

## **OSIRIS V2.3 Release Notes**

6/19/2014

Note: Revision 2 of the OSIRIS User's Guide is available on the OSIRIS Homepage (see below).

### **New features:**

#### **No need to upgrade Operating Procedures**

1. Operating Procedures no longer need to be upgraded to be used with OSIRIS Version 2.3. All analyses use a centralized message book file. As before, OSIRIS 1.x Operating procedures will not work with OSIRIS Version 2.3.

#### **Baseline Normalization**

1. Improved normalization to make baseline normalization more robust and prevent minor undershoot due to pull-down in raw data.
2. A button was added to the Graph View to allow display of the subtracted baseline when normalization is active.
3. Baseline estimation uses raw data where the analyzed data falls below a threshold. Changed threshold from hard coded percentage of Analysis Threshold (minRFU) to a user-specified RFU threshold. Default is 10 RFU (in analyzed data).
4. Allow user to set window width for dynamic baseline estimation. Default changed from 9 to 15.
5. Baseline estimation for normalization improved in high noise situations by optionally prefiltering raw data using a user specified filter window (default = 15). Once baseline is estimated, the filtered data is deleted. The estimated baseline is subtracted from original raw data which is then analyzed. (Does not apply to non-normalization baseline estimation.)

#### **Reference versus possible mixture sample analysis**

1. Added capability to differentially apply artifact filters to known reference samples versus samples that might potentially be mixtures in the same analysis. The user can choose to disable one or more of the following: fractional filter, pull-up fractional filter, stutter filters (positive and negative), and adenylation filter for samples that might be mixtures. The software distinguishes between known single source samples and possible mixtures based on user-specified file name/sample name search strings in the lab settings. The user-specified sample analysis and detection thresholds still apply uniformly across all samples.

## Artifacts

1. Made inter-locus pull-up non-critical and restricting, so the artifacts associated with those peaks will be non-critical regardless of other artifacts associated with the peak. This reduces the number of interlocus artifact peaks that require editing.
2. Cross channel (pull-up & spike) analysis has been improved to be more reliable and more robust, resulting in fewer artifacts that require editing.
  - a. New signals are analyzed including sigmoidal peaks that have a positive and negative component, and negative peaks.
  - b. Potential craters and sigmoids are assessed at the start and eliminated if they do not arise from a pull-up, based on spacing.
  - c. No peak is considered a primary pull-up unless its height exceeds a user-specified threshold.
  - d. Cross-channel peak proximity spacing is now based on ILS-base pairs rather than time, making the new algorithm more accurate and reliable.
  - e. Peaks are assessed as pull-up depending on peak types, based on peak proximity differences.
  - f. No crater peak can also be assessed as a side peak to a different crater.
3. The multi-peak poor morphology message has been made non-critical and such peaks are not called as alleles to reduce the number of artifacts that require editing.
4. Added a metric to estimate the quality of the sample-to ladder fit. This measures the linearity of the mapping between ladder and sample. Added optional test and threshold to warn if metric is “too large”.
5. Added ability to optionally suggest rework if there are too many peaks with excessive residual in the sample.
6. Added ability to distinguish alleles from artifacts based on excessive residual displacement (ERD) within each locus. Peaks may migrate differently than the ladder peaks giving rise to Peak Residual, or “shift,” due to differences in electrophoretic conditions between ladder and sample. This can occur across the entire electropherogram, or be localized to one or more loci. Because Residual, or shift, is due to electrophoretic conditions, all the peaks in a locus should have a Residual of essentially the same magnitude and direction (+/-). The Residual Displacement (RD) metric is a measure of differences in Peak Residual, or shift, between a valid allele and a possible artifact peak in the same locus. The test requires the selection of a “known” valid allele in a locus, typically the tallest allele, and then calculates the difference between the residuals of each other candidate allele in the locus and the residual of the valid allele – the residual displacements. ERD only reports in the presence of one or more secondary artifacts including: unacceptable fit, marginal fit, spike, blob, width unexpectedly large or small, or multi-peak morphology. If the user specifies that the ERD artifact should be non-critical, then the peak is non-critical independent of other artifacts. Displacements in excess of the user-specified ERD threshold (default = 0.17 base pair) indicate an unlikely allele. ERD artifact peaks are not called as alleles, but they can be edited to

override the software recommendation. This reduces the number of low level artifact peaks that require editing.

### **New kits, ILS, and ladder analysis**

1. Added PowerPlex ESX 16 and 17 and PowerPlex ESI 16 and 17 marker sets.
2. Added ILS's for Identifiler and IdentifilerPlus that optionally omit the ILS 340 peak or the ILS 250 and ILS 340 peaks. Added ILS for PowerPlex 16 that omits the ILS 225 peak. Omission of those peaks may reduce the amount of Residual (shifting) seen in allele peaks.
3. Improved ladder locus analysis algorithm using known ILS base pair information to more efficiently select correct peaks. The new algorithm is also more robust and improves peak selection for evenly spaced ladder loci, reducing the number of ladders that fail to analyze.
4. Controls and ladders can now be designated based on sample name as well as on file name – the option is selectable in Lab Settings

### **Bug Fixes:**

Various minor bugs that did not affect accuracy were fixed.

### **Revision 2 of User's Guide Version 2.3**

Revision 2 of the OSIRIS User's Guide, available on the OSIRIS Homepage, has an explanation of differential analysis of reference (single source) versus possible mixed samples (described above) that was not included in revision 1 of the User's Guide.

To update the copy of the User's Guide that is available through the help function of the OSIRIS program, rename the User's Guide (OsirisHelp.pdf) and copy the revised User's Guide into the same directory. (Located in \Osiris on the PC. For the Mac, Open the Osiris Mac folder, control-click or right-click OSIRIS and select 'Show package contents', the file is in /Contents/MacOS).