

# UMAP SUPERVISED TEMPLATE (UST) - TUTORIAL USING CYTOF DATA

This tutorial shows how to use AutoGate's UMAP supervised template (UST) feature. It uses the heavily referenced panoramic data set from Stanford's Gary Nolan lab. This data is in 2 pivotal Nature publications in flow informatics

- Nikolay Samusik's publication on X-shift
- Leland McInnes's publication on UMAP.

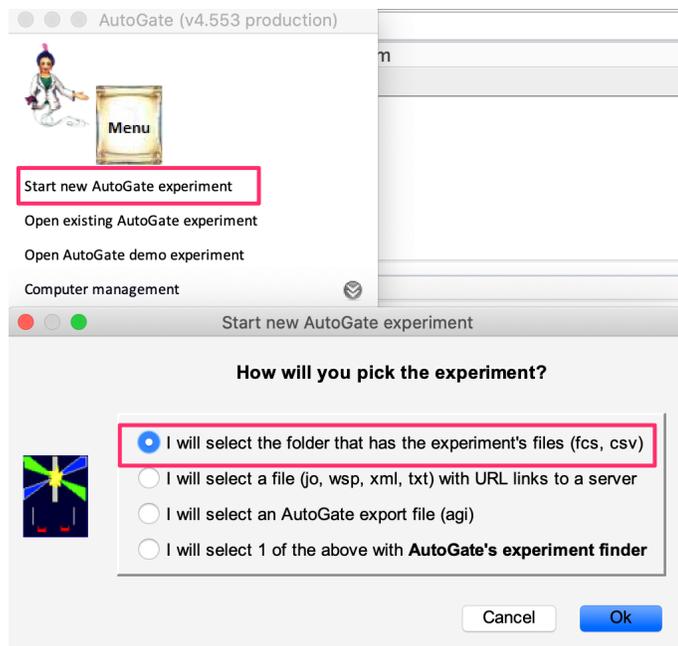
The data has been extensively referenced elsewhere including the FlowCAP publications where different automatic gating methods are tested against the expert subset delineations made by manual gating. These exact delineations are included in this tutorial

## 1 DOWNLOAD RESOURCES FOR TUTORIAL

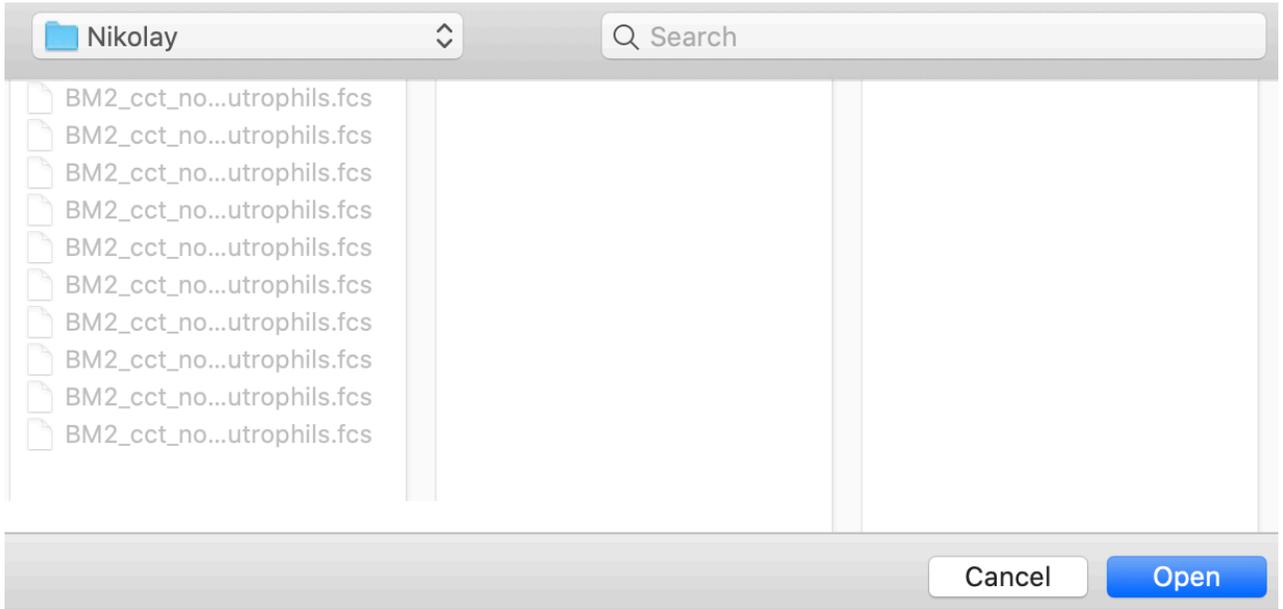
Download the 2 pivotal publications, **FCS data, population assignments** from this URL - [https://1drv.ms/u/s!AkbNI8Wap-7\\_jMBDofUfjs5zZmHQEg?e=RzeCvM](https://1drv.ms/u/s!AkbNI8Wap-7_jMBDofUfjs5zZmHQEg?e=RzeCvM)

