisition, an optional analysis status and FCS file conversion status.

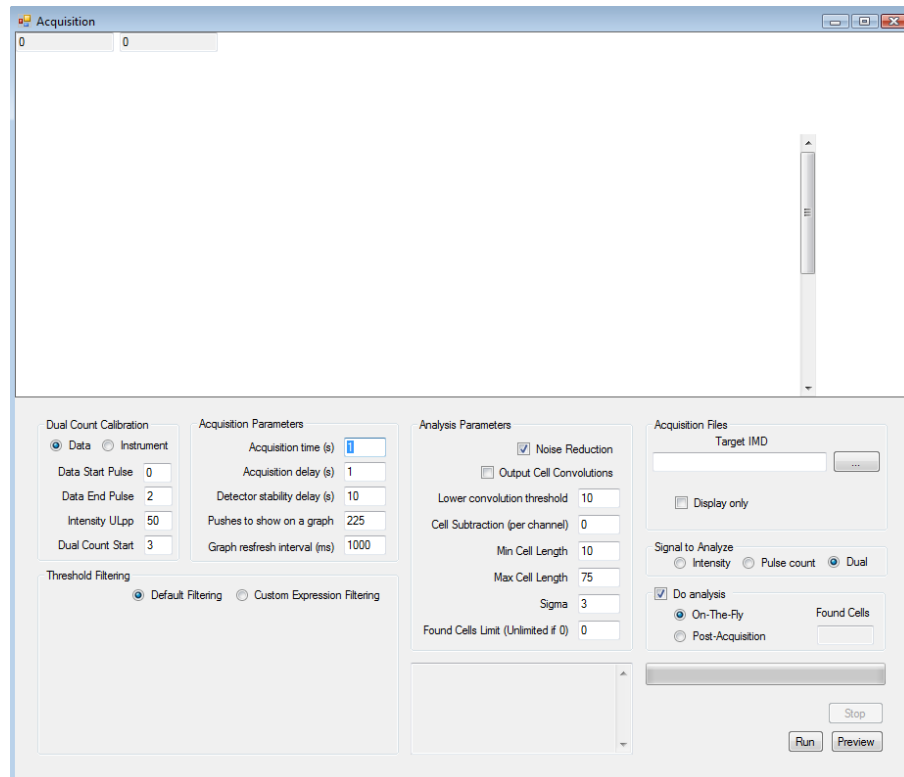


Figure 2.1. Acquisition window

Action controls

The Acquisition window has two action controls: start acquisition process using the “Run” button and cancel a previously started acquisition (Cancel button).

The “Cancel” button is greyed out until the “Start” button is pressed. As soon as the Start button has been pressed, the “Start” button is deactivated and the Cancel button becomes operational.

If the “Cancel” button is pressed, the button itself becomes deactivated and the software tries to shut down a previously started acquisition process. After the cancellation process succeeds, the Start button becomes enabled.

The “Preview” button is used for the 10-seconds preview. The data is shown in the Mass form.

Acquisition Parameters

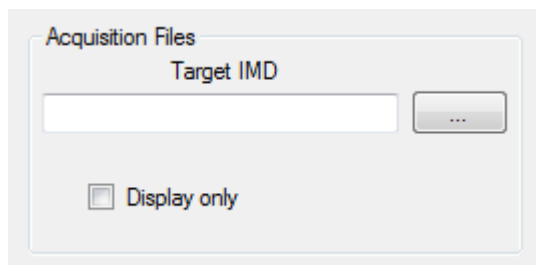
Acquisition parameters are categorized into two groups: “Acquisition Parameters” and “Acquisition Files”.

Acquisition Parameters	
Acquisition time (s)	1
Acquisition delay (s)	1
Detector stability delay (s)	10
Pushes to show on a graph	225
Graph refresh interval (ms)	1000

“Acquisition Parameters” parameter group has the following items:

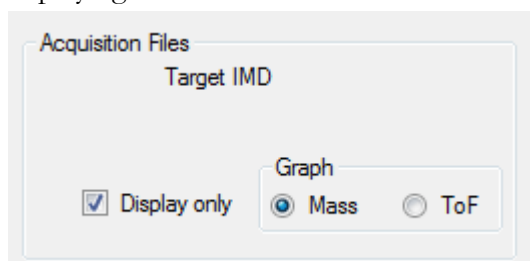
- “Acquisition time (s)” – duration of an experiment in seconds. The time does not include neither “Acquisition delay” nor “Detector stability delay”.
- “Acquisition delay (s)” – a delay in seconds after the start button is pressed and before actual acquisition of data and activation of detector. The detector has not yet been activated.
- “Detector stability delay (s)” – delay in seconds applied after the acquisition delay is over, and the detector has been activated, but no data is being acquired.
- “Pushes to show on a graph” – parameter determines vertical size of the graph, where each pixel corresponds to a triggered event of acquiring a single segment of data (a push). Pushes descend from the top to the bottom; such that the last push is at the very bottom of the graph (graph area may be scrolled if number of pushes exceeds the screen size). Setting this parameter to a high value may lead to low graph refresh rate
- “Graph refresh interval (ms)” – delay in milliseconds between two consequent graphs refreshes. Setting this parameter to a low value may lead to an acquisition buffer overflow error, the situation when a computer has not been able to cope with the incoming data stream, which leads to an on-board buffer overflow

“Acquisition Files” parameter group



The “Acquisition Files” parameter group consists of the following parameters:

- “Display only” checkbox – when selected, greys out all other parameter groups except for the “Acquisition parameters” group. The data is not saved to disk, and no data analysis is done. An internal Graph parameter group becomes visible providing a choice of a signal (Mass or TOF) for displaying.

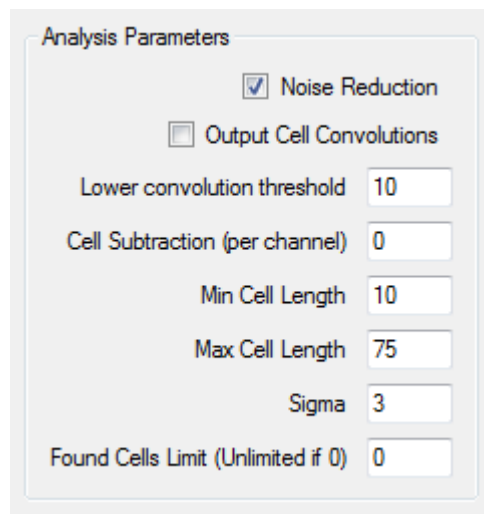


- “Target File”- specifies an IMD file, where the acquired data is saved during an experiment.

Analysis parameters

Analysis parameters consist of groups: “Analysis Parameters”, “Signal to analyze”, “Do Analysis” analysis type (which includes “On-the-fly” analysis and “Post-acquisition” analysis options); “Dual Count Calibration”, and “Threshold Filtering” group.

“Analysis Parameters” group



Analysis Parameters

Noise Reduction

Output Cell Convolutions

Lower convolution threshold

Cell Subtraction (per channel)

Min Cell Length

Max Cell Length

Sigma

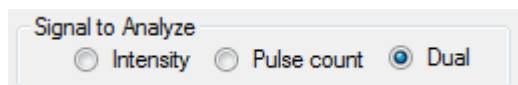
Found Cells Limit (Unlimited if 0)

The “Analysis Parameters” group has the following items:

- “Noise reduction” – activates the noise reduction algorithm during the analysis phase. When chosen, a text file with the noise statistics is generated (the file suffix is “*_cells_found_noise_statistics.txt”)
- “Output cell convolutions” – if this option is marked, two files containing cell integrated convolutions and convoluted noise are generated (the files suffixes are “*_cells_found_convolution.txt” and “*_cells_found_noise_convolution.txt”)
- “Cell subtraction (per channel)” – this parameter allows one to decrease cell analyte values by a common specified background value. If the number is greater than a cell analyte value, the resulting value is set to 0.
- “Min Cell Length”, “Max Cell Length” – these parameters specify a legitimate cell event duration range in terms of number of pushes. Only cells satisfying the specified interval of cell lengths are reported in the resulting files. Longer or shorter events are considered to be anomalies and are not recorded.
- “Lower convolution threshold” – this value is used to determine cell borders when the default algorithm is chosen (related to the “Threshold filtering” parameter group).
- “Sigma” – this is a convolution Sigma parameter, when the default algorithm is used to search for cells.
- “Found cells limit (Unlimited if 0)” – if specified, this parameter limits acquisition to the indicated number of cells. The acquisition stops after the

earlier of the two events occur: acquisition time is reached, or the target number of found cells is reached. The default value is 0.

“Signal to Analyze” parameter group



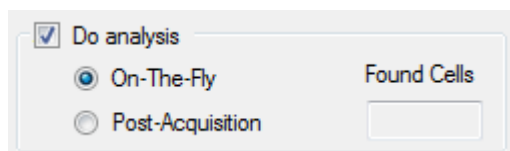
The “Signal to Analyze” parameter group selects the signal type to be processed during data analysis. It has three choices:

- “Intensity”
- “Pulse count”
- “Dual”. When “Dual” is chosen, the conversion of the Intensity signals to Pulse Counts signals is performed using the Slope and Intercept obtained for each analyte mass as described in section on Calibrating Detector for Dual Counts in Chapter 1, or new Slope and Intercept obtained from the data obtained during the currently running acquisition. For the latter, Data Dual Count Calibration should be selected in the “Dual Count Calibration” parameter group (in this case, a file having calibrated analytes with the suffix “*_cells_found.conf” is generated). For the former, “Instrument” should be selected in the “Dual Count Calibration” parameter group. It is recommended that the Pulse Dual Calibration is performed on the day of experiment.

