



CyTOF® Software V 5.1.648 Release Notes

CyTOF software version 5.1.648 should be installed on all CyTOF® Mass Cytometer instruments. This version includes several major improvements and critical revisions to the previous version 5.1.584:

1. Instrument Dual (Di) is now the default method for dual signal intensity conversion, improving data consistency.
2. The pulse counting algorithm has been optimized for Dual Count Calibration, and is no longer valid for measuring sensitivity or oxidation in tuning mode. This change also results in a new workflow for daily instrument tuning that is described within these release notes.
3. Dual Count Calibration window has new notifications (described below)
4. The method for identifying cell event boundaries (e.g., “cell length”) has been improved.
5. An option to skip washing is introduced for Autosampler operation.
6. A critical bug related to the Autosampler 5.1 valve initialization has been addressed and AS5.1 should only be operated with this upgrade moving forward.

A brief discussion of the background and implications of each of these important revisions follows.

Installation Instructions:

1. Make copies of these two folders and place them in a desktop folder named ‘CyTOF Backup files’ (in case DVS needs to access in the future):
 - C:\Program Files (x86)\DVSSCIENCES\CyToF\conf
 - C:\Program Files (x86)\DVSSCIENCES\CyToF\Data
2. Download software version 5.1.648 by clicking [here](#).
3. Double-click the cytof_setup_648.msi installer.
4. Remove the following files from C:\Program Files (x86)\DVSSCIENCES\CyToF\conf folder:
 - Instrument.conf
 - SamplerConfig.conf
5. Start the CyTOF software.

Please contact Customer Support (support@DVSsciences.com or 1-855-387-2986) for further information if required.

