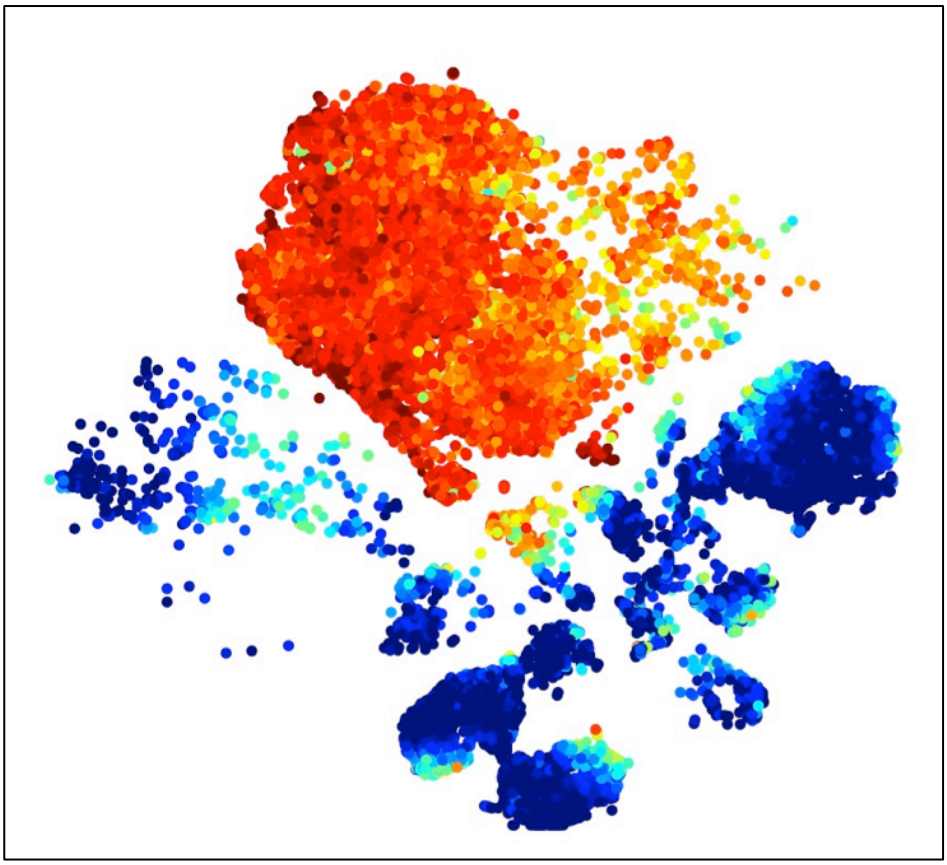
Creating colourised tSNE plots using R

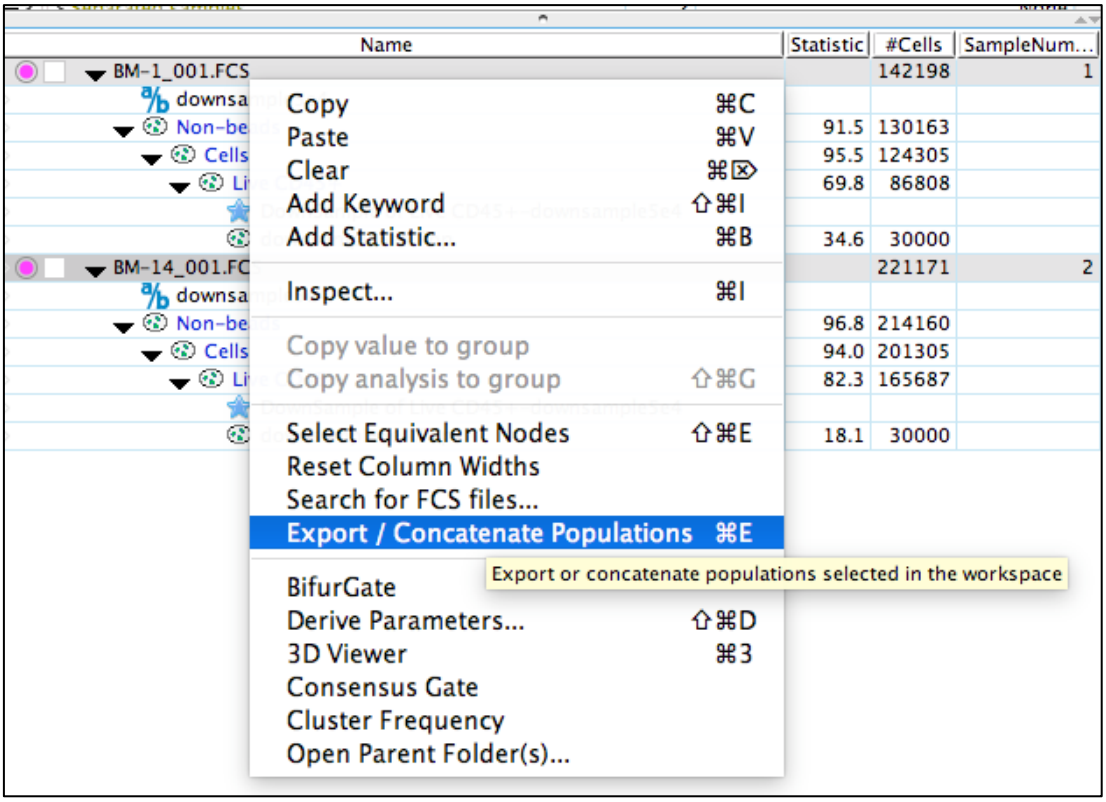


Thomas Myles Ashhurst
April 2017
Sydney Cytometry core facility,
The Centenary Institute and The University of Sydney

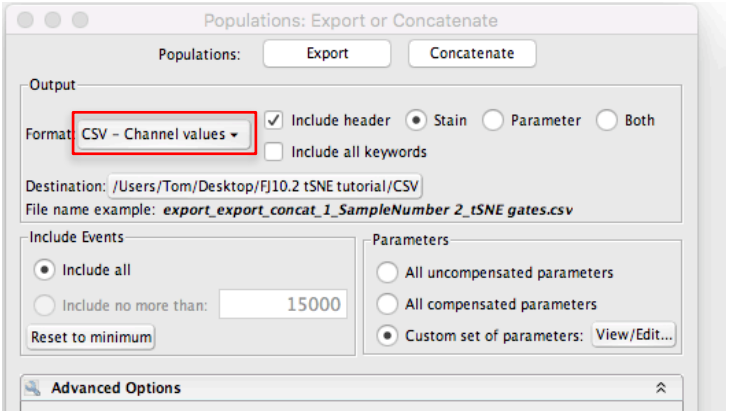
thomas.ashhurst@sydney.edu.au
www.sydneycytometry.org.au

Select all relevant samples in FlowJo workspace

- Select all relevant samples in the FlowJo workspace
- Right click, select ‘Export / Concatenate Populations’



