How to Get the Best Performance out of your CyTOF

Carina Torres

CyTOF User Group Meeting

CYTO 2013

23 May 2013

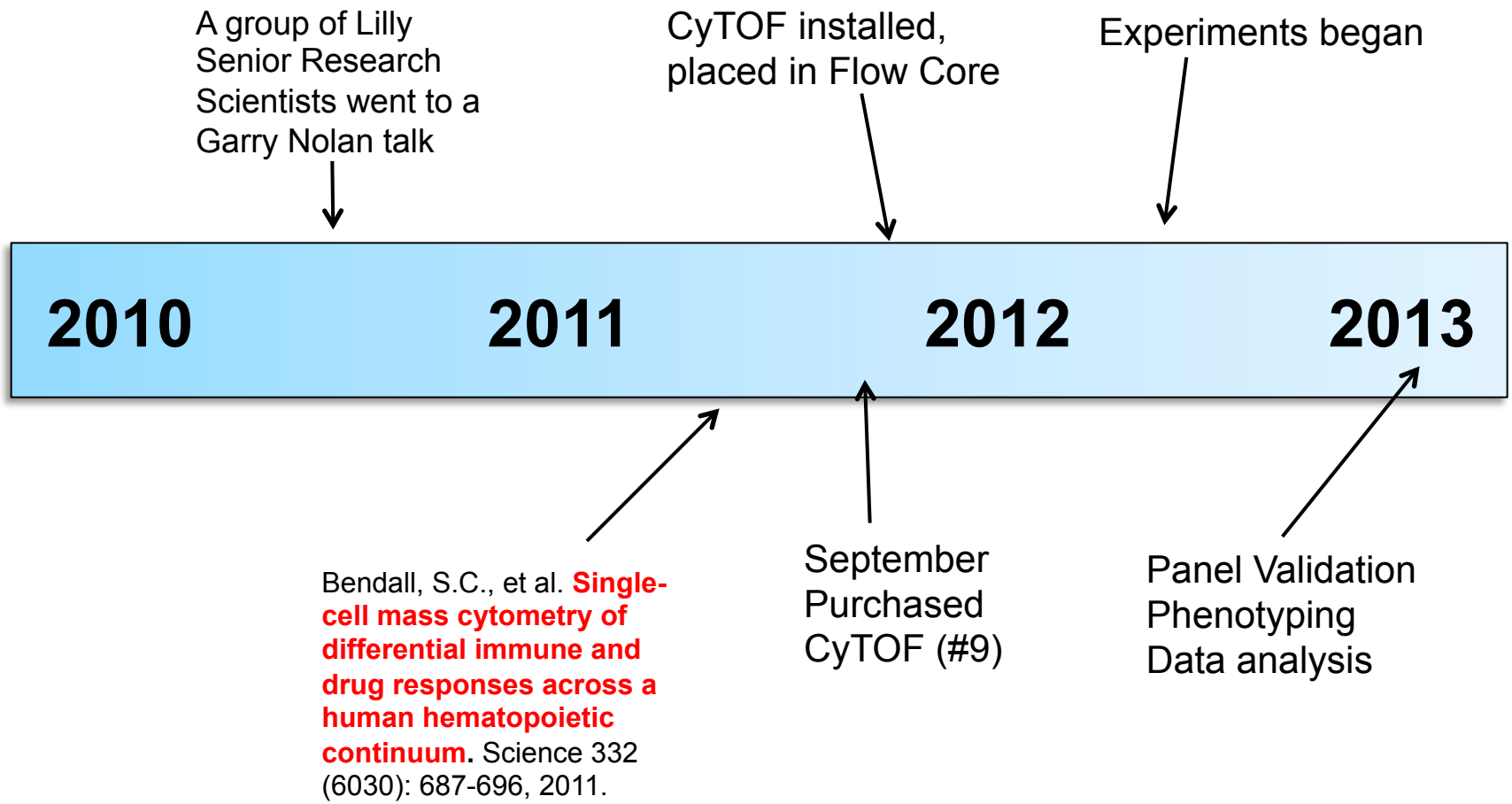
Lilly

Answers That Matter.

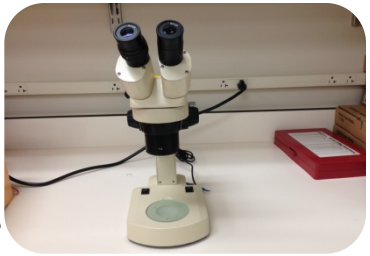
Outline

- History
- CyTOF Workflow
  - Instrument Startup
  - Optimization
  - Running Samples
  - Instrument Shutdown
  - Troubleshooting
- Progress Report…1 ½ years later

CyTOF at Lilly

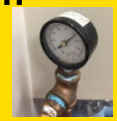


Preparing your Lab



Daily Workflow: Instrument Startup

Make sure Argon is on
- 100 psi at wall
- 50 psi on instrument

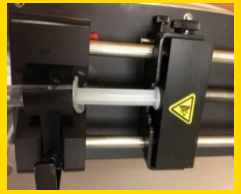


Check lights on instrument

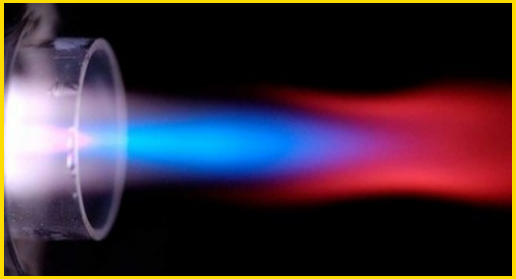
Turn Heater on

Instrument setup → Card Cage Tab → Click Heater on → 200°C

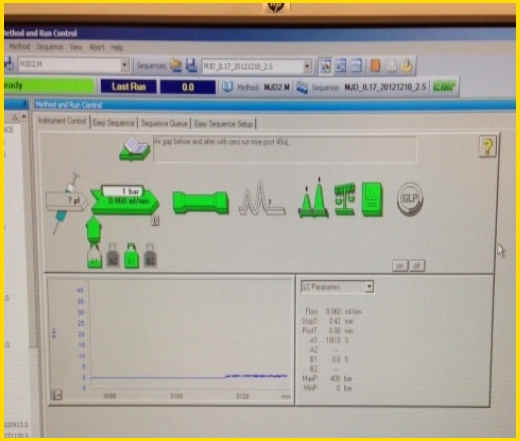
Prepare Nebulizer and Syringe Pump



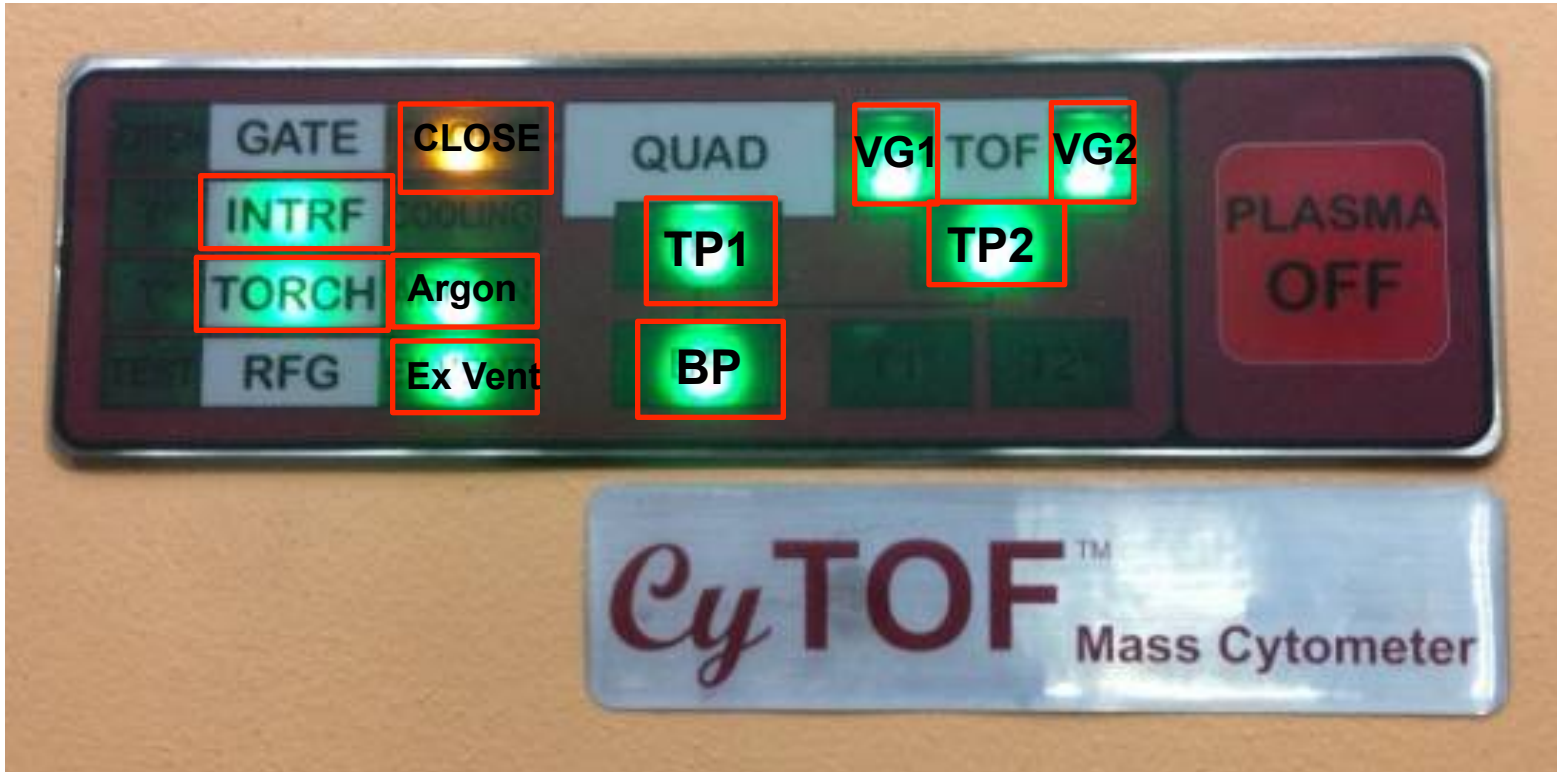
Start Plasma



Start Syringe pump (HPLC)

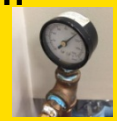


Instrument Startup



Instrument Startup

Make sure Argon is on
- 100 psi at wall
- 50 psi on instrument

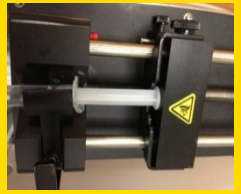


Check lights on instrument

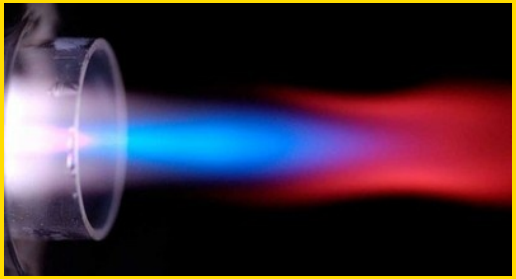
Turn Heater on

Instrument setup → Card Cage Tab → Click Heater on → 200°C

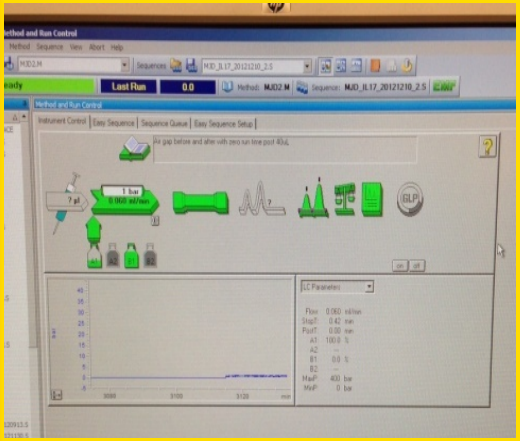
Prepare Nebulizer and Syringe Pump



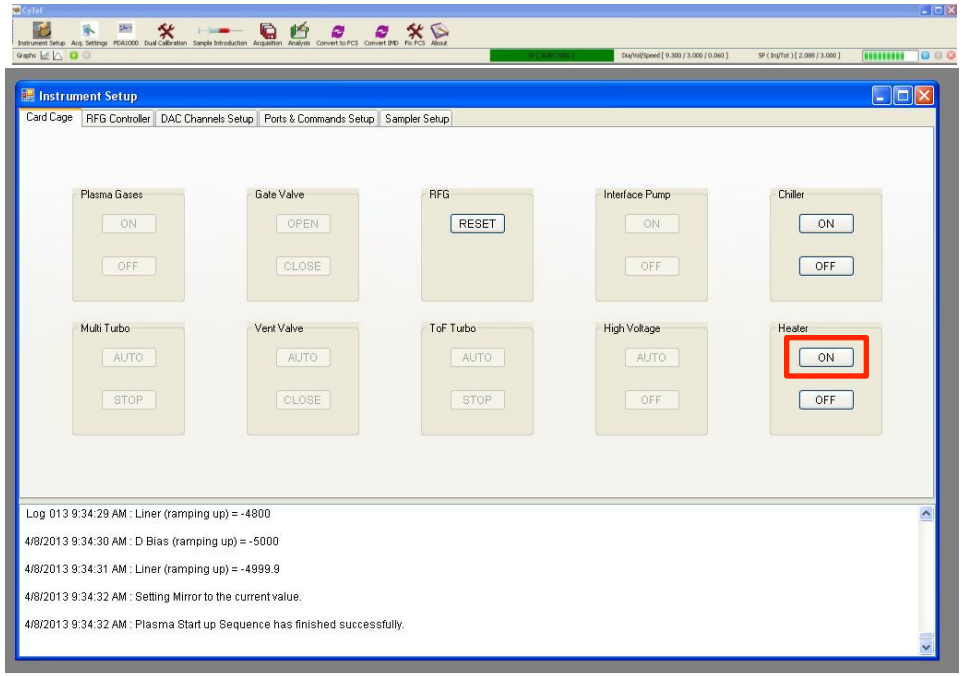
Start Plasma



Start Syringe pump (HPLC)



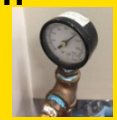
Instrument Startup



20 min

Instrument Startup

Make sure Argon is on
- 100 psi at wall
- 50 psi on instrument

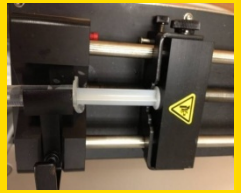


Check lights on instrument

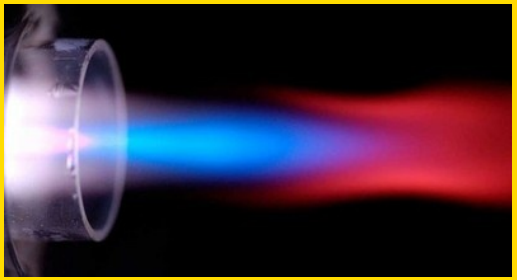
Turn Heater on

Instrument setup → Card Cage Tab → Click Heater on → 200°C

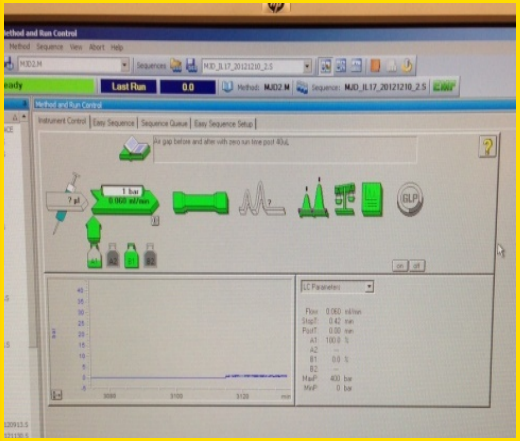
Prepare Nebulizer and Syringe Pump



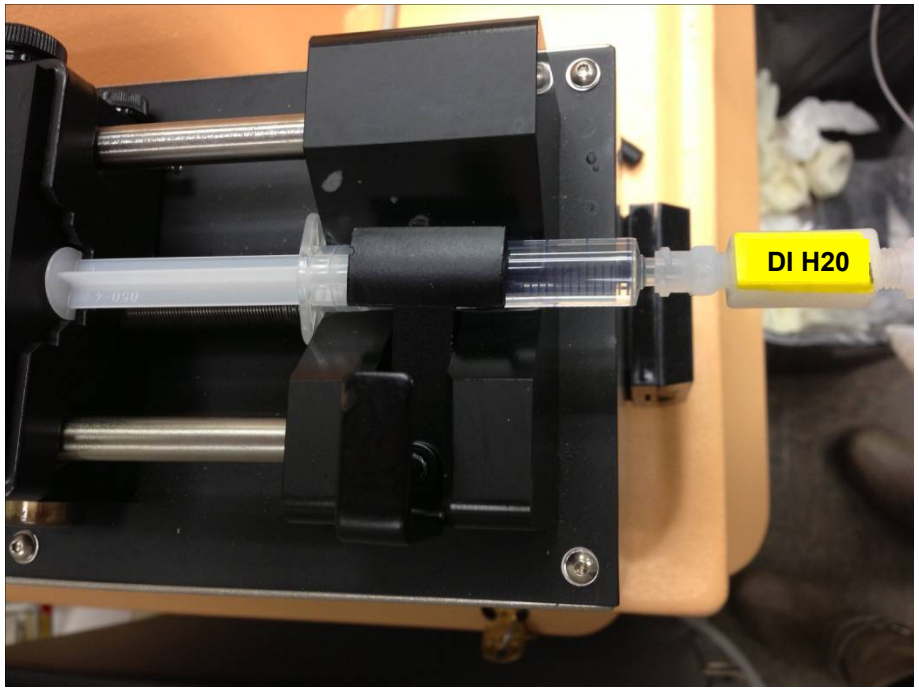
Start Plasma



Start Syringe pump (HPLC)

