How to Get the Best Performance out of your CyTOF

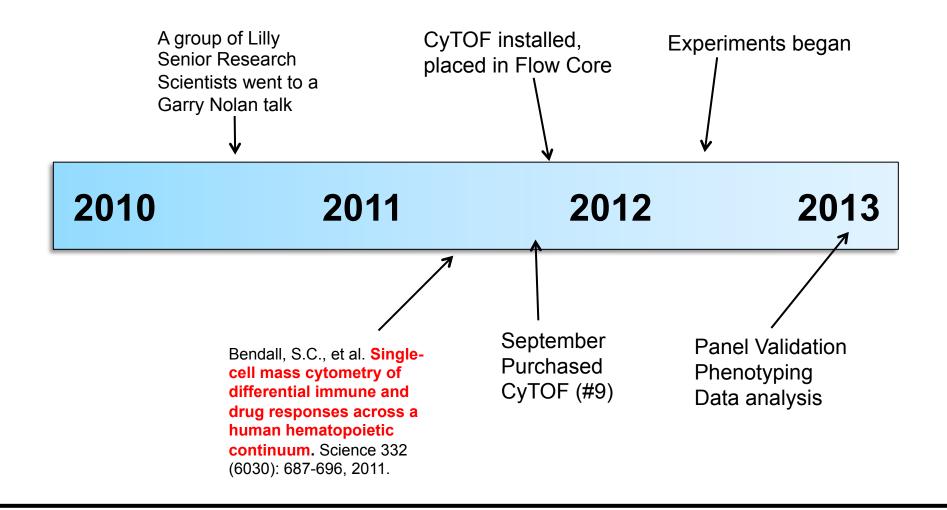
Carina Torres CyTOF User Group Meeting CYTO 2013 23 May 2013



Outline

- History
- CyTOF Workflow
 - Instrument Startup
 - Optimization
 - Running Samples
 - Instrument Shutdown
 - Troubleshooting
- Progress Report...1 ¹/₂ years later