Note: That in case this calibration is not performed before the cells data collection, it is possible to perform it afterwards and re-processed the recorded *.imd files with updated coefficients of the Instrument Pulse Dual Calibration.

The graph always displays Intensity signals

“Do analysis” analysis type parameter group



The “Do analysis” parameter group provides three options: no data analysis (“Do analysis” is not checked), do “Post-acquisition” data analysis or do “On-the-fly” data analysis. If “On-the-fly” analysis is selected, it is possible to specify a target number of found cells (“Found Cells Limit” parameter). The acquisition stops upon reaching the expected cell number or the Acquisition time is reached, whichever occurs earlier.

When the “Do analysis” checkbox is checked, two files are created: an FCS 3.0 format file and a text file, both containing data for the same found cells. FCS file generation uses the default parameters described in the FCS window documentation section.

“Dual Count Calibration” Parameter Group

The Dual Count Calibration parameter group provides values for calibration type and values for the data-based dual calibration.

Data based dual calibration takes place during the analysis time and its execution depends on the analysis type chosen.

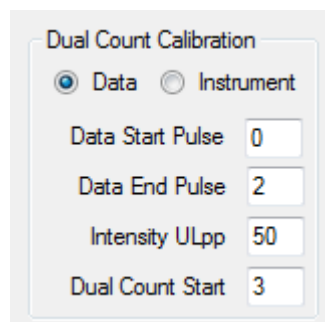
If the analysis is selected as “On-the-fly” analysis, the dual data-based calibration takes place within the first 30 s of an experiment. This time limit is set to provide an acceptable response time when refreshing a real time acquisition graph for the first time.

If “Post-acquisition” analysis type is selected, there is no 30-s limit, and all the collected data are used for deriving the calibration coefficients, which results in better precision of the Data calibration.

In the case that some of the analytes could not be calibrated – the program will generate an error message listing the “problem” analytes and will not do the analysis.

Note: Since the imd data files are saved, the same analysis can be repeated.

The “Dual Calibration” parameter group includes the following:



Dual Count Calibration

Data Instrument

Data Start Pulse 0

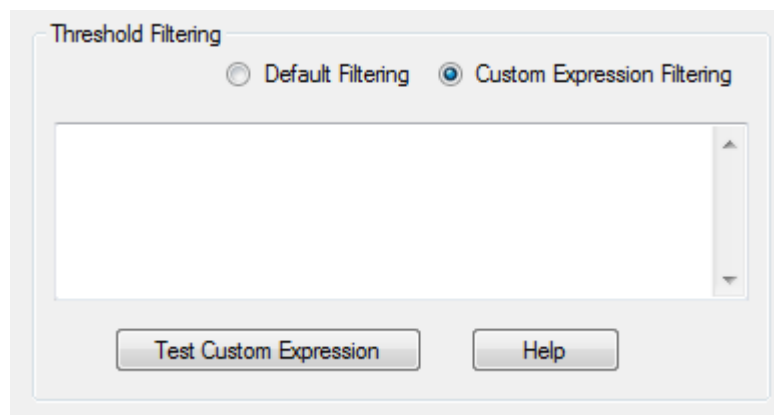
Data End Pulse 2

Intensity ULpp 50

Dual Count Start 3

- Type of calibration (Data-based or Instrument-based)
- “Dual Count Start” – a point at which Pulses are being converted to Duals based on Intensity. Before this value – Dual Counts are equal to Pulse Counts
- “Start Pulse” and “End Pulse” – determine the range for Dual-Pulse calibration
- Intensity upper limit per push (ULpp) is used during data-based dual calibration

“Threshold Filtering” parameter group



This parameter group allows use of either default or custom expressions in the data analysis algorithms of cell detection.

Use of the custom expressions replaces the default algorithm criteria during the analysis phase (“Lower convolution threshold” criterion is bypassed).

When the custom expression option is chosen, two action buttons become available: “Test Custom Expression” and “Help”. The “Test” button performs expression testing and the “Help” button opens a text window with the description of the programming environment.

An example use of a custom expression is the cell selection criterion based on a custom function of values for selected analytes from the list of all experiment analytes.

Custom expression has a data type of Boolean, and the expression evaluation result should be “True” to include a current push into a found cell. The expression language uses the same standards as C# and .Net platform (Microsoft).

EXPRESSION PROGRAMMING NOTES

The following is the description of the programming environment present in the expression window:

- A push number of the current line can be referenced as "pushNumber". The type is ulong.
- A convoluted value for a massesPerPush[0,] "all-masses" integral line may be referenced as "convoluted Value". Its type is double.
- "isotopes" array contains a current push line in-process and has a single dimension showing analyzed masses from the source file.
Note: A convolution is calculated for a sampling point minus "2*sigma" from the current point.

- In Addition to "isotopes" array there are two more double arrays with the same mass mapping but with an extra dimension:
 - massesConvolution[] - array containing convolution values per mass(m) for a massesPerPush[0, m] position
 - massesPerPush[,] - two-dimensional array containing values per Push having 2*sigma rows.
Rows present pushes, columns present masses. A number of rows is equal (2*sigma) if version one is used. The data in "isotopes" array is equal to the data from massesPerPush[2*sigma-1,].
- The expression in the window should return a boolean value.

For example, an expression limiting cell analysis to only the first two analytes of the complete list of analytes would look like this:

$((massesConvolution[0] + massesConvolution[1]) > 40)$. The lower convolution threshold in the example provided would be 40.

Processing messages

When an acquisition is running, two fields indicate the progress of the experiment: the progression bar and the Found Cells field.

The screenshot shows a control panel with the following elements:

- Sigma:** A numeric input field containing the value 3.
- Found Cells Limit (Unlimited if 0):** A numeric input field containing the value 0.
- Do analysis:** A checked checkbox followed by two radio button options: "On-The-Fly" (selected) and "Post-Acquisition".
- Found Cells:** A read-only text field that is currently empty.
- Progress Bar:** A horizontal bar with a grey gradient, currently showing 0% completion.
- Message Field:** A large empty rectangular area on the left side of the panel.
- Buttons:** Three buttons at the bottom right: "Run", "Preview", and "Stop".

If "On-the-fly" analysis is selected, the read-only field "Found Cells" shows the number of found cells.

The message field, on the left from the progress bar, contains messages (error or informational) from the acquisition, analysis, and FCS conversion procedures, as well as current graph refresh number.

FCS Conversion Window

Overview

The FCS conversion window is the interface through which the user can convert text data to FCS format.

The text format is Tab delimited lines of data, with the header specifying column titles in the first line. The number of Tab delimited fields must be the same for all lines. All fields after the first row must have positive or zero number values.

Multiple text files can be converted simultaneously. In this the target names will be based on the source file names. See Figure 2.2.