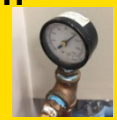


Instrument Startup



Instrument Startup

Make sure Argon is on
- 100 psi at wall
- 50 psi on instrument

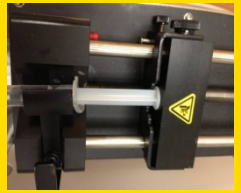


Check lights on instrument

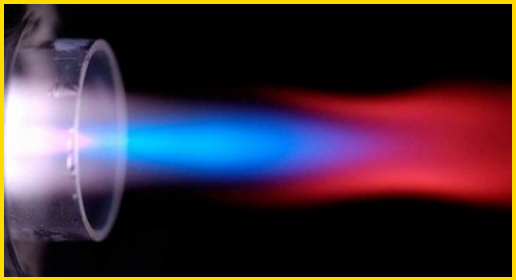
Turn Heater on

Instrument setup → Card Cage Tab → Click Heater on → 200°C

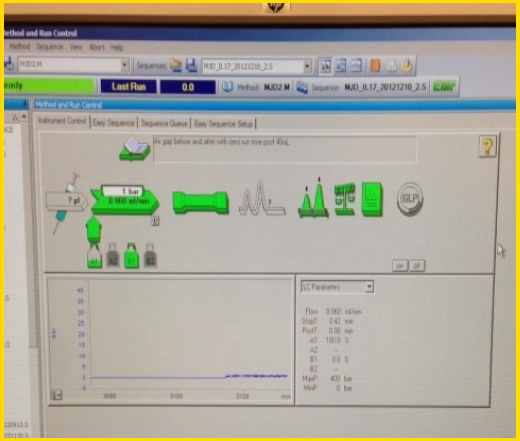
Prepare Nebulizer and Syringe Pump



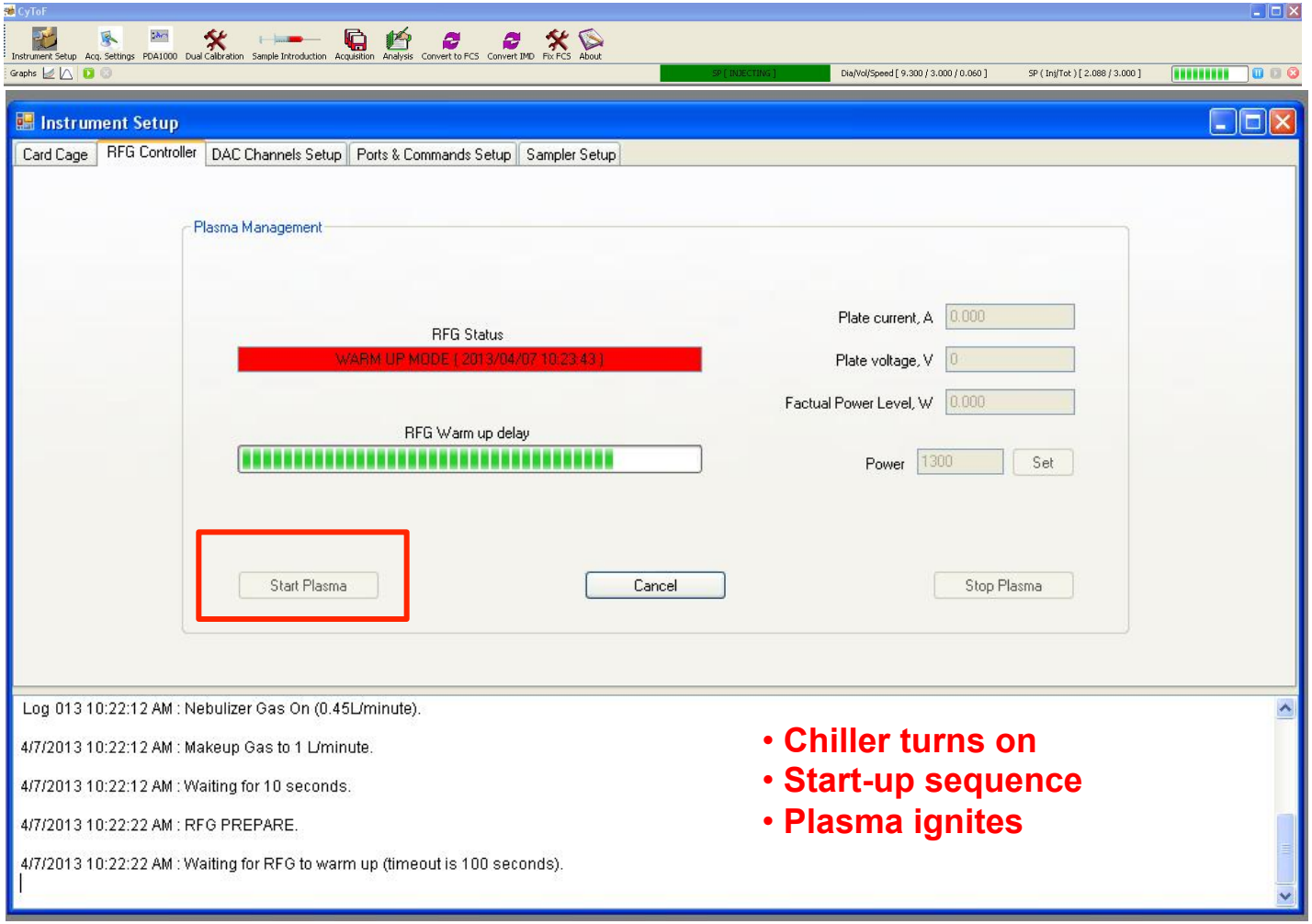
Start Plasma



Start Syringe pump (HPLC)

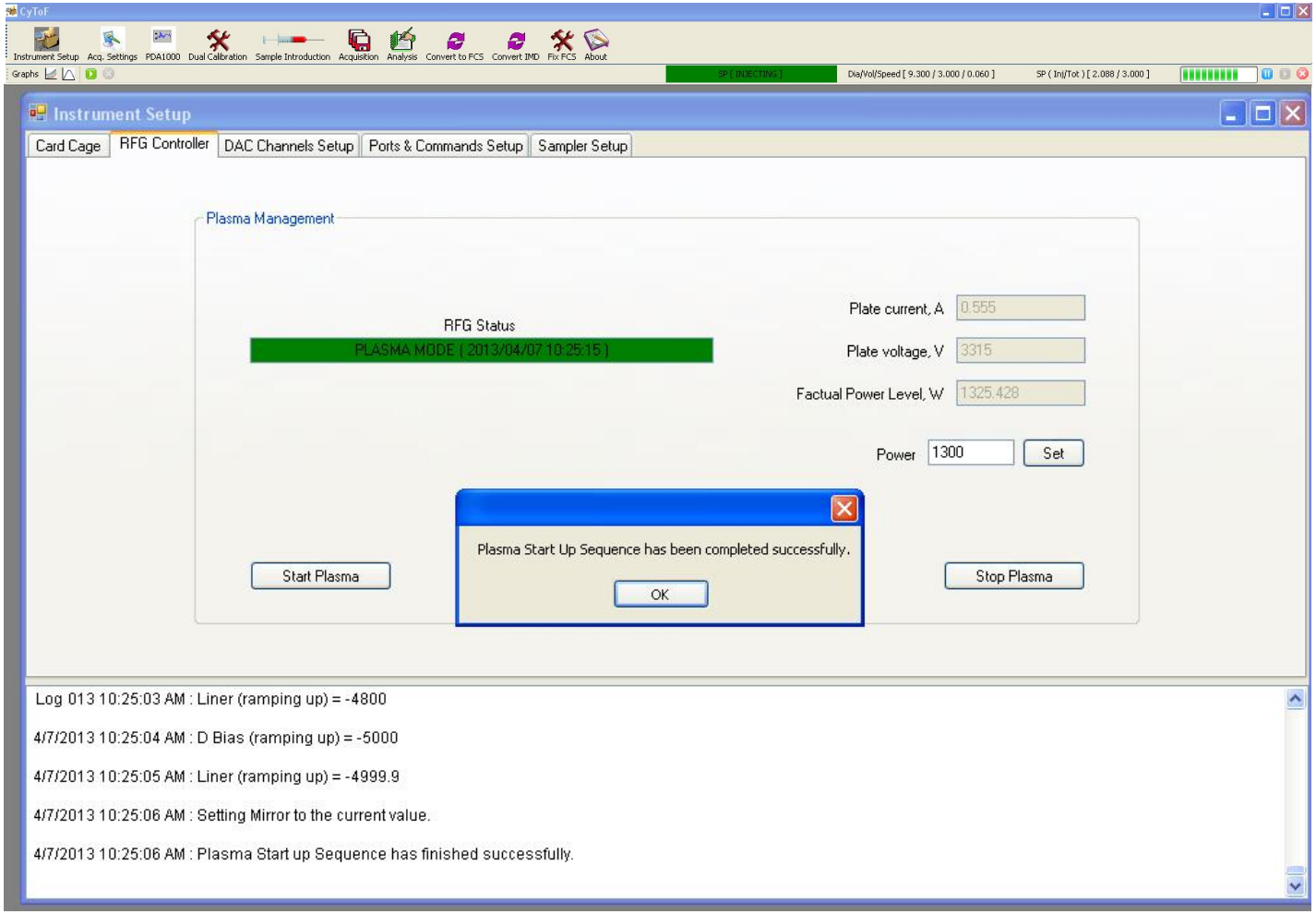


Instrument Startup



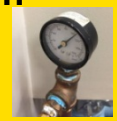
- **Chiller turns on**
- **Start-up sequence**
- **Plasma ignites**

Instrument Startup



Instrument Startup

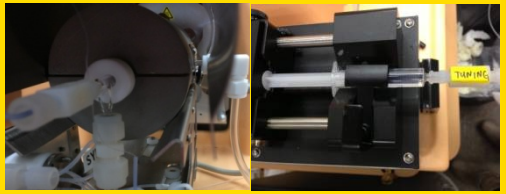
Make sure Argon is on
- 100 psi at wall
- 50 psi on instrument



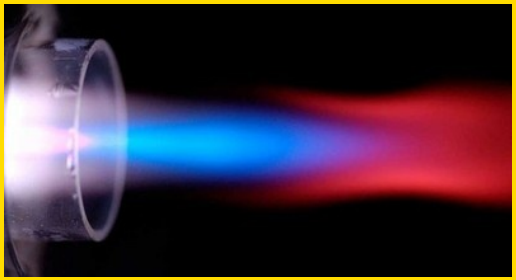
Check lights on instrument

Turn Heater on
Instrument setup → Card Cage Tab → Click Heater on → 200°C

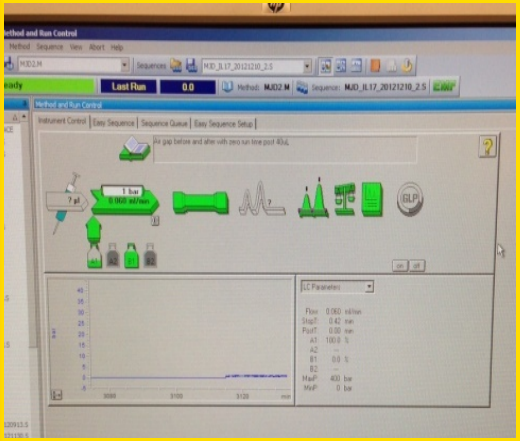
Prepare Nebulizer and Syringe Pump



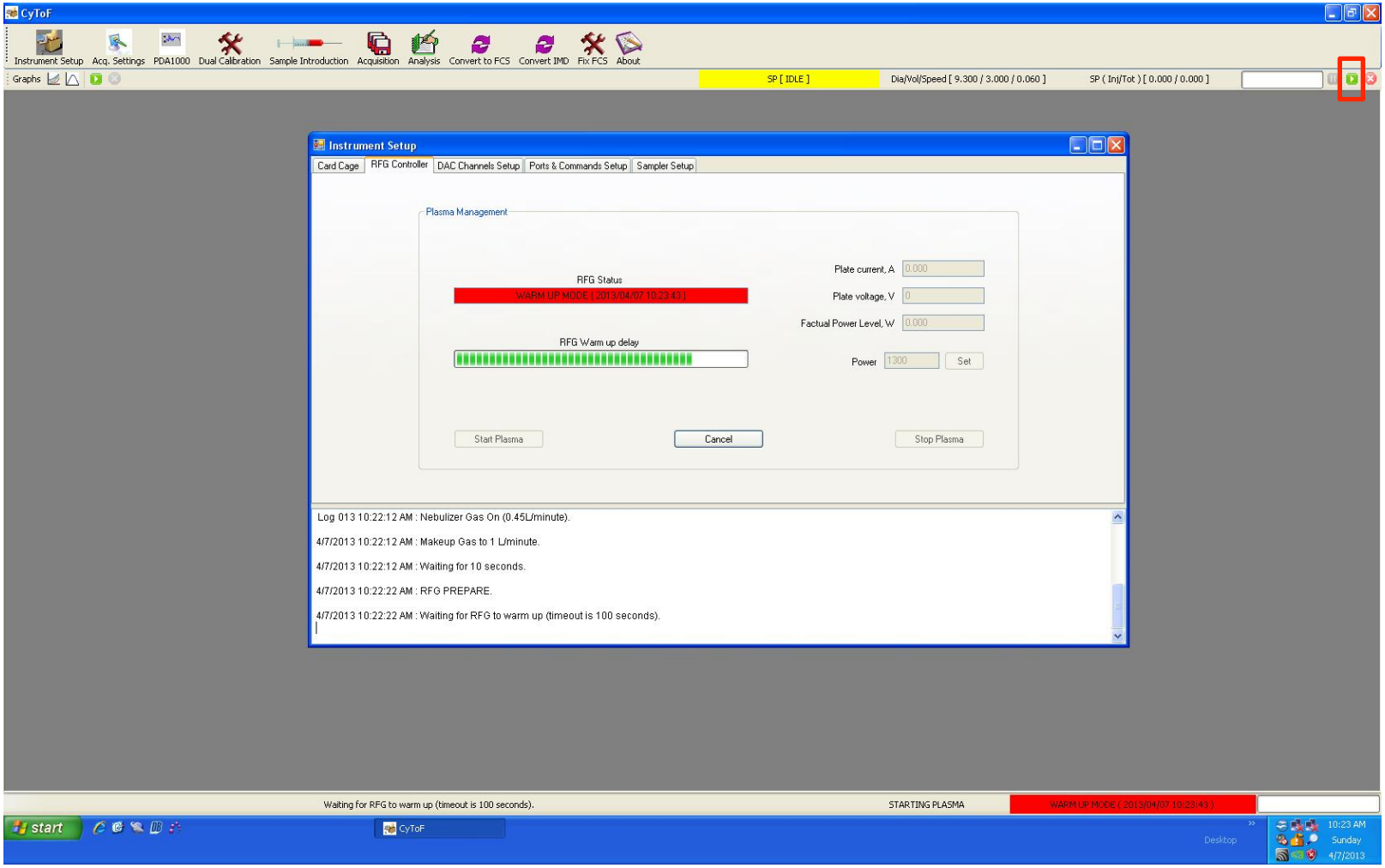
Start Plasma



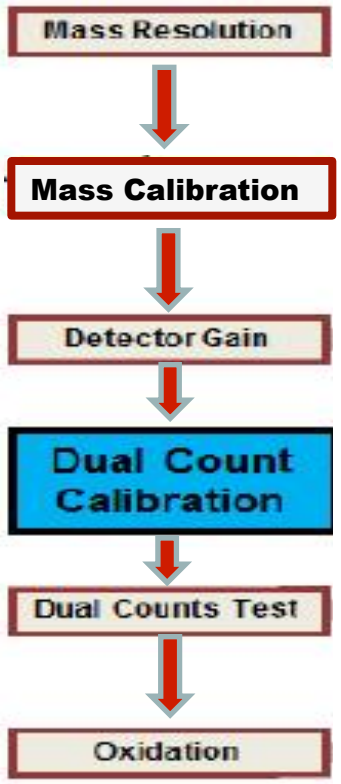
Start Syringe pump (HPLC)



Instrument Startup



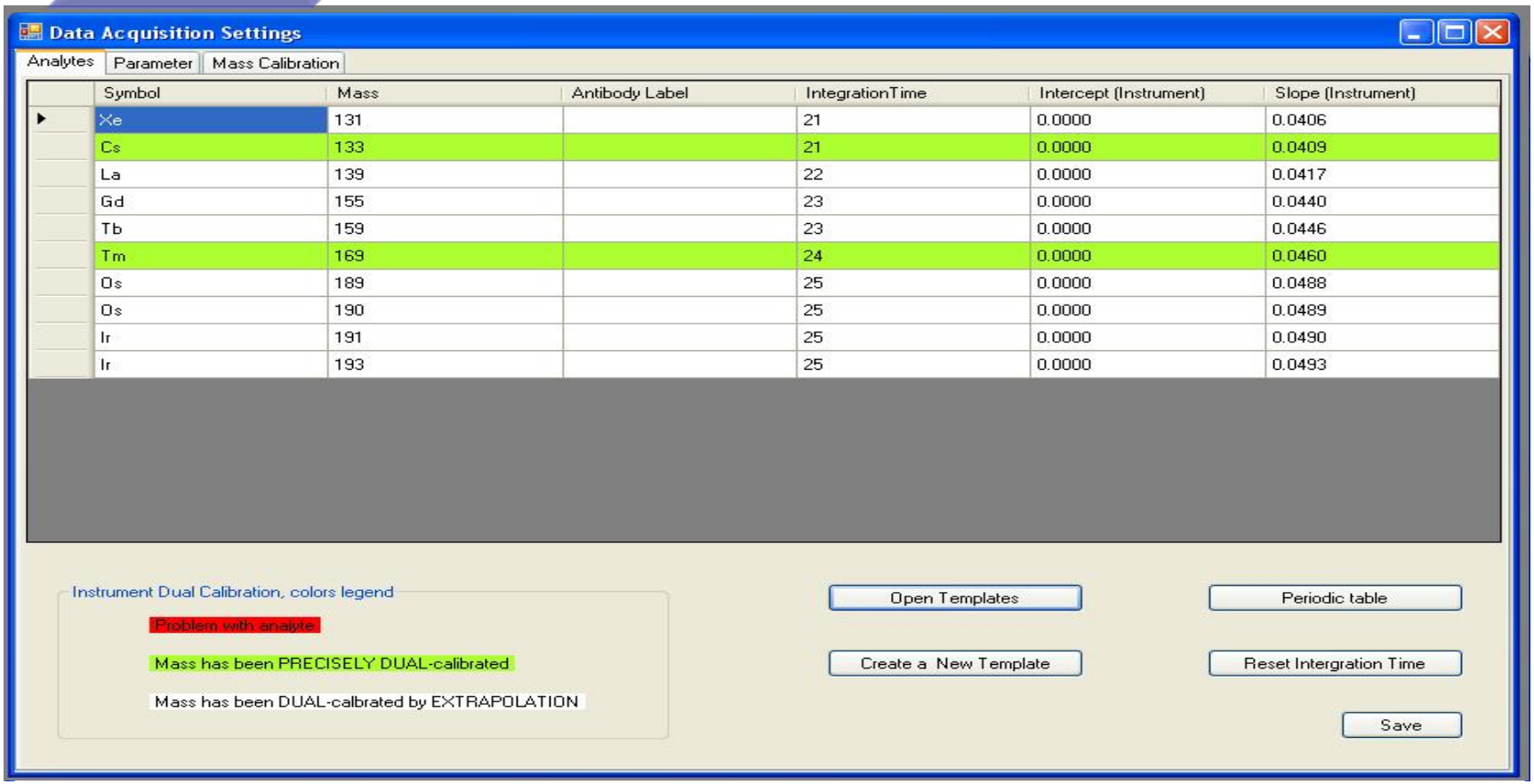
Optimization: Performance Check and Tuning