## 2 OPEN AUTOGATE ON DATA

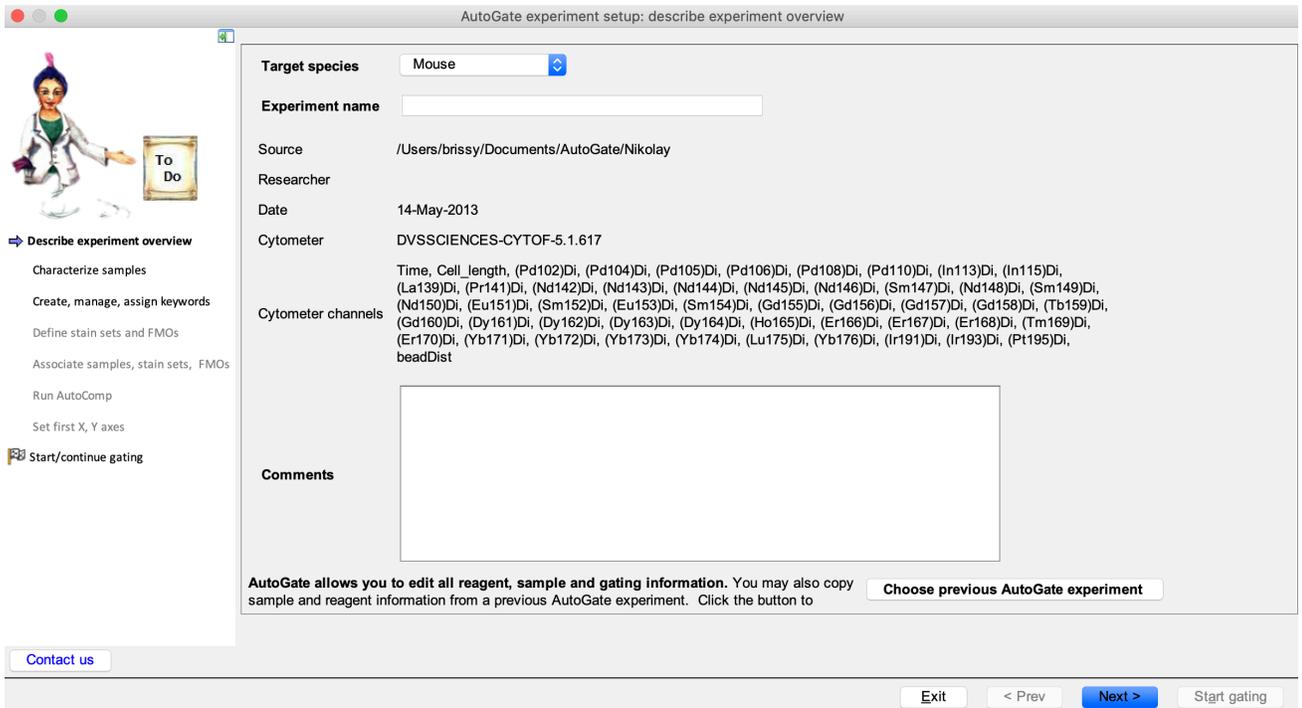
Click **Start new AutoGate experiment** on the opening window and choose **I will select the folder that has the experiment's files (fcs, csv)**. Click OK



Specify the folder that has the samples downloaded from the URL in Step 1.

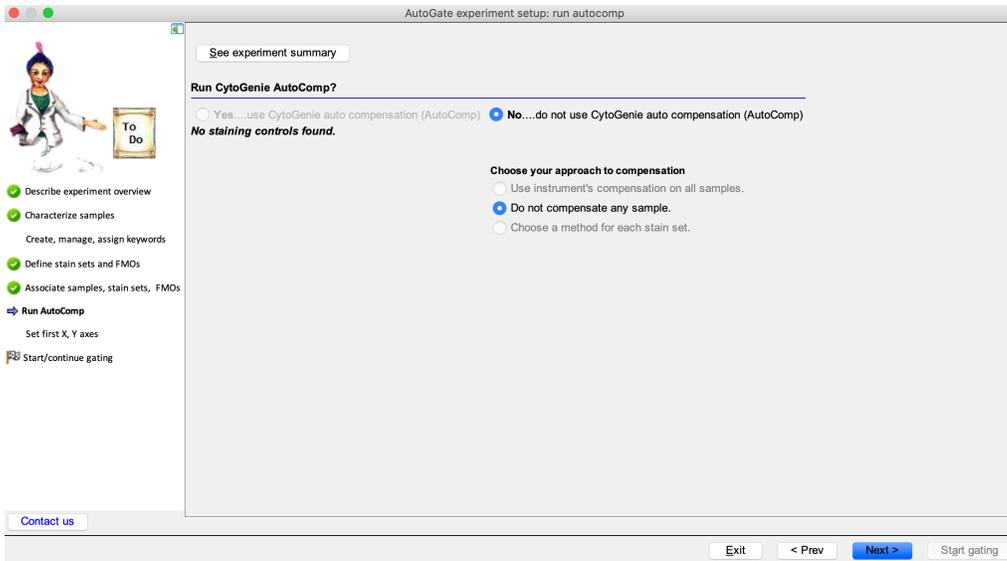


AutoGate opens up the experiment's setup window. Click **Next** and accept the defaults for all the screens (including **stain set** definition) that follow.

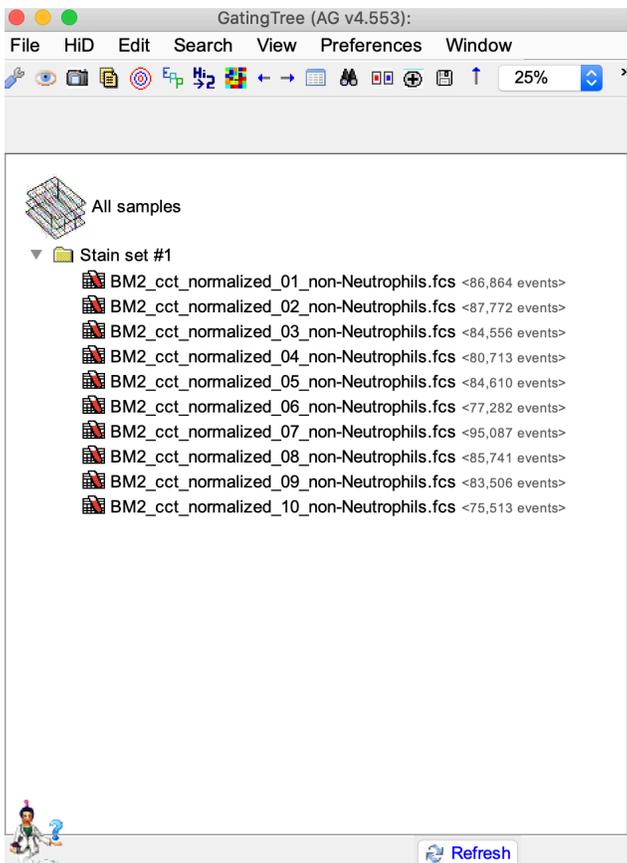


### 3 ACCEPT ALL SETUP DEFAULTS

In the **Run AutoComp** screen, verify **Do not compensate any sample** is chosen, since this is CYTOF data. Click **Next**



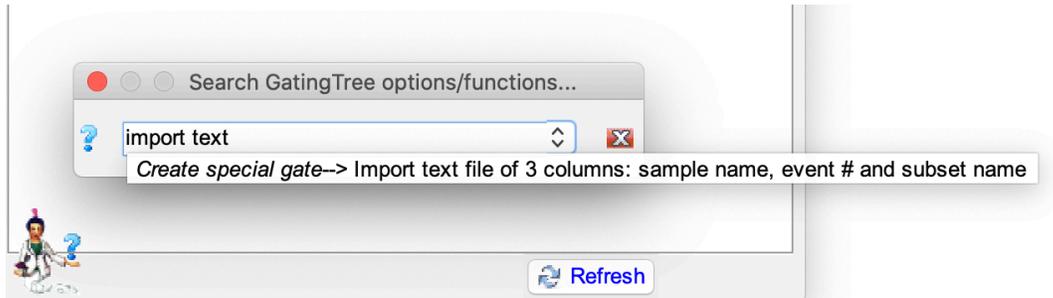
In the last step (**set first X,Y axes**), click **Start gating** to complete the setup. The **GatingTree** window opens showing the 10 ungated CYTOF samples