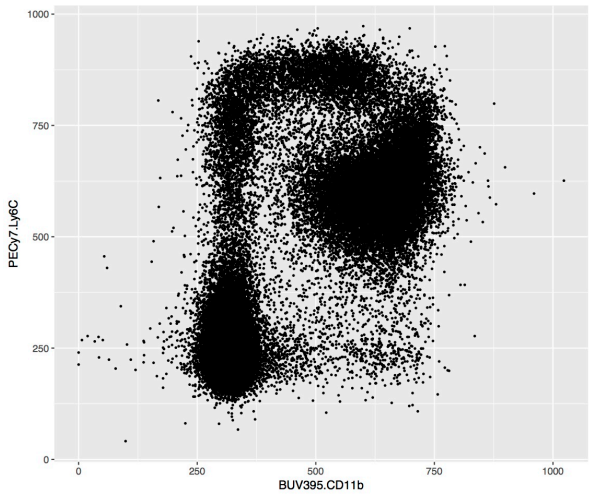
CSVs for colourised images



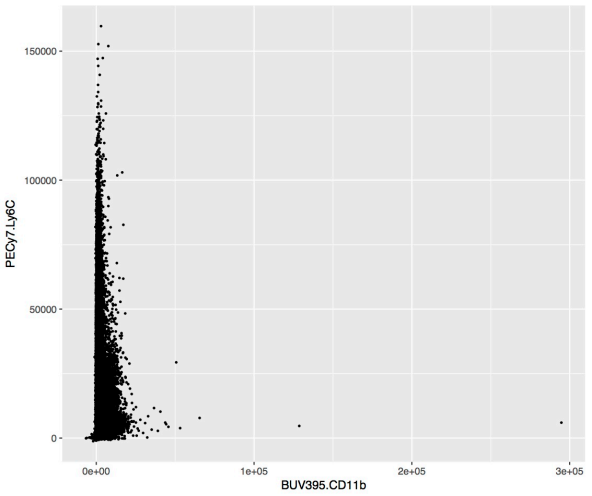
Output

- Select to export CSV files
- Option A: 'CSV – Channel values'
  - **Preferred option**
  - These parameter values are modified so that a bi-exponential plot can be visualized on a linear axis (and a linear colour gradient)
  - Values typically range from 0 to ~1,000
  - This is preferred, as individual bi-exponential transformation settings on different fluorescent parameters are difficult to account for in the R coding for generating plots.
- Option B: 'CSV – Scale values'
  - These are the exact parameter values, unmodified
  - If your data has been transformed in cyt/MATLAB before loading in FlowJo, use this option
  - This will be graphed and coloured on a linear scale by default, which isn't suitable for fluorescence or mass cytometry data
- Fluorescence vs mass cytometry data
  - Fluorescence: it is possible to use scale values and simply transform the axis and colour scale in R into a biexponential format, but it is prohibitive to create individual settings for each parameter
  - Mass cytometry: because all the parameters are transformed with the same settings, scale values can be exported, and the axis scales and colour gradient can be arcsinh transformed with a uniform co-factor (not described in this protocol)

