

# CYTOSWARM

## USER GUIDE



# CytoSwarm User Guide

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## 1 Welcome to CytoSwarm Software

CytoSwarm is a software utility that schedules and remotely runs algorithms on selected FCS files

CytoSwarm software is for research use only.

VenturiOne is a registered trademark of Applied Cytometry. All other trademarks are acknowledged.

## 2 Contact Details

To speak to one of our team email [customersupport@appliedcytometry.com](mailto:customersupport@appliedcytometry.com) or telephone +44 (0)1909 547210

Or contact us by post at the following address:

Applied Cytometry  
Matrix Business Centre  
Nobel Way  
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S25 3QB  
UK

Visit us online at [www.appliedcytometry.com](http://www.appliedcytometry.com)

## 3 Installation of CytoSwarm Software

To run the CytoSwarm software, you need to first follow installation and licencing steps below

### 3.1 Starting Software Installation

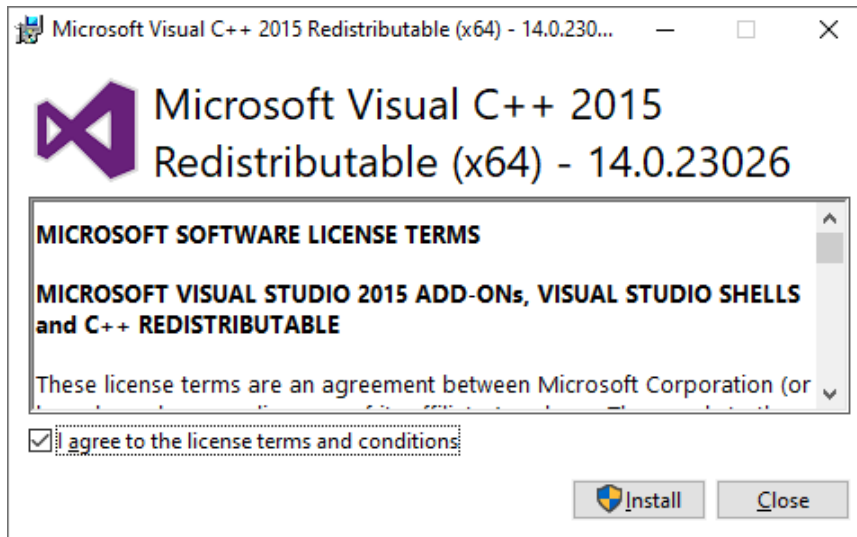
On the USB media drive contained in the USB Media Kit is a folder called

**\Cytoswarm**

Run the **setup.exe** file and installation will begin.

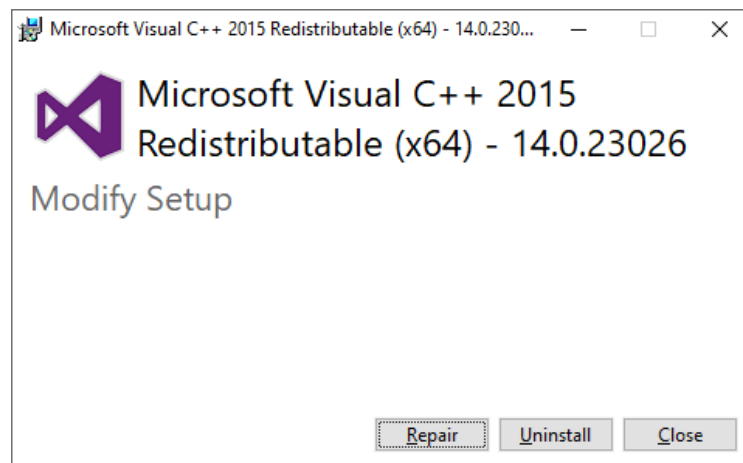
### 3.2 Installation of Pre-Requisites

The software will first install any pre-requisite software, if not already installed the following dialog is displayed



Check the 'I agree to the license terms and conditions' checkbox then select the Install button; you will need administrative privileges to install this program successfully.