CyTOF at Lilly

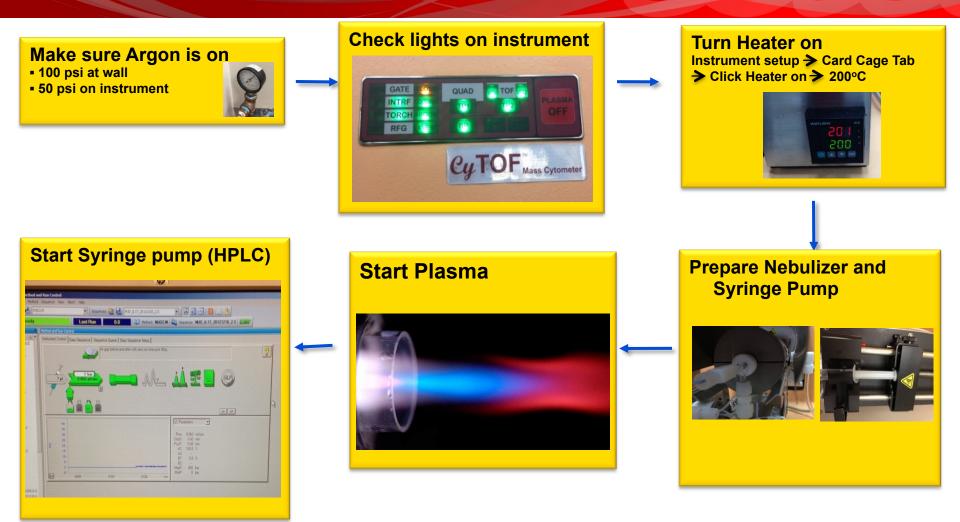


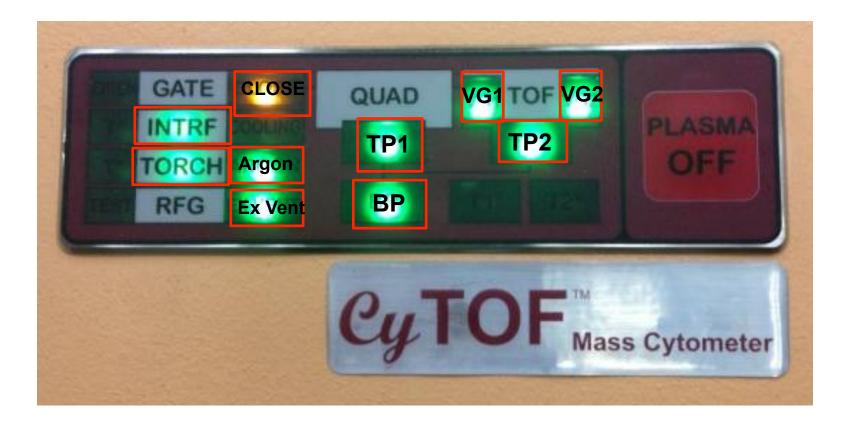
Preparing your Lab

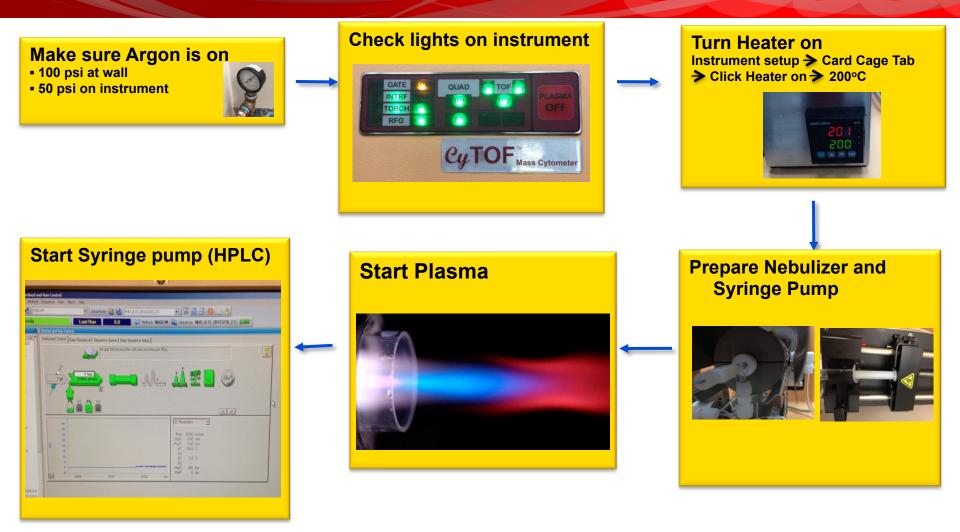




Daily Workflow: Instrument Startup

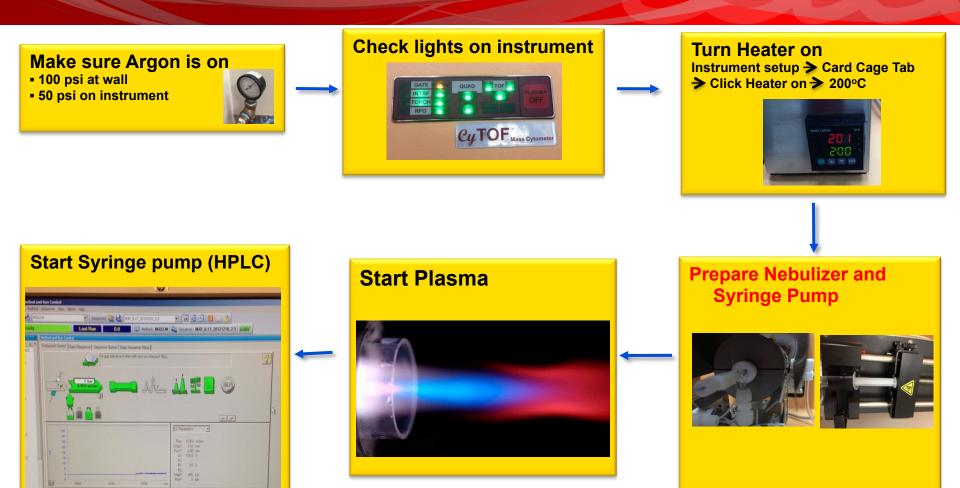


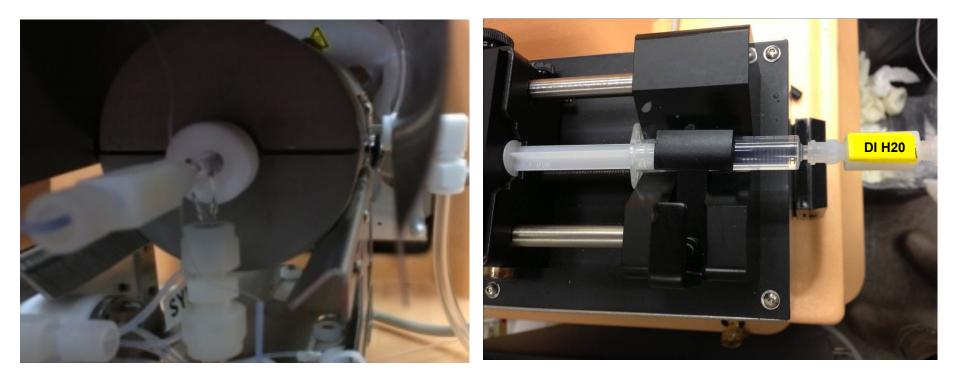


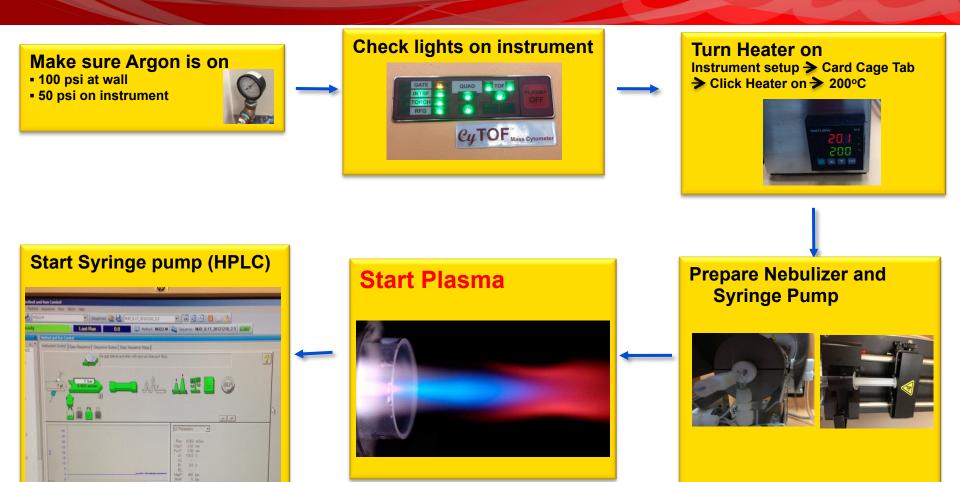


Instrument Setup Acq. Settings PDA1000 Dual Calibratian Sar	mple Introduction Acquisition Analysis Convert to FCS Conv	et IPD ForFCS About			
Graphs 🕍 🔼 🔋		SP (1005	[1995] Dia/Val/Speed [9.300 / 3.000 / 0.060]	SP (Inj/Tot) [2.088 / 3.000]	
Instrument Setup Card Cage RFG Controller DAC Cha	nnels Setup Ports & Commands Setup	Sampler Setup			
Plasma Gases ON OFF	Gate Valve OPEN CLOSE	RFG	Interface Pump ON OFF	Chiller ON OFF)
Multi Tubo AUTO STOP	Vent Valve AUTO CLOSE	Tof Tubo AUTO STOP	High Vohage AUTO OFF	Heater ON OFF	
Log 013 934:29 AM : Liner (rampir 4/8/2013 934:30 AM : D Bias (ramp 4/8/2013 934:31 AM : Liner (rampir 4/8/2013 934:32 AM : Setting Mirror	iing up) = -5000 1g up) = -4999.9				
4/8/2013 9:34:32 AM : Setting Mirror 4/8/2013 9:34:32 AM : Plasma Start		sfully.			





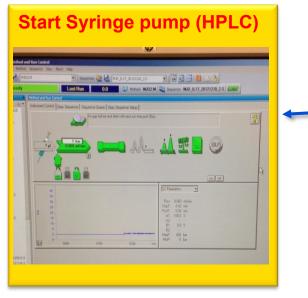


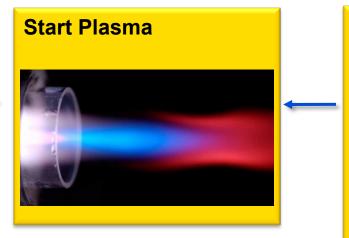


Bilgtaf 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1				- • ×
Instrument Setup Acq. Settings PDA1000 Dual Calibration Sample Introduction Acquisition Analysis Convert to FCS Convert IMD Fix FCS About	ECTING Dia/Vol/Speed [9.300 / 3.000 / 0.060] SP	(Inj/Tot) [2.088 / 3.000]		
Card Cage RFG Controller DAC Channels Setup Ports & Commands Setup Sampler Setup			_	
Card Cage Third Controlled DAC Charmers Setup Forts & Commands Setup Sampler Setup				
RFG Status	Plate current, A 0.000			
WARM-UP MODE [2013/04/07 10:23:43]	Plate voltage, V			
	Factual Power Level, W 0.000			
RFG Warm up delay	- 3000			
	Power 1300 S	Set		
Start Plasma Cancel	Stop Plasma	•		
Log 013 10:22:12 AM : Nebulizer Gas On (0.45L/minute).				^
4/7/2013 10:22:12 AM : Makeup Gas to 1 L/minute.	 Chiller turns on 			
4/7/2013 10:22:12 AM : Waiting for 10 seconds.	 Start-up sequent 	nce		
4/7/2013 10:22:22 AM : RFG PREPARE.	Plasma ignites			
4/7/2013 10:22:22 AM : Waiting for RFG to warm up (timeout is 100 seconds).	-			
1997a 96 97. 67.				~

