# 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 publicatons were 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

#### 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.

Nikolay	٥	Q Search		
<ul> <li>BM2_cct_noutrophils.fcs</li> </ul>				
			Cancel	Open

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

• • •		AutoGate experiment setup: describe experiment overview
<u>م</u>	Torget opening	
R	Experiment name	Widuse v
To	Source	/Users/brissy/Documents/AutoGate/Nikolay
	Researcher	
Star and	Date	14-May-2013
Describe experiment overview	Cytometer	DVSSCIENCES-CYTOF-5.1.617
Characterize samples		Time, Cell_length, (Pd102)Di, (Pd104)Di, (Pd105)Di, (Pd106)Di, (Pd108)Di, (Pd110)Di, (In113)Di, (In115)Di, (In115)Di, (In1440)Di, (In1440)
Create, manage, assign keywords	Cytometer channels	(Rd159)Di, (Pr14)Di, (Rd142)Di, (Rd143)Di, (Rd144)Di, (Rd143)Di, (Rd140)Di, (Sm147)Di, (Rd140)Di, (Sm149)Di, (Rd150)Di, (Eu151)Di, (Sm152)Di, (Eu153)Di, (Sm154)Di, (Gd155)Di, (Gd156)Di, (Gd157)Di, (Gd158)Di, (Tb159)Di,
Define stain sets and FMOs		(Gd160)Di, (Dy161)Di, (Dy162)Di, (Dy163)Di, (Dy164)Di, (Ho165)Di, (Er166)Di, (Er167)Di, (Er168)Di, (Tm169)Di, (Er170)Di, (Yb171)Di, (Yb172)Di, (Yb173)Di, (Yb174)Di, (Lu175)Di, (Yb176)Di, (Ir191)Di, (Ir193)Di, (Pt195)Di,
Associate samples, stain sets, FMOs		beadDist
Run AutoComp		
Set first X, Y axes		
🔊 Start/continue gating	Comments	
	sample and reagent in	to edit all reagent, sample and gating information. You may also copy formation from a previous AutoGate experiment. Click the button to
Contact us		
		Fxit < Prev Next > Start gating

# **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

•	AutoGate expen	iment setup: run autocomp	
e e	See experiment summary Run CytoGenie AutoComp?		
To	Yesuse CytoGenie auto compensation (AutoComp) No staining controls found.	● Nodo not use CytoGenie auto compensation (AutoComp)	
1 A. 1		Choose your approach to compensation	
Oescribe experiment overview		Ouse instruments compensation on all samples.	
🥝 Characterize samples		Choose a method for each stain set	
Create, manage, assign keywords		Choose a method for each stain set.	
🤣 Define stain sets and FMOs			
🤣 Associate samples, stain sets, FMOs			
🔿 Run AutoComp			
Set first X, Y axes			
Start/continue gating			
Contact us			
		Exit < F	Prev Next > Start gating

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

					(	Gati	ngT	Tree	e (A0	G v4	.553	3):						
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16	<b>B</b> P																	
	🗎 Si	tain	set	#1														
		B B	M2_	cct_	norm	naliz	ed_	_01	_nor	n-Ne	utrop	ohils	.fcs	<86,8	364	events>		
		B	M2_	cct_	norm	naliz	ed_	_02	_nor	1-Ne	utrop	ohils	.fcs	<87,7	72	events>		
		B	M2_	cct_	norm	naliz	ed_	_03	_nor	1-Ne	utrop	bhils	.fcs	<84,5	556	events>		
	 €	n Bi	M2_	cct_	nom	naliz	ea_	04	_nor	1-INC	utrop	oniis	.ics	<80,7	13	events>		
	Ē	BR	M2	cct	nom	ializ vəliz	.eu_ od	00_ 00	_nor		utrop	ohile	fce.	<84,0	010	events>		
			M2_	cct	norm	ializ ializ	.eu_ ed	00	_nor		utror	hile	fre	<05 (	187	events>		
	Ē	B	M2	cct	norm	naliz	ed.	08	_nor	n-Ne	utror	ohils	fcs	<85.7	741	events>		
	Ē	B	M2	cct	norm	naliz	ed	09	_nor	n-Ne	utro	ohils	.fcs	<83.5	506	events>		
	É	B	M2	cct	norm	naliz	ed	10	nor	n-Ne	utrop	ohils	.fcs	<75,5	513	events>		
1																		
2	,																	
15 ×													æ F	Refre	sh			