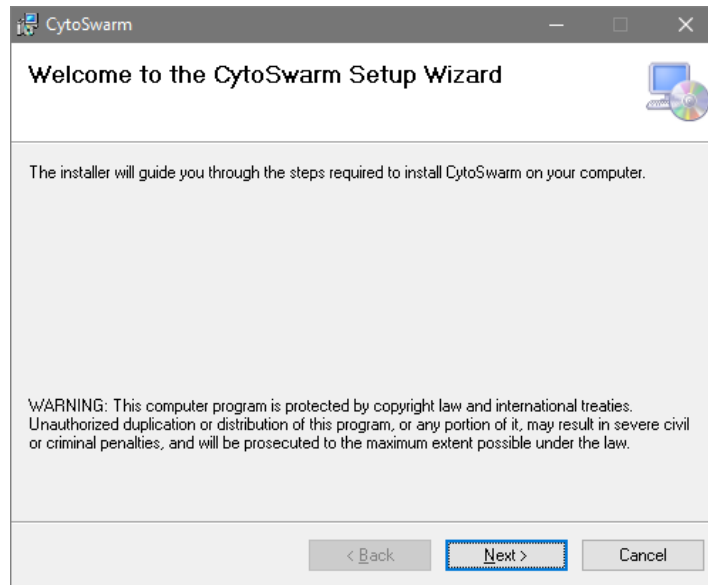
If the Modify Setup dialog is displayed, the drivers are already installed, and you can cancel installation of the pre-requisites.



Press close to move on to installing the CytoSwarm software.

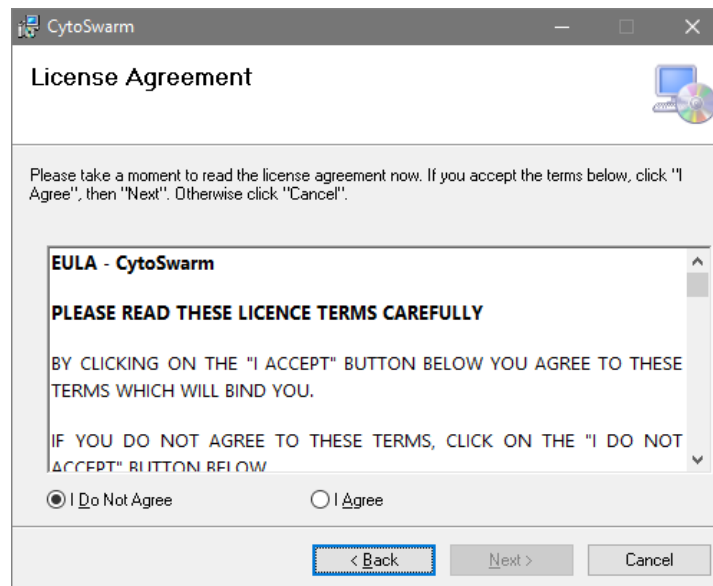
### 3.3 CytoSwarm Installation

The welcome screen is displayed first



Select Next to move on.

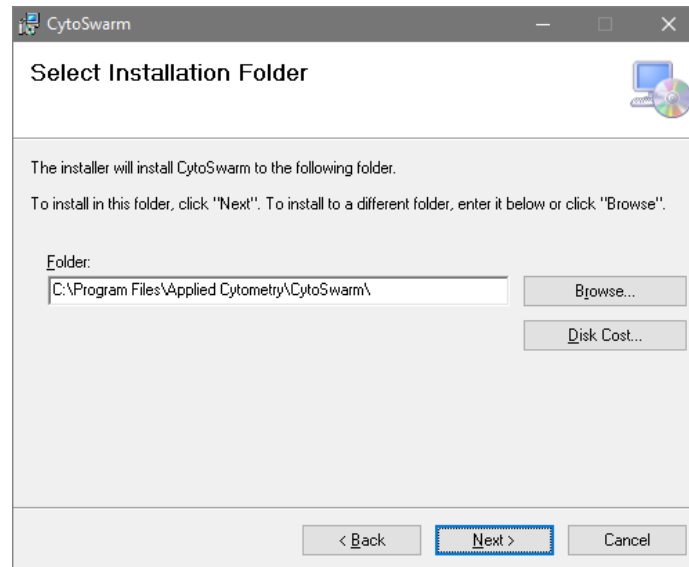
The End User License Agreement is the displayed.