The DVS Sciences Team

1. Dual Signal Calibration Using Instrument Dual Method

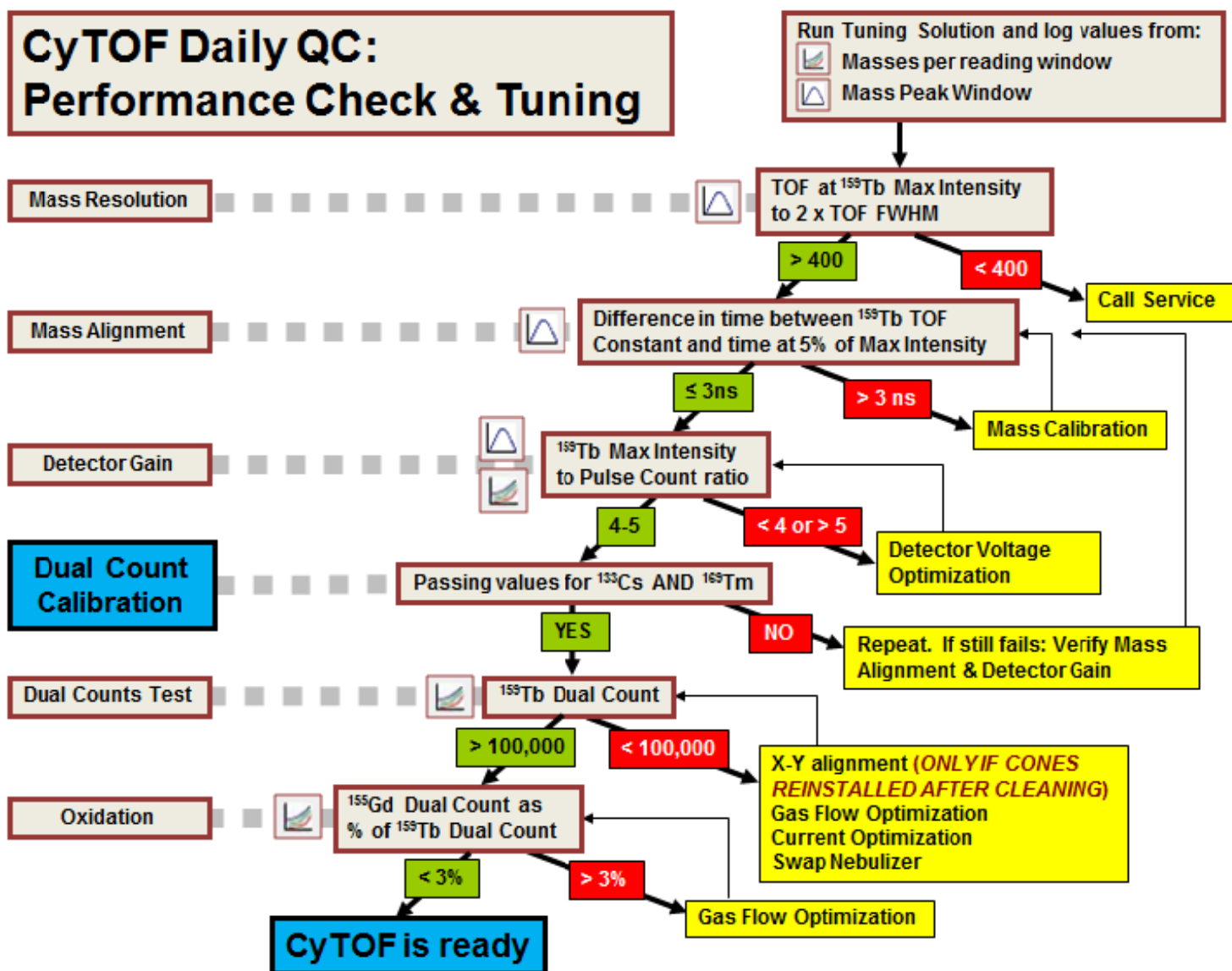
- Ion signals are quantified using digital pulse count at the low end and analog intensity at the high end of the dynamic range. These signals are cross-correlated to form a single 'Dual Signal' scale, such that at the low end, Pulse count is recorded, and at the high end, counts derived from applying a Dual Count Coefficient to the analog Intensity is recorded. Derivation of an accurate cross-correlation Dual Count Coefficient requires data that spans the range of intensities. CyTOF has employed two methods to determine the Coefficient necessary to generate the Dual Signal scale. The first, Instrument Dual (Di), uses data collected from the Dual Count Calibration, which systematically creates and measures data that spans the range of intensities and creates regions of 'dual validity' (i.e., both Pulse and Intensity signals are valid) to accurately determine the correlation coefficient, independent of the characteristics of the sample. The second, called Data Dual (Dd), identifies regions of dual validity within the same data set to determine the correlation coefficient. This method is accurate for data that contain stains that span the range of intensities, but results in overestimation of signal intensity for data with bright stains, and a variable estimation of signal intensity for data with uniformly dim signal. Extensive testing has revealed that Dd does not provide optimal calibration when ion signals change rapidly within some datasets, while Di produces accurate and stable results independent of sample characteristics. **As a result, v5.1.648 (and future releases) collects dual count data in Di mode as the default.**
- **NOTE: You will notice differences in intensities between data processed in Dd vs Di mode since they generate and utilize different Dual Count Coefficients. This difference in intensities has no impact on sensitivity, since the Coefficients are applied to the data after collection by the detector. Di is superior since it provides a consistent Coefficient, as opposed to Dd Coefficients which vary from data set to data set.**
- The values of the Di Dual Count Coefficients are expected to be between 0.03 and 0.06, depending on mass resolution and detector gain settings of the system. As long as Mass Resolution and Detector Gain are within spec, the Di Coefficient is valid.
- Di conversions are only valid when the Detector Gain and Dual Count Calibration are valid. You can re-process old *.imd data with newly generated Di Coefficients provided you know that Detector Gain was properly calibrated at the time of data collection.
- For applications where Dd conversions may be useful or to allow consistency with earlier Dd results, this software version optionally allows reprocessing in Dd format.
- If you reprocess *.imd data recorded with a prior version of the CyTOF software (e.g., to generate the corresponding Di or Dd data), make sure to re-set the cell detection threshold parameter to 200. Please contact your local Field Applications Scientist for assistance if required.

