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CyTOF®2 Daily Quality Control

CyTOF®2 Daily QC: Auto-Tuning

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- Daily QC involves performance checking and tuning the CyTOF®2.
- Auto-Tuning automatically and simultaneously performs the following:
  - Performance Check: Testing instrument performance
  - Tuning: Calibration of CyTOF®2 for maximum performance
  - **Prior to QC: Ensure CYTOF®2 is properly cleaned and assembled.**
- Auto-Tuning is performed using **Tuning Solution.**

QC Product

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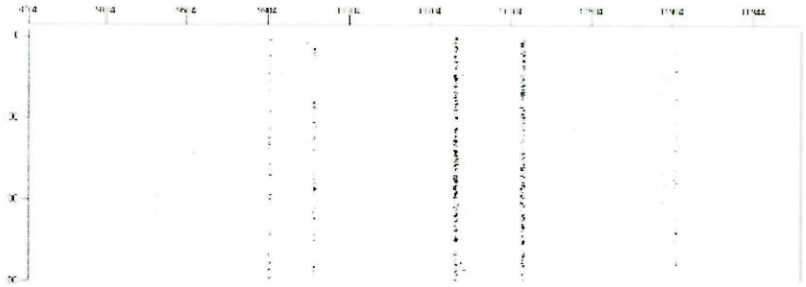

CyTOF® Tuning Solution, E-Pure (250 ml), PN 201072: consists of six isotopes in liquid phase, each at the given concentration.

Isotopes Assessed in Auto-Tuning

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Isotope:	Source:
^{133}Cs	Tuning Solution/Environmental contaminant
^{139}La	Tuning solution
^{155}Gd	Oxide formed from ^{139}La
^{159}Tb	Tuning solution
^{169}Tm	Tuning solution
^{193}Ir	Tuning solution

Auto-Tuning Results

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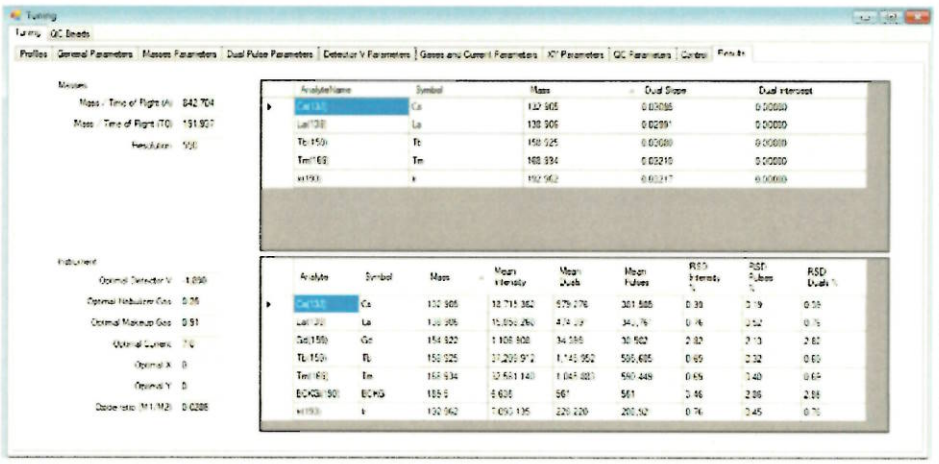

CyTOF® 2 Auto-Tuning

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CyTOF® 2 Daily QC: Auto-Tuning

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- The purpose of QC is to check and optimize the following parameters:
  - Mass Calibration (Automatically run)
  - Mass Resolution (Automatically run)
  - Detector Voltage (selectable)
  - Dual Pulse Calibration (Automatically run)
  - X-Y Alignment (selectable)
  - Gases and Current Optimization (selectable)
  - QC Report (selectable)

Metal Signal Measurement

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- Two different measurements are recorded for each metal in a 13 µsec push:

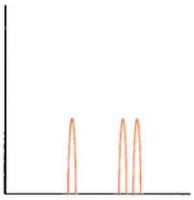
➢ Pulse count

- Most accurate when ion counts are low enough that discreet ion pulses can be counted.
- Detects the exact number of ion pulses per push.