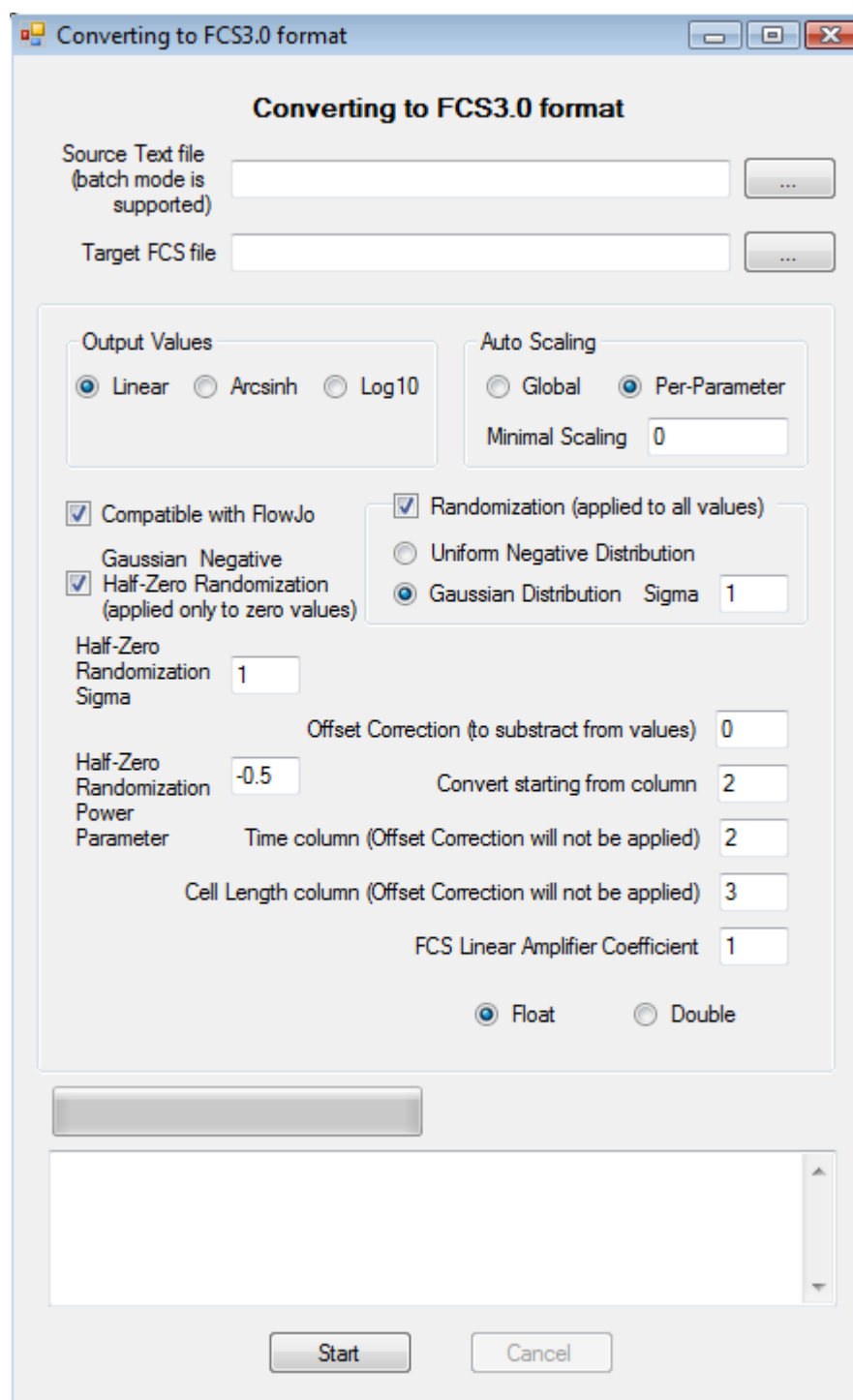


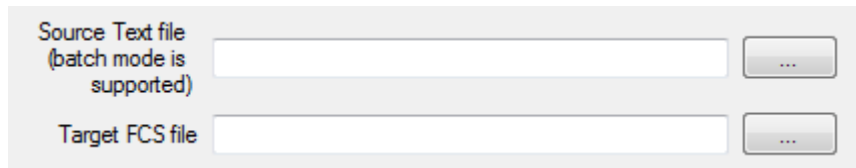
Figure 2. 2. FCS Conversion Window

Description

FCS Conversion Parameters

The FCS conversion window contains the following parameter groups:

File Parameter Group



The image shows a software dialog box titled "File Parameter Group". It contains two rows of controls. The first row has the label "Source Text file (batch mode is supported)" followed by a text input field and a button with three dots "...". The second row has the label "Target FCS file" followed by a text input field and a button with three dots "...".

- “Source Text file” – a file containing cell data to be converted to the FCS3.0 format. Multiple file selections are available. The field accepts a quoted string. If multiple files are entered, the file names should be separated by a comma.
- “Target FCS file” – an FCS3.0-format file which will contain converted cell data from the Source Text file. If multiple files are provided as the source, the Target FCS file field becomes disabled, and resulting FCS file names will have corresponding source file names used in a prefix and an “*.fcs” extension.

Conversion parameter group

- “Output values (Linear, Arcsinh, Log10)” – value type used to generate the target file. If “Log10” or “Arcsinh” are chosen, the corresponding function is applied to values. If “Arcsinh” is chosen, the upper border value is set to the maximum value depending on the data type selected (explained later in this parameter list).
- “Auto Scaling” – this parameter determines the upper range value set for each channel. The selections available are “Global” (the maximum value per file) and “Per-Parameter” (the maximum value per channel)
- Minimal scaling – It’s a minimal value set for a top range border in the FCS per parameters. However, if a larger value is found during a conversion – the parameter is ignored and the top range border is set to the max value (rounded accordingly, if FlowJo compatibility is checked).
- “Randomization” –The parameter is applied to the data only if the data type selected is “Linear”.

- When Uniform Negative Distribution is selected a real random value from $[-1 - 0]$ range is added to each value.
 - If Gaussian distribution is selected, a new parameter is made visible (Sigma). A random value according to the distribution is added to each value.
- Optional Gaussian Negative Half Zero Randomization (with a positive distribution is being negated) can be applied to zero values, if checked. When selected, two parameters become available – Half Zero Randomization Sigma and Half Zero Randomization Power. A random value according to the distribution is added to 0 data values.
- “Compatible with FlowJo” – when chosen, special formatting rules are applied during the FCS conversion. The data itself is not changed.
- “Zero correction” – this value is subtracted from data values if “Arcsinh” is chosen for output values. If cell data value is less than the specified parameter, the resulting converted value is set to 0.
- “Threshold correction” - specifies a value which is subtracted from each cell number from the source file. If the result becomes negative, 0 is assigned.
- “Convert starting from column” – a start column when doing a conversion. The preceding columns are skipped.
- “Time Column” – a time column. This column has special treatment when an FCS file is loaded into FlowJo. The threshold correction is not applied to the column when converting.
- “Cell Length Column” – a column representing cell length. The threshold correction is not applied to the column when converting.
- “FCS Linear Amplifier Coefficient” – a multiplier of cell values, applied at the very end, to “Linear” data only.
- FCS data type (Float, Double) – a required parameter specifying FCS data type.

Action Controls

Two buttons are available: “Start” –to initiate a conversion action, and “Cancel” - to stop the process. In this case data conversion will not be completed and you cannot rely on FCS data.

Analysis Window

The Analysis window is intended for processing experiment data (in the form of IMD files) acquired earlier in the Acquisition Window.

Input and Output

As a source of information, the analysis window takes IMD files (integrated mass data), generated during data acquisition in the Acquisition Window.

The output data is represented by the following files, created through the process of the analysis:

- “*_cells_found.txt” – found cells file
- “*_cells_found_long_cells.txt” – found long cells file
- “*_cells_found.conf” – dual calibration coefficients
- “*_cells_found_noise_statistics.txt” – noise statistics
- “*_cells_found_convolution.txt” – convoluted found cells values
- “*_cells_found_noise_convolution.txt” – convoluted noise data
- “*_cells_found.fcs” – found cells in the FCS3.0 format
- “*_cells_found.tcs” – found cells in the txt format, matching the FCS3.0 file values

Description

The Analysis window is shown in Figure 2.3.

It has several analysis parameter groups, two action controls (“Start” and “Cancel”), “Logging” area showing processing messages, and a progress bar indicating the running status of the analysis.

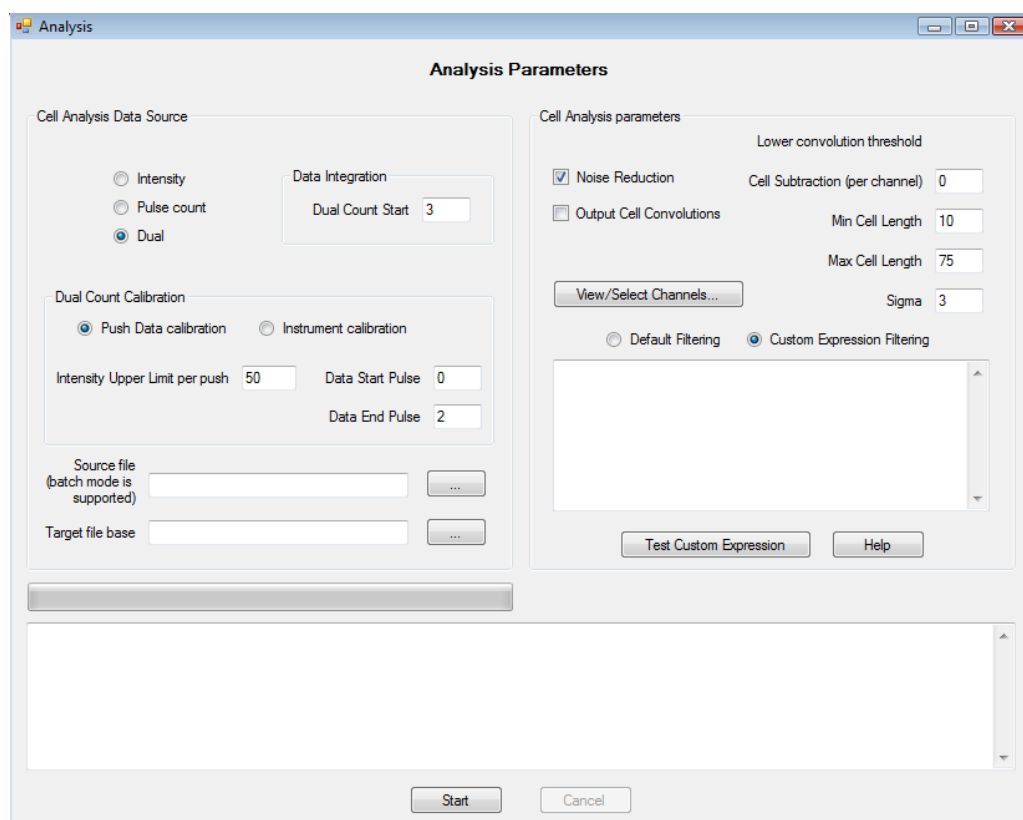
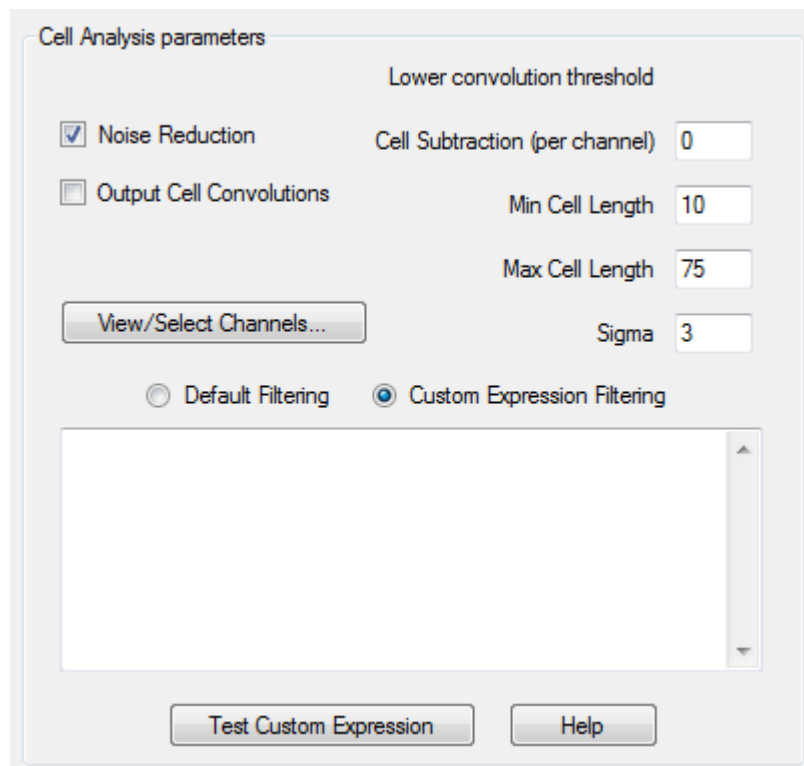