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Instrument Setup Acq. Settings F0A1000 Dual caloration Sample Introduction Acquisition Analysis Convert IND Fix FCS About	
Graphs ⊮ 🛆 🔋 SP [1006/111/63] Dia/Vol/Speed [9.300 / 3.000 / 0.060] SP (111/110k) [2.088 / 3.000]	000
🖳 Instrument Setup	
Card Cage RFG Controller DAC Channels Setup Ports & Commands Setup Sampler Setup	
Plasma Management	
Plate current, A 0.555	
RFG Status	
PLASMA MODE (2013/04/07 10:25:15) Plate voltage, V 3315	
Factual Power Level, W 1325,428	
Power 1300 Set	
Plasma Start Up Sequence has been completed successfully. Start Plasma Stop Plasma	
Start Plasma OK Stop Plasma	
Log 013 10:25:03 AM : Liner (ramping up) = -4800	~
4/7/2013 10:25:04 AM : D Bias (ramping up) = -5000	
4/7/2013 10:25:05 AM : Liner (ramping up) = -4999.9	
4/7/2013 10:25:06 AM : Setting Mirror to the current value.	
4/7/2013 10:25:06 AM : Plasma Start up Sequence has finished successfully.	
	~



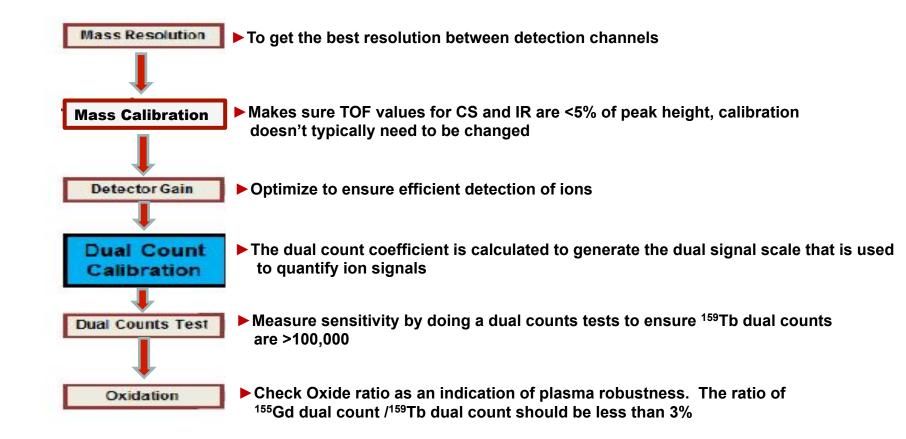




Prepare Nebulizer and Syringe Pump

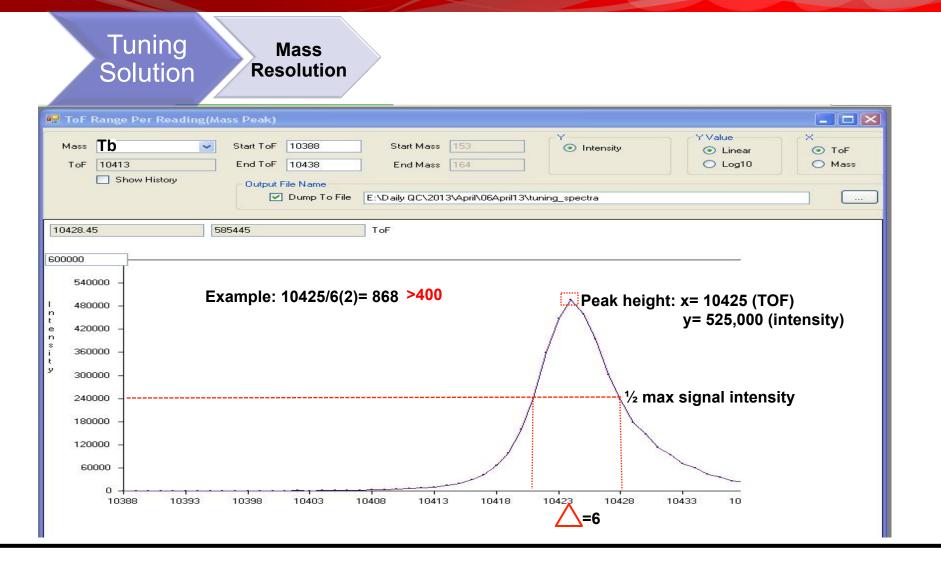


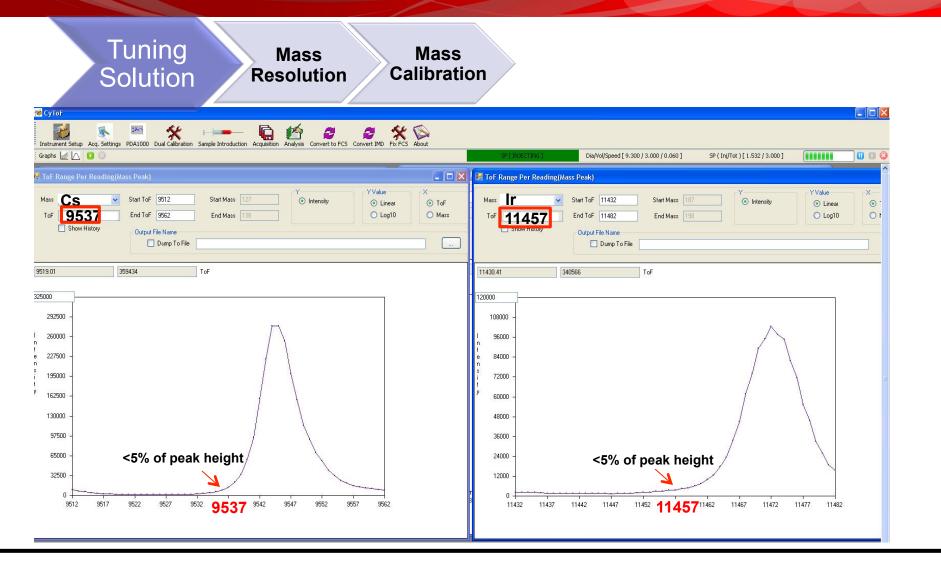
🖬 CyToF 💕 🕵 🔤 🛠 🛏 🖛					
Instrument Setup Acq. Settings PDA1000 Dual Calibration Sample Introduction Acquis	ion Analysis Convert to FCS Convert IMD Fix FCS About	SP [IDLE]	Dia/Vol/Speed [9.300 / 3.000 / 0.060]	SP (Inj/Tot) [0.000 / 0.000]	
Card Cage PF	Setup Controller DAC Channels Setup Ports & Commands Setup Sampler Setup Flasma Management FFG Status FFG Status FFG Warm up delay FFG Warm up delay	Plate or Plate vo Factual Power L	arent, A 0.000 itage, V 0 evel, W 0.000 wer 1300 Set		
4/7/2013 10:22 4/7/2013 10:22 4/7/2013 10:22	12 AM : Nebulizer Gas On (0.45L/minute). 12 AM : Nebulizer Gas On (0.45L/minute). 12 AM : Makeup Gas to 1 L/minute. 12 AM : Waiting for 10 seconds. 22 AM : RFG PREPARE. 22 AM : Waiting for RFG to warm up (timeout is 100 seconds).	Cancel	Stop Plasma		
	to warm up (timeout is 100 seconds).		STARTING PLASMA	APA UF MODE (2013/04/07 10:23:41)	
				Desktop	