- **Mass Resolution** ► To get the best resolution between detection channels
- **Mass Calibration** ► Makes sure TOF values for CS and IR are <5% of peak height, calibration doesn't typically need to be changed
- **Detector Gain** ► Optimize to ensure efficient detection of ions
- **Dual Count Calibration** ► The dual count coefficient is calculated to generate the dual signal scale that is used to quantify ion signals
- **Dual Counts Test** ► Measure sensitivity by doing a dual counts tests to ensure ^{159}Tb dual counts are >100,000
- **Oxidation** ► Check Oxide ratio as an indication of plasma robustness. The ratio of ^{155}Gd dual count /^{159}Tb dual count should be less than 3%

Optimization: Performance Check and Tuning

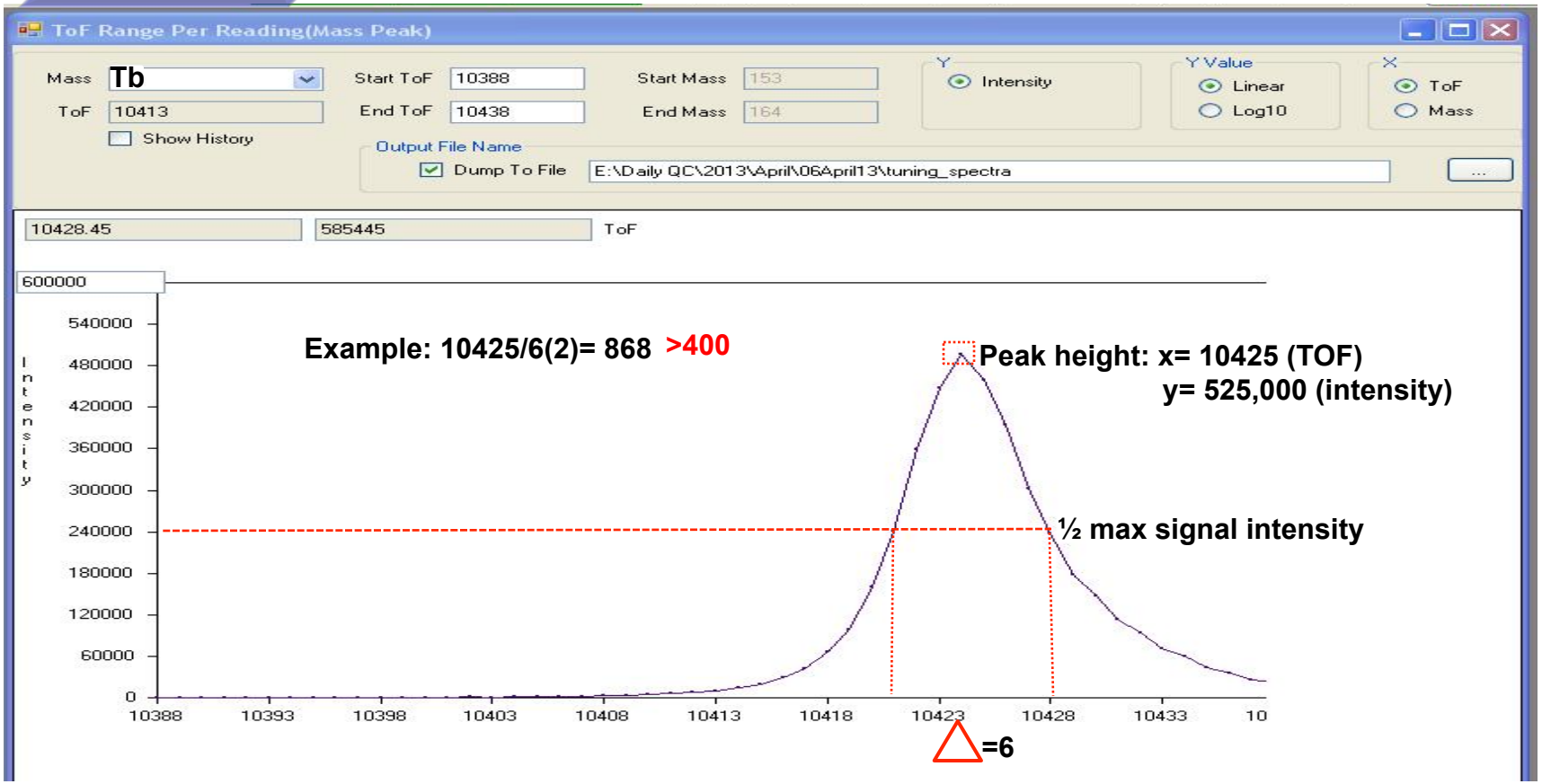
Tuning Solution



Optimization: Performance Check and Tuning

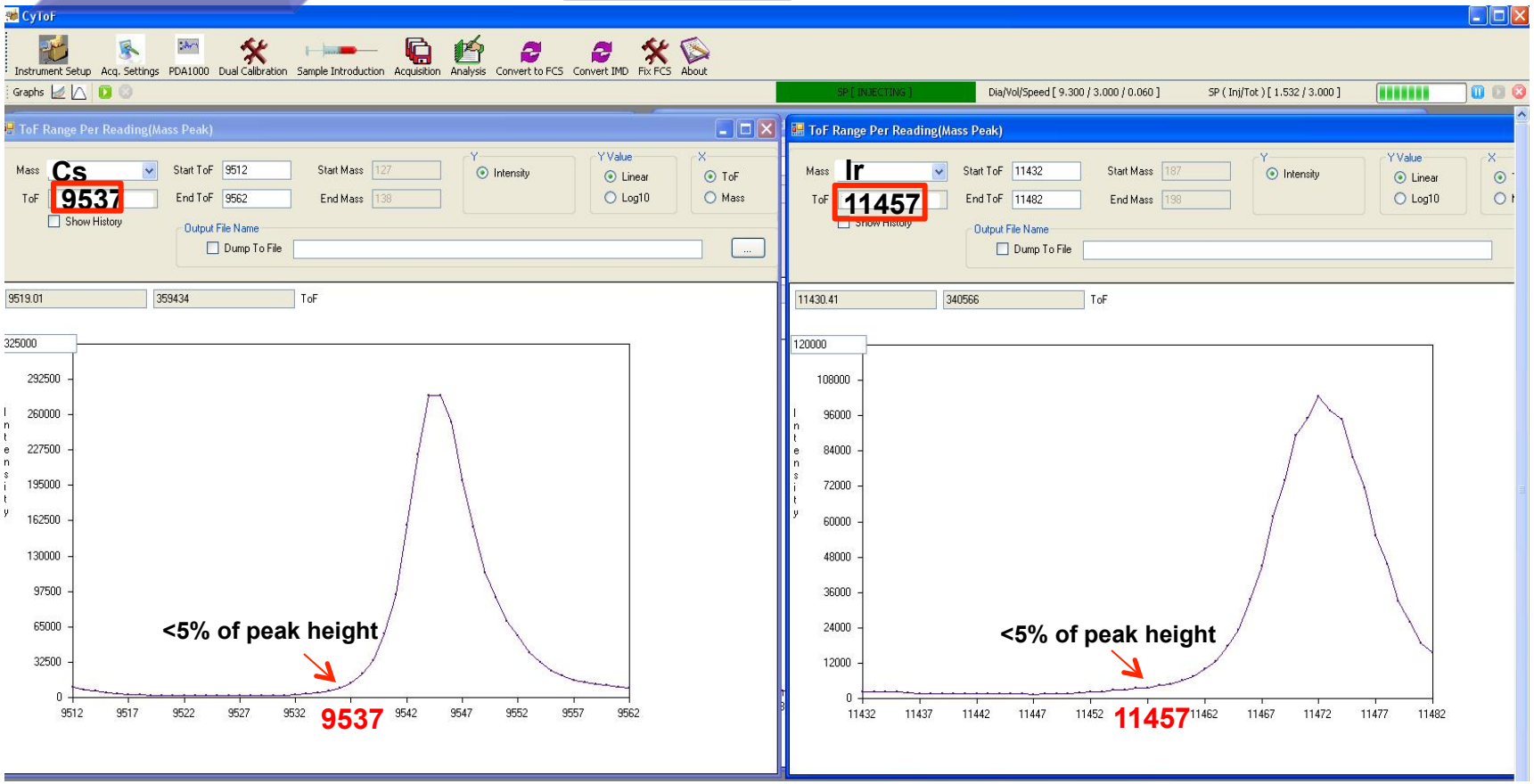
Tuning Solution

Mass Resolution



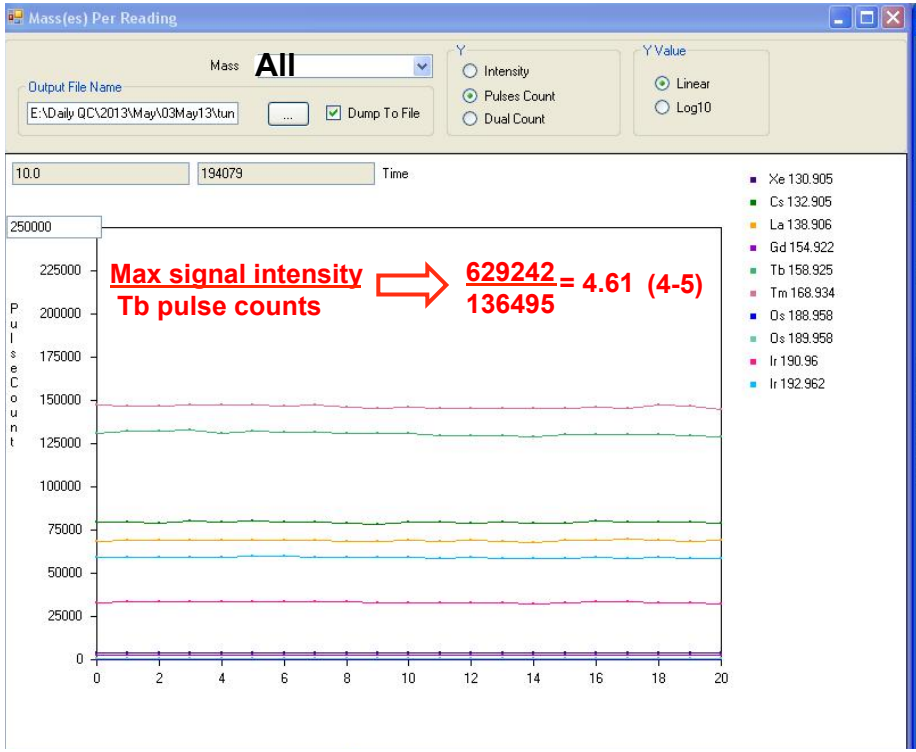
Optimization: Performance Check and Tuning

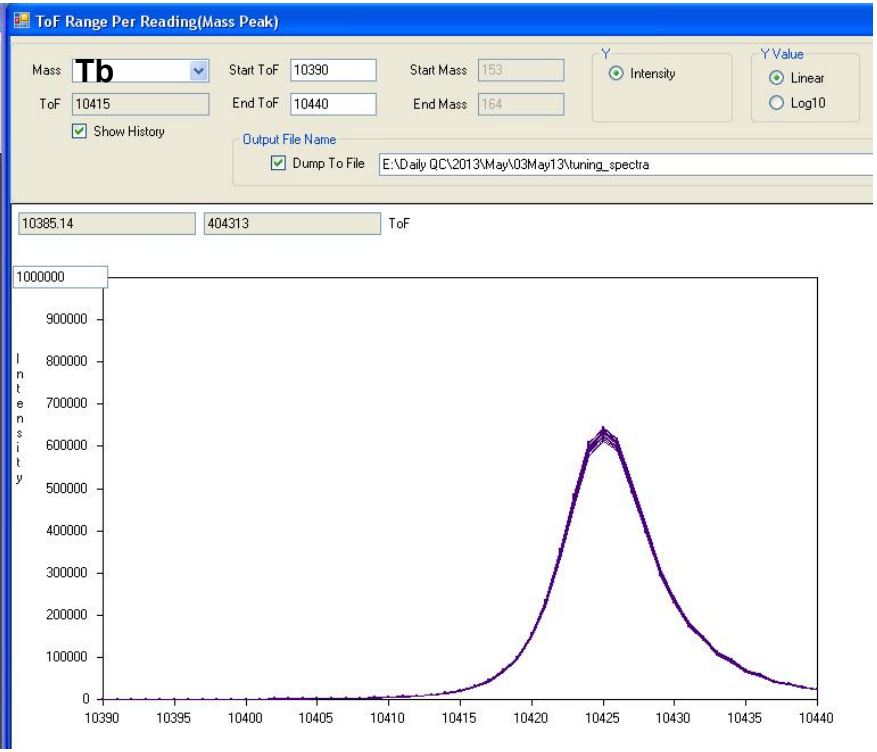
Tuning Solution → Mass Resolution → Mass Calibration



Optimization: Performance Check and Tuning

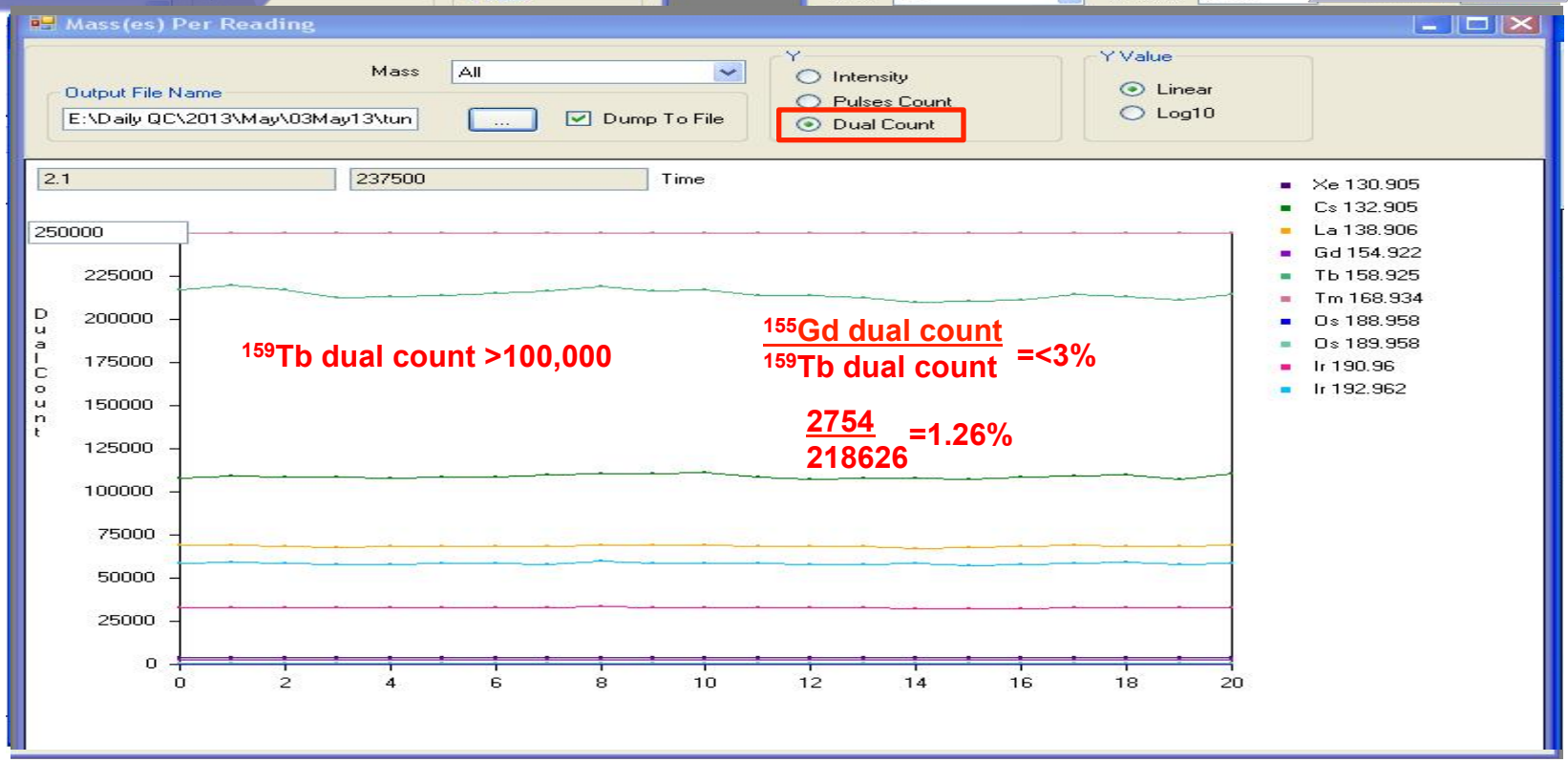
Tuning Solution → Mass Resolution → Mass Calibration → Detector gain





Optimization: Performance Check and Tuning

Tuning Solution → Mass Resolution → Mass Calibration → Detector gain → Dual Count Calibration → Dual Count test Oxidation



CYTOF is Ready!