Figure 2.3. Analysis Window

Analysis parameters consist of the “Files” section and following three groups: “Cell Analysis parameters”, “Dual Count Calibration” and “Cell Analysis Data Source”.

“Cell Analysis parameters” group



The “Cell Analysis parameters” group contains the following items:

- “Noise reduction” – activates the noise reduction algorithm during the analysis phase. When chosen, a text file with the noise statistics is generated (the file suffix is “*_cells_found_noise_statistics.txt”).
- “Output cell convolutions” – if this option is checked, two files containing cell integrated convolutions and convoluted noise are generated (the files suffixes are “*_cells_found_convolution.txt” and “*_cells_found_noise_convolution.txt”).
- “Cell subtraction (per channel)” – this parameter allows one to decrease cell values per channel by a specified number. If the number is greater than the cell channel value, the resulting value is set to 0.
- “Min Cell Length”, “Max Cell Length” – these parameters specify a legitimate cell size range in terms of pushes. Only cells satisfying the specified interval of cell lengths are reported in the resulting files. Longer or shorter cell events are considered to be anomalies.

- “Lower convolution threshold” – this value is used to determine cell borders when the default algorithm is chosen (related to the “Threshold filtering” parameter group).
- “Sigma” – this is a convolution Sigma parameter, when the default algorithm is used to search for cells.
- View/Select channels feature allows excluding of certain channels from the analysis. The channels are still shown in the output files, but all values in such channels are set to 0. Please, note that the feature is not available in the batch mode. The channels are to be selected after an imd file is loaded (the list of analytes will change according to the ones from the imd file loaded).
- “Custom Expression” –Use of the custom expressions replaces the default algorithm criteria during the analysis phase (“Lower convolution threshold” criterion is bypassed). When the custom expression option is chosen, two action buttons become available: “Test Custom Expression” and “Help”. The “Test” button performs expression testing and the “Help” button opens a text window with the description of the programming environment. An example of use of a custom expression is the cell selection criterion is based on a custom function of values for selected analytes from the list of all experiment analytes. Custom expression has a data type of Boolean, and the expression evaluation result should be “True” to include a current push into a found cell. The expression language uses the same standards as C# and .Net platform (Microsoft).

EXPRESSION PROGRAMMING NOTES

The following is the description of the programming environment present in the expression window:

- A push number of the current line can be referenced as "pushNumber". The type is ulong.
- A convoluted value for a massesPerPush[0,] "all-masses" integral line may be referenced as "convolutedValue". Its type is double.
- "isotopes" array contains a current push line in-process and has a single dimension showing analyzed masses from the source file. Note that a convolution is calculated for a sampling point minus "2*sigma" from the current point.
- In Addition to "isotopes" array there are two more double arrays with the same mass mapping but with an extra dimension:
 - massesConvolution[] - array containing convolution values per mass(m) for a massesPerPush[0, m] position
 - massesPerPush[,] - two-dimensional array containing values per Push having 2*sigma rows.
Rows present pushes, columns present masses. A number of rows is equal (2*sigma) if version one is used. The data in "isotopes" array is equal to the data from massesPerPush[2*sigma-1,].
- The expression in the window should return a boolean value.
- Example: an expression limiting cell analysis to only the first two analytes of the complete list of analytes would look like this:
((massesConvolution[0]+ massesConvolution[1]) > 40). The lower convolution threshold in the provided example would be 40.

“Dual Count Calibration” parameter group

The dual calibration parameter group allows one to choose the calibration type and the values for data-based dual calibration.

In the case that some of the analytes could not be calibrated, the program will generate an error message listing the “problem” analytes and will not do the analysis.

The group includes the following:

- Type of calibration (Data-based or Instrument-based)
- “Start Pulse”, “End Pulse” determine the range for Dual-Pulse calibration
- Intensity upper limit per push is used during data-based dual calibration

“Cell Analysis Data Source” parameter group

The “Cell Analysis Data Source” parameter group selects a signal type which is processed during analysis.

There are signal types:

- “Intensity”
- “Pulse count”
- “Dual”. When “Dual” is chosen, the conversion of the Intensity signals to Pulse Counts signals is performed using the Slope and Intercept obtained for each analyte mass as described in the section on Calibrating the Detector for Dual Counts in Chapter 1, or new Slope and Intercept obtained from the data present in the selected file. For the former, Instrument should be selected in the “Dual Count Calibration” parameter group. It is recommended that the Pulse Dual Calibration is performed on the day of experiment. For the latter, “Push Data calibration” should be selected in the “Dual Count Calibration” parameter group (in this case,

a file having calibrated analytes with the suffix “*_cells_found.txt.conf” is generated).

- “Dual Count Start” – a point at which Pulses are being converted to Duals based on Intensity. Before this value – Dual data are the same as Pulse Counts

Files parameter group

The group specifies names input/output files.

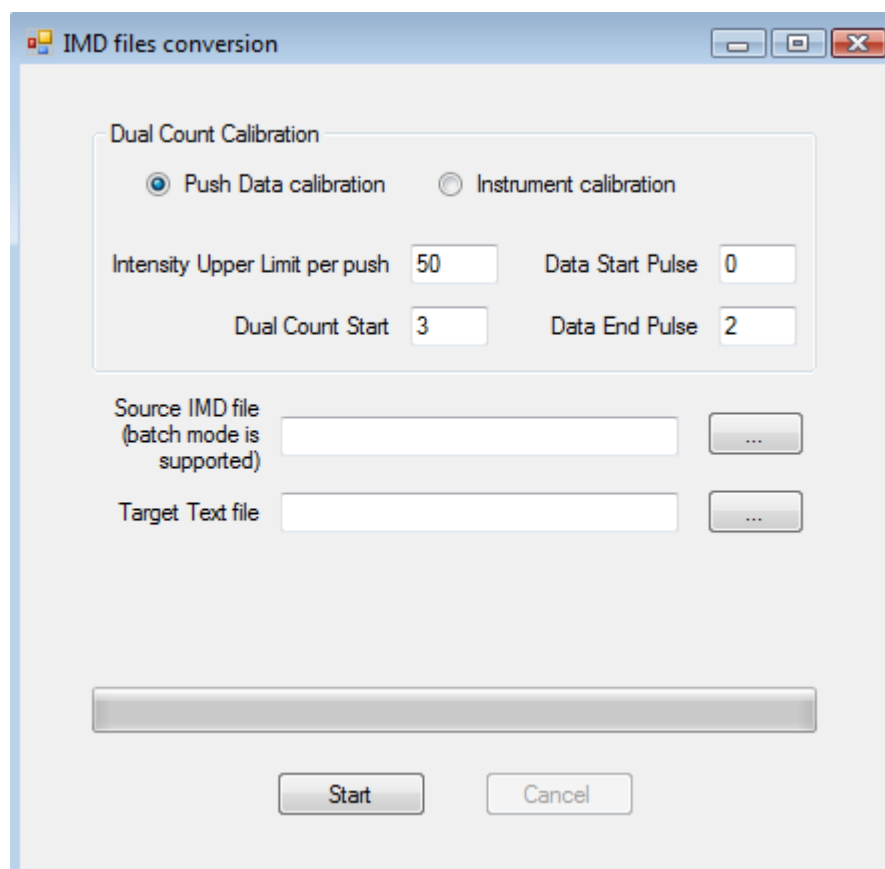
The group has the following items:

- “Source file” – a file containing integrated mass data to be analyzed. Multiple file names can be entered. The field accepts a quoted string (if multiple files are entered, the file names should be separated by a comma)
- “Target file base” – a text file containing found cells data. If multiple files are provided as the source, the target file base field becomes disabled, and the resulting file names will have corresponding source file names as a prefix with “*.cells_found.txt.*” added as a suffix.
A number of auxiliary files (except for the FCS type file, which is always created) may be generated during the analysis, depending on the analysis parameters:
 - “*_cells_found.txt” – found cells file
 - “*_cells_found_long_cells.txt” – found long cells file
 - “*_cells_found.conf” – dual calibration coefficients
 - “*_cells_found_noise_statistics.txt” – noise statistics
 - “*_cells_found_convolution.txt” – convoluted found cells values
 - “*_cells_found_noise_convolution.txt” – convoluted noise data
 - “*_cells_found.fcs” – found cells in the FCS3.0 format
 - “*_cells_found.tcs” – found cells in the txt format, matching the FCS3.0 file values

IMD files Conversion Window

Overview

The IMD files conversion window allows to convert binary experiment IMD files into the txt format.