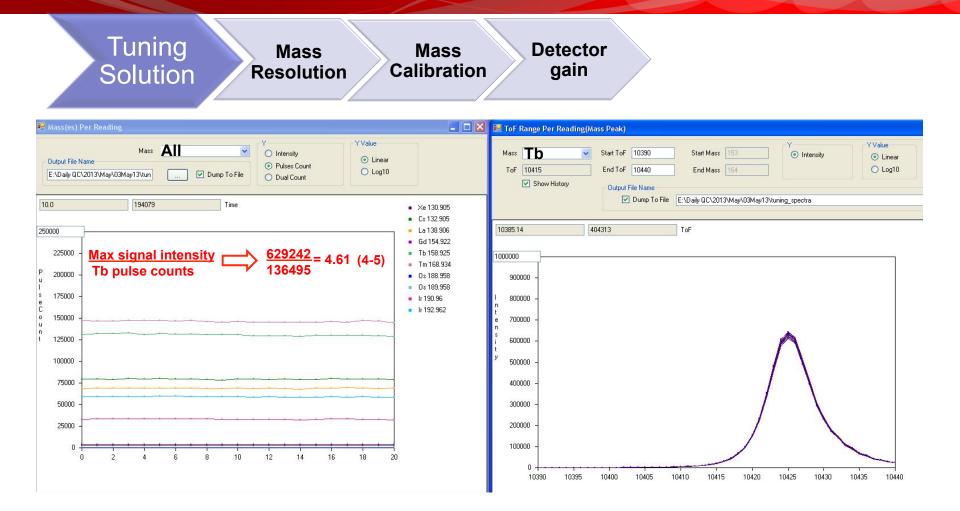


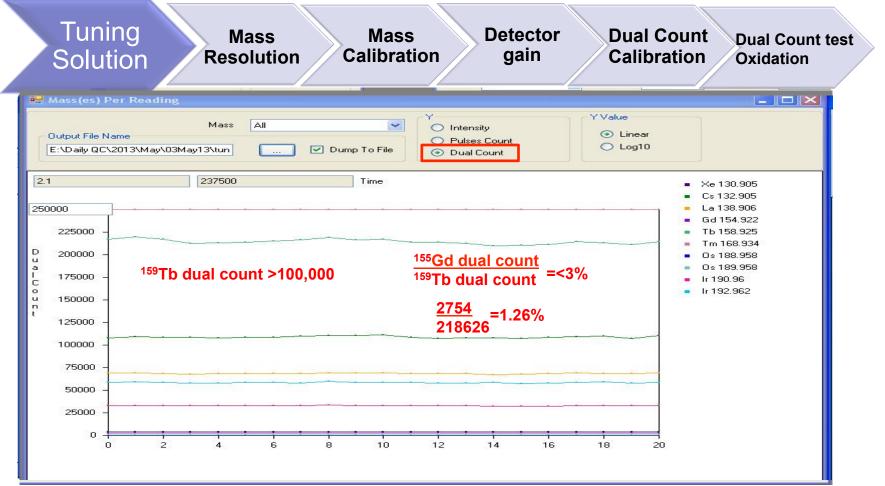
Tuning Solution

Data Acquisition Settings Analytes Parameter Mass Calibration								
	Symbol	Mass	Antibody Label	IntegrationTime	Intercept (Instrument)	Slope (Instrument)		
•	Xe	131		21	0.0000	0.0406		
	Cs	133		21	0.0000	0.0409		
	La	139		22	0.0000	0.0417		
	Gd	155		23	0.0000	0.0440		
	ТЬ	159		23	0.0000	0.0446		
	Tm	169		24	0.0000	0.0460		
	Os	189		25	0.0000	0.0488		
	Os	190		25	0.0000	0.0489		
	Ir	191		25	0.0000	0.0490		
	Ir	193		25	0.0000	0.0493		
Instrument Dual Calibration, colors legend Open Templates Periodic table Periodic table								
		een PRECISELY DUAL-calibra een DUAL-calbrated by EXTR/		Create a New	Template	Reset Intergration Time		











CyTOF Calculations for Instument Performance/Tuning

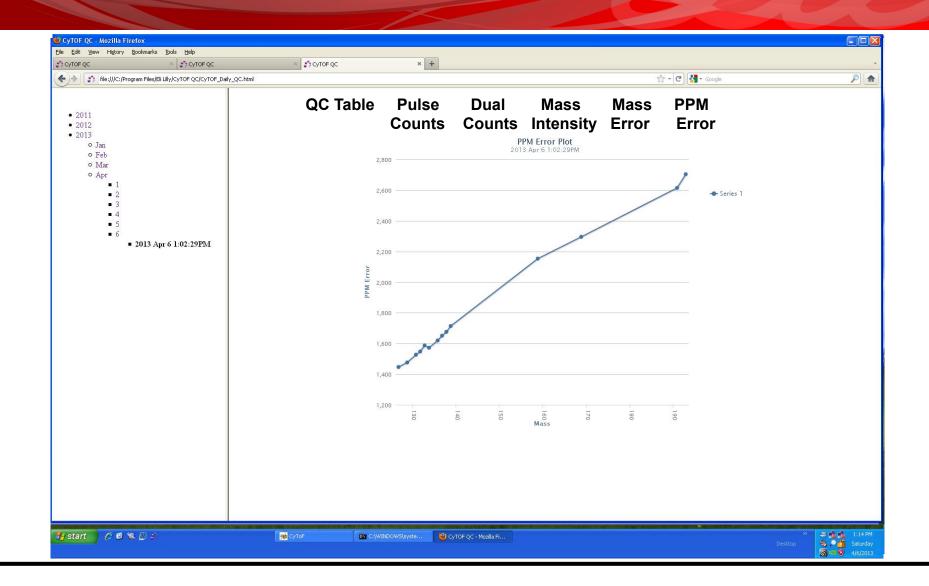
Date:

1.		<mark>tio of Tb:</mark> Intensity of Tb o r troubleshooting, optimiz		/Tb pulse counts	= (3.0-5.0)
2.	Re	solution of Tb:			
	a.	Recorded x value at peak	height =		
	b.	Record y value at peak h	eight =	/2 =	
	c.	x- intercept 1 (at ½ peak	height (y))=	x-intercept 2=	difference=
	d.	recorded x value at peak	height/2/difference =	s (should be >	-400)
3.	Ma	ass Calibration Values for	Cs and Ir2:		
	a.	Cs: peak height= (check against values in (; x value at .03 peak height = Acq settings)	·
	b.	Ir2: peak height= (check against values in (; x value at .03 peak height : Acq settings)	
4.	Ox	ide Ratio:			
	a.	Record level of Tb	and Gd		
	b.	$Gd/Tb \times 100 = <3 \rightarrow$	/	=	