2. Pulse Counting Algorithm

- **Change:** At very low signal intensities, pulse counting is more accurate than analog intensity. As signal increases, the probability of pulse overlap increases, and analog intensity becomes more accurate. CyTOF software has a dual switchover threshold which dictates the signal level at which dual data switches from pulse counts to analog intensity. In previous software versions, the threshold was set to 3 counts per push in tuning mode. At 3 counts per push, there is some signal that overlaps, and the software would split overlapping signals in an effort to maintain accuracy. In v648, the threshold is set to 0.1 counts per push in tuning mode, which virtually eliminates the possibility of signal overlap, and the software no longer attempts to split overlapping signals. This improves the accuracy of the Dual Count Calibration, but requires collection of sensitivity and oxidation ratio tuning data in dual count mode (see next section).
- **Impact on Calibration Workflow:** Since tuning solution contains more than 2 counts per push of Tb signal, sensitivity and oxidation data in tuning mode must be collected in dual count mode (instead of pulse count

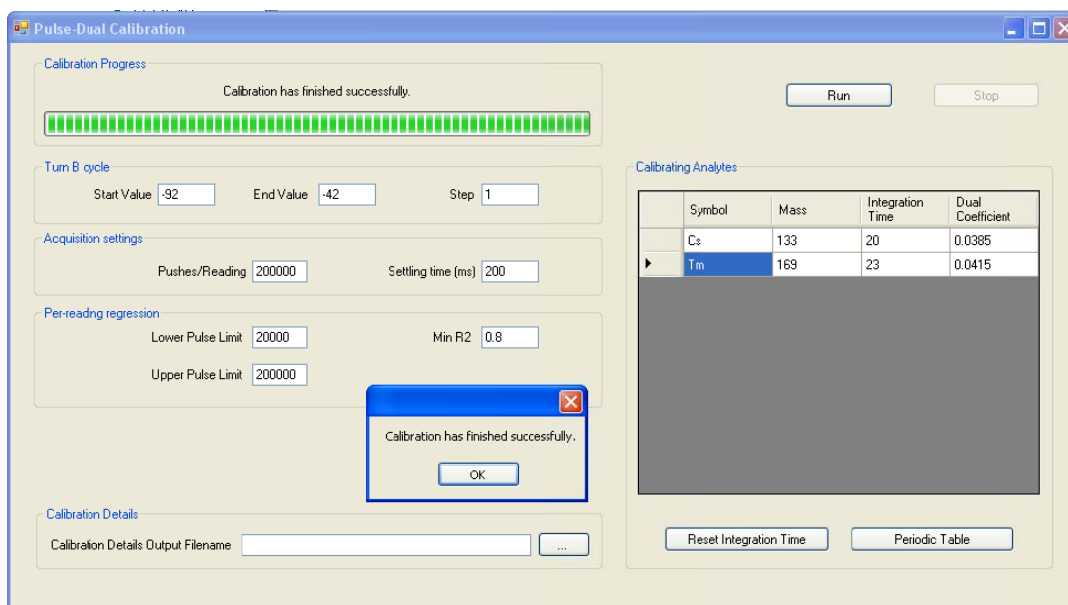
mode which will result in underestimated sensitivity and over-estimated oxidation ratios). Because dual counts are required for these steps, they must be performed after Dual Count Calibration is performed. Dual Count Calibration requires optimal detector gain. Therefore, follow the recommended procedure to perform Dual Count Calibration each day during Tuning (outlined in detail below):

- a. Check Mass Resolution and Mass Alignment
- b. Optimize Detector Voltage
- c. Run Dual Count Calibration
- d. Optimize Sensitivity and Oxidation in dual count mode



3. Dual Count Calibration

- In v648, the Pulse-Dual calibration window shows the default settings appropriate for your instrument (see below). Take a screenshot of the settings for future reference. Note that the Turn B cycle settings are instrument-specific factory settings, and thus may have different values than shown in the screenshot below. These should only be changed by DVS Service. The Acquisition settings and Per-reading regression values should be set as shown in the screenshot below to ensure proper calibration.
- Select Cs-133 and Tm-169 as the two Calibrating Analytes for the Dual Count Calibration. Dual count Coefficients for all other mass channels are extrapolated from these two values, and it is required that these two Coefficients pass (ie have correlation coefficients > 0.8) in order for this extrapolation to be valid.
- If either of these two analytes doesn't pass, a popup will inform you of this, and you should, repeat the calibration. If they fail again, you need to optimize your detector gain.
- **IMPORTANT: You must close the Data Acquisition Settings window in order to save and apply the new Dual Count Coefficients.**