The feature includes generation of dual signals based on the intensity and pulses collected from the IMD files.

Dual calibration group parameters are the following:

- Type of calibration (Data-based or Instrument-based)
- “Start Pulse”, “End Pulse” determine the range for Dual-Pulse calibration
- Intensity upper limit per push is used during data-based dual calibration

The screenshot shows a dialog box titled "Dual Count Calibration". It contains two radio buttons: "Push Data calibration" (which is selected) and "Instrument calibration". Below the radio buttons are four input fields: "Intensity Upper Limit per push" with the value 50, "Data Start Pulse" with the value 0, "Dual Count Start" with the value 3, and "Data End Pulse" with the value 2.

The file group parameters support batch conversion mode.

The screenshot shows a dialog box for file group parameters. It has two rows of input fields: "Source IMD file (batch mode is supported)" and "Target Text file". Each row has a text input field and a button with three dots (indicating a file selection dialog). Below these fields is a large, empty rectangular area. At the bottom of the dialog are two buttons: "Start" and "Cancel".

Fix FCS Window

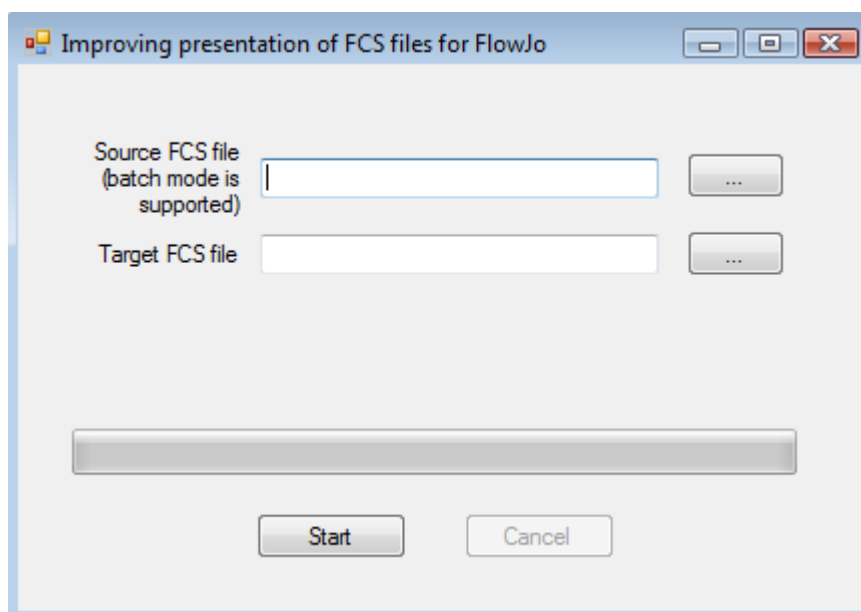
Overview

The Fix FCS window allows improving of the presentation of FCS files in the FlowJo software.

The routine corrects FCS headers in order to help FlowJo to display certain graphs properly. The routine doesn't modify data values.

The feature preserves the original file and generates a new target file, thus allows returning to the original FCS source.

The window supports batch mode by allowing a selection of multiple source files.



D V S S C I E N C E S I N C .

USER NOTES:

Maintenance

Spray Chamber

The spray Chamber needs to be removed from the Heater Module in order to be cleaned. The frequency of cleaning depends on the sample load. Daily cleaning is expected for heavy use.

WARNING : After the heater is turned off, the spray chamber and other heater module parts remain hot. Please allow sufficient time (~ 30 min) for the Heater Module to cool down before attempting to remove the spray chamber.

Removing the Spray Chamber from the Heater Module

To remove the spray chamber, the Heater Module needs to be opened.

- 1) Loosen the four Screen Screws (see Figure 3.1 below).
- 2) Remove the Screen.
- 3) Remove the Nebulizer Adapter from the Spray Chamber opening, using twisting and pulling motion.
- 4) Remove the ball joint clamp which secures the spray chamber to the torch.
- 5) Slide the Heater Module by pulling the Platform away from the torch. The front roller height may need to be adjusted by rotating it, in order to align all three rollers for easier sliding of the Heater Module.
- 6) Remove the three thumb screws that secure triangular tabs (x3) to the top of the Heater Box.
- 7) Open the heater box.

- 8) Lift the Heater Body slightly and slide the Spray Chamber out.

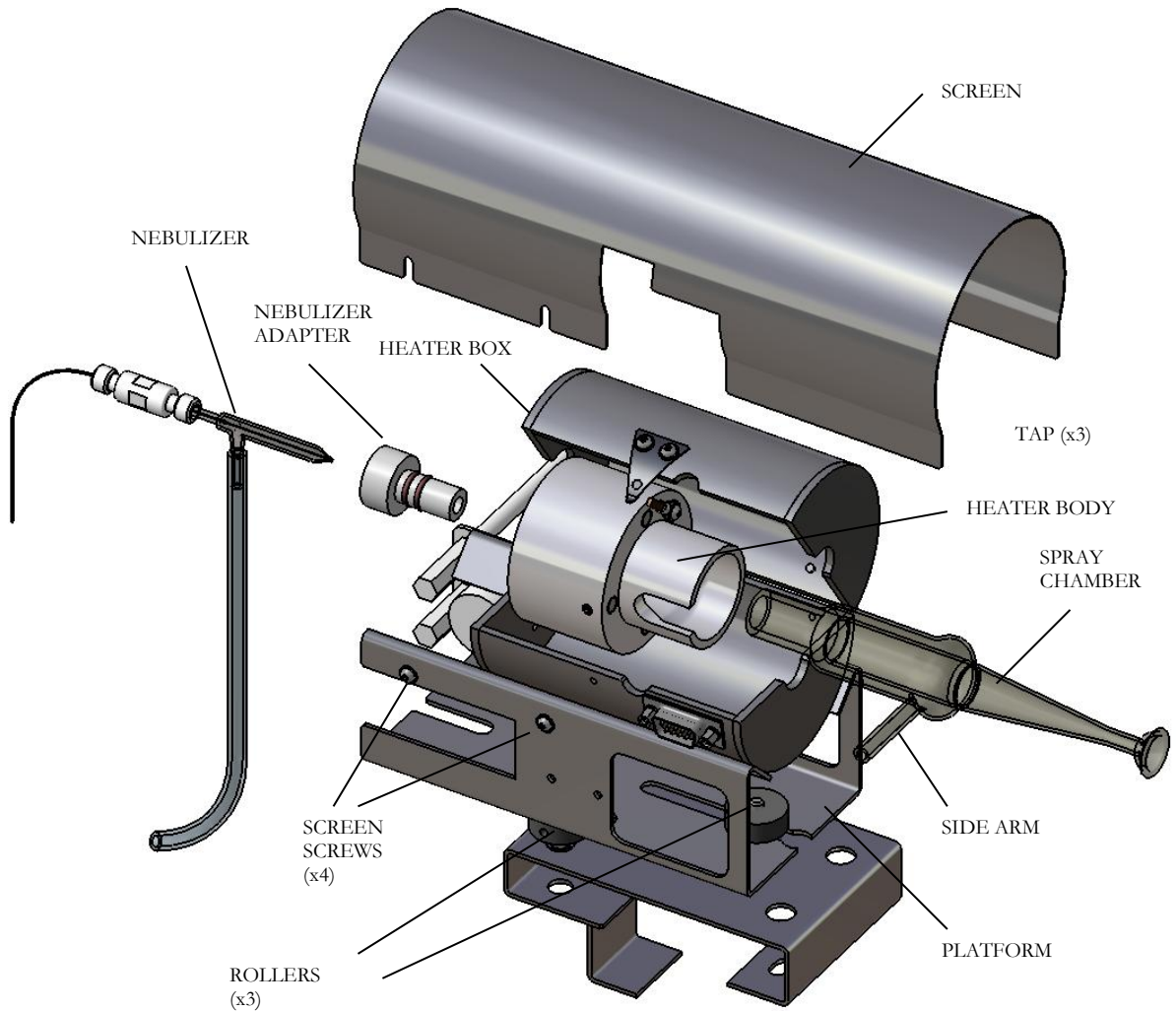


Figure 3.1. Heater Module details

Cleaning the Spray Chamber

If the Spray Chamber is cleaned regularly, cleaning in warm tap water with the use of a small bottle brush is sufficient for removal of organic matter and buffer residue from the chamber walls. After that, rinsing in deionised water is required.