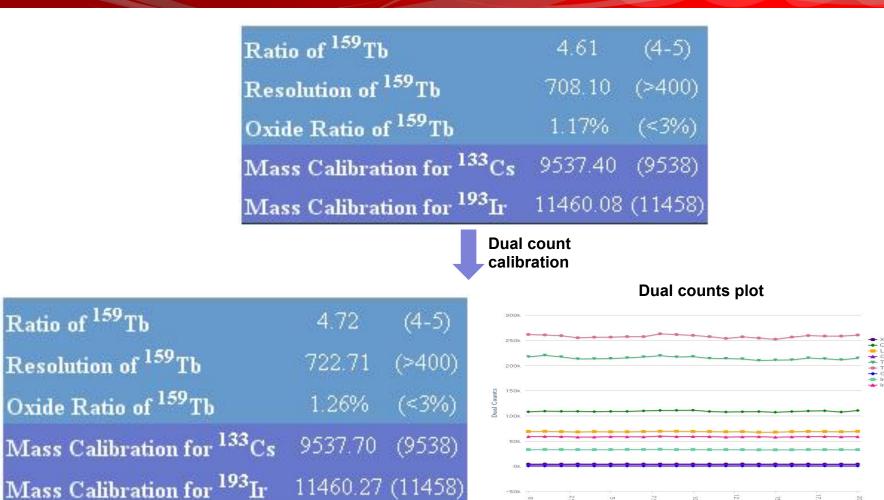
Automated QC at Lilly

ile:///C:/Program Files/Eli Lilly/CyTOF QC/CyTOF_Da	QC Table Pulse Dual Mass Counts Counts Intensit	Mass PPM y Error Error
n eb ar pr ay • 1	Ratio of ¹⁵⁹ Tb	4.61 (4-5)
• 2 • 3 • 2013 May 3 10:23:35AM	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