# **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'

📄 Nikolay	C Searc	ch	
Name	<ul> <li>Date Modified</li> </ul>	Size	Kind
population_assignments.txt	7:34 PM	36.6 MB	Plain Text Document
BM2_cct_normalized_10_non-Neutrophils.fcs	11/10/19	15.4 MB	Document
BM2_cct_normalized_09_non-Neutrophils.fcs	11/10/19	17 MB	Document
BM2_cct_normalized_08_non-Neutrophils.fcs	11/10/19	17.5 MB	Document
BM2_cct_normalized_07_non-Neutrophils.fcs	11/10/19	19.4 MB	Document
BM2_cct_normalized_06_non-Neutrophils.fcs	11/10/19	15.8 MB	Document
BM2_cct_normalized_05_non-Neutrophils.fcs	11/10/19	17.3 MB	Document
BM2_cct_normalized_04_non-Neutrophils.fcs	11/10/19	16.5 MB	Document
BM2_cct_normalized_03_non-Neutrophils.fcs	11/10/19	17.3 MB	Document
BM2_cct_normalized_02_non-Neutrophils.fcs	11/10/19	17.9 MB	Document
BM2_cct_normalized_01_non-Neutrophils.fcs	11/10/19	17.7 MB	Document
			Cancel Open

#### 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.



#### 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** 

🛑 😑 🔵 Pa	arameter	Reducti	on (AG	v4.55	3): BM	2_c	ct_normalized_01_non-Neutrophils.fcs
🌢 🔳 🤌	+ 🖌	©1 ÿ2	Σ ∂	0	D 🚠	Ū	?
	Computation progress						
Pseudocolor	ers?		U	MAP su 86,8	ipervise 364 cells	d ga X 50	ates <sup>(86,864</sup> ) ) dim.
	Parameter r UMAP Euclidea	eductio an	Unused All stain Unused All stain Choose Use Gat	stains s stains & s & scatt stains/s tingTree	k scatter ter catter paramet	ers	
	Quick	c & dir	2019 ve	ersion (s	slower, :	safe	Run

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.

Choose parameters					
Select 3 or more					
🗌 All <mark>(19</mark> /51)					
CCR7:(Dy163)Di (AKA: CD197, CMKBR7)					
CD3:(Nd144)Di (AKA: T3)					
CD4:(Eu153)Di (AKA: Leu3, OKT4, T4)					
CD5:(Gd160)Di (AKA: Leu1, Ly1, OX19, T1, Tp67)					
CD8:(Eu151)Di (AKA: Leu2CD8, Lyt2CD8, OKT8, T8CD8)					
CD11b:(Sm154)Di (AKA: CR3A, ITGAM, MAC-1, MAC1A, MO1A, integrin, αM)					
CD11c:(Nd142)Di (AKA: CR4, ITGAX, integrin, p150.95, aX)					
✓ > CD16_32:(Gd156)Di					
CD19:(Sm149)Di (AKA: B4, Leu12, MGC12802)					
CD23:(Nd146)Di (AKA: B6, CD23A, FCER2, Fc&RII, IGEBF, Leu20, Ly42CD23)					
Cancel Ok					

Confirm all the UMAP settings shown below

😑 😑 🗣 ParameterReduction (AG v4.553): BM2_cct_normalized_01_non-Neutrophils.fcs
🎍 🖪 🥒 🕂 🖍 🛍 💺 ∑ 20 🥥 🧥 🗑 🤶
Computation progress
Show clusters?
Pseudocolor C UMAP supervised gates (86,864 ) 86,864 cells X 19 dim.
Parameter reduction settings
Euclidean Neighbors 15
Randomize Min dist. 0.3
Quick & dir 2019 version (slower, safe
3D output