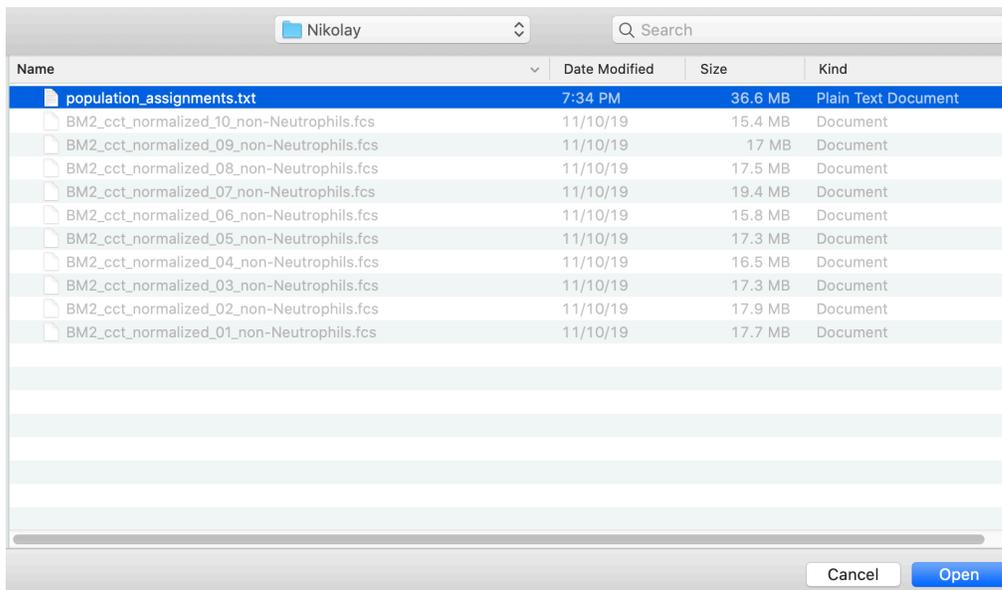
## 4 IMPORT THE EXPERT SUBSET DELINEATIONS

4.1 CLICK THE GENIE AT THE BOTTOM LEFT OF THE GATINGTREE WINDOW

4.2 TYPE "IMPORT TEXT" AND PRESS RETURN KEY

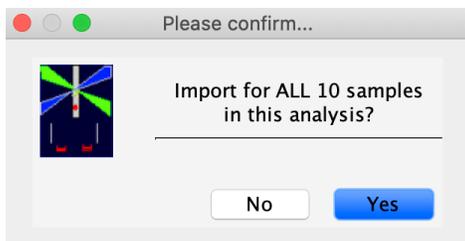


4.3 FROM THE FILE WINDOW SELECT THE FILE POPULATION\_ASSIGNMENTS.TXT'

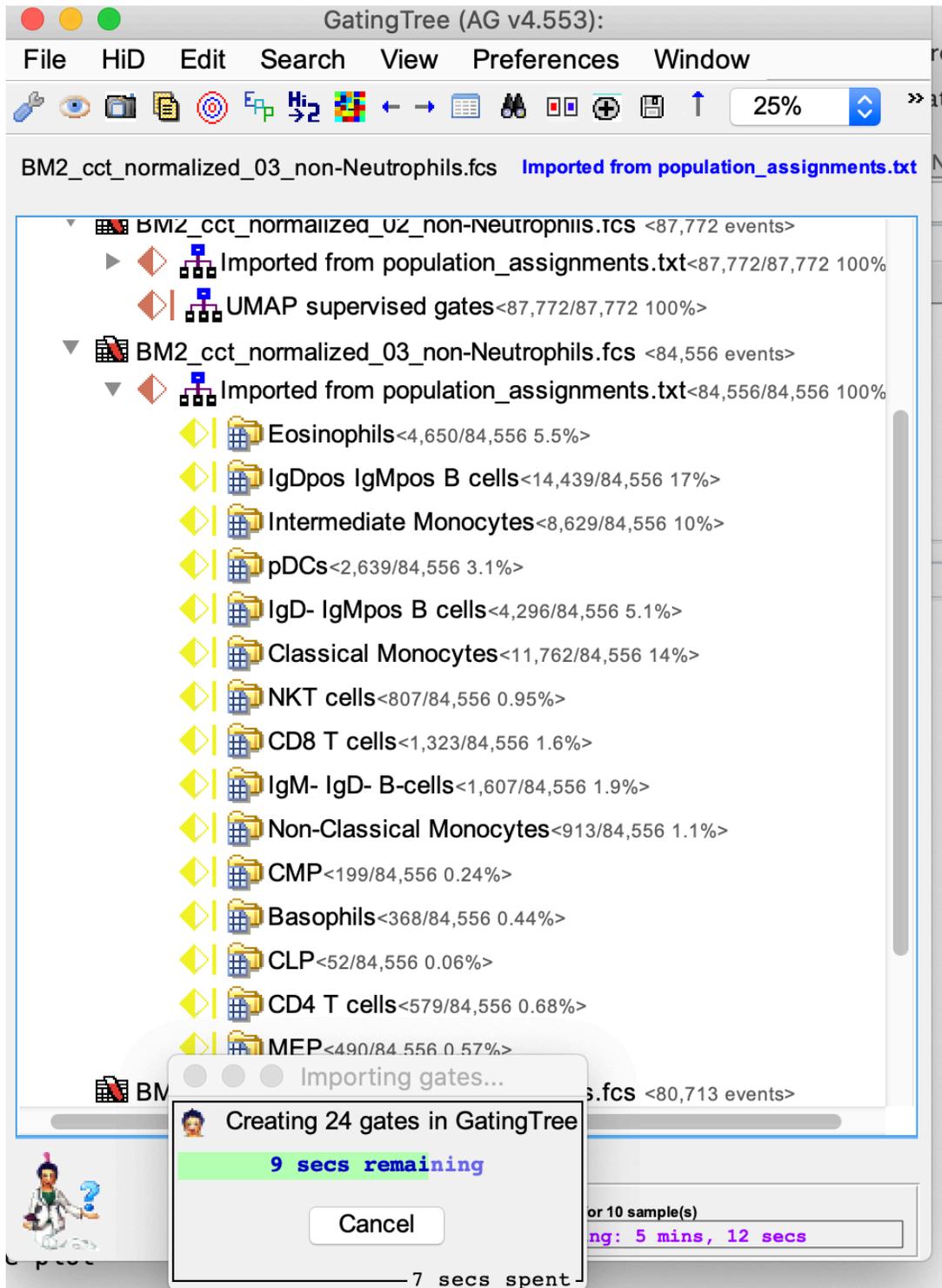


4.4 SAY YES TO IMPORT FOR ALL 10 SAMPLES

If you do not see this question then press No and repeat prior steps **without anything selected in GatingTree**



#### 4.5 AUTOGATE WILL IMPORT GATES



All samples are opened and have the logicle biexponential transform applied to them and then have the subset delineations added to the GatingTree.