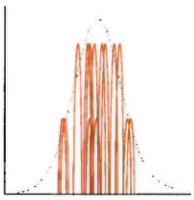
➢ Intensity

- Used when pulse count is saturated, i.e. when ion count is high.
- Detects the total intensity in the channel by measuring the area under the curve.

Pulse count:



Intensity:



Check Mass Resolution

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- Mass Resolution

  PURPOSE: To ensure that ions are tightly segregated by mass. Intensity curves should NOT overlap.

Time of Flight Separation of Ions

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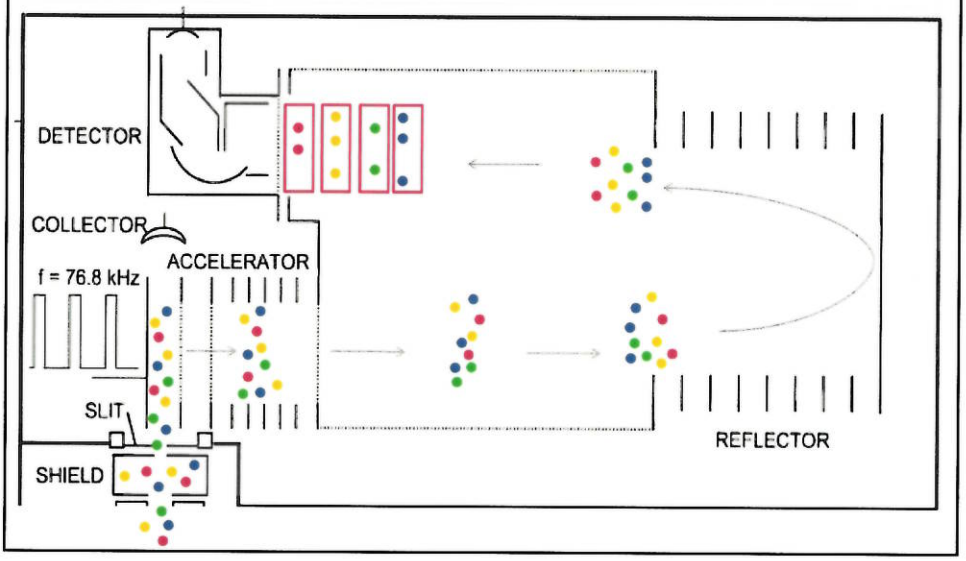
All ions of a particular mass reach the detector over a time range of 21 to 26 ns. This is known as the integration time. It is metal-specific.



Integration Time: metal specific

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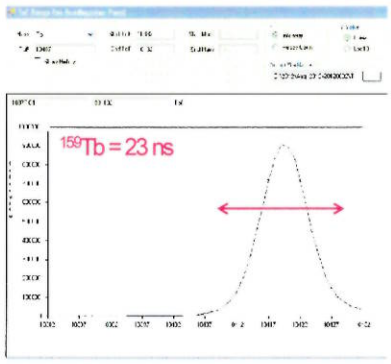
Symbol	Mass	Integration Time
Xe	131	21
Cs	133	21
Nd	142	22
Sm	147	22
Eu	151	23
Eu	153	23
Sm	154	23
Tb	159	23
Ho	165	24
Er	167	24
Yb	171	24
Yb	172	24



ToF values are in ns.

Check Mass Resolution

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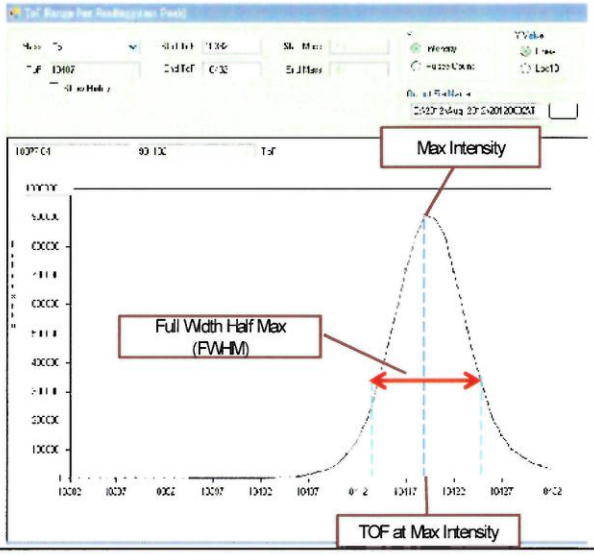
$$\text{Mass Resolution} = \frac{TOF\ at\ 159Tb\ Max\ Intensity}{2\ x\ FWHM}$$



Mass Resolution above 400 = PASS

Check Mass Calibration

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- Mass Calibration

  PURPOSE: To ensure that ions are being detected in the correct detection channels on the TOF scale.