CyTOF Calculations for Instument Performance/Tuning

Date:

1. **Ratio of Tb:** Intensity of Tb on mass peaks graph_____ /Tb pulse counts _____= _____ (3.0-5.0)
   For troubleshooting, optimize detector voltage

2. **Resolution of Tb:**
   a. Recorded x value at peak height =_____

   b. Record y value at peak height =_____ /2 =_____

   c. x- intercept 1 (at ½ peak height (y))=_____ x-intercept 2=_____ difference=____

   d. recorded x value at peak height/2/difference = _____ (should be >400)

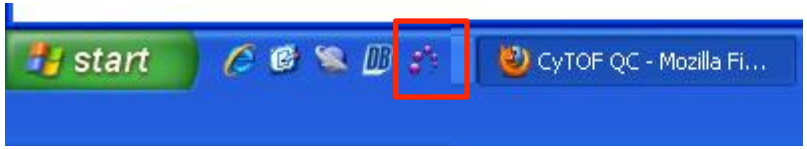
3. **Mass Calibration Values for Cs and Ir2:**
   a. Cs: peak height=_____ x .03= _____; x value at .03 peak height = _____
   (check against values in Calibration tab or Data Acq settings)

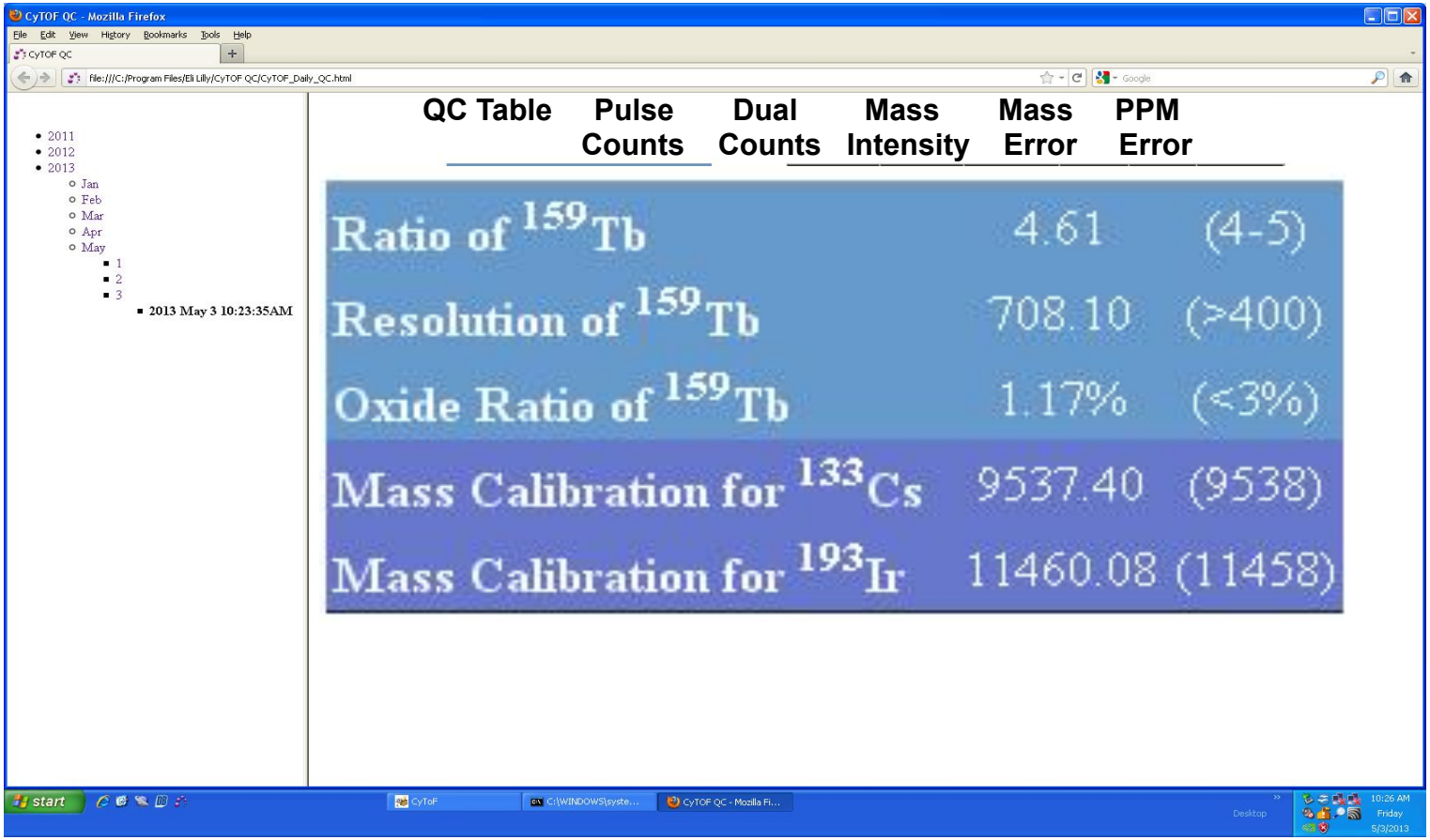
   b. Ir2: peak height=_____ x .03= _____; x value at .03 peak height = _____
   (check against values in Calibration tab or Data Acq settings)

4. **Oxide Ratio:**
   a. Record level of Tb _____ and Gd _____

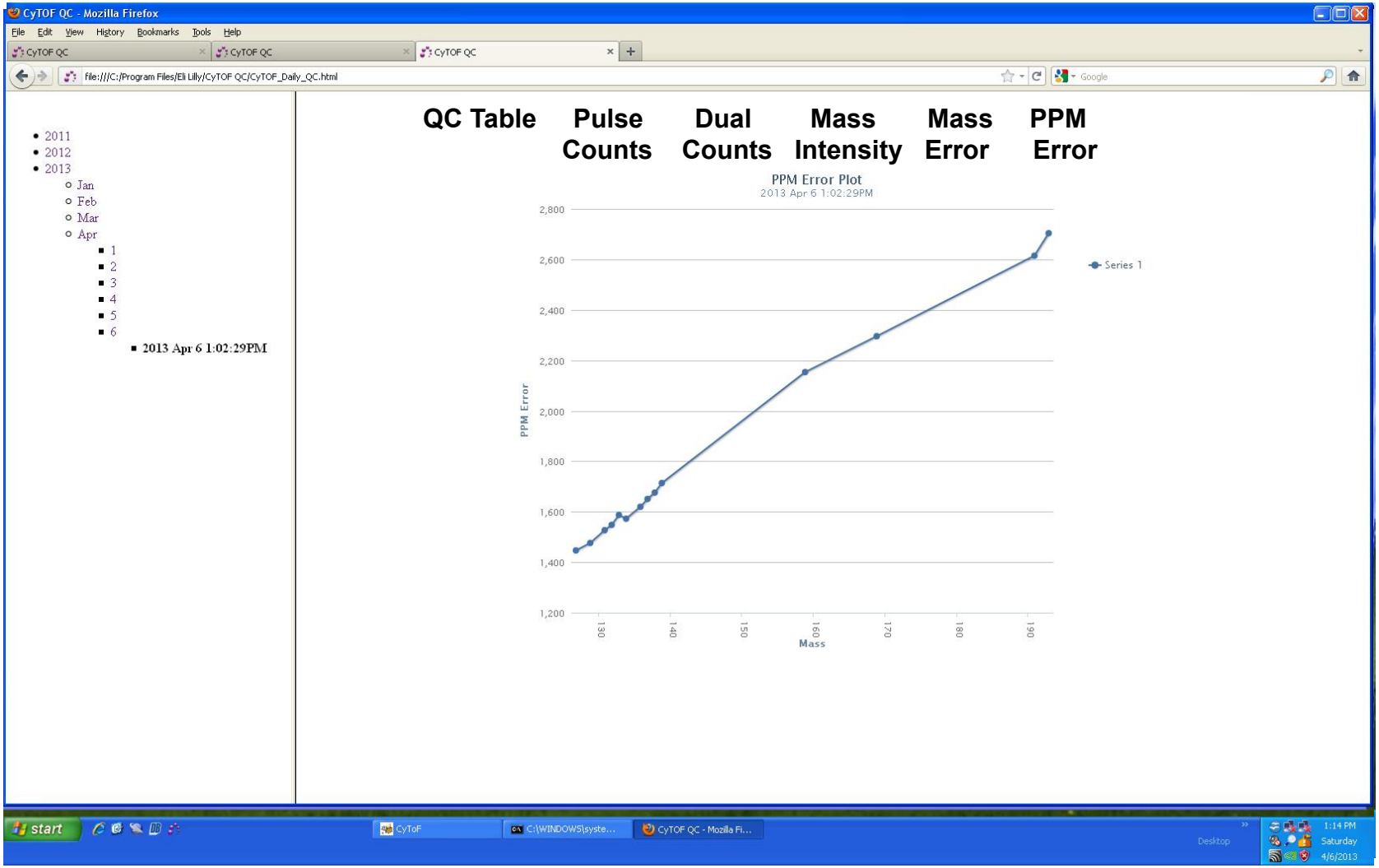
   b. Gd/Tb x 100= <3 → _____ / _____ =_____

Automated QC at Lilly





Automated QC at Lilly



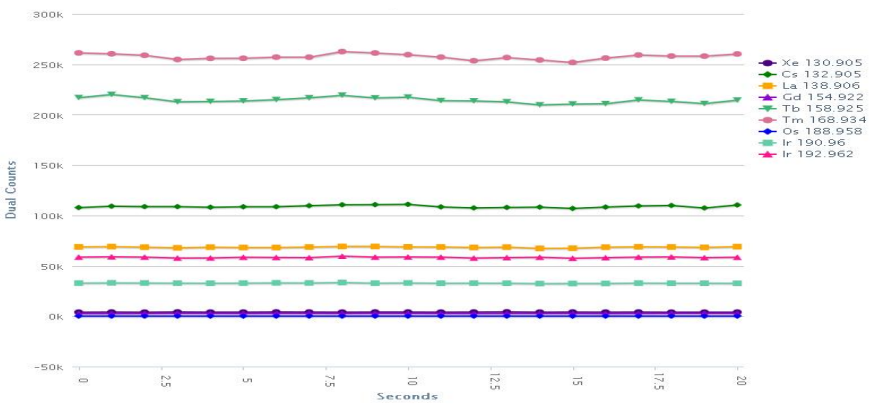
Automated QC at Lilly

Ratio of ^{159}Tb	4.61	(4-5)
Resolution of ^{159}Tb	708.10	(>400)
Oxide Ratio of ^{159}Tb	1.17%	(<3%)
Mass Calibration for ^{133}Cs	9537.40	(9538)
Mass Calibration for ^{193}Ir	11460.08	(11458)

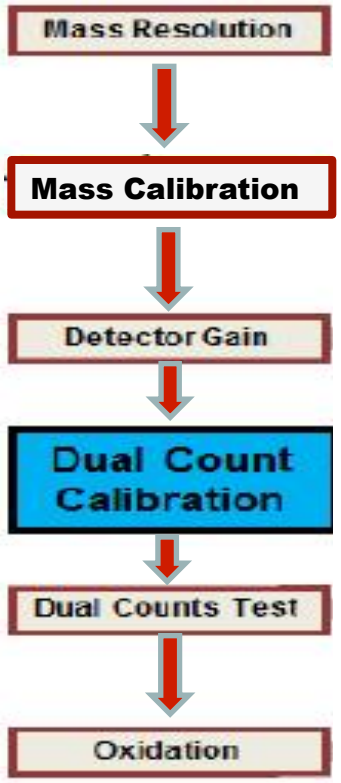
Dual count calibration

Ratio of ^{159}Tb	4.72	(4-5)
Resolution of ^{159}Tb	722.71	(>400)
Oxide Ratio of ^{159}Tb	1.26%	(<3%)
Mass Calibration for ^{133}Cs	9537.70	(9538)
Mass Calibration for ^{193}Ir	11460.27	(11458)

Dual counts plot

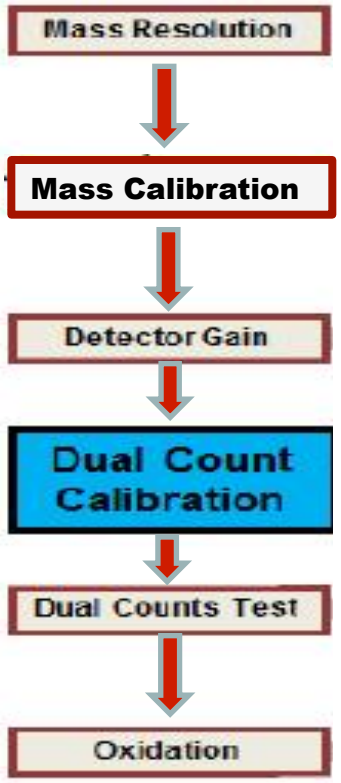


When Specs Are Not Met



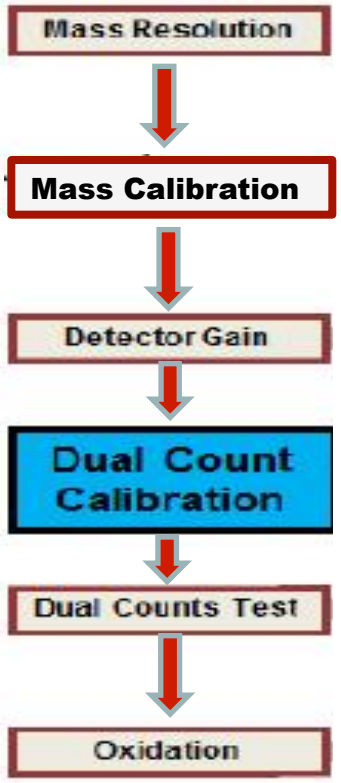
Parameter	Observation	Possible Solution
Mass Resolution	<400	Call for service
Mass Calibration	ToF values for Cs and Ir at beginning of peak are >5% of peak height	Verify ToF values and enter new values in the mass calibration tab
Detector Gain	^{159}Tb ratio is<4 or >5	optimize detector voltage
Dual Count Calibration	^{159}Tb dual count <100,000	Check X-Y alignment Optimize gas flows Optimize current Swap nebulizer
Dual Counts Test	$\frac{^{155}\text{Gd dual count}}{^{159}\text{Tb dual count}}$ >3%	Optimize gas flows
Oxidation		

When Specs Are Not Met



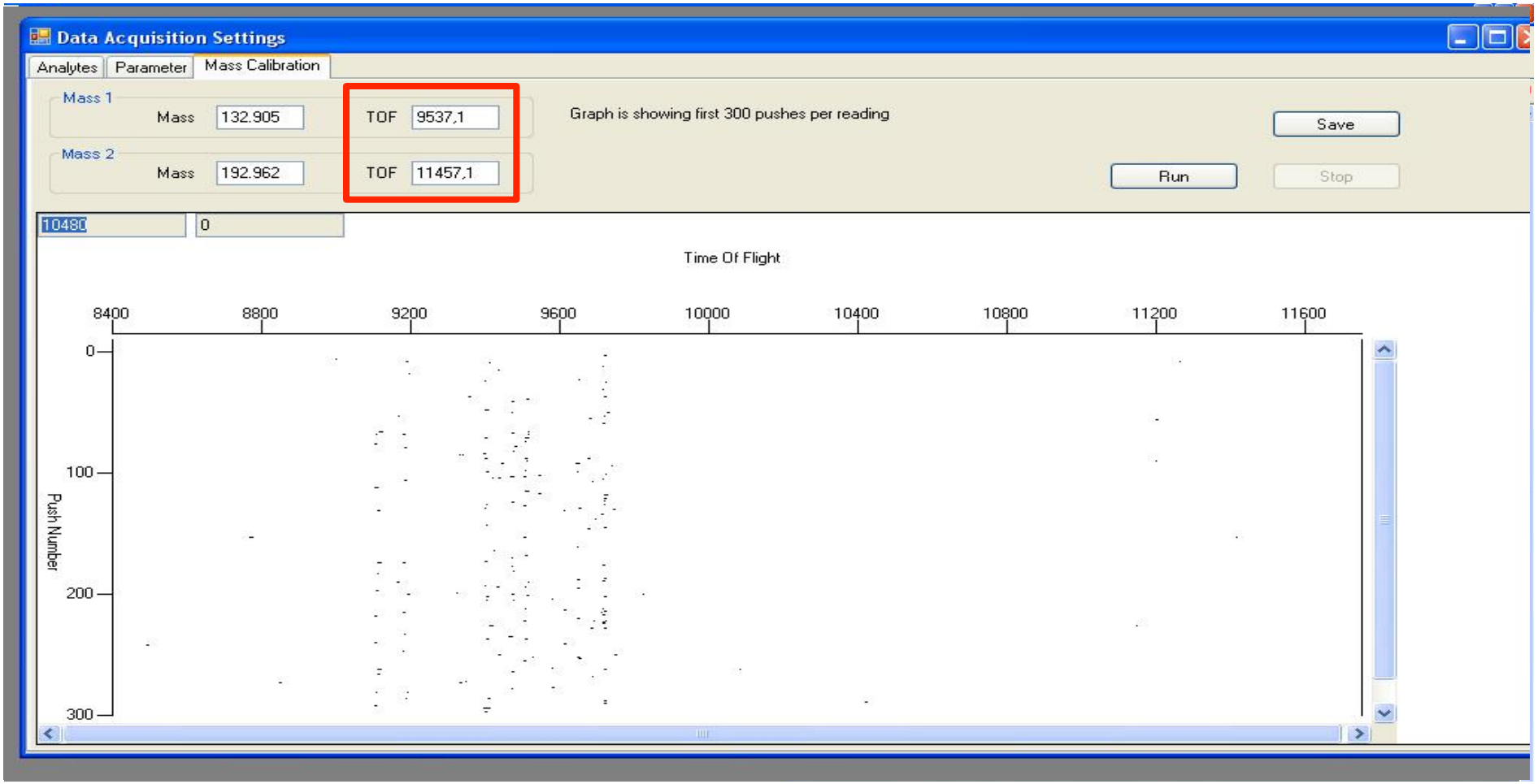
Parameter	Observation	Possible Solution
Mass Resolution	<400	Call for service
Mass Calibration	ToF values for Cs and Ir at beginning of peak are >5% of peak height	Verify ToF values and enter new values in the mass calibration tab
Detector Gain	^{159}Tb ratio is<4 or >5	optimize detector voltage
Dual Count Calibration	^{159}Tb dual count <100,000	Check X-Y alignment Optimize gas flows Optimize current Swap nebulizer
Dual Counts Test	$\frac{^{155}\text{Gd dual count}}{^{159}\text{Tb dual count}}$ >3%	Optimize gas flows
Oxidation		