GatingTree (AG v4.553):

File HiD Edit Search View Preferences Window

25%

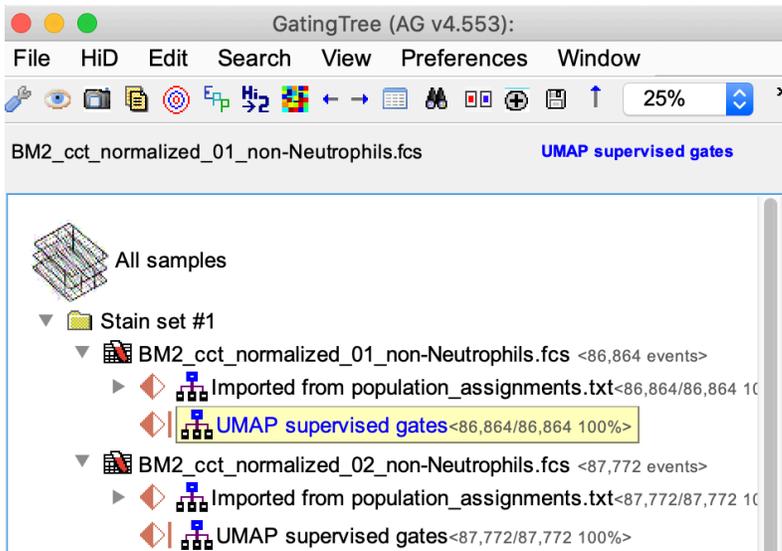
Experiment=

- All samples
  - Stain set #1
    - BM2\_cct\_normalized\_01\_non-Neutrophils.fcs <86,864 events>
      - Imported from population\_assignments.txt<86,864/86,864 100%>
      - UMAP supervised gates<86,864/86,864 100%>
    - BM2\_cct\_normalized\_02\_non-Neutrophils.fcs <87,772 events>
      - Imported from population\_assignments.txt<87,772/87,772 100%>
      - UMAP supervised gates<87,772/87,772 100%>
    - BM2\_cct\_normalized\_03\_non-Neutrophils.fcs <84,556 events>
      - Imported from population\_assignments.txt<84,556/84,556 100%>
      - UMAP supervised gates<84,556/84,556 100%>
    - BM2\_cct\_normalized\_04\_non-Neutrophils.fcs <80,713 events>
      - Imported from population\_assignments.txt<80,713/80,713 100%>
      - UMAP supervised gates<80,713/80,713 100%>
    - BM2\_cct\_normalized\_05\_non-Neutrophils.fcs <84,610 events>
      - Imported from population\_assignments.txt<84,610/84,610 100%>
      - UMAP supervised gates<84,610/84,610 100%>
    - BM2\_cct\_normalized\_06\_non-Neutrophils.fcs <77,282 events>
      - Imported from population\_assignments.txt<77,282/77,282 100%>
      - UMAP supervised gates<77,282/77,282 100%>
    - BM2\_cct\_normalized\_07\_non-Neutrophils.fcs <95,087 events>

Refresh

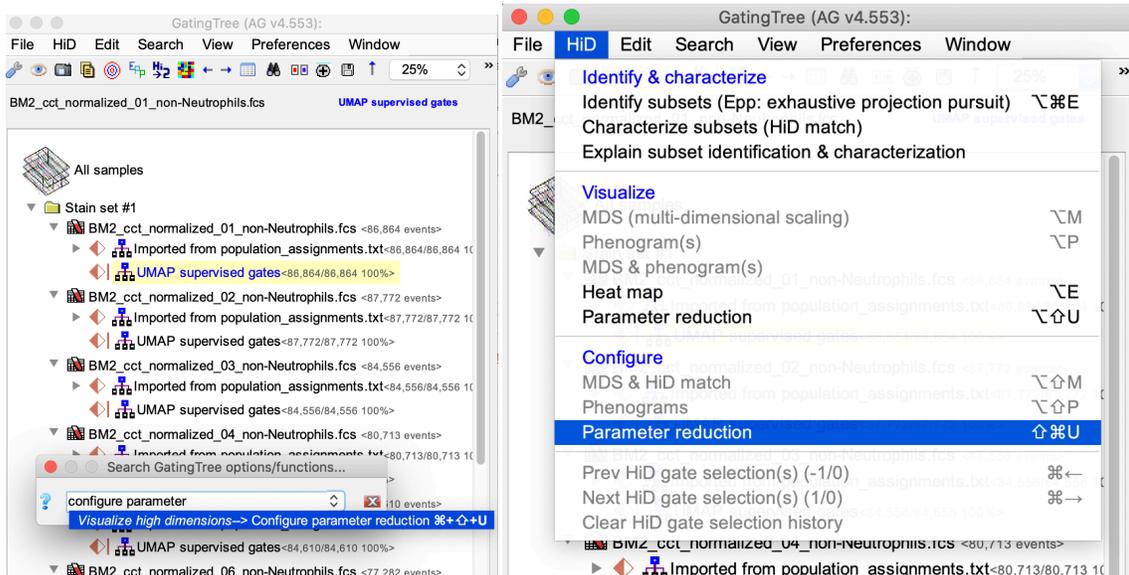
## 5 SETUP THE UST PROCESSING

### 5.1 SELECT THE FIRST TREE NODE WITH “UMAP SUPERVISED GATES”



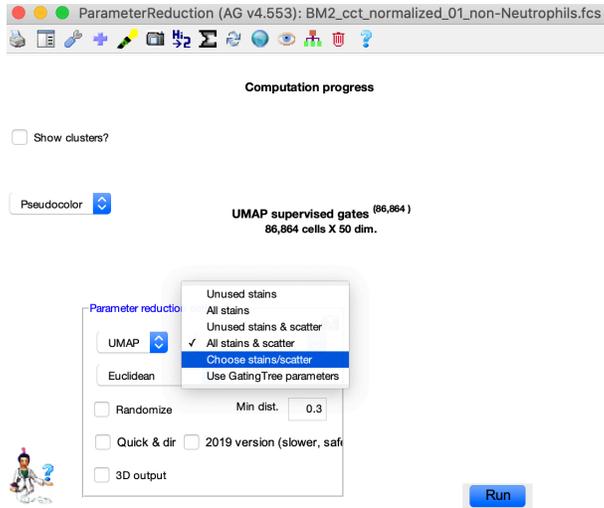
### 5.2 CHOOSE “CONFIGURE PARAMETER REDUCTION”