For heavy residue removal, use of laboratory detergent may be required, with soaking for one hour or overnight. A good example of suitable laboratory detergent is 2 % volume percent (v/v) solution in water of RBS 35 laboratory detergent concentrate, used at 50° C.

WARNING: Please observe all safety precautions recommended by the detergent manufacturer.

After soaking the Spray Chamber, clean it in warm tap water with the use of a small bottle brush and rinse with de-ionised water.

ICP Torch and Injector

The Injector (narrow center tube of the ICP Torch) needs to be cleaned at the same frequency as the Spray Chamber. To clean the Injector, the Torch Holder Assembly needs to be removed from the Torch Box, and the Torch needs to be removed. Before removal of the Torch Holder Assembly, free up the room in front of the Torch Box by sliding out the Heater Module Platform (refer to Fig. 3.1). To do so:

- 1) Loosen the four Screen Screws (see Figure 3.1).
- 2) Remove the Screen.
- 3) Remove the ball joint clamp which secures the spray chamber to the torch.
- 4) Slide the Heater Module by pulling the Platform away from the torch. The front roller height may need to be adjusted by rotating it, in order to align all three rollers for easier sliding of the Heater Module.

Removing the Torch Holder Assembly

To remove the Torch holder Assembly, undo the two Thumb Screws (see Fig. 3.2), then slide the Holder out of the Torch Box (not shown). The Torch Holder Assembly can be placed securely on a horizontal surface for further handling.

WARNING: Wear powder-free gloves when touching the Torch and Injector quartz surfaces. Contaminating the quartz may cause problems with starting and sustaining plasma, as well as premature deterioration of the glassware.

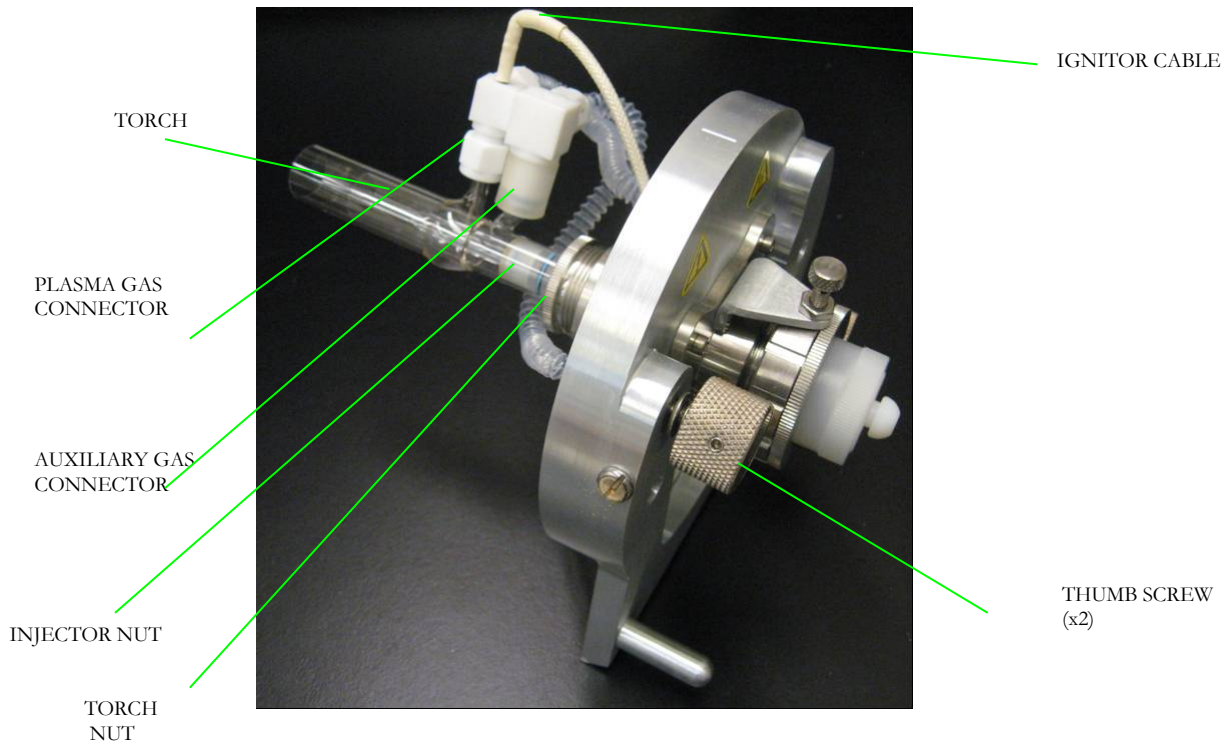


Figure 3.2. Photo of the removed Torch Holder Assembly

Removing the Torch

To remove the Torch:

- Undo the Plasma Gas Connector and Auxiliary Gas connector (see Fig.3.2.). You may need to slightly rotate the torch counter-clockwise around its axis by twisting motion in order to point the Plasma Gas side arm in such a way that both the Ignitor Cable and the Plasma Gas corrugated tubing allow to slide off the Plasma Gas Connector
- loosen the Torch Nut by unscrewing it counter- clockwise
- slide the torch out using a pulling and twisting motion.

WARNING! The Torch is fragile, handle with care.

Removing the Injector

To remove the Injector, loosen the Injector Nut, then slide the Injector out using a pulling and twisting motion.

WARNING! The Injector is fragile, handle with care.

Cleaning the Injector

To clean the Injector, soak it for 1 hr in 3 % nitric acid solution in water (v/v), then rinse with deionised water. If deposit persists, replace injector.

Cleaning the Torch

Inspect the torch for any deposits or traces. Some discoloration and faint brown deposits on the torch are normal. Heavy deposits need to be removed by cleaning.

To clean the torch, soak it for 1 hr in 3 % nitric acid solution in water (v/v), then rinse with deionised water. If deposit persists, replace torch.

Installing the Injector

To install the Injector, loosen the Injector Nut, then slide the Injector in until it stops completely, using a pulling and twisting motion. Some lubrication of the outside surface of the injector with methanol is advisable.

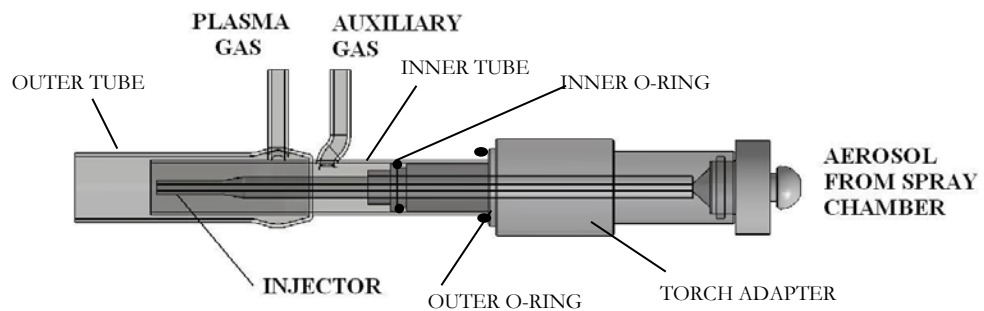
Installing the Torch

To install the Torch:

- Unscrew and remove the Torch Nut
- Slide the Torch Nut around the torch base
- Lubricate the Outer O-ring or the outside of the Inner Tube with little deionised water
- Slide the Outer O-ring onto the outside of the Inner Tube
- Lubricate Inner O-ring or the inside of the Inner Tube with little methanol
- slide the torch onto the inner O-ring using a pushing and twisting motion, until the torch Inner Tube is completely against the lexane Torch Adapter
- Screw in the Torch Nut (which will move the Outer O-Ring down and compress it)
- Check that the distance between the injector end and the edge of the inner tube is approximately $1.5 \text{ mm} \pm 0.5 \text{ mm}$ (see the Figure below).
- Connect the Plasma Gas Connector to the Plasma Gas side-arm of the torch, and Auxiliary Gas connector – to the Auxiliary Gas sidearm.

WARNING! The Torch is fragile, handle with care.

ICP TORCH



Sampler Cone and Skimmer-Reducer Assembly

Sampler Cone and Skimmer-Reducer Assembly cleaning frequency depends on instrument use. Weekly cleaning is expected for moderate use. For cleaning, the cones need to be removed from the interface.

Under heavy use, over time cones can degrade beyond acceptable limits. Careful handling and proper cleaning will typically enable you to get about 500 hours of operational use from each set. Some common symptoms of cone degradation are elevated oxide levels (eg. LaO/Tb ratio), and in severe cases, poor vacuum levels.

WARNING! The tips and surfaces of the cones can be easily damaged and should not be touched. Mishandling of the cones will cause deterioration of the instrument performance

Removing the Sampler

Sampler removal is done with the use of the Cones Installation and Removal Tool (see Fig.3.3).