Check Mass Calibration

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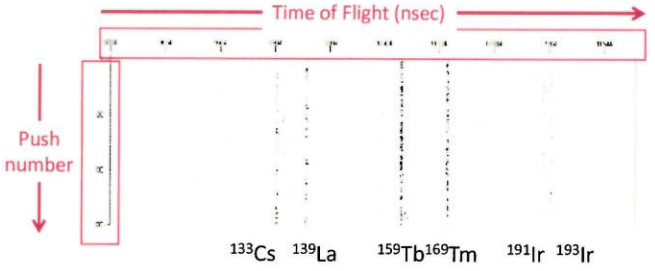

- The TOF for a particular ion is dependent on its mass to charge ratio (M/z). Because all ions have the same charge, the TOF for each ion is directly proportional to mass.
- The TOF value for each mass is **calibrated at install** to ensure optimal measurement in the appropriate detector channel.
- Auto-Tuning checks Time of Flight for the ^{133}Cs and ^{193}Ir peaks and then calculates values for additional isotopes.

  PURPOSE: To ensure that ions are being detected in the correct detection channel on the TOF scale.

Check Mass Calibration

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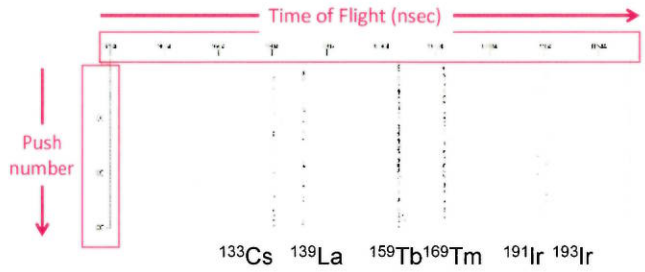

Element	Mass (Da)	TOF (peak) (nsec)
Cs	133	9,533
La	139	9,786
Tb	159	10,591
Tm	169	10,975
Ir	191	11,781
Ir	193	11,852

Detector Voltage Optimization

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- Detector Voltage

  PURPOSE: To ensure that the system is optimized for detector sensitivity and longevity.

Detector Voltage Optimization

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- The detector must be optimized for the correct ratio between pulse count and intensity: this is important for instrument dual pulse calibration, absolute quantification and dynamic range.
- The detector must also be powered with the correct number of volts: this will increase as the detector ages and sensitivity decreases.

Detector Voltage Optimization

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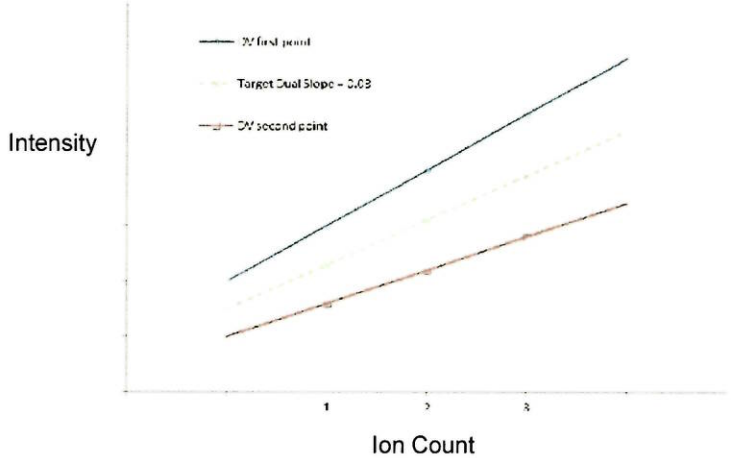
Dual Slope Stability Method

- Intensity is determined for one, two and three ion counts at Detector V1 and Detector V2.
- The optimal voltage is then interpolated to give the target dual slope of 0.03.