You can either do this typing “configure parameter” in the Genie entry window (as shown in LHS below) or can Click the HiD->Configure Parameter reduction (as in RHS below)

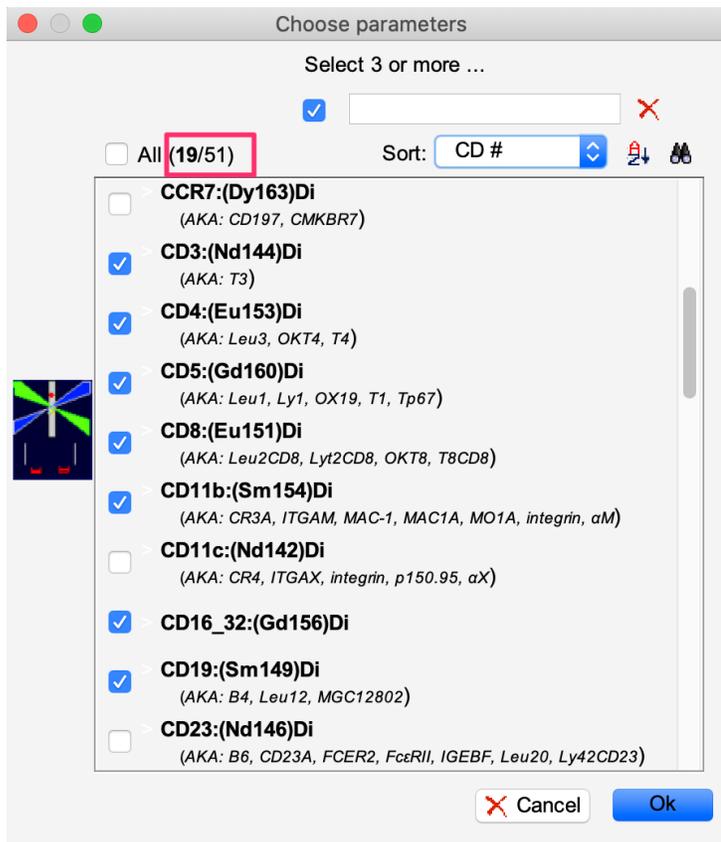


### 5.3 CONFIGURE UMAP PROCESSING

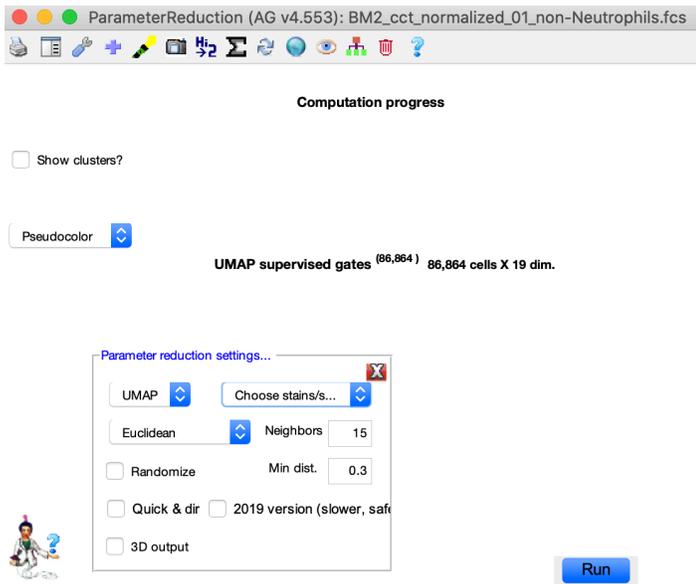
In the **ParameterReduction** window, click on the dropdown at the top right of the configuration panel to select **Choose stains/scatter**



From the **Choose parameters** popup window, select the 19 markers used for manual gating: 120g8, B220, CD3, CD4, CD5, CD8, CD11b, CD16\_32, CD19, CD27, CD34, CD43, CD44, CD49b, cKit, F480, IgD, IgM, Ly6C.



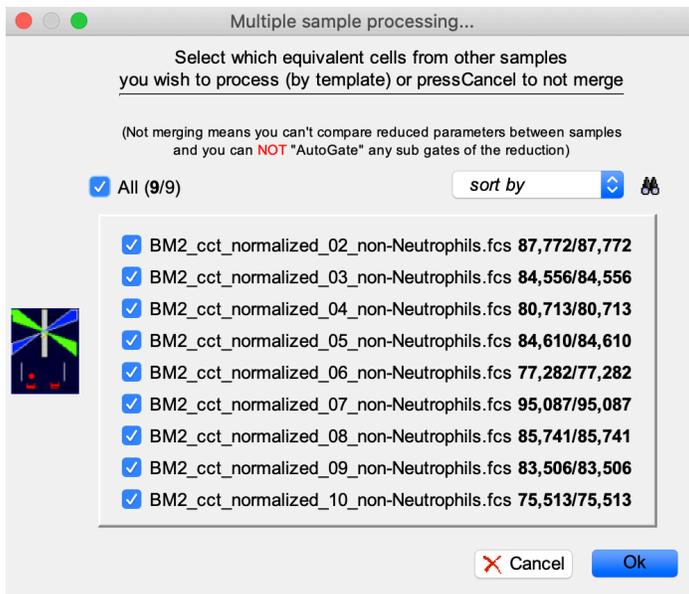
Confirm all the UMAP settings shown below