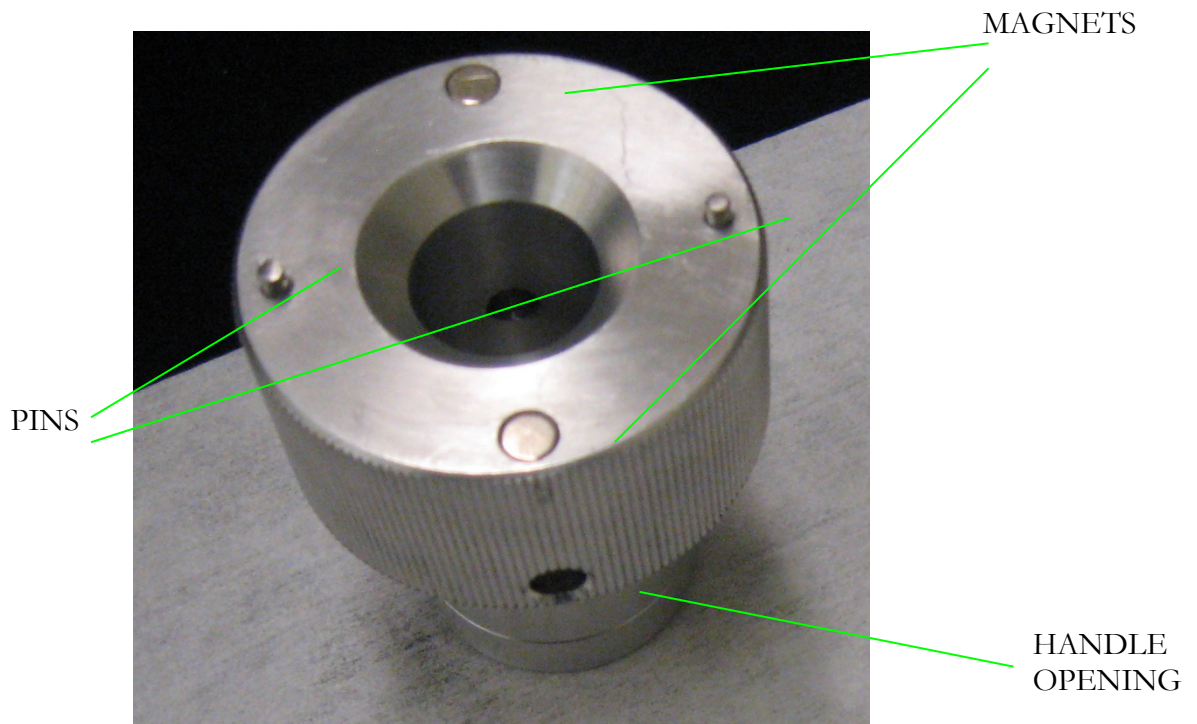


Figure 3.3. Sampler end of the Cones Removal and Installation Tool

The sampler has two blunt holes into which the pins on the tool should be inserted. The magnets keep the tool attached to the sampler.

To remove the sampler, open the back cover of the instrument and move the vacuum chamber to the back by pulling the z- positioning lever.

Attach the Cones Removal and Installation Tool to the sampler as shown in Figure 3.4, and insert the Handle into the Handle Opening.

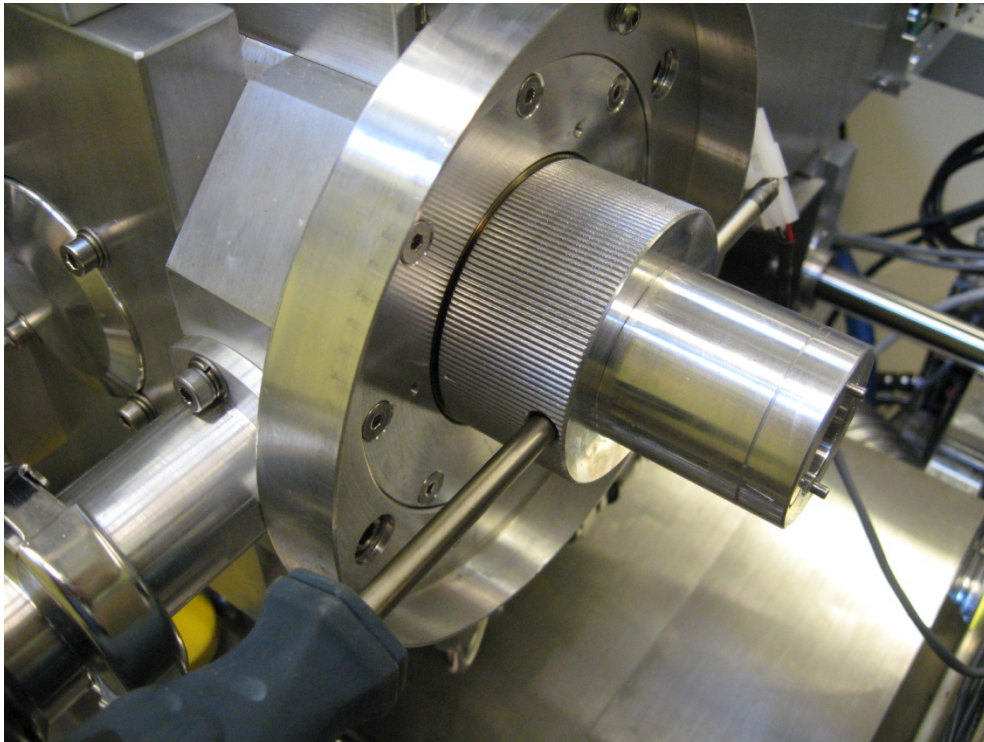


Figure 3.4. Sampler removal tool attached to the sampler

To remove the sampler, rotate the tool using the handle, in a **CLOCKWISE** direction, by $\sim 90^\circ$, then pull the tool away from the interface. The sampler should stay attached (see Figure 3.5.)



Figure 3.5. The sampler attached to the Cones Removal and Installation Tool

Removing the Skimmer-Reducer Assembly

Skimmer-Reducer Assembly removal is performed via the sampler opening of the interface with the use of the other end of the Cones Installation and Removal Tool by rotating the tool COUNTERCLOCKWISE to undo the Skimmer thread. The pins need to be aligned and inserted into the blunt holes on the skimmer. The magnets will keep the assembly attached to the tool (see Fig.3.6).



Figure 3.6. Skimmer-reducer Assembly attached to the Cones Removal and Installation Tool

Cleaning the Sampler and the Skimmer-Reducer Assembly

We recommend cleaning your cones under mild conditions, without use of mechanical removal of deposits.

Ultrasonic cleaning is recommended, however, care must be taken to prevent build-up of air bubbles around the cone tips during sonication. To do so, use special Cone Cleaning Containers and always place the cones into the washing solution with the tips pointing downwards.

The following is a common recommended MILD cones cleaning procedure:

- Soak in 1:20 Citranox : H₂O for 2 hours (sonicate for last 15 min.).
- Rinse thoroughly with tap water.
- Soak in deionised water for 10 min. (sonicate).
- Replace deionised water and repeat 10 min. soak (sonicate).
- Dry thoroughly (Argon or Nitrogen, if available), or place in glassware oven (40-50°C).

For heavier contaminated cones, STANDARD cleaning procedure using Acid / Detergent method is recommended:

- Soak in 1:20 Citranox :H₂O for 2 hours (sonicate for last 15 min.).
- Rinse thoroughly with tap water.
- Invert cone into Cone Cleaning Container containing 2% HNO₃.
- Let sit for 10 minutes, or place in bath sonicator.
- Wipe off residue with cotton swab, then rinse in tap water.
- Return Cone Cleaning Container for 10 minutes.

- Rinse thoroughly with tap water.
- Rinse thoroughly with dH₂O (sonicate for 15 min.).
- Replace dH₂O and repeat soaking/sonication.
- Dry with clean, dust free cloth and then let air dry, blow dry with Argon or Nitrogen, or place in glassware oven (40-50°C).

Nebulizer

Frequency of nebulizer cleaning and its longevity depends on the care which is taken to prevent its clogging. As discussed in Chapter 1, the nebulizer is recommended to be stored soaked in deionised water between CyTOF[®] instrument operating sessions.

The state of the nebulizer directly affects the quality of the produced cell-containing spray and the efficiency of cell introduction into the plasma. Properly maintained, the nebulizer should yield stable performance indefinitely. However, failure to apply a few simple preventive measures can lead to obstructed gas and liquid passages in the nebulizer which can seriously impair operation.

The smallest opening of the Nebulizer is that of the annular gas nozzle. An argon gas filter is used on the input of the CyTOF instrument in order to filter out particulate matter from the gas supply. The sample capillary is more tolerant of particulate matter than the gas annulus. We suggest, however, that you filter the cell samples through a 40 micrometers filter right before loading into the instrument. A good example of a suitable filtering is the use of BD Falcon 5 mL tubes with Cell-Strainer Cap (part # 9226803).

