CrossPlate is a software application developed at Hauptman-Woodward Institute for analyzing the performance of crystallization conditions (cocktails) across multiple high-throughput reads. These reads may be representative of different proteins, or a single protein screen over time. The program produces output indicating which cocktails matched a target result (e.g. "Crystal"), and which read(s) produced the target result; and graphical output to aid the user in identifying trends.

This document will walk the user through use of the program and explain the available options.
(Some information in examples is blurred out to protect privacy.)

Using the Program

After starting *CrossPlate*, a splash screen will appear to introduce the program, followed by the main interface (Figure 1). The user must enter information about the analysis before running the program:

Group Name: This is used to identify the outputs from the analysis; they are grouped together under this name.

Output Location: The user chooses a path in which the outputs are placed; a new folder, named with the Group Name, is created at this location.

MSO/RAR list: The user selects 2-16 read files on which to perform the analysis. ([Shift] or [Ctrl] may be held to select multiple files.) These reads can be MSO files (scored or "marked") or RAR files (unscored). However, when selecting an MSO file, the related RAR file must be present in the same directory. The first file in the list must always be an MSO file, even if the other files are RARs. It is the user's responsibility to ensure that all reads are from the same generation (composite list). The user must also select an alias for each read, which will appear in the image outputs.

Match Settings: A combination of scores is selected to represent a "match". Any combination of scores can be used; the default is [Crystal]: only cocktails with crystal hits will be matched. The ["Marked" Only] option, however, is exclusive; this option instructs the program to match against any conditions that have been marked (by *MacroScope*) or selected (by *MacroscopeJ*).

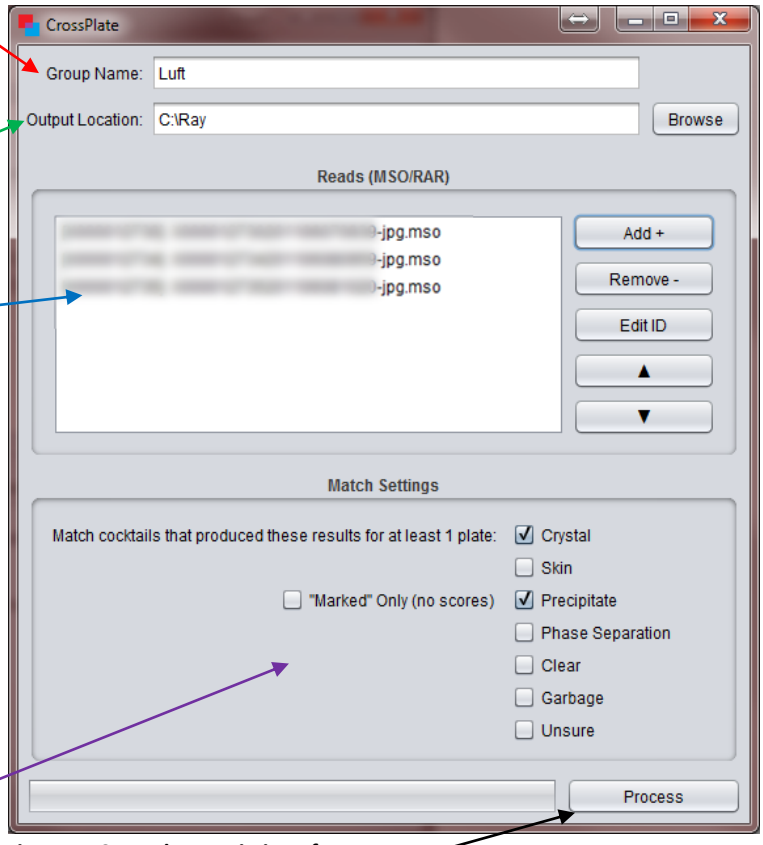

Figure 1: CrossPlate main interface.

Process: Clicking this button starts processing selected reads to produce the output. The user will have the option to cancel processing.

Analysis

CrossPlate examines every MSO file in the list, comparing each cocktail's scores with the pattern indicated by the user. Whenever a score match is found for a cocktail *in at least one MSO*, an image output is created for that cocktail (Figure 2).