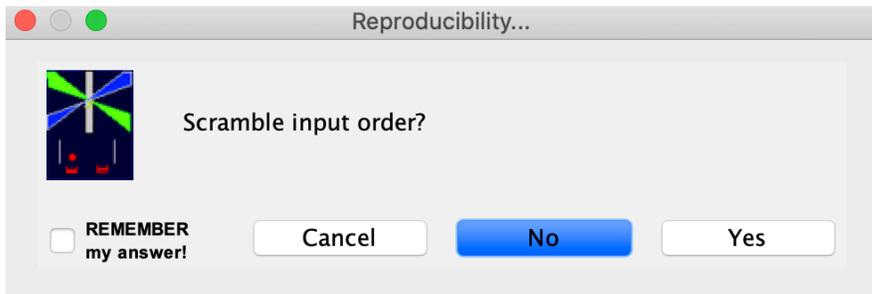
## RUN UST

5.4 CLICK THE **RUN** BUTTON AT THE BOTTOM RIGHT OF PARAMETERREDUCTION WINDOW

5.5 SELECT ALL SAMPLES IN EXPERIMENT

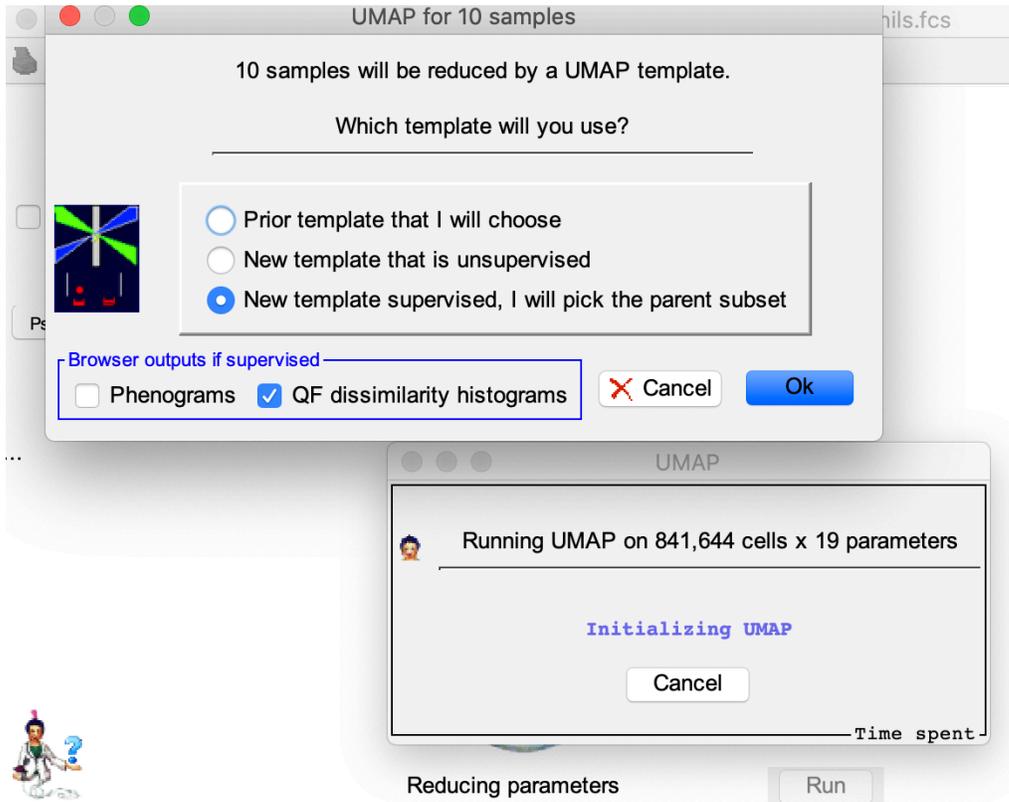


Click **No** to the below prompt



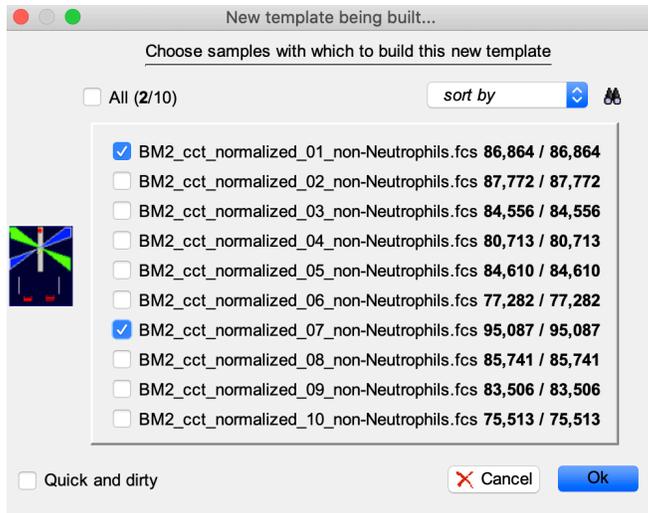
## 5.6 CREATE A NEW SUPERVISED TEMPLATE

In the window that follows, ensure that you select **QF dissimilarity histograms** at the bottom right in order to see a summary of how good the processing is.



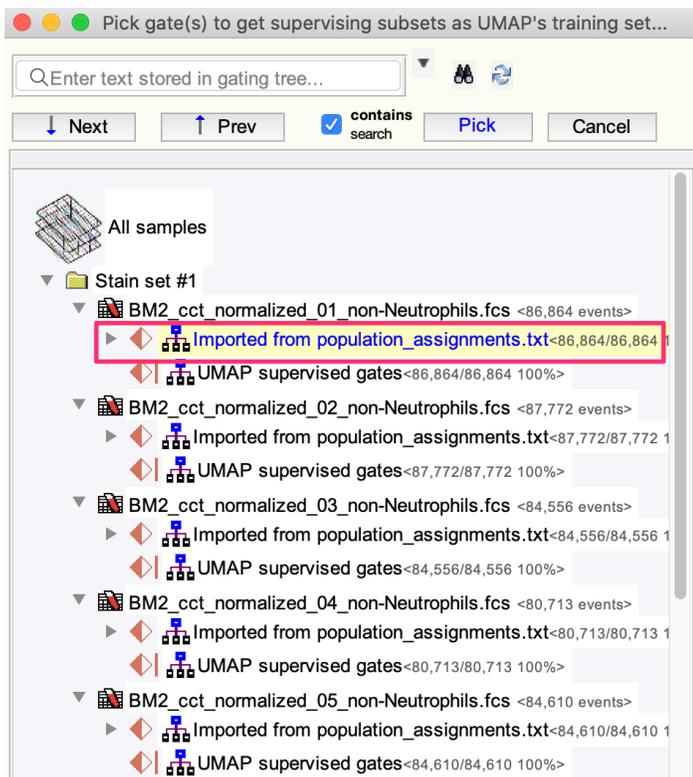
## 5.7 USE 2 REPRESENTATIVE SAMPLES FOR THE TEMPLATE

