nCounter Troubleshooting Tool – Quick Start

**Basic Workflow**:

1. Click “Import”
2. Import via whole directory or by selecting individual files
3. Choose a table or SLAT output available given the files imported (i.e. MTX, RCCs, or both).
4. If running **SLAT on ONLY the run logs**, instead directly select the RunLogs directory within a set of Sprint user run logs and the relevant parts of the SLAT will be run automatically

**Importing Files**:

The “Import” button in the upper left corner is the starting place for any of the functions of the tool. Next to it you’ll see two radio buttons labeled “Folder” and “Files” which determine the mode of import when clicking the import button. The behavior is as follows:

**Folder**: Clicking this will allow you to browse directories only. Clicking “OK” after selecting a directory will recursively open all folders and zips within the directory and import any RCCs or MTX files found.

**Files**: Clicking this will allow you to browse and select one or more files. Selected RCCs or MTX files will be imported as you’d expect and selected zip files will be extracted and searched recursively for any MTX or RCC files.

Next to the “Folder” radio button are two checkboxes, “RCC” and “MTX”, which allow you to specify what file types you want imported (useful in cases where importing large amounts of files bogs down when both are selected).

**Cartridge and Lane view windows**:

After importing files you’ll see any cartridges represented within those files in the window on the left, labeled “Cartridges Represented”, and any lanes included (the tool merges RCCs and MTX files for the same lane into a single object) will be shown in the window on the right labeled “Lanes Imported”. Whether MTX or RCC files are imported for each lane is indicated by checkbox columns for each.

Whole cartridges or individual lanes can be selected or deselected for generating tables, SLATs, etc. using the checkbox columns labeled “selected” in both windows. Unchecking or checking a cartridge’s “selected” checkbox will do the same for the imported lanes from that cartridge.

**Running the SLAT report:**

The SLAT report is an output used by instrument sustaining, instrument services, and Tech services for Sprint run diagnoses. If MTX files from a Sprint run have been imported the “SLAT Report” button to the lower left will become enabled. Clicking that button will have the following behavior: if only one cartridge is selected the SLAT output will open directly. If multiple cartridges are selected a dialog will open allowing you to run SLAT for each cartridge one at a time at the touch of a button. If a Sprint run did not generate MTX files, SLAT can still be run to obtain pressure traces, run history, etc. by using the Directory import mode, and directly selecting the RunLogs folder within the user run logs. The SLAT should open automatically with no further button clicks.

**Table Output:**

Depending on file types imported, one or more of the “Table” buttons will become enabled. FOV Lane Averages, gives imaging metrics recorded for each field of view, averaged across all FOV. The String Class Sums table gives sums across all FOV of the binned classes of fluorescent spots detected on the lane. The Code Summary table gives summed counts by reporter (i.e. genes, proteins, or DSP IDs). Finally, the troubleshooting table is a quick table the nCounter side of Tech Services uses for initial diagnosis of a lot of issues, as it captures what’s needed to diagnose the most common issues.

The FOV Lane Averages, String Class Sums, and TroubleShooting tables are only available if MTX files are included, whereas Code Summary table is available for both.

**RCC/MTX merging:**

When MTX files and RCCs for the same lane are imported, the tool checks that the files match the same lane and then merges them into the same Lane object. The main impact of this as far as output is concerned is that, for the Code Summary table, targets only available on RCCs or MTX files will be merged into the same table.

**Cross Codeset:**

All versions of RCCs and MTX files are compatible as are all of the RLF types, including DSP and PlexSet. For situations where files imported include more than one RLF (i.e. cross RLF) the tool automatically imports both RLFs from the available repositories on the networked drives (Z codeset drive, etc. assuming the drives have been mapped on the user’s computer) or pops up a dialog box to allow the user to import the RLF. Once RLFs are imported, the tool matches all overlapping content (i.e. using the same probeID) such that the Code Summary table will only output those probes that overlap.

**DSP:**

All nCounter readout for DSP can be interpreted with correct target assignments using this tool. If a Lab Worksheet is imported, the computer networked, and the DSP drive mapped on the user’s computer, Probe Kit Config (PKC) files will be automatically imported to correctly assign DSP\_IDs to targets and wells. If not, a dialog box will pop up allowing all needed PKCs to be imported.

**On the horizon:**

A quick plotting function is currently in the works to more easily review metrics, string classes, counts in bar plot, bubble plot, or scatter plot format. Stay tuned (on hold at the moment). Also I will be looking to add something like a SLAT for Gen2 to make these diagnoses more standardized as well.

Let me know what you would like to see added/removed/altered and thanks for reviewing this tool!!