Automated QC at Lilly



Automated QC at Lilly

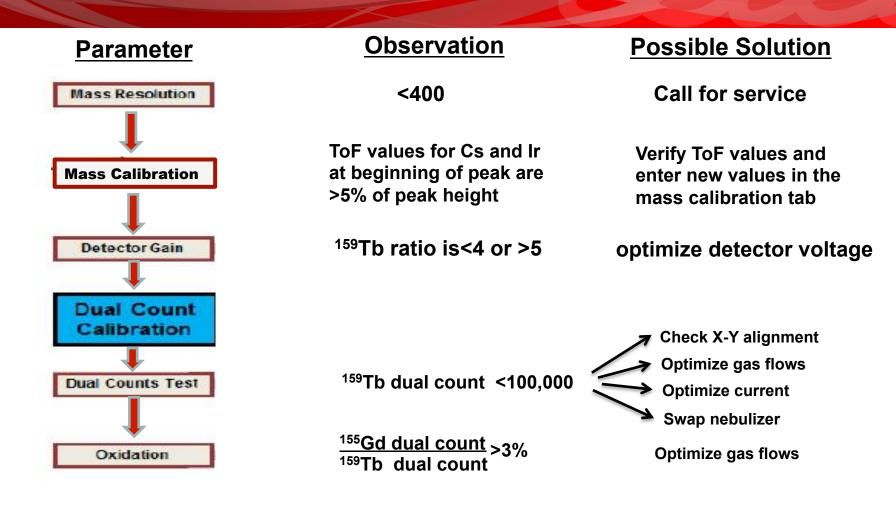


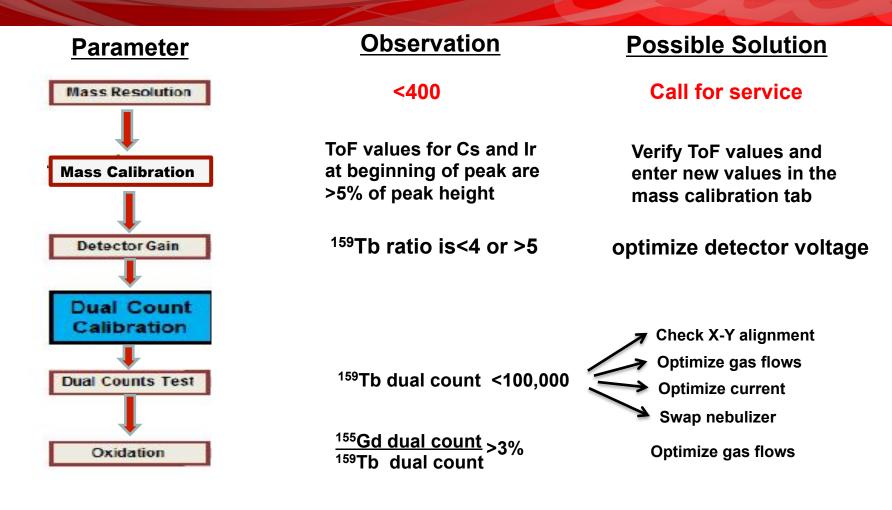
12

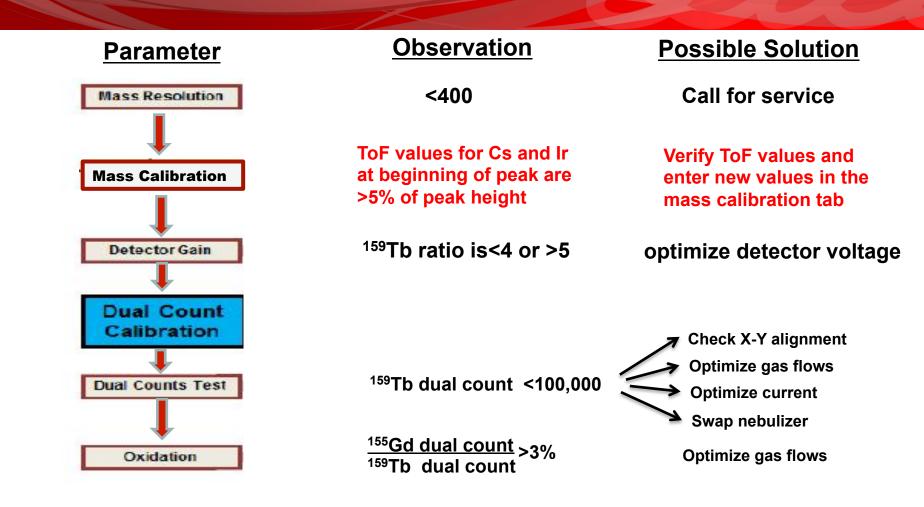
3

Second

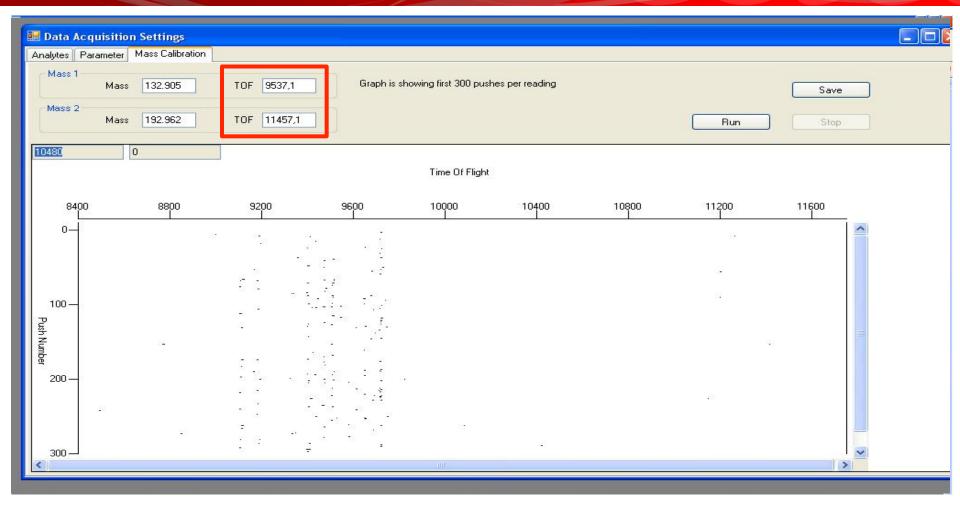
20

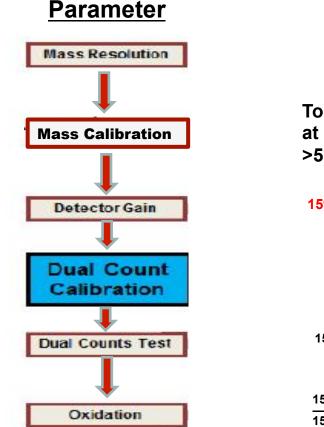






Verify Mass Calibration





Observation

<400

ToF values for Cs and Ir at beginning of peak are >5% of peak height

¹⁵⁹Tb ratio is<4 or >5

Possible Solution

Call for service

Verify ToF values and enter new values in the mass calibration tab

optimize detector voltage

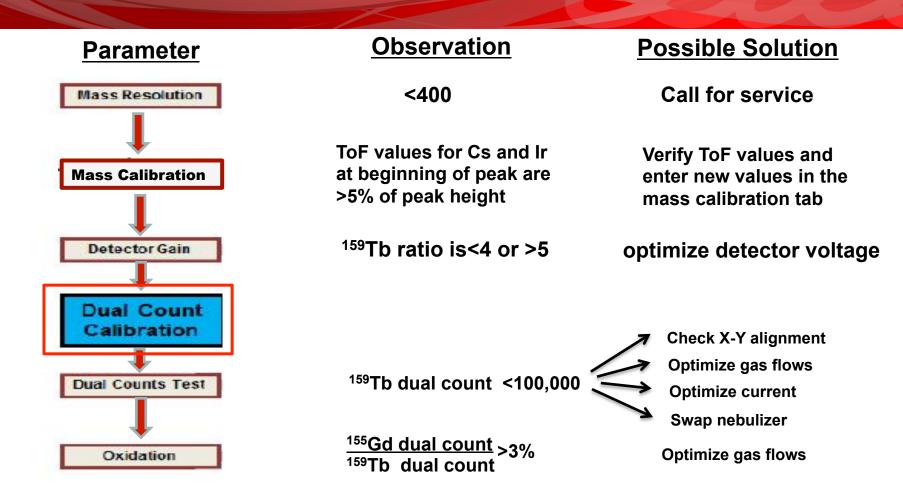
¹⁵⁹Tb dual count <100,000

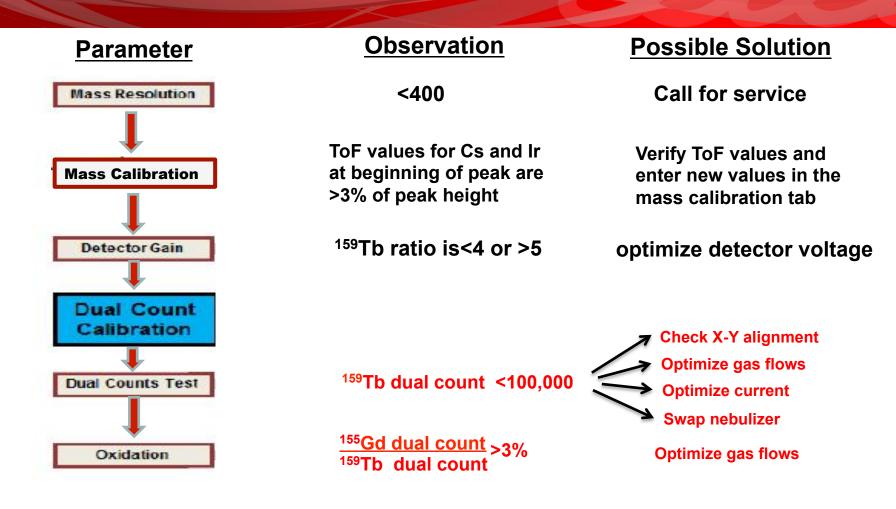
¹⁵⁵Gd dual count >3% ¹⁵⁹Tb dual count Check X-Y alignment Optimize gas flows Optimize current Swap nebulizer

Optimize gas flows

Optimize Detector Voltage

Parameter Name	Actual Min	Actual Max	Actual Current Value	Update	F1	F2	
Middle Point	-154.5	0	-120	Set Actual Current Value			
Nebulizer Gas	0	1.5	0.3	Set Actual Current Value			
Detector Bias	-5000	0	-5000	Set Actual Current Value			
Detector Voltage	-5000	0	-1825	Set Actual Current Value			
D02	0	40	20	Set Actual Current Value			
PulserP	0	713	430	Set Actual Current Value			
PulserN	-703	0	-700	Set Actual Current Value			
Liner	-5000	0	-4999.9	Set Actual Current Value			
Mirror	0	1000	940	Set Actual Current Value			
Makeup Gas	0	2	0.85	Set Actual Current Value			
Current	0	25	3	Set Actual Current Value			
400V1	-410	0	-120	Set Actual Current Value			
Slit	-209	n	-120	Set Actual Current Value			
alize Save							
3 1:38:33 PM : Please, note	e that when Initialize	button is pr	ressed - Deti	ector Voltage, Liner, Bias an	d Mirror will a	II be set to 0.	