Pick the samples shown. Ensure that **Quick and dirty** at the bottom left is NOT selected



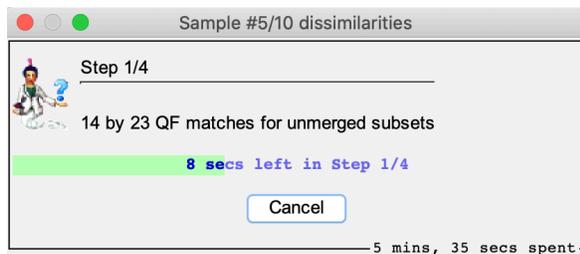
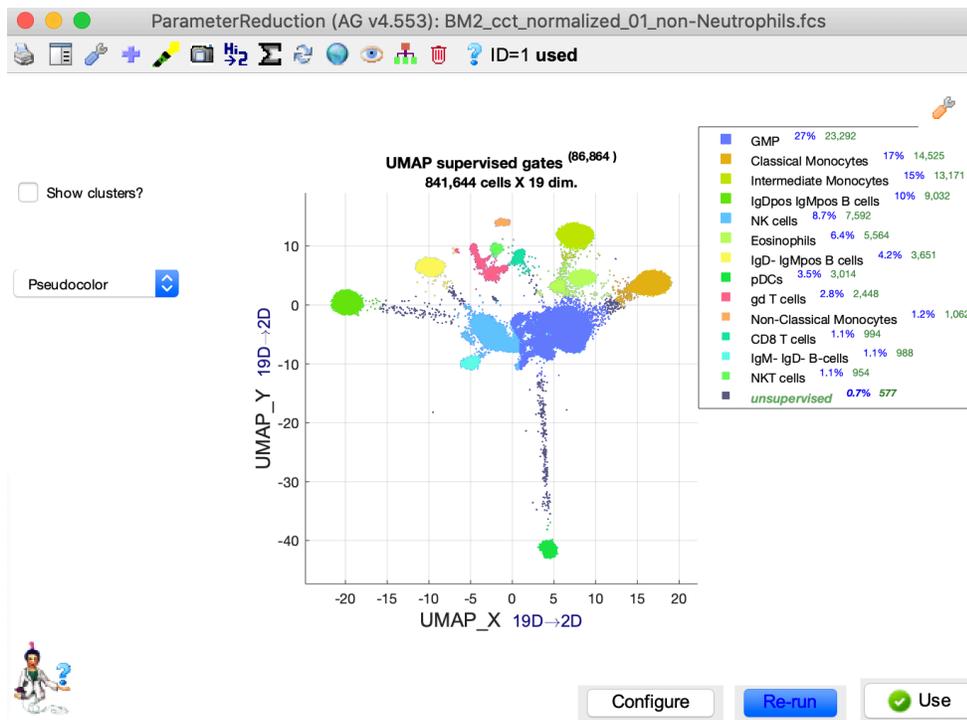
## 5.8 SELECT THE DELINEATED SUBSETS AS SUPERVISORS

**Double click** the tree node for sample 1 that contains the expert's imported subsets (highlighted below).

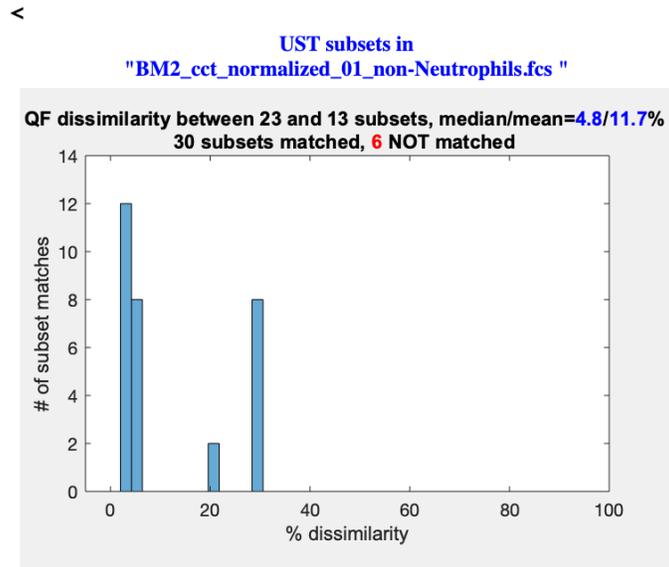
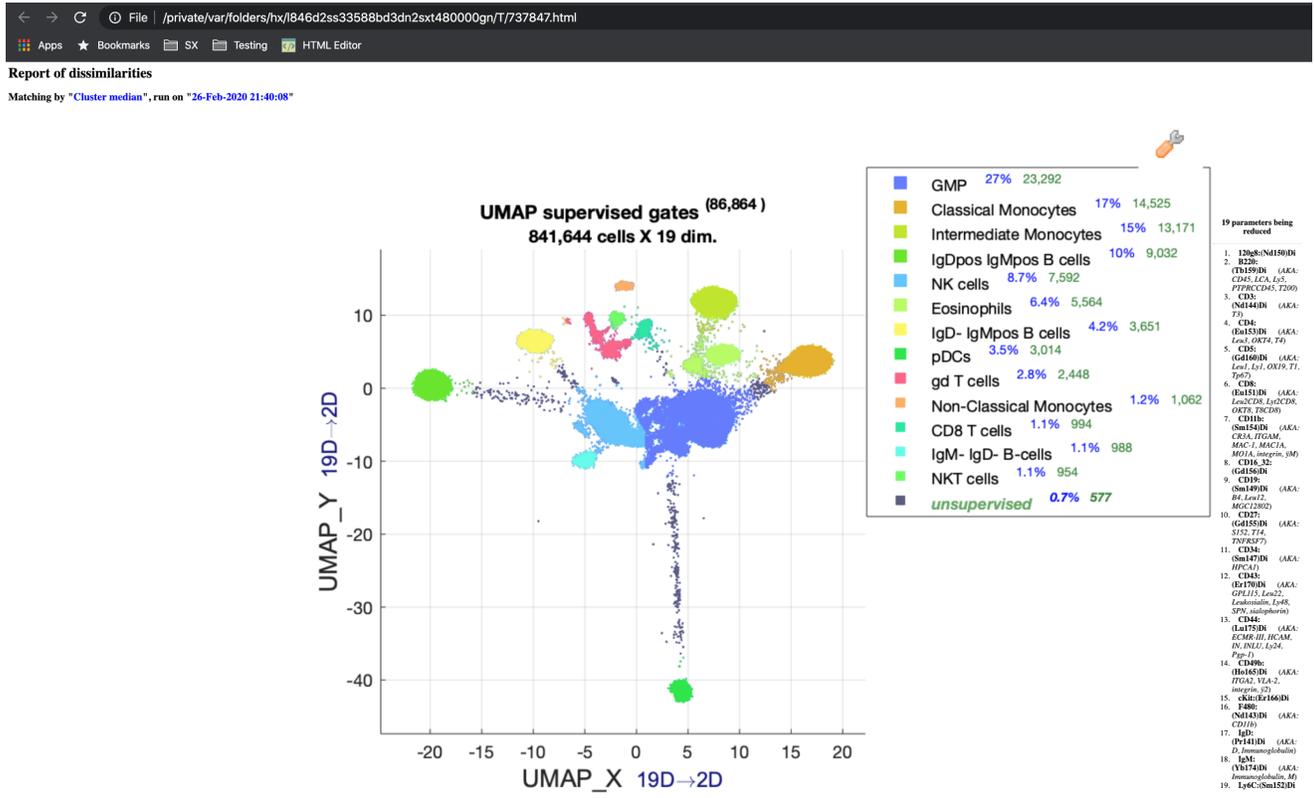