When Specs Are Not Met

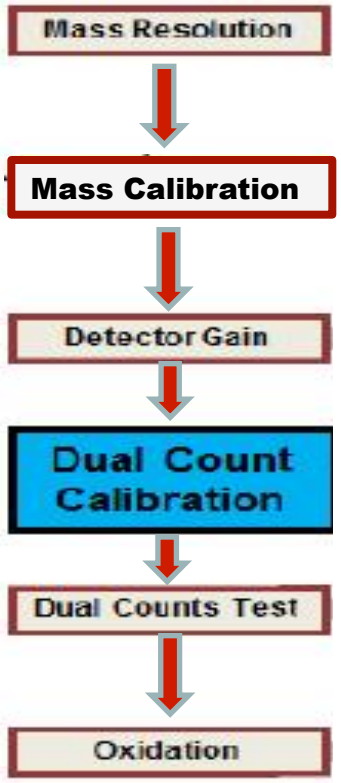


Parameter	Observation	Possible Solution
Mass Resolution	<400	Call for service
Mass Calibration	ToF values for Cs and Ir at beginning of peak are >5% of peak height	Verify ToF values and enter new values in the mass calibration tab
Detector Gain	^{159}Tb ratio is<4 or >5	optimize detector voltage
Dual Count Calibration	^{159}Tb dual count <100,000	Check X-Y alignment Optimize gas flows Optimize current Swap nebulizer
Dual Counts Test	$\frac{^{155}\text{Gd dual count}}{^{159}\text{Tb dual count}}$ >3%	Optimize gas flows
Oxidation		

Verify Mass Calibration

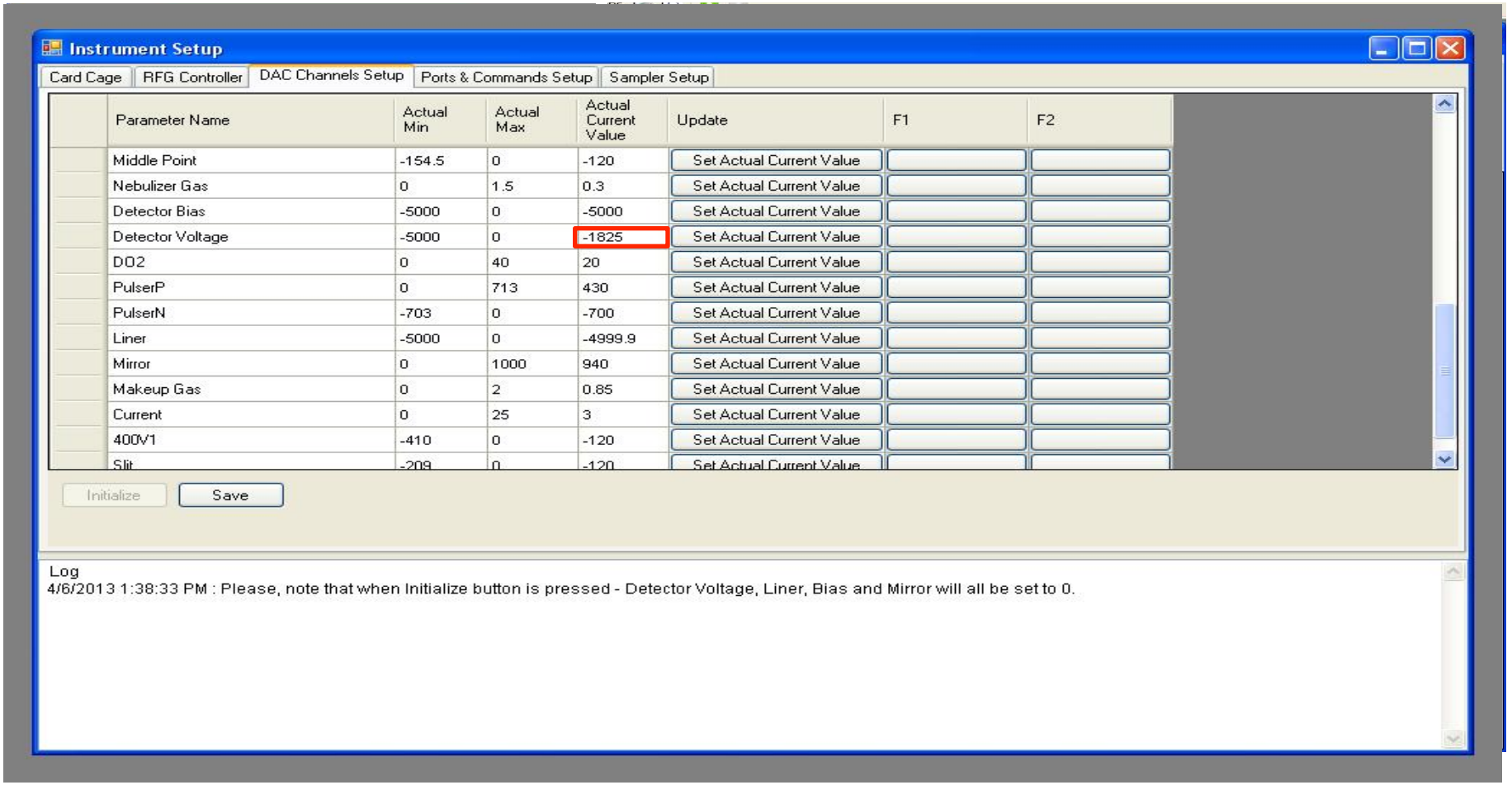


When Specs Are Not Met

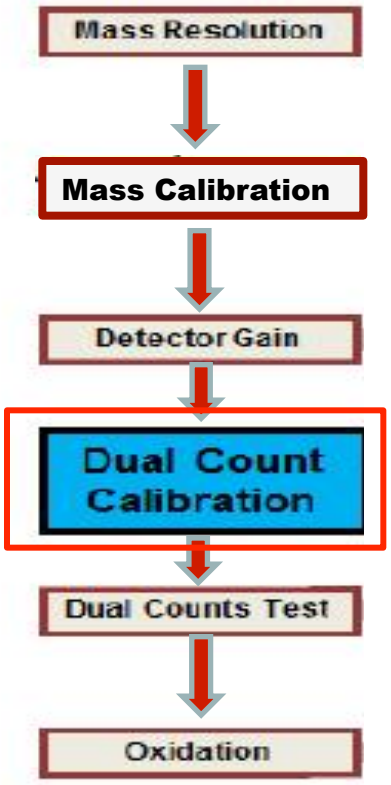


Parameter	Observation	Possible Solution
Mass Resolution	<400	Call for service
Mass Calibration	ToF values for Cs and Ir at beginning of peak are >5% of peak height	Verify ToF values and enter new values in the mass calibration tab
Detector Gain	^{159}Tb ratio is<4 or >5	optimize detector voltage
Dual Count Calibration	^{159}Tb dual count <100,000	Check X-Y alignment Optimize gas flows Optimize current Swap nebulizer
Dual Counts Test	$\frac{^{155}\text{Gd dual count}}{^{159}\text{Tb dual count}}$ >3%	Optimize gas flows
Oxidation		

Optimize Detector Voltage

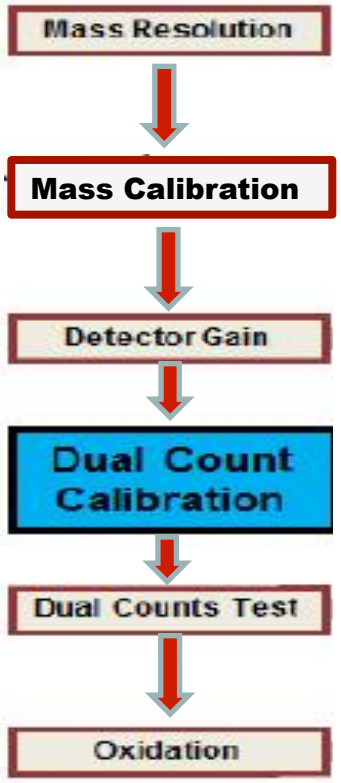


When Specs Are Not Met



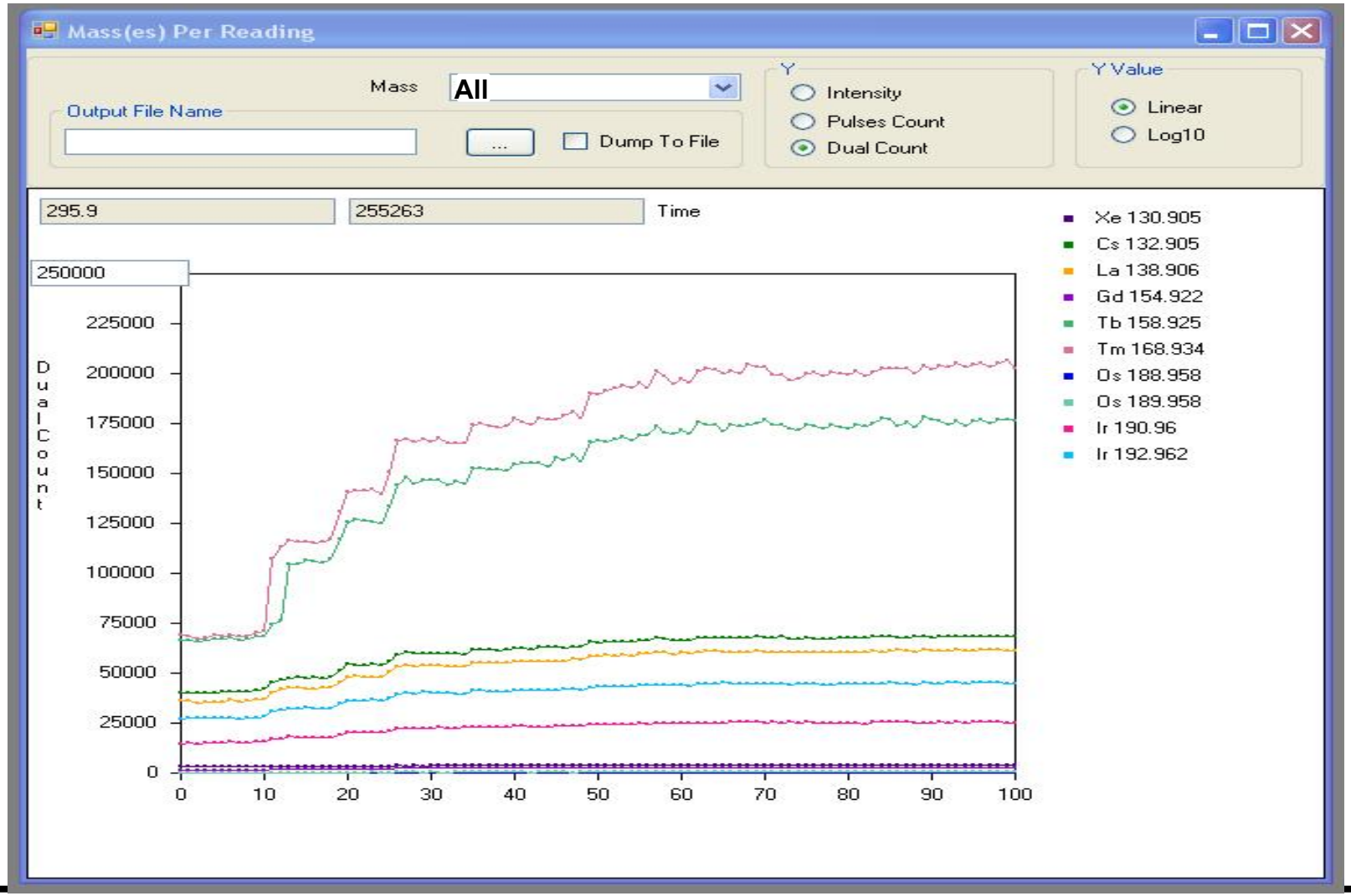
Parameter	Observation	Possible Solution
Mass Resolution	<400	Call for service
Mass Calibration	ToF values for Cs and Ir at beginning of peak are >5% of peak height	Verify ToF values and enter new values in the mass calibration tab
Detector Gain	^{159}Tb ratio is<4 or >5	optimize detector voltage
Dual Count Calibration	^{159}Tb dual count <100,000	Check X-Y alignment Optimize gas flows Optimize current Swap nebulizer
Dual Counts Test	$\frac{^{155}\text{Gd dual count}}{^{159}\text{Tb dual count}}$ >3%	Optimize gas flows
Oxidation		

When Specs Are Not Met

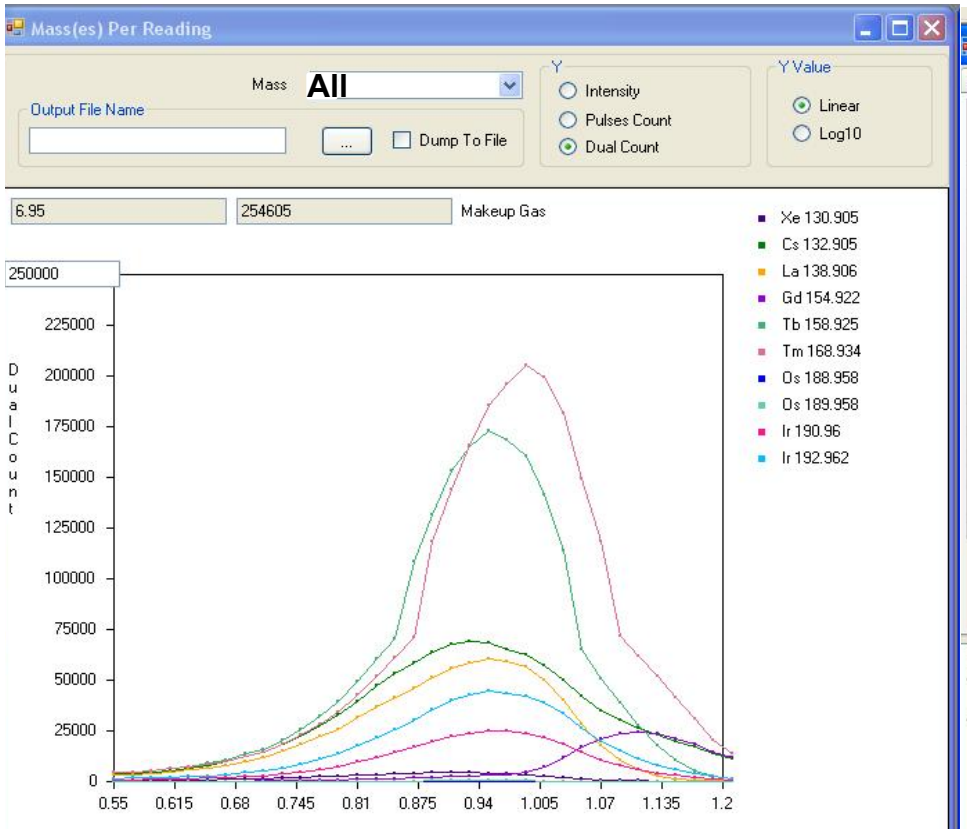


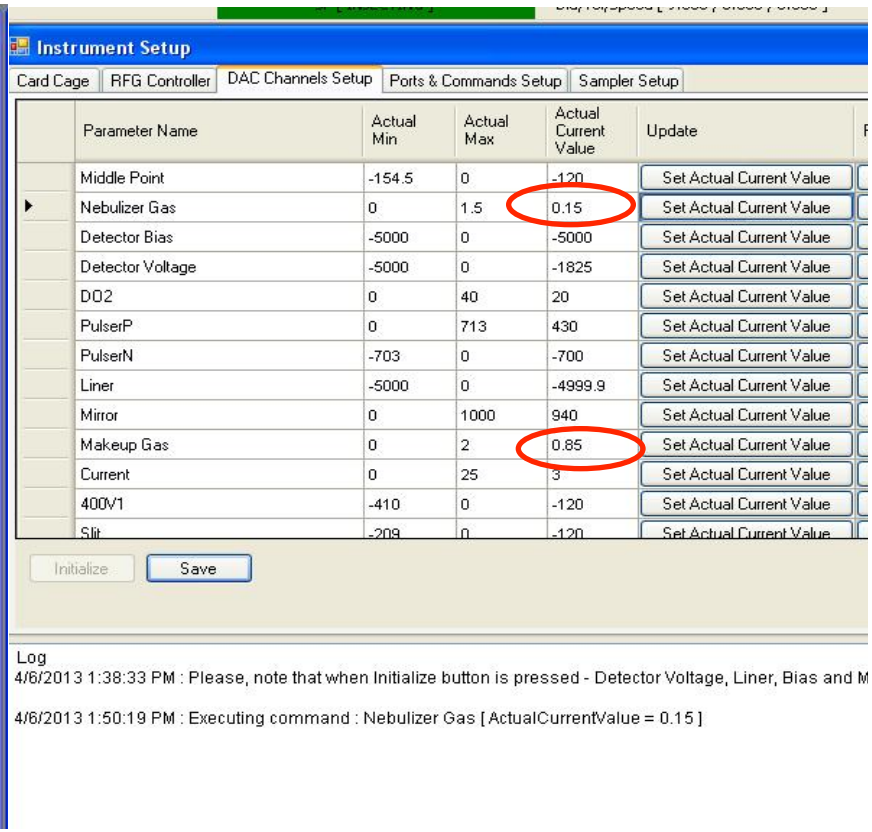
Parameter	Observation	Possible Solution
Mass Resolution	<400	Call for service
Mass Calibration	ToF values for Cs and Ir at beginning of peak are >3% of peak height	Verify ToF values and enter new values in the mass calibration tab
Detector Gain	^{159}Tb ratio is<4 or >5	optimize detector voltage
Dual Count Calibration	^{159}Tb dual count <100,000	Check X-Y alignment Optimize gas flows Optimize current Swap nebulizer
Dual Counts Test	$\frac{^{155}\text{Gd dual count}}{^{159}\text{Tb dual count}}$ >3%	Optimize gas flows
Oxidation		