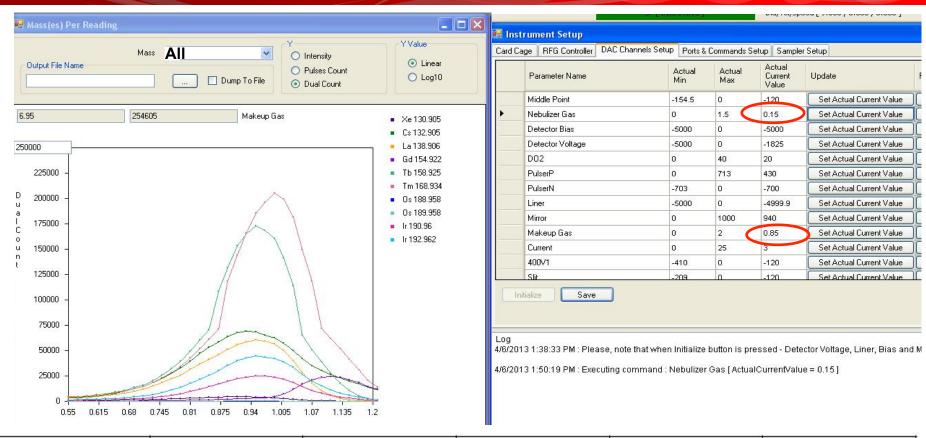




X-Y Alignment of the Interface

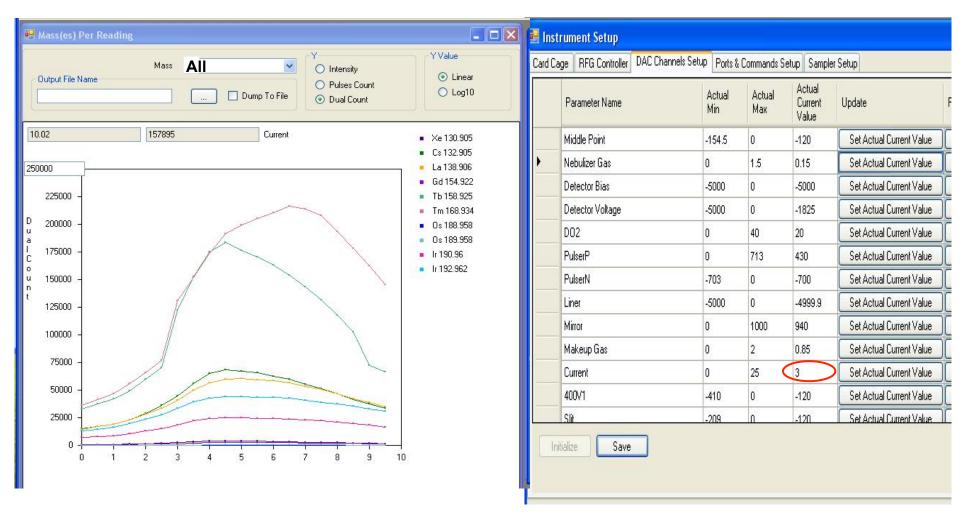
	Mass(es) I	Per Reading	
	Dutput File N	Mass All I Intensity Iame I Dump To File Dual Count	Y Value
	5.9 000 225000 -	255263 Time	 Xe 130.905 Cs 132.905 La 138.906 Gd 154.922 Tb 158.925
Dualc	200000 - 175000 -	and the second	 Tm 168.934 Os 188.958 Os 189.958 Ir 190.96
l C o u n t	150000 - 125000 -		 Ir 192.962
	100000 -	T	
	75000 - 50000 -		
	25000 -		
	0 -) 10 20 30 40 50 60 70 80 90 100	

Optimize Gas Flows

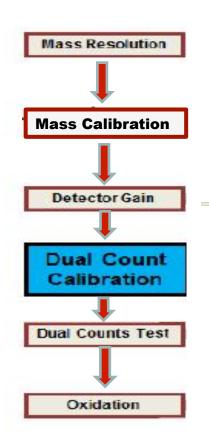


Date	Nebulizer Gas Value	Optimal Makeup Gas Value	Tb159 dual count	Gd155 dual count	Mass155/Tb159
2-May-13	0.15	1.008	208553	3947	0.018925645
	0.2	0.95	219079	3947	0.018016332
	0.25	0.88	219421	3947	0.017988251
	0.3	0.85	225000	4605	0.020466667
	0.35	0.77	225658	3289	0.014575154

Optimize Current



Re-Check QC

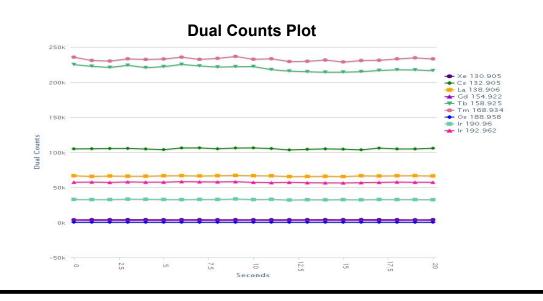


QC Tables

Pulses Count Plot

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)

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

🔜 Data Acquisition Setti	ngs								
Analytes Parameter Mass C	Analytes Parameter Mass Calibration								
Mass 1 Mass 132.9	905 TOF 9537,1	Graph is showing first 300 pus	hes per reading			Save			
Mass 2 Mass 192.5	962 TOF 11457,1				Run	Stop			
10480		Time Of Flight							
8400 88	300 9200	9600 10000 I I	10400	10800 	11200 	11600			
0					а ч				
100 — Push Number									
हूँ 200 —					18				
300			21			×			

Running Samples: User Guidelines

🔛 Acquisition	
	Analysis Parameters Noise Reduction Lower convolution threshold 200 Cell Subtraction (per channel) 0 Min Cell Length 10 Max Cell Length 15 Sigma 3 Found Cells Limit (Unlimited if 0) 0 Found Cells Limit (Unlimited if 0) 0
	Graph Refresh Found Cells Stop Run Preview

Running Samples: User Guidelines

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

🔚 Data Acquisition Settings							
Analytes Parameter Mass Calibration							
Mass 1 Mass 132.905 TOF	Graph is showing first 300 pushe	ss per reading	Run	Save			
0	Time Of Flight						
8400 8800 9	9200 9600 10000	10400 10800	11200 1	1600			
0- 100 - Push Number 200 - 			10. 11. 12. 13.				
300							

- Clean with H₂0 between samples to minimize carryover
- Clean instrument when done running samples (Wash solution and H₂0)

Instrument Shutdown

🔜 Instrument Setup						
Card Cage RFG Controller DAC Channels Setup Ports & Commands Setup Sampler Setup						
Plasma Management						
RFG Status Plate current PLASMA MODE (2013/04/06 14:30:12) Plate voltage						
Factual Power Level,	w 1318.567					
Power	1300 Set					
Start Plasma Cancel	Stop Plasma					
Log 013 2:05:44 PM : Executing command : Nebulizer Gas [ActualCurrentValue = 0.3]						
4/6/2013 2:07:55 PM : Executing command : Nebulizer Gas [ActualCurrentValue = 0.25]						
4/6/2013 2:09:35 PM : Executing command : Nebulizer Gas [ActualCurrentValue = 0.35]						
4/6/2013 2:11:51 PM : Executing command : Nebulizer Gas [ActualCurrentValue = 0.3]						
4/6/2013 2:11:54 PM : Executing command : Makeup Gas [ActualCurrentValue = 0.85]						

Instrument Shutdown

🖶 Instrument Setup	
Card Cage RFG Controller DAC Channels Setup Ports & Commands Setup Sampler Setup	
Plasma Management	
RFG Status Plate current, A 0.000 NO RESPONSE FROM RFG (2013/04/06 14/32:44) Plate voltage, V 0	
Factual Power Level, W 0.000 Power 1300 Set	
Start Plasma OK	
Log 013 2:32:42 PM : Nebulizer Gas Off.	
4/6/2013 2:32:42 PM : Chiller off.	
4/6/2013 2:32:42 PM : High Voltage Off.	
4/6/2013 2:32:42 PM : Plasma Stop Sequence has been finished successfully.	
4/6/2013 2:32:42 PM : Executing command : Current [ActualCurrentValue = 0]	