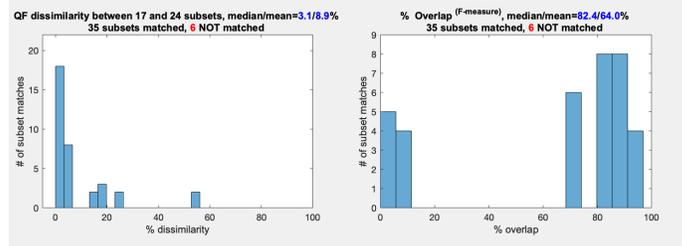
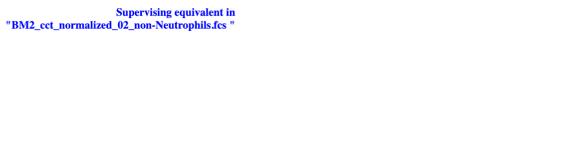
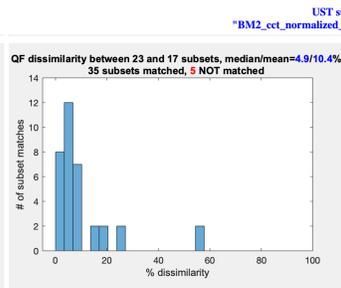
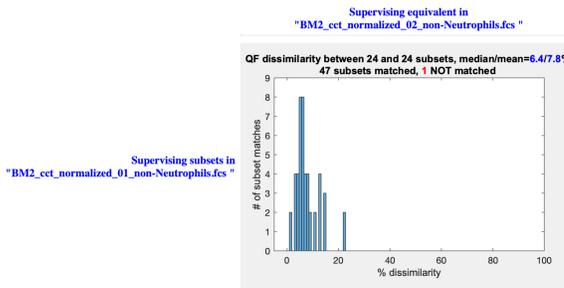
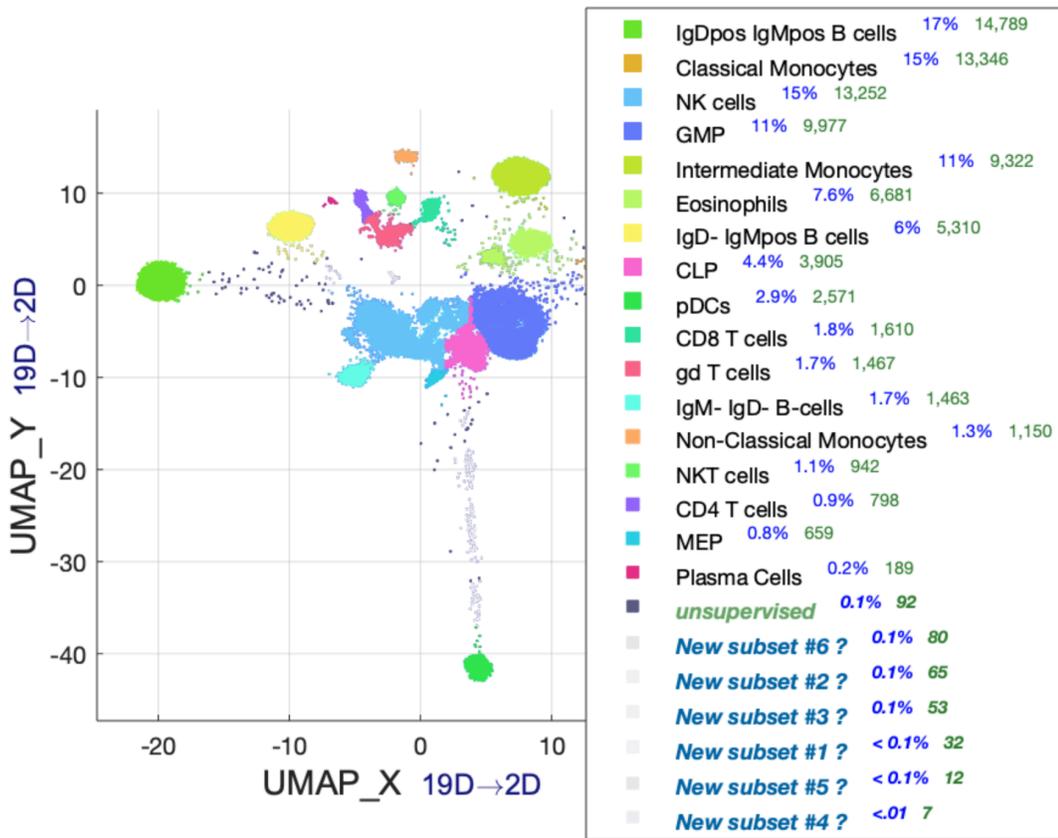
The full processing starts and runs through the following steps;

- Imported subset delineations for the 2 chosen samples
- Build a representative template from the 2 sample's subsets
- Apply the template to all 10 samples
- Compute the QF histograms relevant to UST for all 10 samples
- Put the histogram plots and UMAP output plots into a single html file and open in browser
- Offer the opportunity to save template to use on future compatible experiments.



The browser output will appear like this automatically at the end of the processing





## 5.9 SAVE THE TEMPLATE

Click “Save UMAP template” and save the result in your file system where you can find it to save time on future UST processing for compatible data.