It is especially important to rinse the Nebulizer before turning the Nebulizer Gas off. Depending on the chemistry of your samples it may also be advisable to rinse periodically throughout the testing. Solids may deposit in the nozzle as sample solvent evaporates, further constricting the flow passages and reducing the analytical signal. We recommend using Deionised Water rinsing after each sample, and Washing Solution rinsing before and after each operating session of the CyTOF instrument. Rinsing will minimize or eliminate the deposits. Thus, although some experiments can be conducted without thorough wash between samples (for example, when the analytes are different between the running and the next sample, or when the expected concentration of the analytes in the subsequent sample are much higher than in the running sample), it is advisable to do the wash in between anyway: the salt in the buffer can accumulate if the rinsing is infrequent.

WARNING ! Do not use ultrasonic cleaning! Resonance vibrations may be set in the capillary causing it to bounce against the inside of the nozzle and chip. Nebulizer performance can decline severely as a result.

The state of the nebulizer can be checked by examining its tip under a microscope. A “healthy” nebulizer should have no particles obstructing either the annular gas nozzle or the sample capillary (see Figure 3.7).

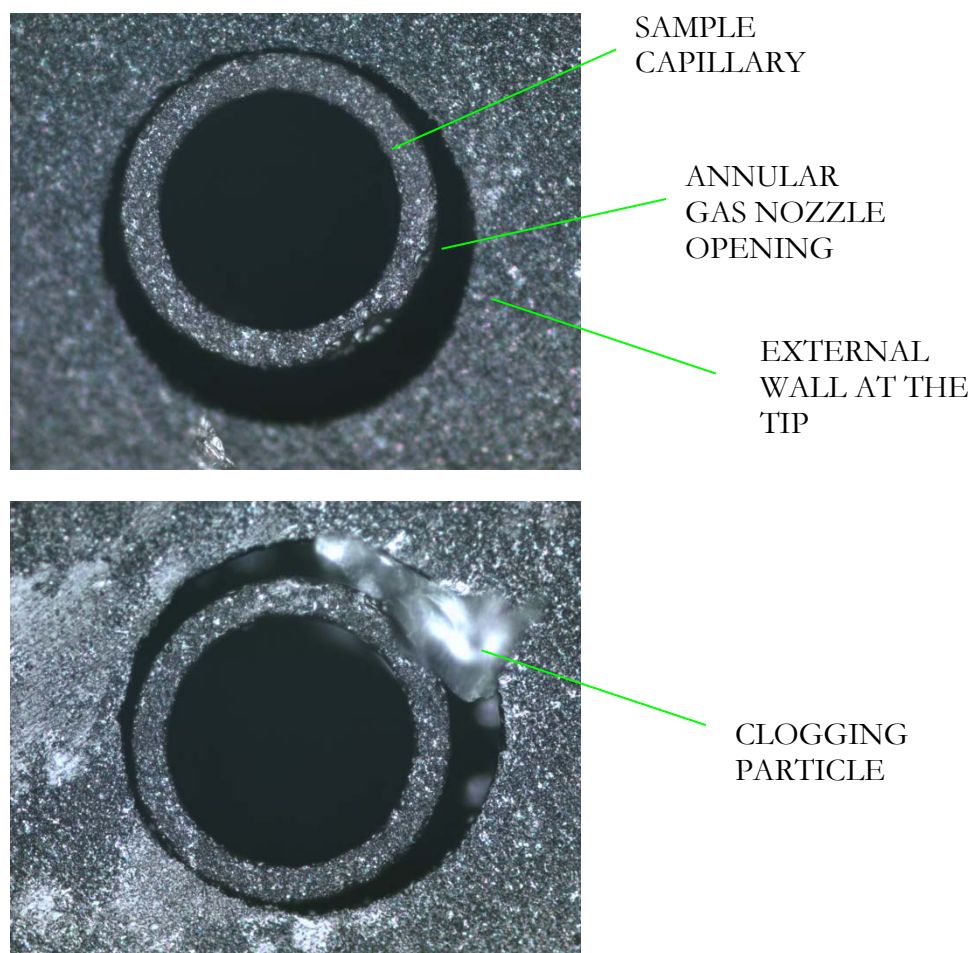


Figure 3.7. Microphotographs of satisfactory (top) and unsatisfactory (bottom) states of the nebulizer tip

Nebulizer Cleaning

The following is a recommended cleaning procedure for de-clogging the capillary from the tip.

Materials needed for this procedure include:

- Detergent: Contrad 100 (from Decon Labs)

- Deionized water
- Methanol
- Syringe (Recommended: Norm-Ject 3mL, 5mL, or 10 mL can all be used)
- Flexible tubing that will fit the syringe, side arm and sample inlet of Nebulizer (Recommended: R-3603 Tygon tubings with 3/16 OD and 1/18 ID)
- 5mL tubes (Round bottom) *Note: the type of tubes used depends on the physical depth of the tube, as tubes are used for containing the liquid in which the nebulizer tip will immerse. Hence, this depth must be greater than the distance from the side arm to the tip of the nebulizer such that the tip of the nebulizer will not touch the base of the tube when submerged into the liquid in the tube

Procedure:

- 1) Soak tip in detergent in 5mL tube for 15 minutes
- 2) Attach the flexible tubing to the syringe as shown in Figure 3.8.



Figure 3.8. Syringe attached to flexible tubing

- 3) Lubricate the end of the tubing with water and connect the side arm of the nebulizer to the flexible tubing attached to the syringe as shown in Figure 3.9. With tip still submerged in detergent, apply vacuum from syringe to infuse the detergent through the nebulizer tip via the side arm into the syringe. This will clean the annular gas nozzle. Repeat as many times as necessary. Note that the liquid taken up into the syringe should be dumped and should not be injected back into the nebulizer.



Figure 3.9. Flexible tubing attached to both Syringe and Side Arm of Nebulizer

- 4) Lubricate the end of the tubing with water and connect the sample inlet of the nebulizer to the flexible tubing attached to the syringe as shown in Figure 3.10. With tip still submerged in detergent, apply vacuum with syringe to infuse the detergent through the tip via the sample inlet into the syringe. This will clean the sample capillary. Repeat as many times as necessary. Note that the liquid taken up into the syringe should be dumped and should not be injected back into the nebulizer.

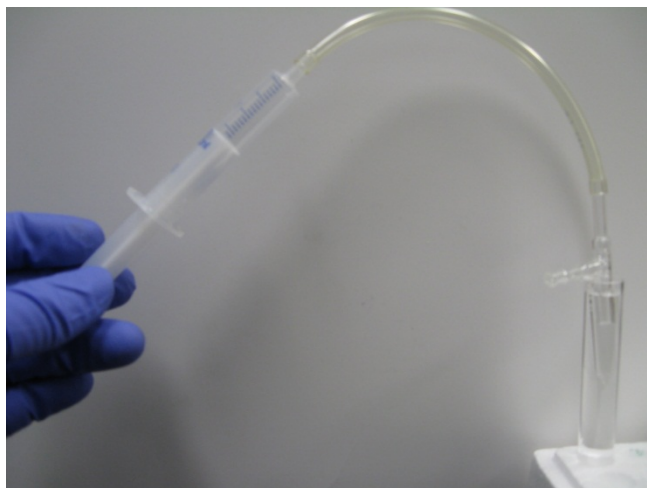


Figure 3.10. Flexible tubing attached to both Syringe and Sample Inlet of Nebulizer

- 5) Soak tip in DIW in a fresh tube for 15 minutes
- 6) With tip still submerged in DIW, apply vacuum to the sidearm and the sample inlet as in steps 3) and 4). This will rinse the parts that were in contact with the detergent. Repeat as many times as needed.

- 7) If nebulizer is to be used immediately, dry with compressed air. Alternatively, repeat step 6 with methanol.

Timely removal of the nebulizers from the elevated temperature environment of the Heater Module (as suggested by the instrument software after each Plasma Off sequence) and keeping Nebulizers soaked in DIW between operational sessions will help to avoid nebulizer clogging.

Rotary Pumps

The frequency of the oil change depends on the use of the instrument. First change of oil is performed during the instrument installation (the instrument is shipped with no oil in the pump). Please refer to the Oil Condition Chart supplied with the Interface Pump.

The second oil change is performed by DVS personnel during the warranty period on-site visit. On-site training will be provided on the oil change procedures during the visit.

Instrument Inputs and Outputs

For instrument operation, the following inputs and services are necessary:

Gas : Argon, 345 ± 7 kPa (50 ± 1 psi) at 20 L/min flow

Cooling : Coolant circulation of at least 3.8 L/min at an operating pressure of 345 ± 14 kPa (50 ± 2 psi)

Electrical Power:

Maximum Volt Amperes (for each line) 4500

Operating Voltage 200/208/220/230V AC

Operating Frequency 50 or 60 Hz

Exhaust vents:

100 mm (4") 70 L/s (150 ft³/min)

150 mm (6") 210 L/s (450 ft³/min)

Outputs:

Trigger: connected to the Trigger Input of the computer PDA 1000 board via coaxial BNC-SMA cable

Output 2 : connected to the Analog Input of the computer PDA 1000 board via coaxial BNC-SMA cable

Instrument – Computer communications connection:

25 – pin connector on the instrument, connected via multi-wire cable to a computer RS232 board

Please refer to “Preparing your laboratory for the DVS Sciences CyTOF Mass Cytometer” Guide for detailed information on connections and services.