Instrument Shutdown

🔛 Instrum	nent Setup							
Card Cage	RFG Controller	DAC Channels Setup	Ports & Commands Setup	Sampler Setup				
	Plasma Gases	_	Gate Valve	RFG	Interfac	ice Pump	Chiller	
	ON		OPEN	RESE		ON	ON	
	OFF		CLOSE			OFF	OFF	
	Multi Turbo		Vent Valve AUTO	ToF Turbo	High V	AUTO	Heater	1
	STOP		CLOSE	STOP		OFF	OFF	
,								
	2:32:42 PM : Chil							~
4/6/2013 2:32:42 PM : High Voltage Off. 4/6/2013 2:32:42 PM : Plasma Stop Sequence has been finished successfully.								
4/6/2013 2	2:32:42 PM : Exe	cuting command : C	urrent [ActualCurrentVal	ue = 0]				
4/6/2013 2	2:33:21 PM : Exe	cuting command : H	eater - OFF					~

Turn off HPLC
Remove nebulizer and store in 3% Citranox

Troubleshooting: Common Issues

Problem

1. Plasma won't ignite

<u>Cause</u>

Argon off Argon not pure enough

Incorrect Gas?

Air Leak

Solution

Turn it on Use Ultra High Purity Argon

Double check gas

Localize source



Plasma ignites

Look for air leak in the area of sample introduction

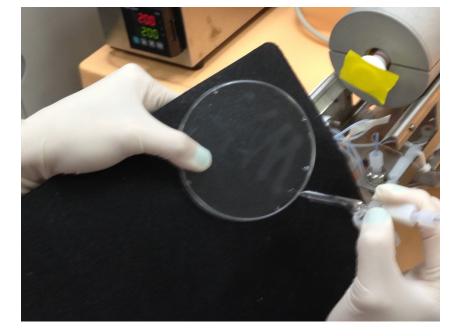
Plasma does not ignite

Check the torch, injector, coil for condensation

Troubleshooting: Common Issues

Problem

2. No/low signal



<u>Cause</u>

Sample line capillary pinched

Sample line capillary inserted improperly

Clogged nebulizer

Solution

Inspect capillary line

Reinsert capillary

Check spray/swap nebulizer

Insufficient gas flows

Check gas flows

Problem



<u>Cause</u>

 Leak in one of the argon lines

Solution

- 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

Problem

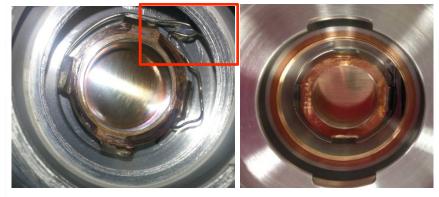
Output File Name	Mass All Mass All Intensit Pulses Dump To File Dual Co	Count
9.20 200000 180000 - 180000 - 140000 - C 120000 - 0 100000 -	205848 Current	 Xe 130 Cs 132 La 138 Gd 154 Tb 158 Tm 164 Os 188 Os 188 Ir 190.9 Ir 192.9 Pb 203
80000 - 60000 - 40000 - 20000 -		9 10

<u>Cause</u>

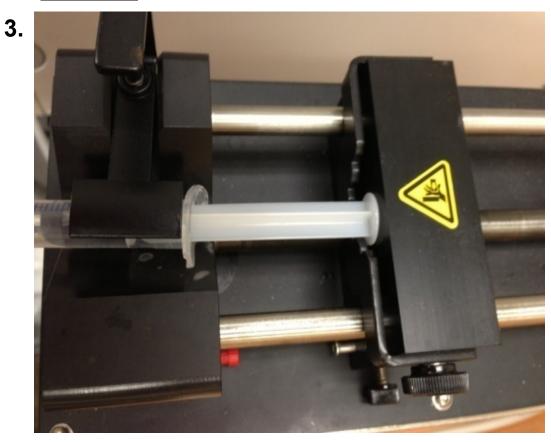
Poor contact of skimmer/reducer with the contact ring on the interface. This may be due to oxidation on the contact ring or on the reducer itself.

Solution

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



Problem



<u>Cause</u>

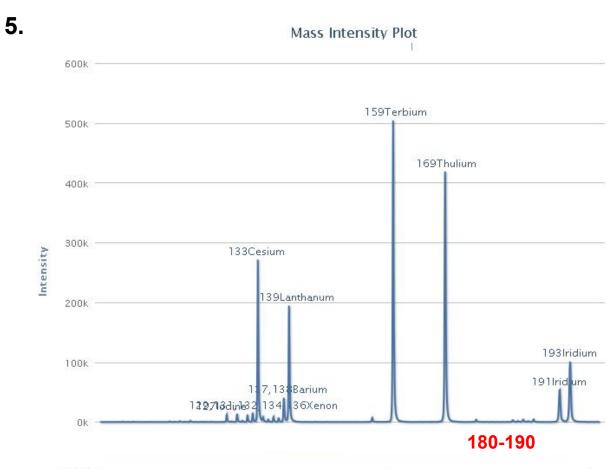
Originally thought it Replaced the entire was a faulty syringe pump. Turned out to be a bad valve.

Solution

valve assembly

	<u>Problem</u>	<u>Cause</u>	Solution
4.	Mass All Value Output File Name E:\Daily QC\2012\Sep 2012\12Sep1 Dump To File Dump To File	Partially clogged/ dirty nebulizer	Clean/swap nebulizer
	177.8 205848 Time ×e 130.905 • Cs 132.905 • Cs 132.905	Sample capillary not positioned properly	Reinsert capillary
	200000 180000 180000 160000 140000 140000 100000 100000 0 0 100000 0 0 0 0 0 100000 0 0 0 0 0 0 0 0 0 0 0	Bad syringe	Replace syringe

Problem



<u>Cause</u>

Tungsten contamination; but from where?

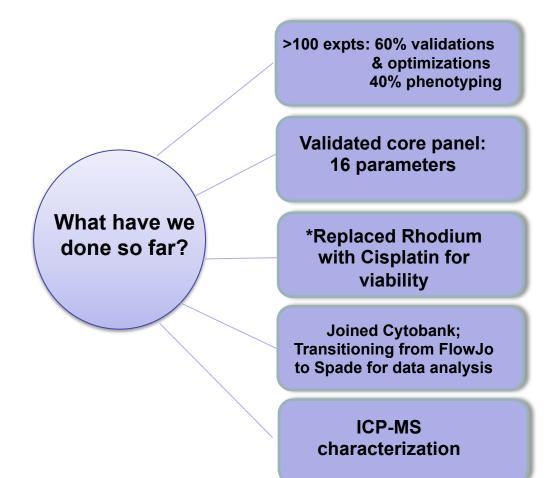
Solution

Cleaned ignitor pin with sandpaper

Progress Report: 1 ¹/₂ 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