X-Y Alignment of the Interface



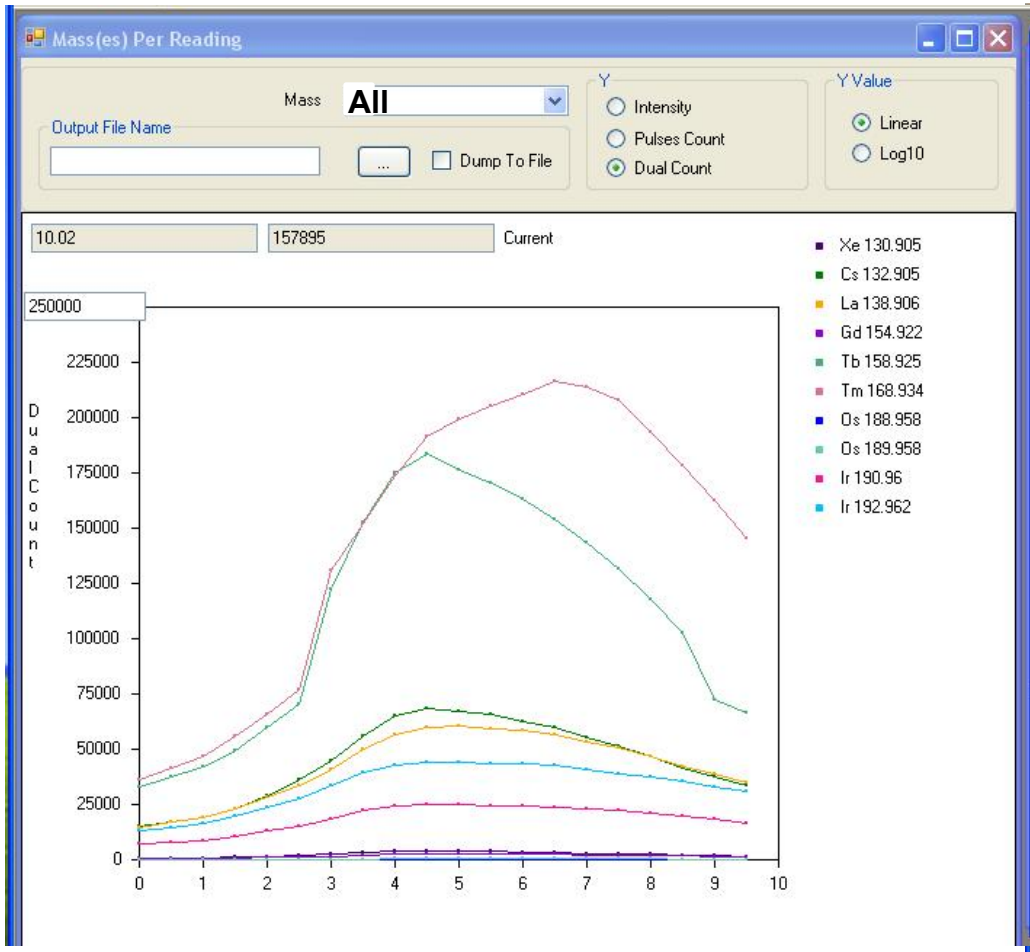
Optimize Gas Flows

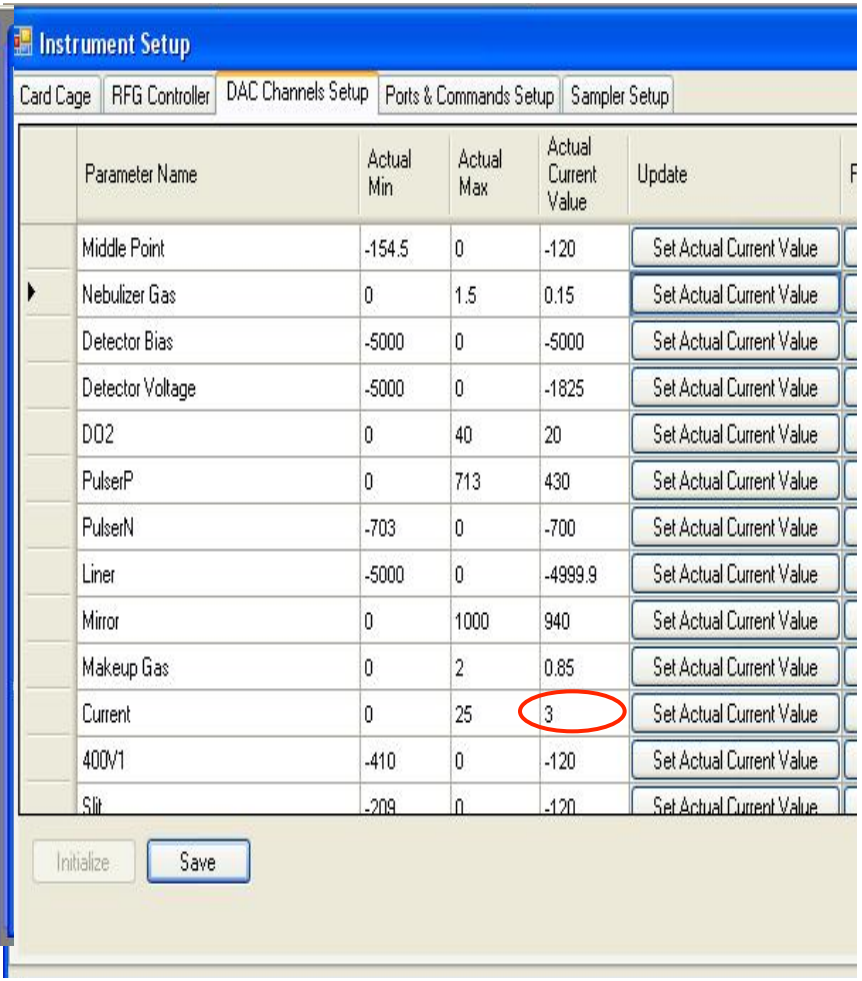




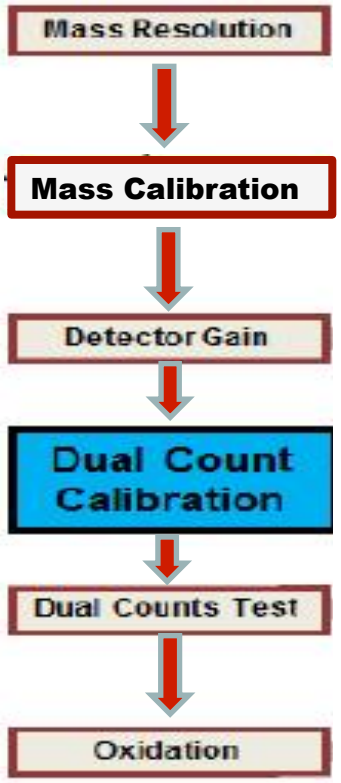
Date	Nebulizer Gas Value	Optimal Makeup Gas Value	Tb159 dual count	Gd155 dual count	Mass155/Tb159
2-May-13	0.15	1.008	208553	3947	0.018925645
	0.2	0.95	219079	3947	0.018016332
	0.25	0.88	219421	3947	0.017988251
	0.3	0.85	225000	4605	0.020466667
	0.35	0.77	225658	3289	0.014575154

Optimize Current



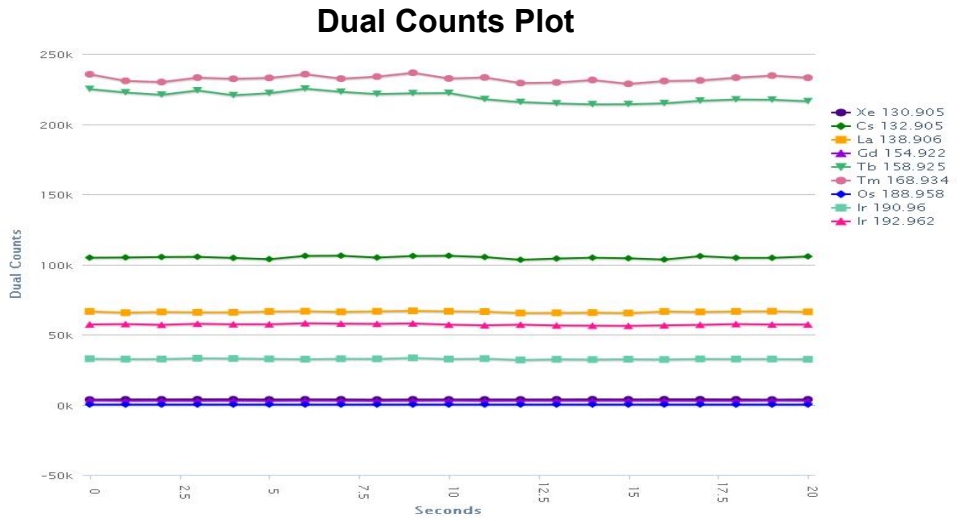


Re-Check QC



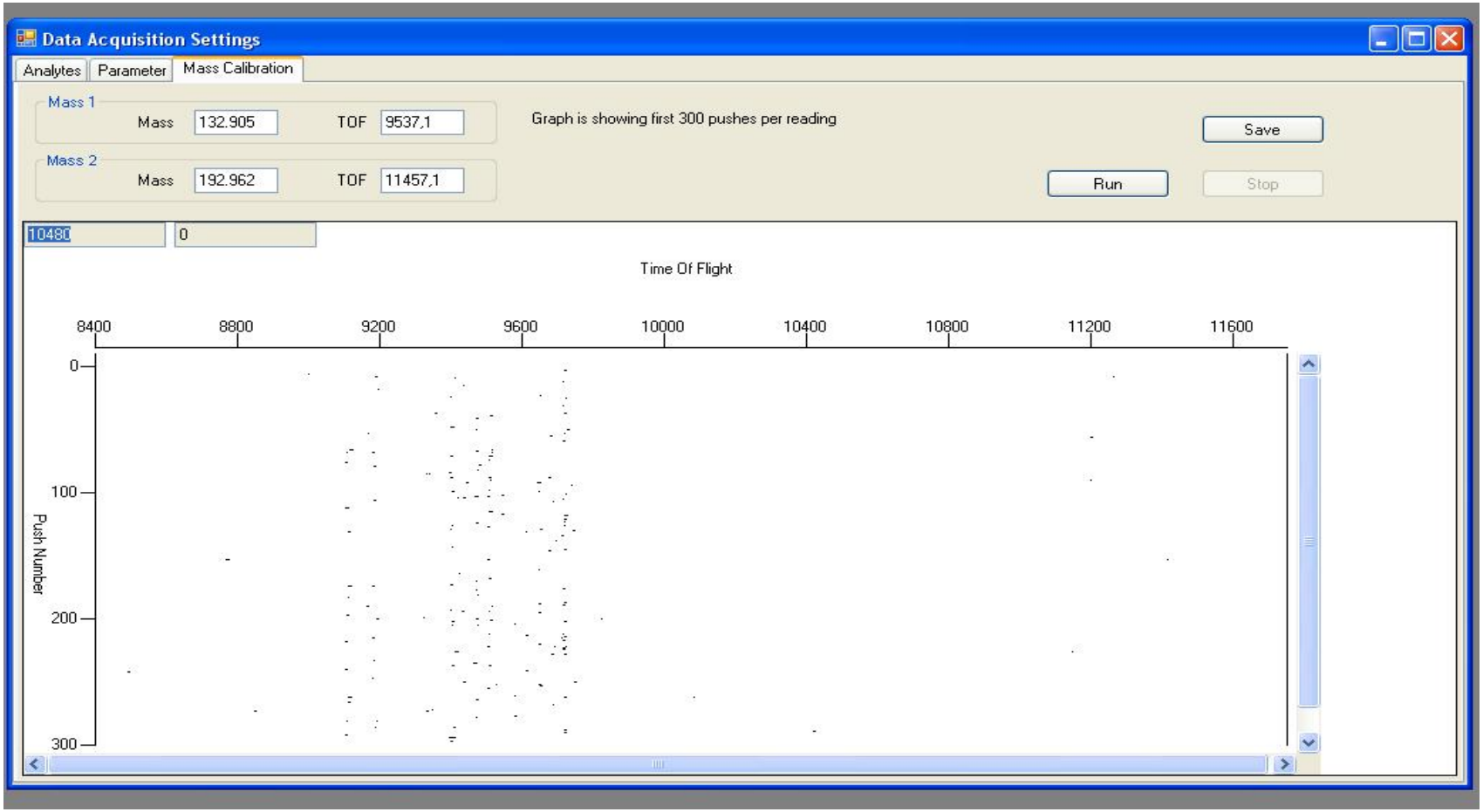
Ratio of ^{159}Tb	4.58	(4-5)
Resolution of ^{159}Tb	727.77	(>400)
Oxide Ratio of ^{159}Tb	2.05%	(<3%)
Mass Calibration for ^{133}Cs	9537.28	(9538)
Mass Calibration for ^{193}Ir	11459.98	(11458)

QC Tables | Pulses Count Plot | **Dual Count Plot** | Mass Intensity Plot | Mass Error Plot | PPM Error Plot