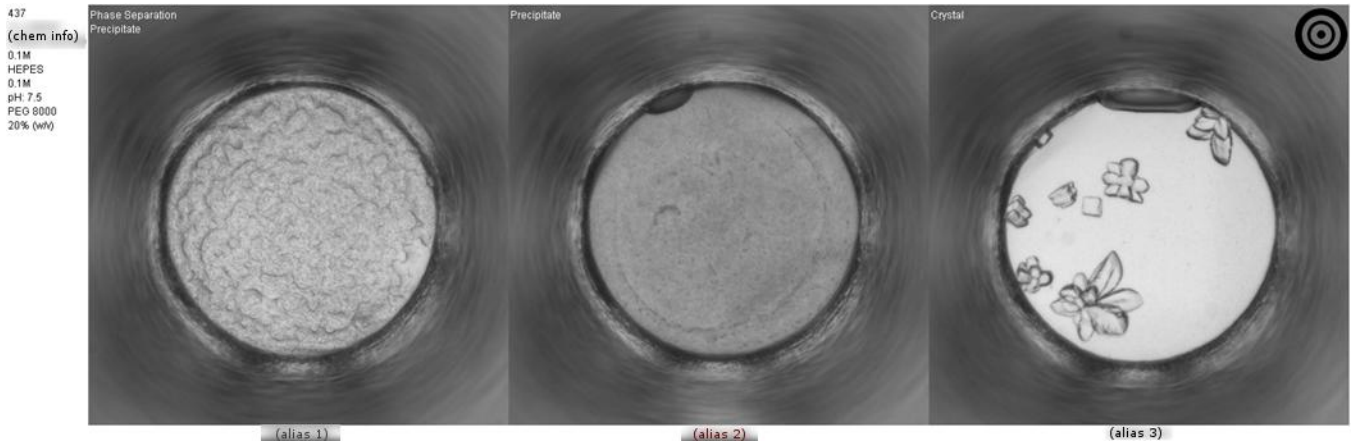

Figure 2: Image output (per matching cocktail). At left is a listing of the cocktail composition being represented. An image of the cocktail's result from each read is shown, along with the scores that were given to each image. A "bulls-eye" icon is drawn on the image(s) that matched the selected condition – in this case, "Crystal".

As mentioned before, reads can be included using either MSO (MacroScope Output) files **or** RAR (image archive) files. Only MSO files are "scored", containing information about the experiments that can be used to find matches. Any RAR-only reads included are ignored for purposes of matching; but images are still displayed in the case that the cocktail in question had a match in one of the MSO reads. Because only MSO files contain the score information required to match, one of the reads must always be an MSO file. Also note that an MSO file does not contain any images; therefore, each MSO file selected must have a corresponding RAR file in the same folder.

MSO files may also be saved from MacroScope or MacroscopeJ without being scored; these MSOs contain cocktails that were "marked" or "selected" as being interesting (vs. other cocktails). Therefore, CrossPlate contains an option to match marked conditions and ignored unmarked ones. Since the match is not dependent on scores, this option is exclusive (cannot be chosen along with a "score" pattern).

Here are some examples of how the analysis can be set up:

1.) The user chooses "aaa.mso", "bbb.mso", "ccc.mso" – 3 *scored* MSO files; and opts to identify "Crystal". Whenever CrossPlate comes across a cocktail that had a crystal in at least 1 read, an output image (Figure 2) will be produced, indicating which read had the crystal. (Note that "aaa.rar", "bbb.rar", and "ccc.rar" must all be present in the same folder with the MSO files.)
2.) The user chooses "aaa.mso" – a *scored* MSO file – and "ddd.rar". CrossPlate will create an image output for any cocktail that had a match in "aaa.mso"; and will also include the corresponding cocktail image from "ddd.rar". Nothing from the RAR, however, can be used to produce a match.
3.) The user chooses "xxx.mso" and "yyy.mso" – 2 *unscored* MSO files; and selects the ["Marked" Only] option. All entries from either MSO file (i.e., which were marked in MacroScope) will be counted as a match; and an output image will be produced, indicating which image was marked.

Summary Outputs

There are 2 additional types of output which serve to provide a summary/overview of the matches. One is a textual report (Figure 3) which gives a listing of all matches, sorted by which read(s) produced the match.

Group Name & Match Pattern

Read Key (indicates which reads are being referred to in the first column of the listing)

Match Listing (shows each match; which reads contained the match; and the cocktail's composition)

Distinct Combinations of reads, and how many matches occurred for each combination.