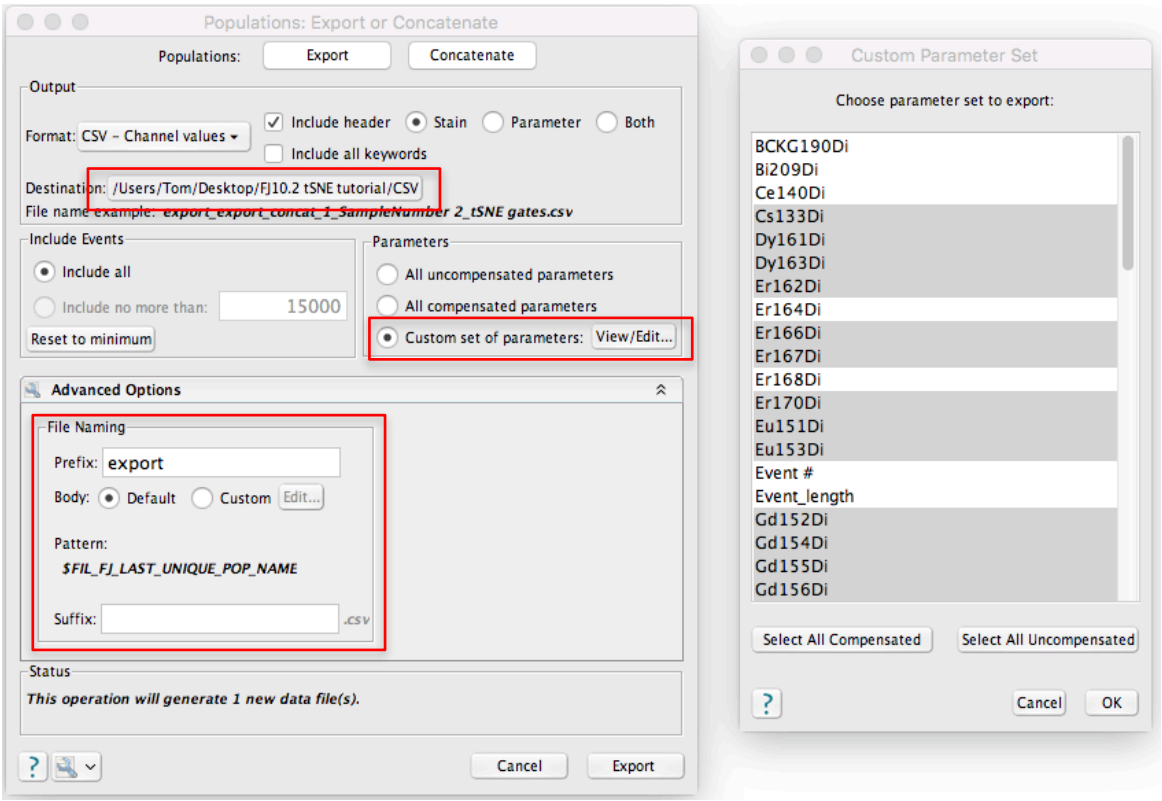
Channel values



Scale values



Exporting CSV files



- **Destination**
  - Select a destination for the CSV files
- **Parameters**
  - Select ‘Custom set of parameters: View/Edit’
  - Select the parameters that you wish to be included for colouration
  - For fluorescence data, select the ‘COMP-fluorophore/marker’ option
- **Advanced options: File Naming**
  - Prefix: keep or delete ‘export’
  - Suffix: write ‘tSNE_embedded’
- **Click ‘Export’**
  - Once export is COMPLETE, click cancel to close the window

Modifying R script in Rstudio

Download ‘R’ and ‘R studio’

- R: https://cran.r-project.org/mirrors.html
- R studio: https://www.rstudio.com/

Download ‘tSNEplots’ script

- Go to: https://github.com/sydneycytometry/tSNEplots
- Download the R script ‘tSNEplots.R’
- Open in Rstudio

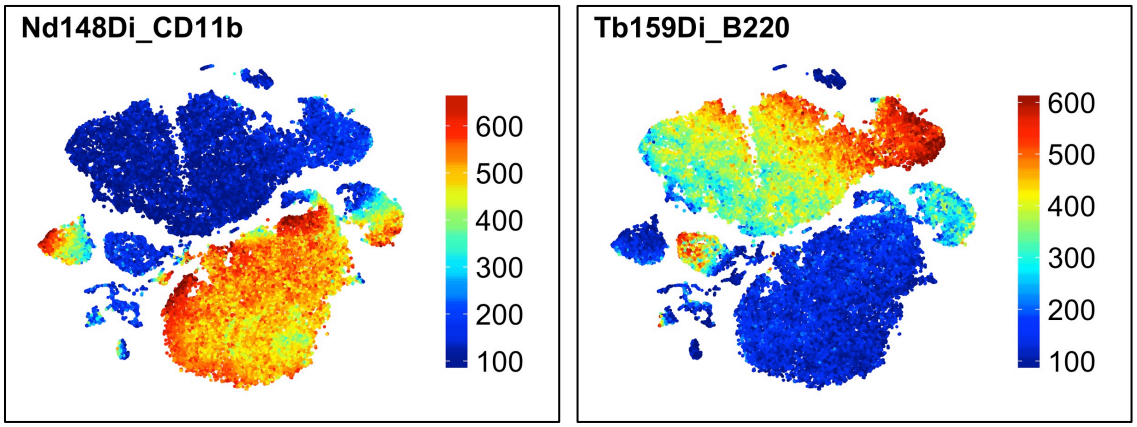
Follow instructions

- Follow the comment instructions in the script to generate colourised tSNE images
- Run ‘Step 1’ to setup the script packages
- Go through ‘Step 2’ line-by-line to set up the samples and parameters
- Modify tSNE parameter names in ‘Step 3’,

Run script (step 3)

- Run the entirety of step 3
- Images will be created in sub-folders in your working directory

Check images



Please reference us if you found this script helpful