Dual Count Calibration

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- Dual Pulse Calibration

  Purpose: To determine a Dual Pulse Coefficient (Dual Slope) that correlates Pulse Count and Intensity.

What are Dual Counts?

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- The TOF detector sees a wide range of signal (low to high analyte density)
- **Pulse Count** (for low concentrations) and **Intensity** (for high concentrations) values are collected for every channel.
- CyTOF plots the entire data range on a single Dual Signal scale, the *units of which are actual counts of particles that hit the detector*. This requires:
  - Determination of a Dual Count Coefficient which relates Pulse Count and Intensity values.
  - Application of the following equation to determine actual counts:

    Counts = Intensity X Dual Count Coefficient
- The instrument-derived (Di) Dual Count Coefficient is determined using the Dual Pulse Calibration during Auto-Tuning of the instrument.

Dual Pulse Calibration

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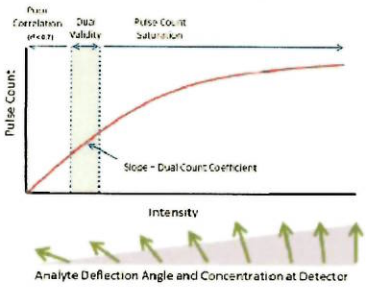

Figure 3: Derivation of the Dual Count Coefficient using Instrument Dual Count Calibration

During the calibration, tuning solution dual count data are collected across a wide range of quadrupole deflection voltages, from least to most optimal (left to right). At least optimal deflection, analyte concentration is too low to collect reliable data (Pulse Count – Intensity R^2 < 0.7). At optimal deflection, analyte concentration causes Pulse Count saturation. In between lies a zone of dual validity in which Pulse Count and Intensity are linearly related. The slope of the line in this region is the Dual Count Coefficient.

- Dual Count Coefficients (DCC) for two analytes at opposite ends of the mass range (^{133}Cs and ^{169}Tm) are determined.
- An equation is determined relating mass and DCC.
- The DCC for all other masses are extrapolated from this equation.

X-Y Alignment

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- X-Y Alignment

  PURPOSE: To fine tune the position of the interface to ensure that ion clouds are being optimally pulled into the cones.

X-Y Alignment

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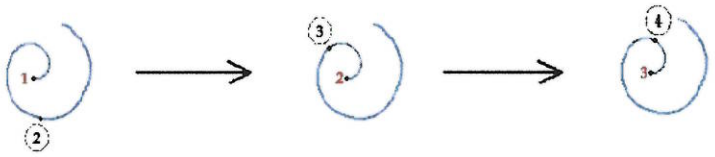
- Auto-Tuning runs an algorithm which uses spiral cycles that begin from a pre-determined starting point.
- During each spiral cycle, the software determines the point where the highest ^{159}Tb signal is achieved.
- The next spiral cycle begins from the point where the high ^{159}Tb signal was found.



Gases and Current Optimization

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- Gases and Current Optimization

  PURPOSE: To ensure that plasma temperature is high enough and that conditions are optimal for ion clouds to travel into the cones.

Gases Optimization

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- The plasma temperature is 5500° K.
- This must be maintained for optimum vaporization, atomization and ionization.
- The formation of metal oxides (M + 16) increases when the plasma temperature drops.

PURPOSE: To ensure that the plasma temperature is high enough.

Gases Optimization

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- A percentage of Lanthanum oxide (measured in the 155 channel) above 3% indicates the plasma temperature is too low.

PURPOSE: To ensure that the plasma temperature is high enough.

Current Optimization

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- Current is checked at optimized Make-up Gas and Nebulizer Gas values.
- The optimum current is where the maximum ^{159}Tb signal is achieved and oxides are still below 3%.

PURPOSE: To ensure that the current on cones is optimal to drive ion clouds through the interface .

QC Report

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- QC Report – ^{159}Tb Dual Mean and RSD %*
  PURPOSE: To ensure that CyTOF®2 is optimally detecting ions .

  * RSD = Relative Standard Deviation (equivalent to CV)