Individual MSO Contributions (how many cocktails contained a match for each particular MSO)

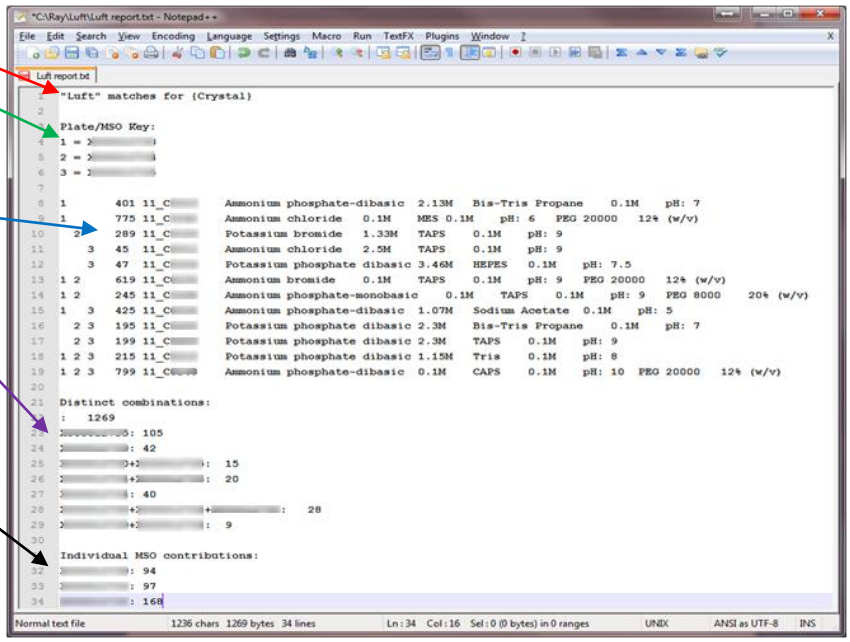

Figure 3: Textual report. The report is best viewed in a text editor with "word wrap" turned off; or pasted into a spreadsheet program like Microsoft Excel.

The other summary output is a graphical table (Figure 4) listing all matches (as in the text report) and connecting them by color-coded lines to the read(s) which produced each match. Note that the dimensions of this image can be ***VERY*** large. It is usually not possible to see the detail of the list and the trends of the reads at the same time; you will have to zoom in and out.

All outputs are placed in the output folder chosen by the user. The image outputs can be most easily viewed using an image preview program (e.g. *Windows Photo Viewer*: right-click and image and select "Preview" from the context menu) or a slideshow. The text report is best viewed in a text editing program with any "word wrap" or "line wrap" features turned off; or pasted/imported into a spreadsheet program such as Microsoft Excel.

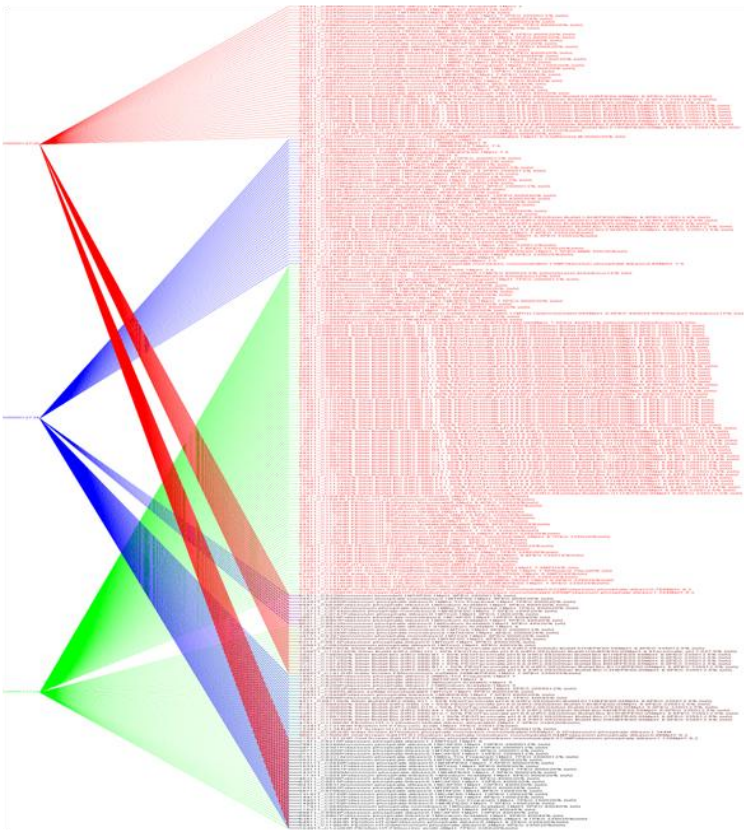

Figure 4: Match trends by read(s). Each read is color-coded. Matches are listed from top (least reads) to bottom (most reads).