# **RUN UST**

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

5.5 SELECT ALL SAMPLES IN EXPERIMENT

Select which equivalent cells from	other samples	
you wish to process (by template) of pres	sCancel to not merge	
(Not merging means you can't compare reduced par and you can NOT "AutoGate" any sub gate	rameters between samples s of the reduction)	
All ( <b>9</b> /9)	sort by ᅌ	8
<ul> <li>BM2_cct_normalized_02_non-Neutrop</li> <li>BM2_cct_normalized_03_non-Neutrop</li> <li>BM2_cct_normalized_04_non-Neutrop</li> <li>BM2_cct_normalized_05_non-Neutrop</li> <li>BM2_cct_normalized_06_non-Neutrop</li> <li>BM2_cct_normalized_07_non-Neutrop</li> <li>BM2_cct_normalized_08_non-Neutrop</li> <li>BM2_cct_normalized_09_non-Neutrop</li> <li>BM2_cct_normalized_10_non-Neutrop</li> </ul>	hils.fcs 87,772/87,772 hils.fcs 84,556/84,556 hils.fcs 84,556/84,556 hils.fcs 80,713/80,713 hils.fcs 84,610/84,610 hils.fcs 77,282/77,282 hils.fcs 95,087/95,087 hils.fcs 85,741/85,741 hils.fcs 83,506/83,506 hils.fcs 75,513/75,513	
	<ul> <li>you wish to process (by template) or press</li> <li>(Not merging means you can't compare reduced pai and you can NOT "AutoGate" any sub gate</li> <li>All (9/9)</li> <li>BM2_cct_normalized_02_non-Neutrop</li> <li>BM2_cct_normalized_04_non-Neutrop</li> <li>BM2_cct_normalized_05_non-Neutrop</li> <li>BM2_cct_normalized_06_non-Neutrop</li> <li>BM2_cct_normalized_06_non-Neutrop</li> <li>BM2_cct_normalized_07_non-Neutrop</li> <li>BM2_cct_normalized_08_non-Neutrop</li> <li>BM2_cct_normalized_08_non-Neutrop</li> <li>BM2_cct_normalized_09_non-Neutrop</li> <li>BM2_cct_normalized_10_non-Neutrop</li> <li>BM2_cct_normalized_10_non-Neutrop</li> </ul>	<ul> <li>Select which equivalent cens nonn other samples you wish to process (by template) or pressCancel to not merge (Not merging means you can't compare reduced parameters between samples and you can NOT "AutoGate" any sub gates of the reduction)</li> <li>All (9/9) sort by ?</li> <li>BM2_cct_normalized_02_non-Neutrophils.fcs 87,772/87,772</li> <li>BM2_cct_normalized_03_non-Neutrophils.fcs 84,556/84,556</li> <li>BM2_cct_normalized_04_non-Neutrophils.fcs 80,713/80,713</li> <li>BM2_cct_normalized_05_non-Neutrophils.fcs 77,282/77,282</li> <li>BM2_cct_normalized_06_non-Neutrophils.fcs 77,282/77,282</li> <li>BM2_cct_normalized_07_non-Neutrophils.fcs 85,741/85,741</li> <li>BM2_cct_normalized_09_non-Neutrophils.fcs 75,513/75,513</li> </ul>

Click No to the below prompt

	Reprodu	cibility	
Scram	ble input order?		
REMEMBER my answer!	Cancel	No	Yes

#### 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.

	UMAP for 10 samples	hils.fcs
6	10 samples will be reduced by a UMAP template.	
	Which template will you use?	
Pt Browser outp Pheno	<ul> <li>Prior template that I will choose</li> <li>New template that is unsupervised</li> <li>New template supervised, I will pick the parent subset</li> <li>Duts if supervised</li> <li>grams QF dissimilarity histograms</li> </ul>	
	Running UMAP on 841,644 cells x 19 p	parameters
	Initializing UMAP Cancel Reducing parameters	Time spent

#### 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

	New template being built
	Choose samples with which to build this new template
	All (2/10) Sort by
Ж	<ul> <li>BM2_cct_normalized_01_non-Neutrophils.fcs 86,864 / 86,864</li> <li>BM2_cct_normalized_02_non-Neutrophils.fcs 87,772 / 87,772</li> <li>BM2_cct_normalized_03_non-Neutrophils.fcs 84,556 / 84,556</li> <li>BM2_cct_normalized_04_non-Neutrophils.fcs 80,713 / 80,713</li> <li>BM2_cct_normalized_05_non-Neutrophils.fcs 84,610 / 84,610</li> <li>BM2_cct_normalized_06_non-Neutrophils.fcs 77,282 / 77,282</li> <li>BM2_cct_normalized_07_non-Neutrophils.fcs 95,087 / 95,087</li> <li>BM2_cct_normalized_08_non-Neutrophils.fcs 83,741 / 85,741</li> <li>BM2_cct_normalized_09_non-Neutrophils.fcs 83,506 / 83,506</li> <li>BM2_cct_normalized_10_non-Neutrophils.fcs 75,513 / 75,513</li> </ul>
Quic	ck and dirty

#### 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.



• •	Sample #5/10 dissimilarities
ò _	Step 1/4
₹ E	14 by 23 QF matches for unmerged subsets
	8 secs left in Step 1/4
	Cancel
	5 mins, 35 secs spent







<









# 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.

Save UMAP template	Done