Select I Accept to move on and begin installation.

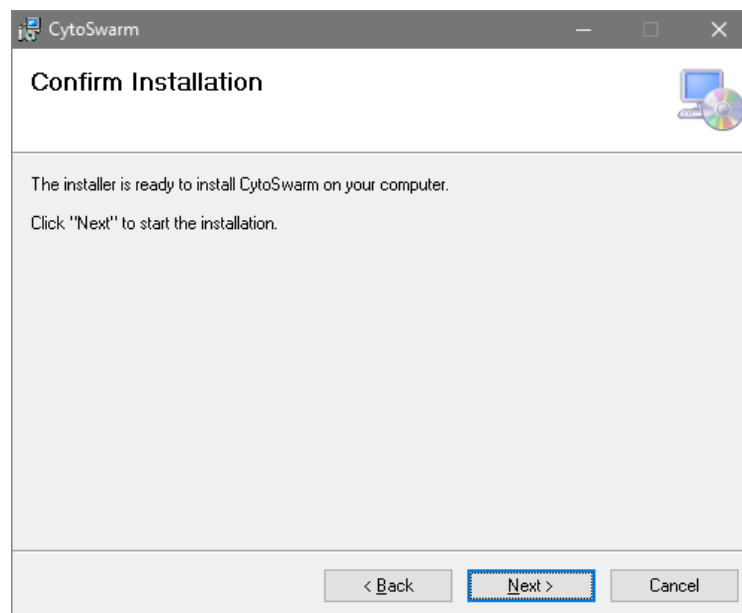
If you do not Agree, installation will not proceed, and you should press cancel.

The Select Installation folder will then be displayed

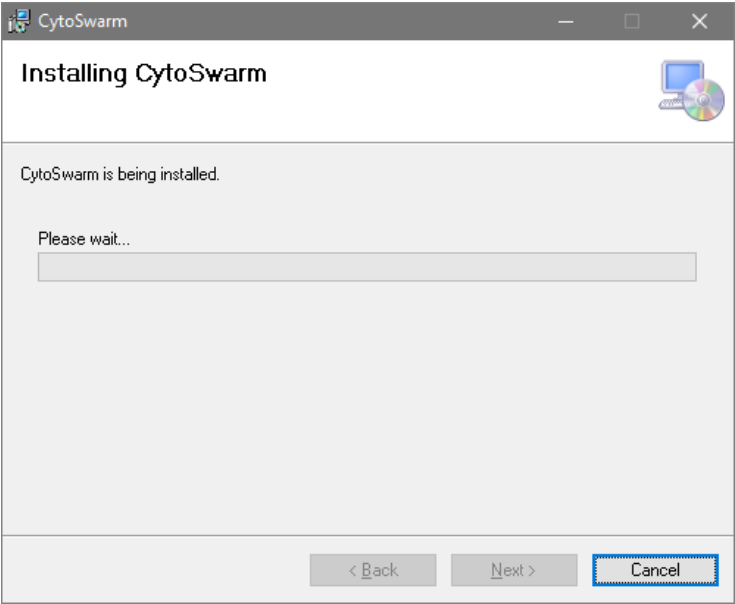


If desired change the folder where the software will be installed, select next to proceed.