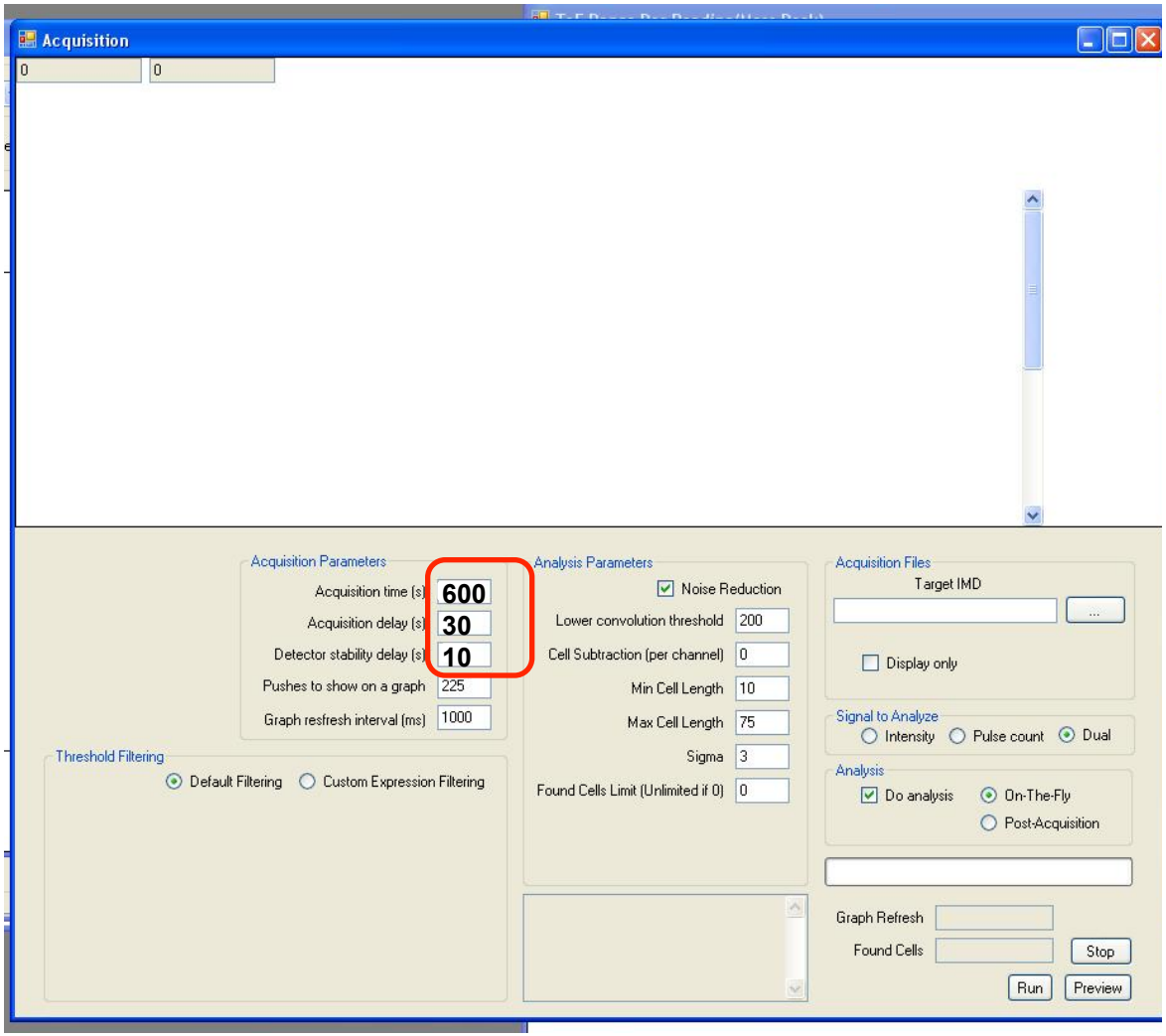


Running Samples: User Guidelines

- **Make sure sample loop is clean: run H20, check Mass Cal screen to verify**

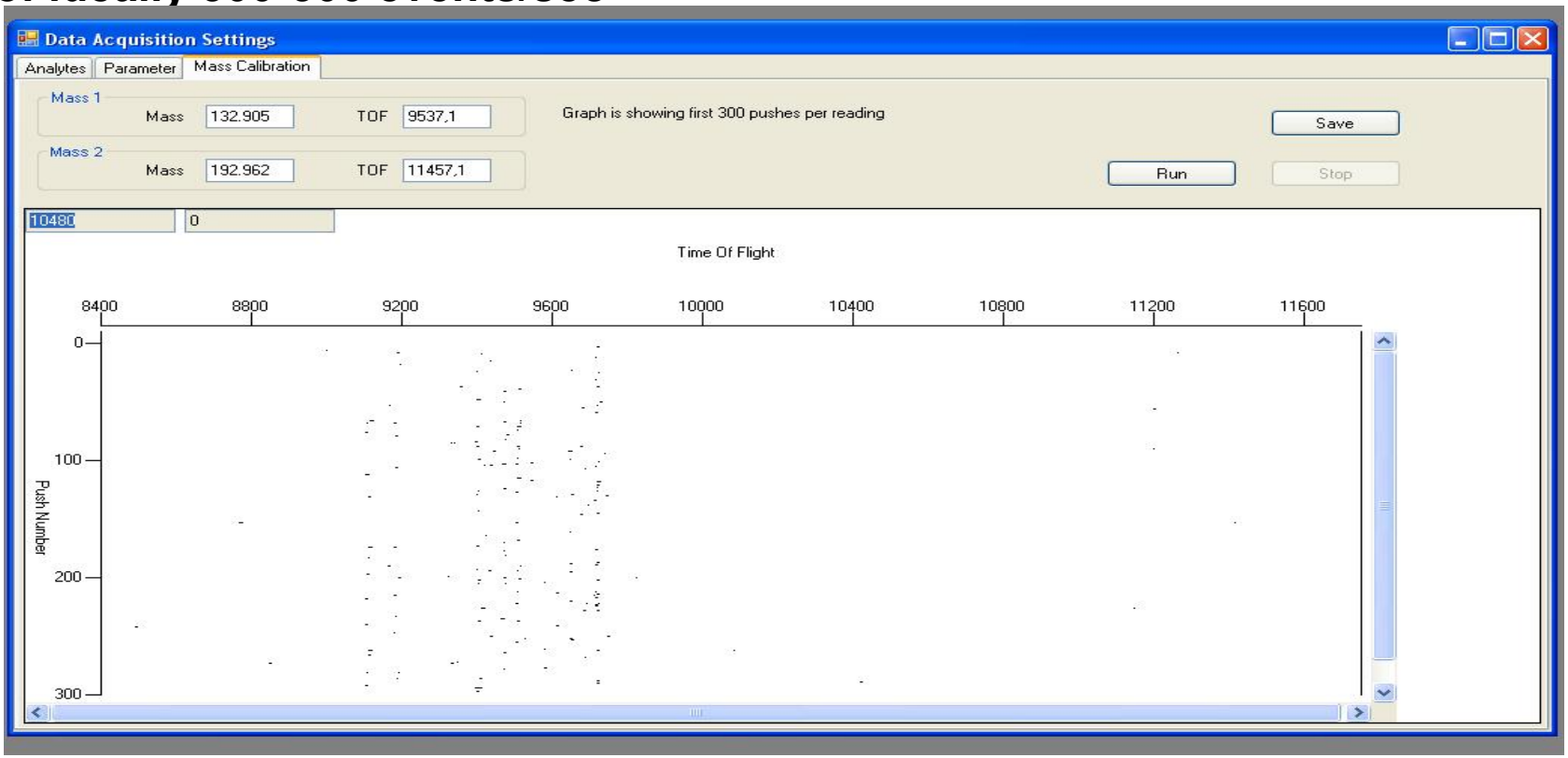


Running Samples: User Guidelines



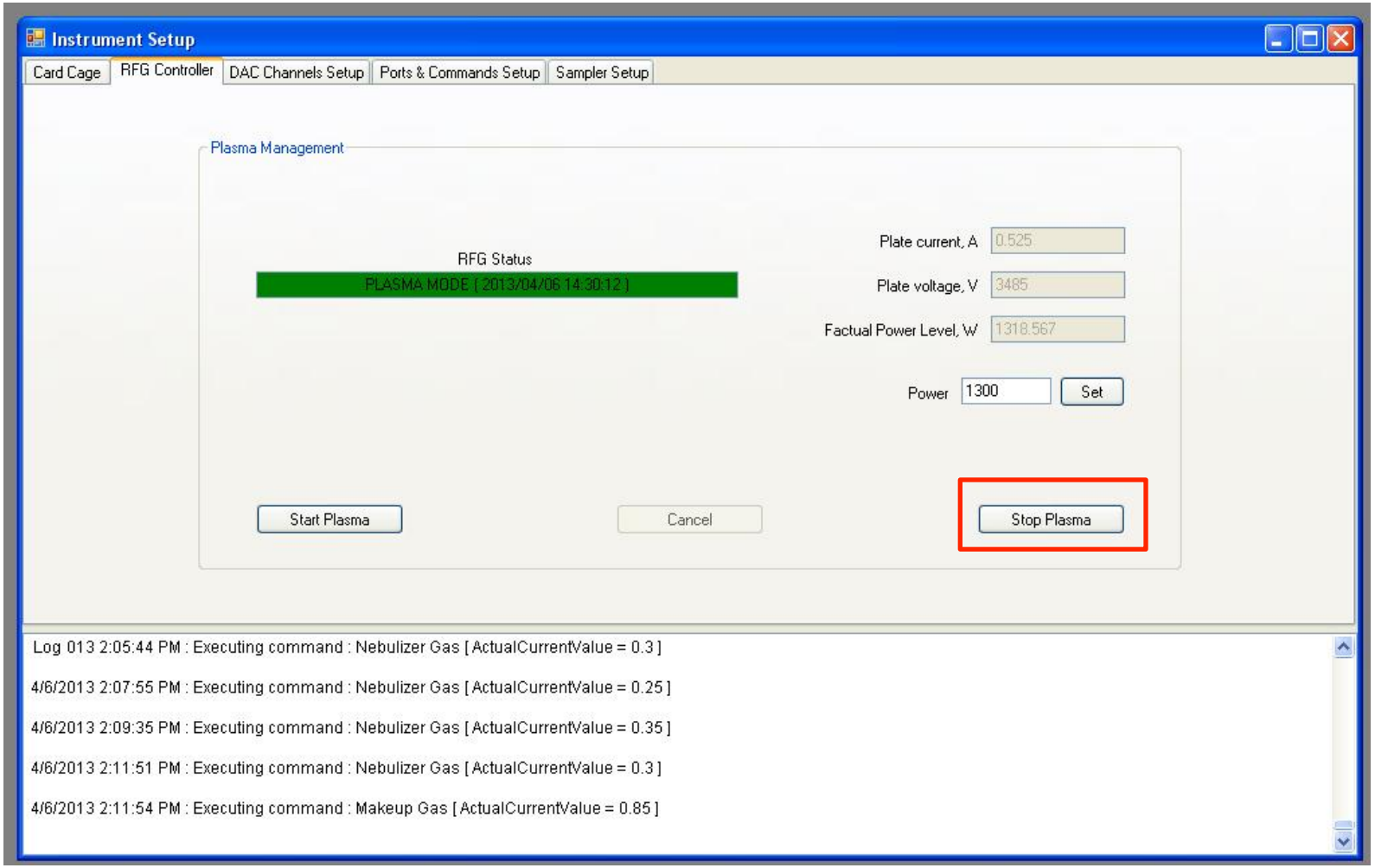
Running Samples: User Guidelines

- **Filter samples and count cells. Resuspend cells to achieve collection rate of ideally 300-500 events/sec**

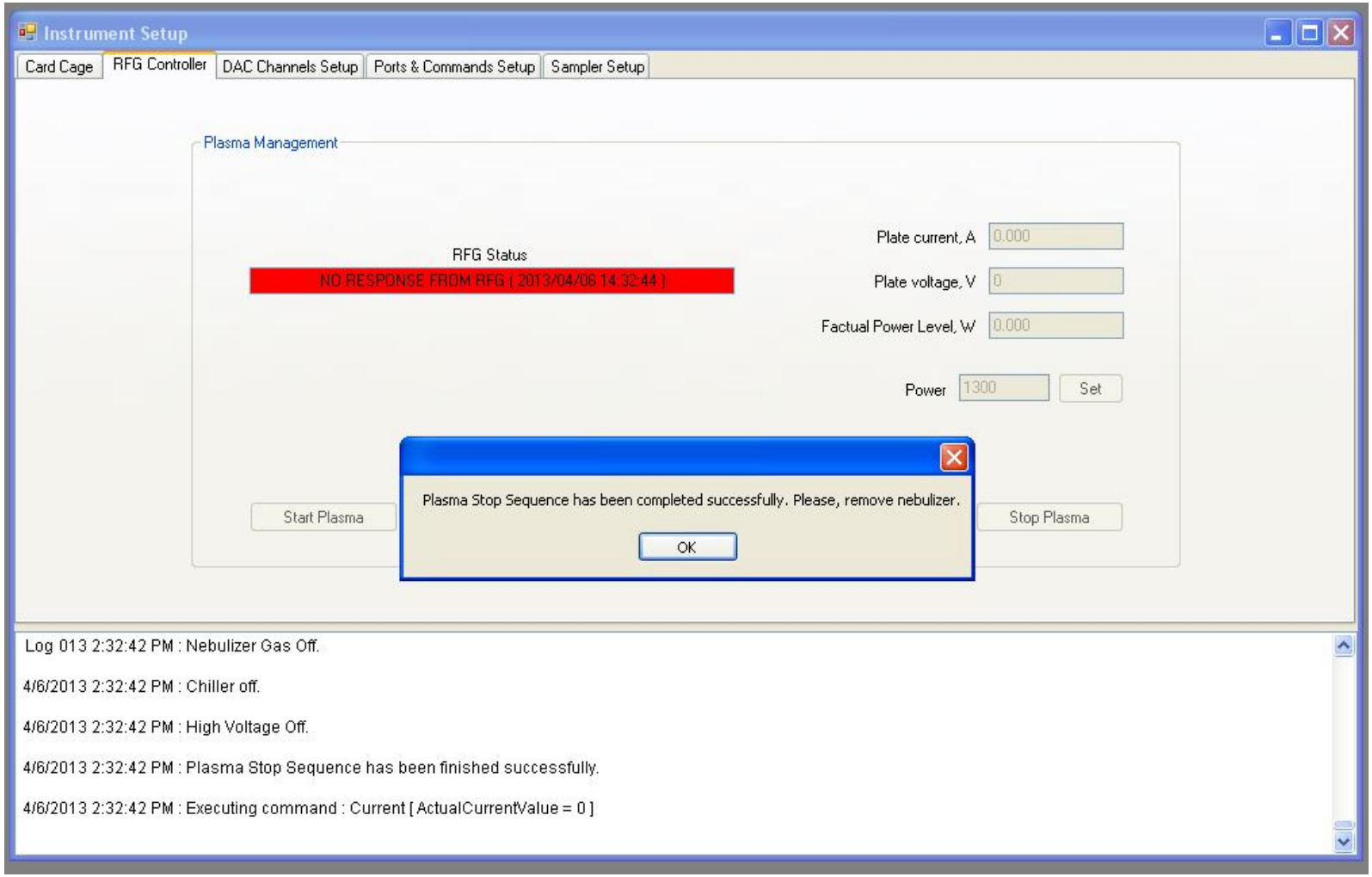


- **Clean with H_20 between samples to minimize carryover**
- **Clean instrument when done running samples (Wash solution and H_20)**

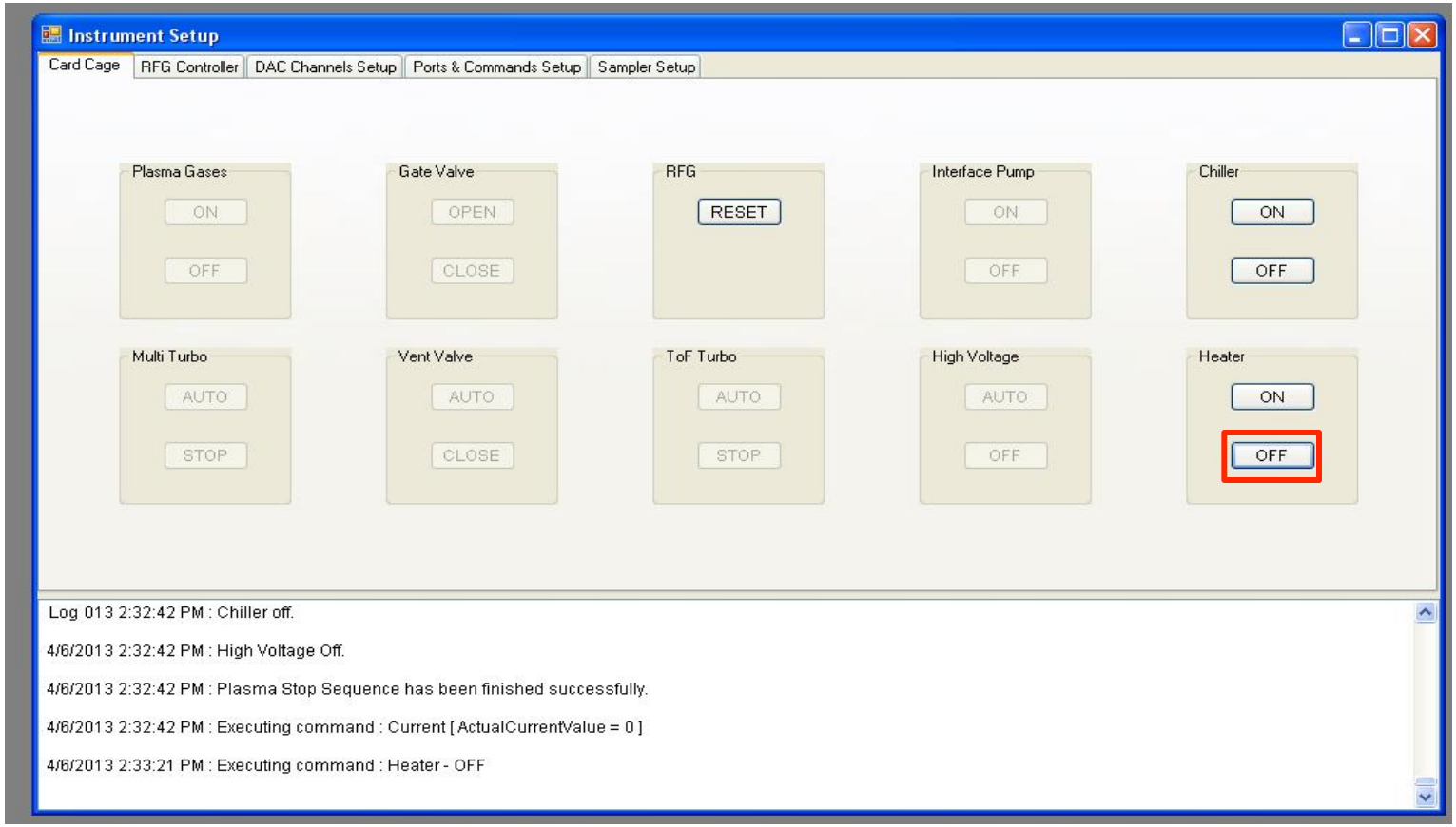
Instrument Shutdown



Instrument Shutdown



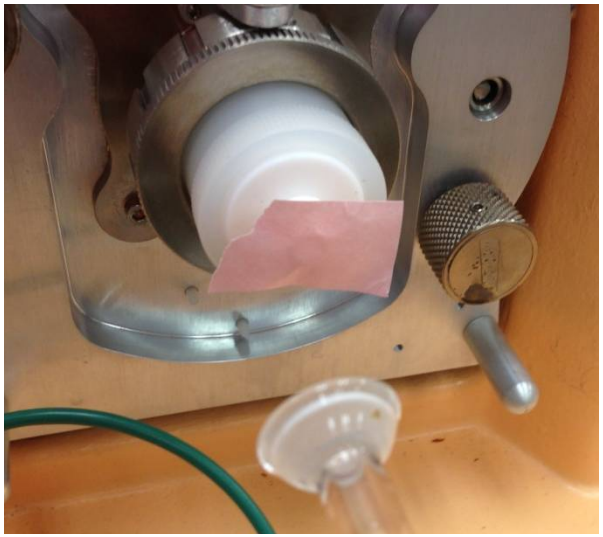
Instrument Shutdown



- **Turn off HPLC**
- **Remove nebulizer and store in 3% Citranox**

Troubleshooting: Common Issues

Problem	Cause	Solution
1. Plasma won't ignite	Argon off	Turn it on
	Argon not pure enough	Use Ultra High Purity Argon
	Incorrect Gas?	Double check gas
	Air Leak	Localize source

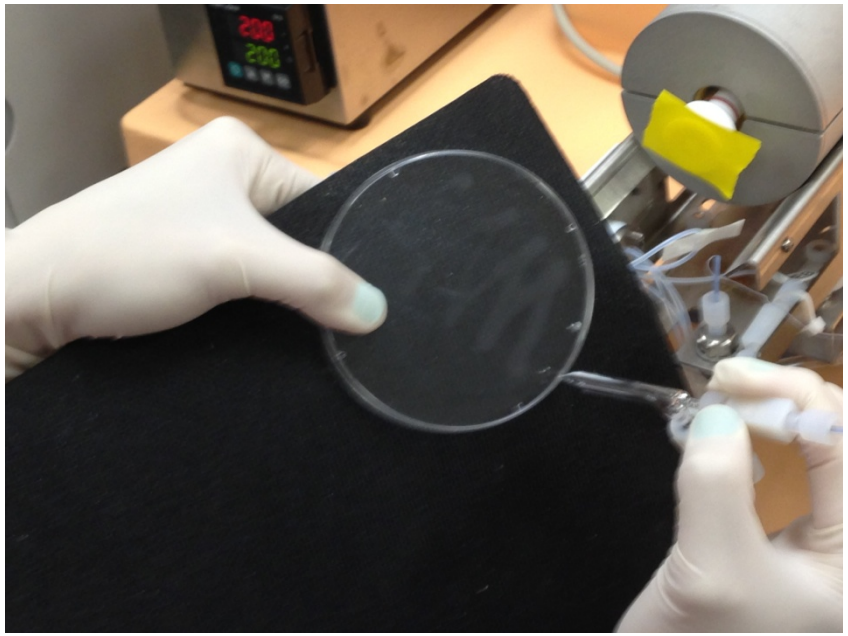


Plasma ignites → Look for air leak in the area of sample introduction

Plasma does not ignite → Check the torch, injector, coil for condensation

Troubleshooting: Common Issues

Problem	Cause	Solution
2. No/low signal	Sample line capillary pinched	Inspect capillary line
	Sample line capillary inserted improperly	Reinsert capillary
	Clogged nebulizer	Check spray/swap nebulizer
	Insufficient gas flows	Check gas flows