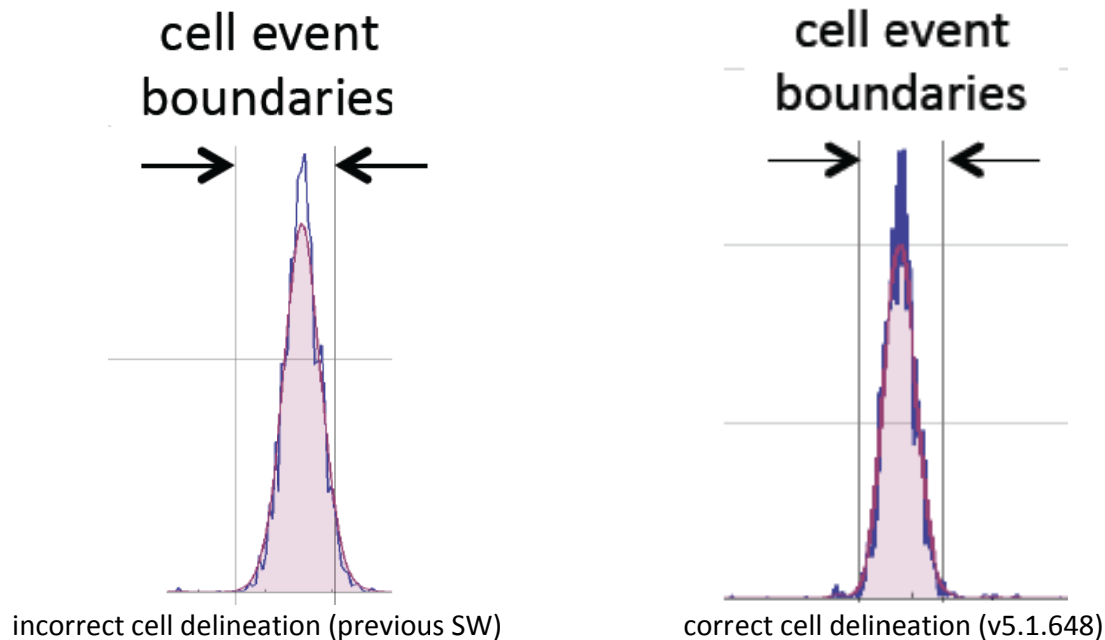


- Detailed explanation of values in the Calibration window:
 - Turn B cycle: The range of voltage applied to the Turn Block where ions are deflected into the quadrupole mass filter. During the calibration, readings are made at each of 50 voltages, spanning the range of suboptimal to optimal deflection of tuning solution analytes, thereby generating data that falls within the zone of dual validity. The Turn B value is a factory setting and should only be changed by DVS personnel.
 - Acquisition settings: sets the number of Pulses per reading to 200,000
 - Per-reading regression: sets the criteria for a valid Dual Count Coefficient. The zone within which both pulse counts and analog intensity are linearly related to one another (ie the zone of dual validity) occurs for readings that have between 0.1 and 1 pulses per push. Because 200,000 pushes are made per reading, those readings that have between 20,000 pulses (lower limit, 0.1 pulses per push) and 200,000 pulses (upper limit, 1 pulse per push) are included in the Pulse-analog Intensity regression analysis. Only those analytes with Pulse-analog Intensity correlation coefficients (R2) greater than 0.8 are considered

'passing' analytes, and the slope of the linear regression is equal to the Dual Count Coefficient for that analyte.

4. Cell Event detection

- V5.1.648 improves the alignment of the cell integration window to the Gaussian kernel used to distinguish single cells from background, debris and multicellular events, resulting in more effective discrimination of single cell events from other events (Fig 1).



5. Skip washing option for Autosampler

- By default, the Autosampler washes the probe and tubing between wells to minimize sample-to-sample carryover. An option to save time by skipping this wash has been added for cases in which large volume individual samples are split into multiple wells.

6. Autosampler 5.1 initialization

- Autosampler 5.1, and the corresponding upgrade from Autosampler 5.0, uses a different valve than the predecessor product. The v648 software upgrade provides for proper operation of this valve.