

WORK-FLOW

Specialized Flow Cytometry Solutions

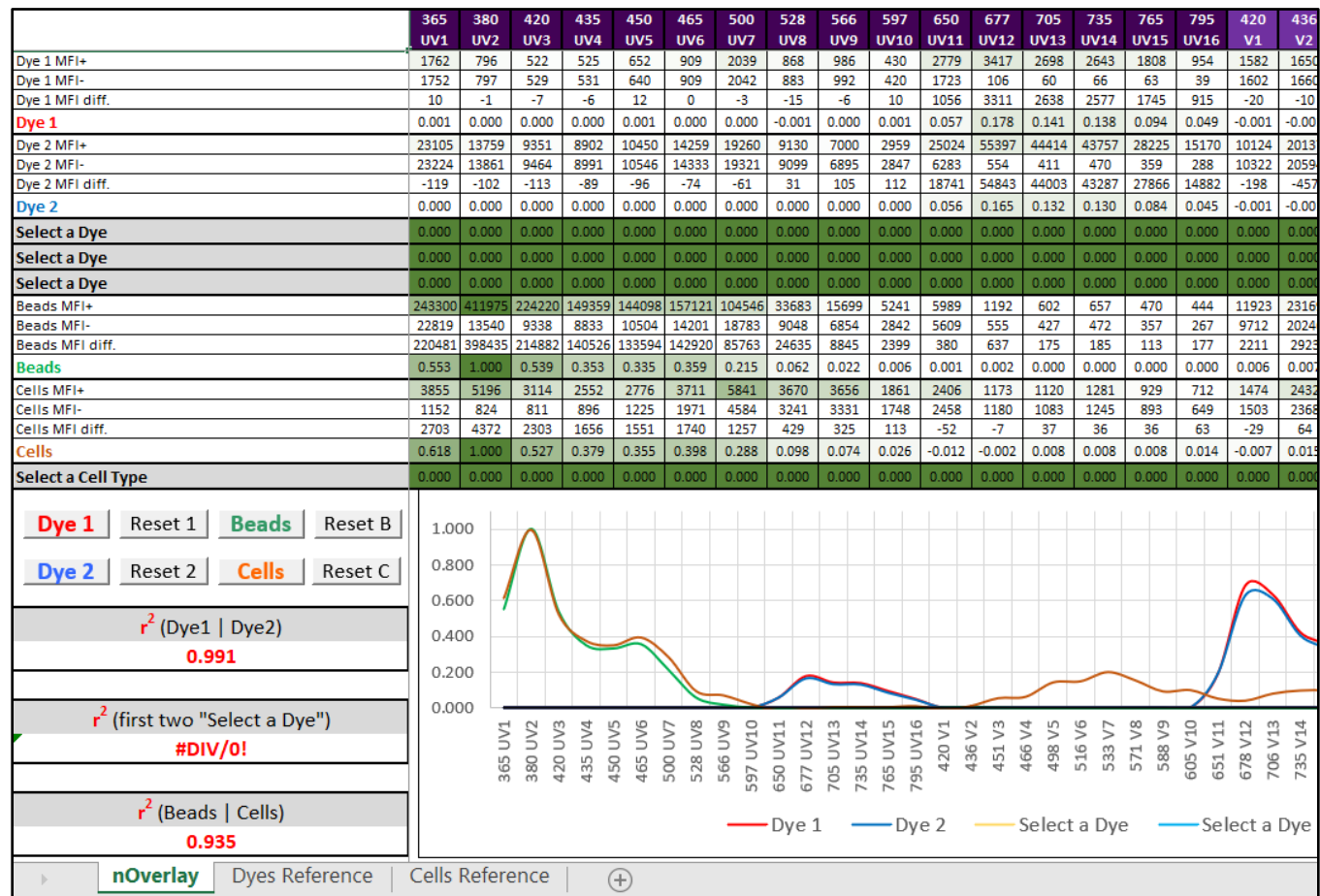
Spectral nOverlay Instructions

1. Preface

This tool enables the comparison of normalized spectral signatures to gauge fluorochrome uniqueness, to determine if dye alterations occurred, or simply to visualize autofluorescence contributions.

Data from exported statistics csv files are loaded using an embedded macro and can be stored in the “Dye Reference” or “Cell Reference” sheets of the document for future use.

This tool can also be modified to overlay non-normalized signatures, a practice that can sometimes be useful when comparing raw MFI values.



nOverlay

Dyes Reference

Cells Reference

+

2. Navigating and Using the nOverlay Tool

- Open the nOverlay Excel spreadsheet. If warned that the macros have been disabled, click the “Enable Content” button.



SECURITY WARNING Macros have been disabled.

Enable Content

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- Go to the Developer tab > View Code > double-click Module 1, and modify the path in the first line for Button1 through Button4 to reflect the location of your statistic files (an example is provided).

```
Sub Button1_Click()  
    Workbooks.Open ("C:\Files\Work\WORK-FLOW\3 Resources\FlowPublic\2 - Upcoming Update\nOverlay Reference csv\1.csv")  
    Workbooks("nOverlay.xlsm").Worksheets("nOverlay").Range("B2:BM3").Value = _  
    Workbooks("1.csv").Worksheets("1").Range("B3:BM4").Value  
    Workbooks("1.csv").Close SaveChanges:=False  
End Sub
```

- The csv statistics files provided in this package were generated with Cytex™ SpectroFlo® on a 5-laser 64-parameter Aurora and must be labelled as follows:

1 for Dye 1 | **2** for Dye 2 | **B** for Beads | **C** for Cells

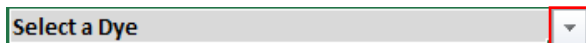
- Click the desired button to load the content of a statistics file and display them on the graph.



- The normalized values can be copied and pasted to the “Dye Reference” sheet for future use.

Dye 1	0.001	0.000	0.000	0.000	0.001	0.000	0.000	-0.001	0.000	0.001	0.057	0.178	0.141	0.138	0.094	0.049	-0.001
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- To recall a saved reference, click the “Select a Dye” drop-down menu.



Note: using the “Select a Cell Type” one can recall an autofluorescence signature from the “Cells Reference” sheet.

- To remove a spectral signature from the graph, simply delete the value in the “Select Dye” or “Select a Cell Type” (column A) or click the Reset button associated with the signature.
- Values for the similarity metric, r^2 , can be found to the left of the graph for several pairs of signatures.