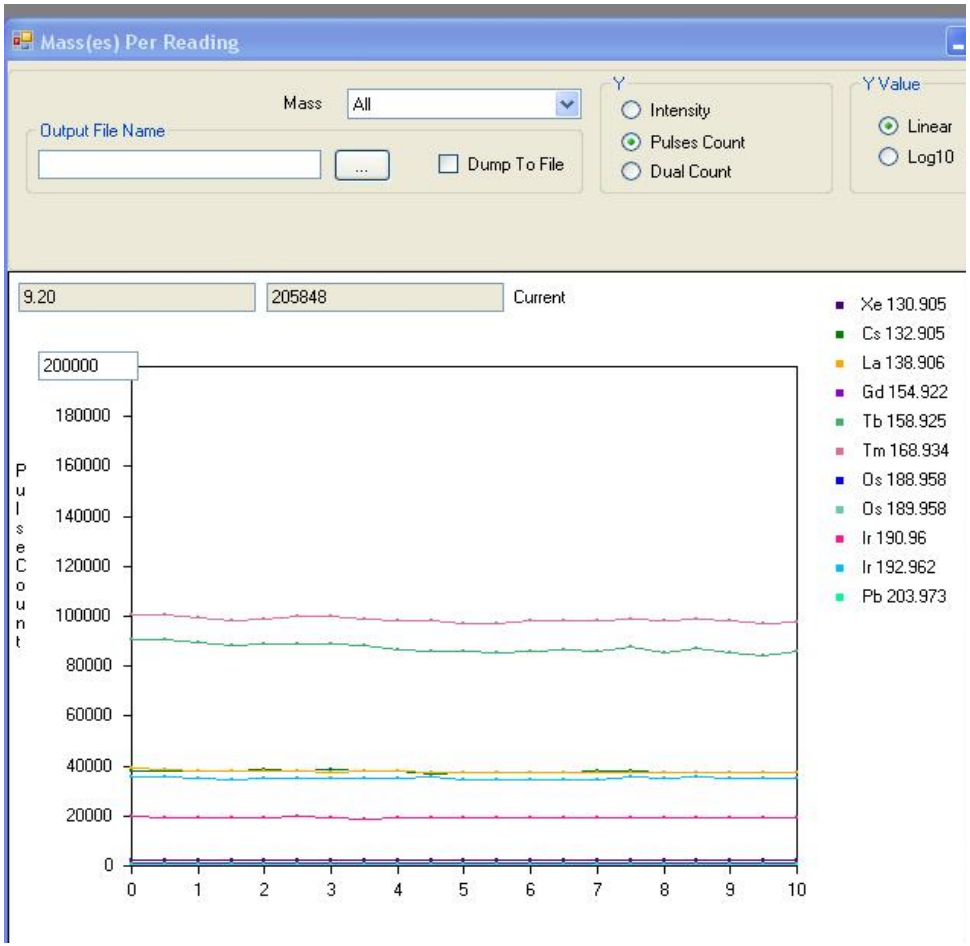
Troubleshooting: Unusual Circumstances

Problem	Cause	Solution
1. 	• Leak in one of the argon lines	•Pankaj came out and found the leak. Replaced torch and replaced RFG coil
	• Arcing because the coil not aligned	•Clean/Replace coil
	• Oxidation on the coils	

Troubleshooting: Unusual Circumstances

Problem

2.

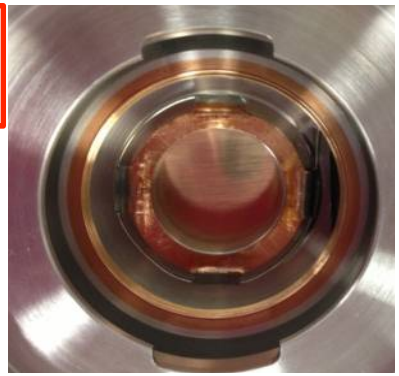


Cause

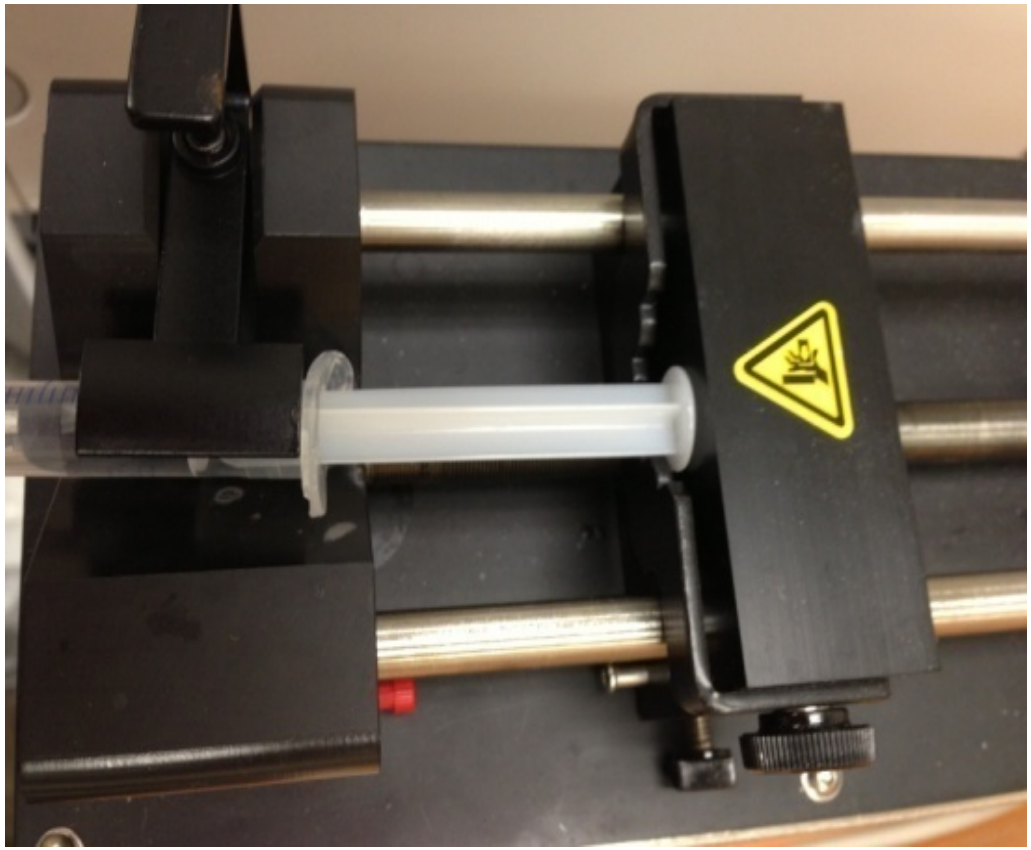
Poor contact of skimmer/reducer with the contact ring on the interface. This may be due to oxidation on the contact ring or on the reducer itself.

Solution

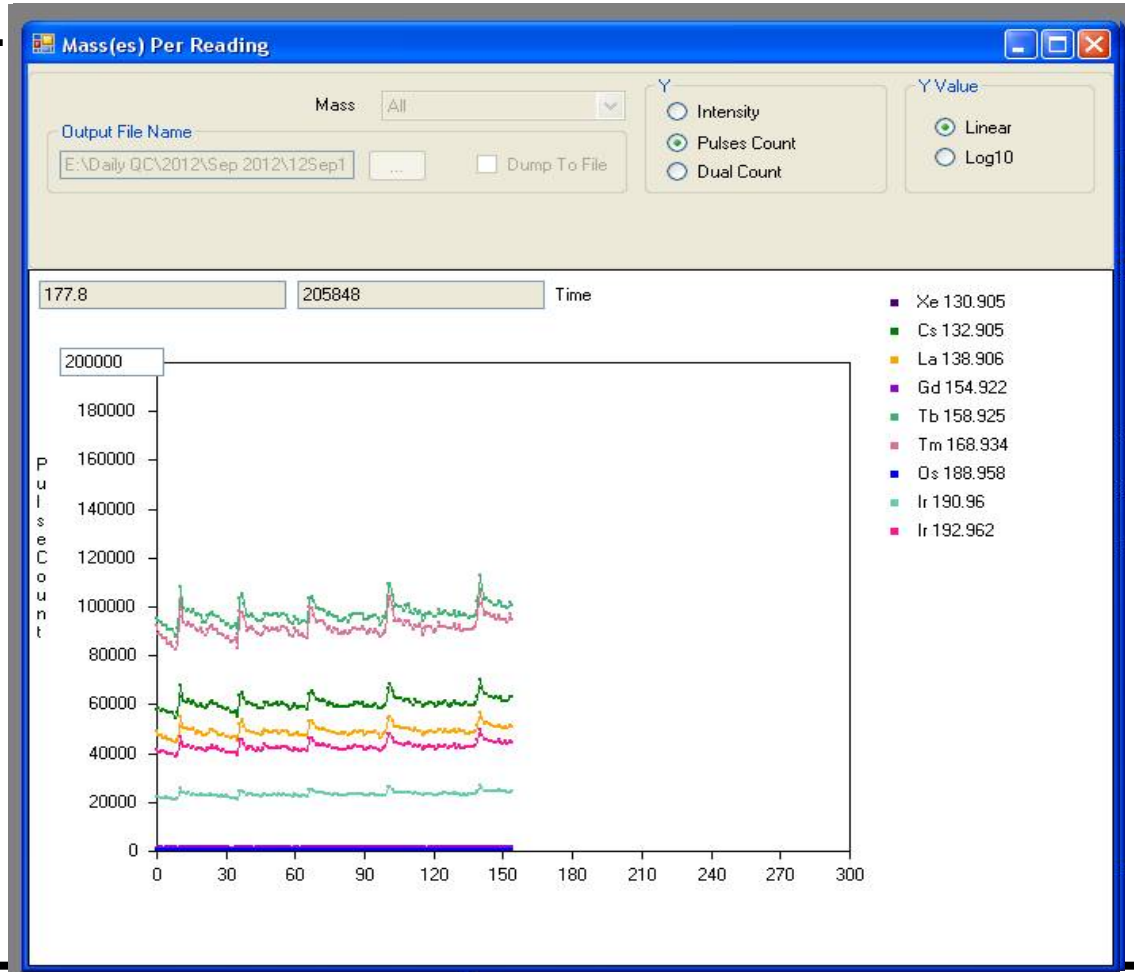
Vigorously cleaned the contact ring on the interface with methanol. Eventually, Daniel came out to replace the interface



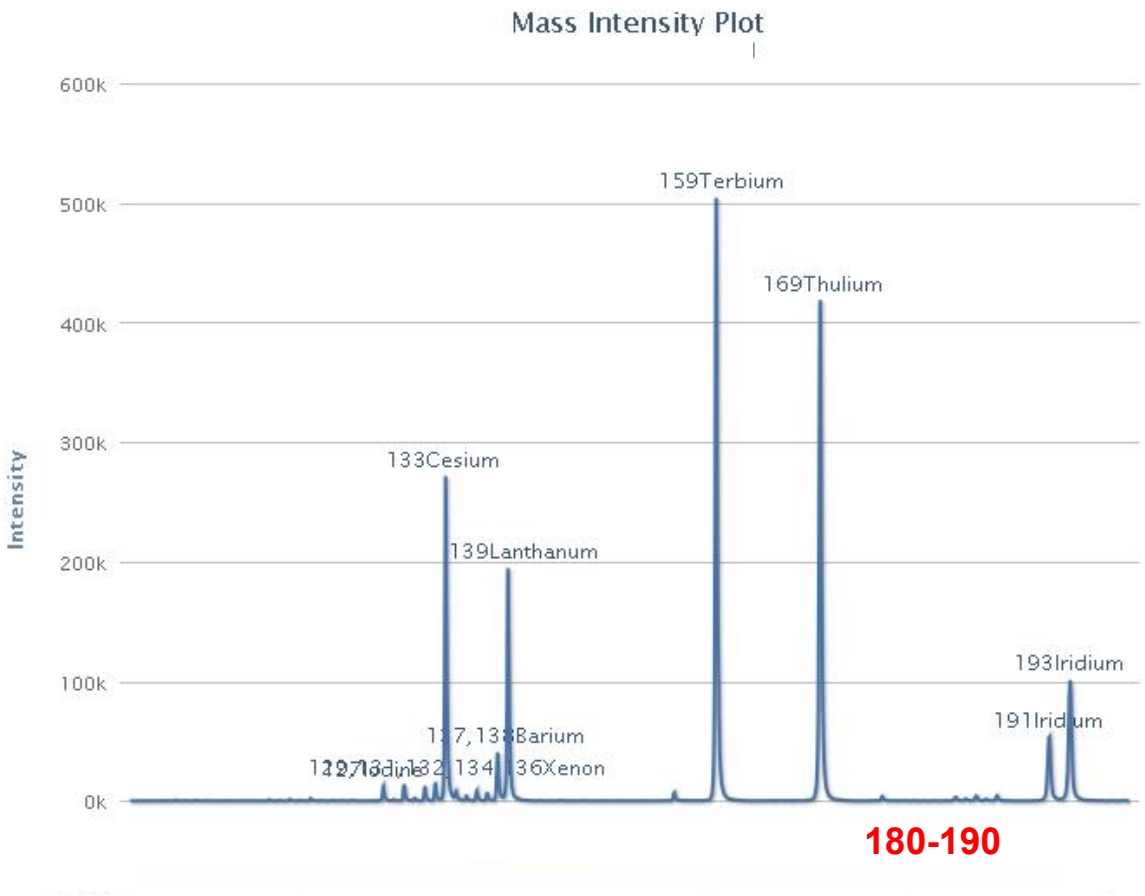
Troubleshooting: Unusual Circumstances

	Problem	Cause	Solution
3.		Originally thought it was a faulty syringe pump. Turned out to be a bad valve.	Replaced the entire valve assembly

Troubleshooting: Unusual Circumstances

	Problem	Cause	Solution
4.		Partially clogged/ dirty nebulizer	Clean/swap nebulizer
		Sample capillary not positioned properly	Reinsert capillary
		Bad syringe	Replace syringe

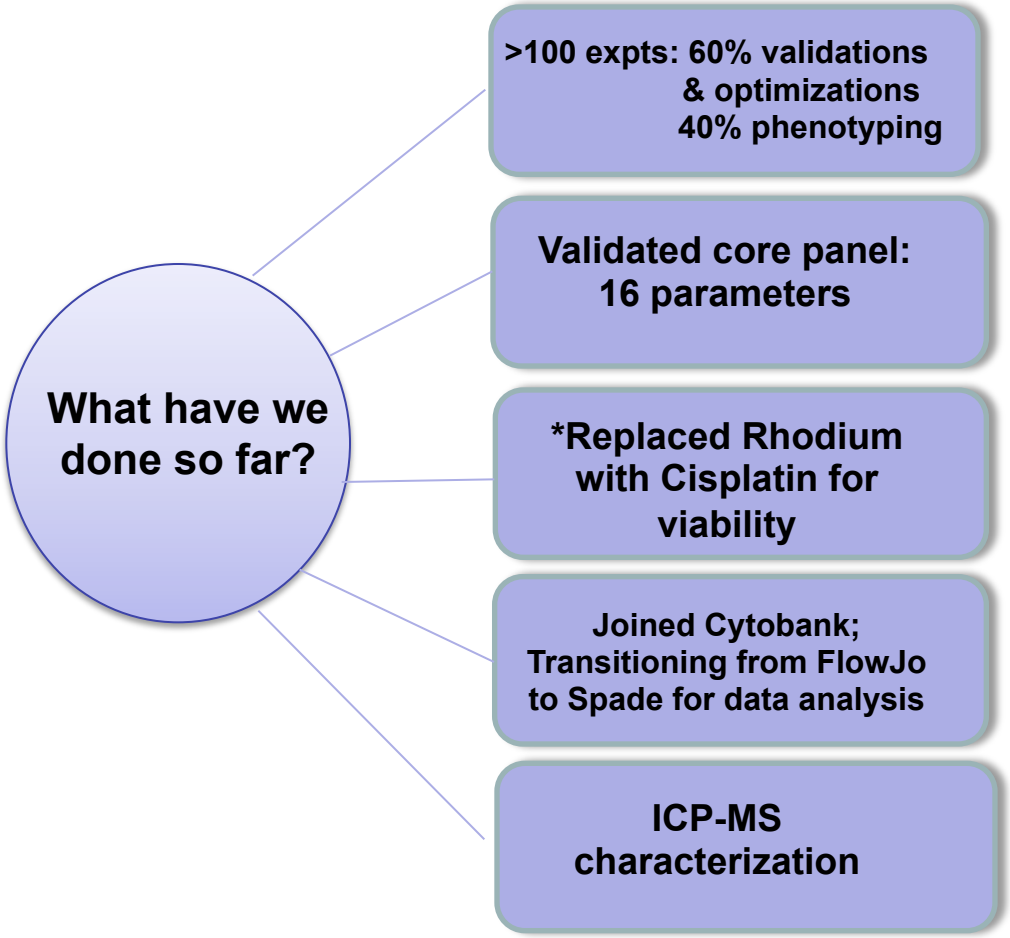
Troubleshooting: Unusual Circumstances

	Problem	Cause	Solution
5.		Tungsten contamination; but from where?	Cleaned ignitor pin with sandpaper

Progress Report: 1 ½ years later

Lilly CyTOF Projects:

- Multi-parametric, high content immunophenotyping
- LRAP with the Sanford Burnham Institute to investigate signaling pathways



*Reference: Feinberg, H.G., et al. A platinum-based covalent viability reagent for single-cell mass cytometry. Cytometry A 81 (6): 467-475, 2012

Acknowledgements

Andrew Glasebrook

Mark Daniels
John Fitchett

Elaine Conner
Marian Mastrangelo
Han He

DVS Sciences
Pankaj Chaudhari
Leslie Fung
Ted Young
Tad George
Ron Cohn
Daniel Tong