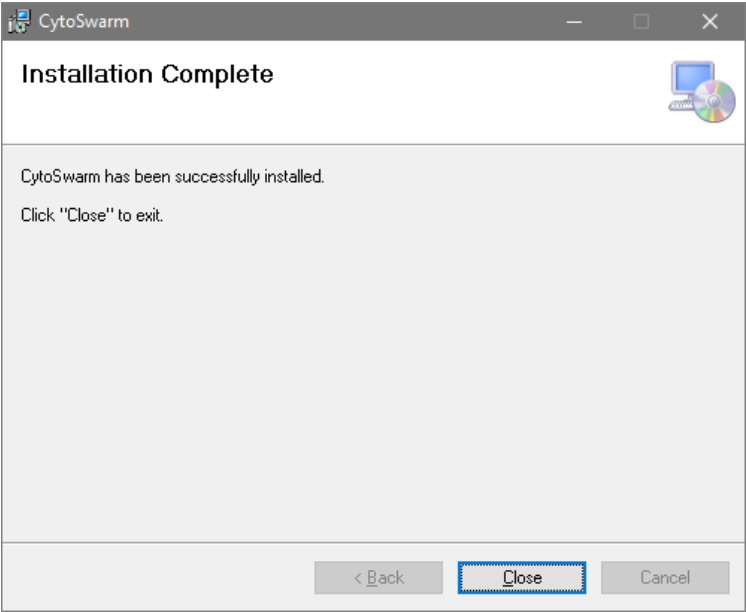
The Confirm installation screen is displayed. If you wish to proceed select Next



During installation, the installation progress dialog is displayed

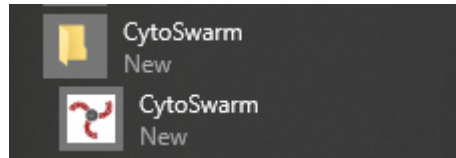


Once installation is complete press the close button to close the final dialog.

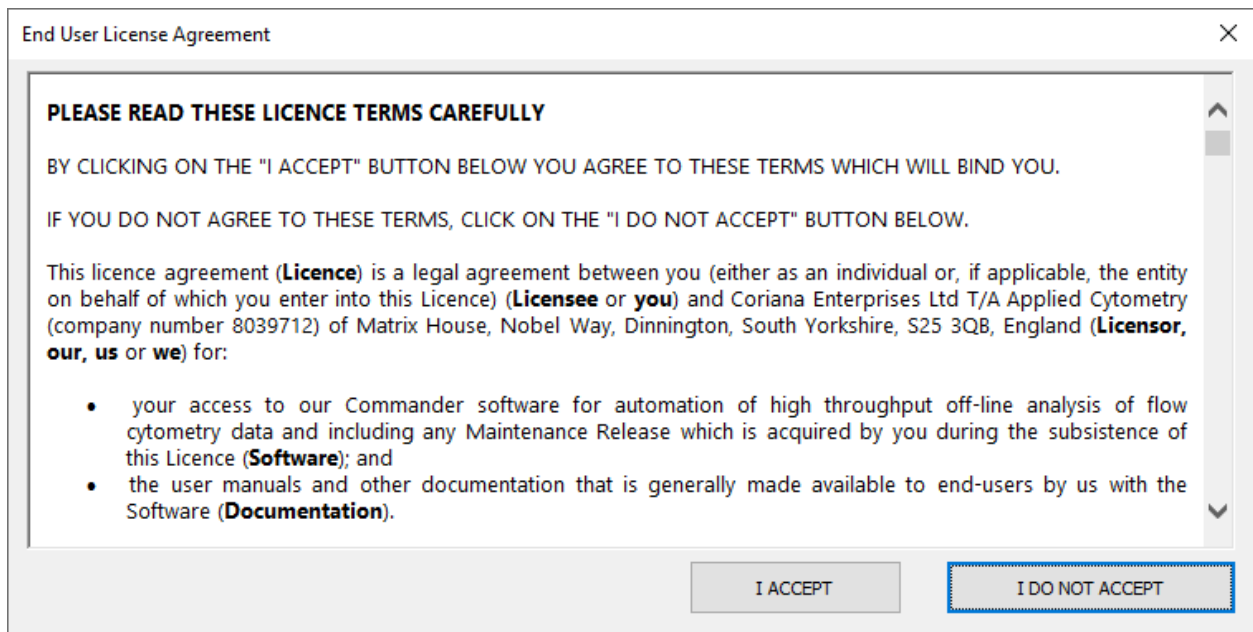


## 4 CytoSwarm Software

To run the software open the CytoSwarm item on the start menu and click on the CytoSwarm icon start the software.



On first running the software the License agreement will be displayed

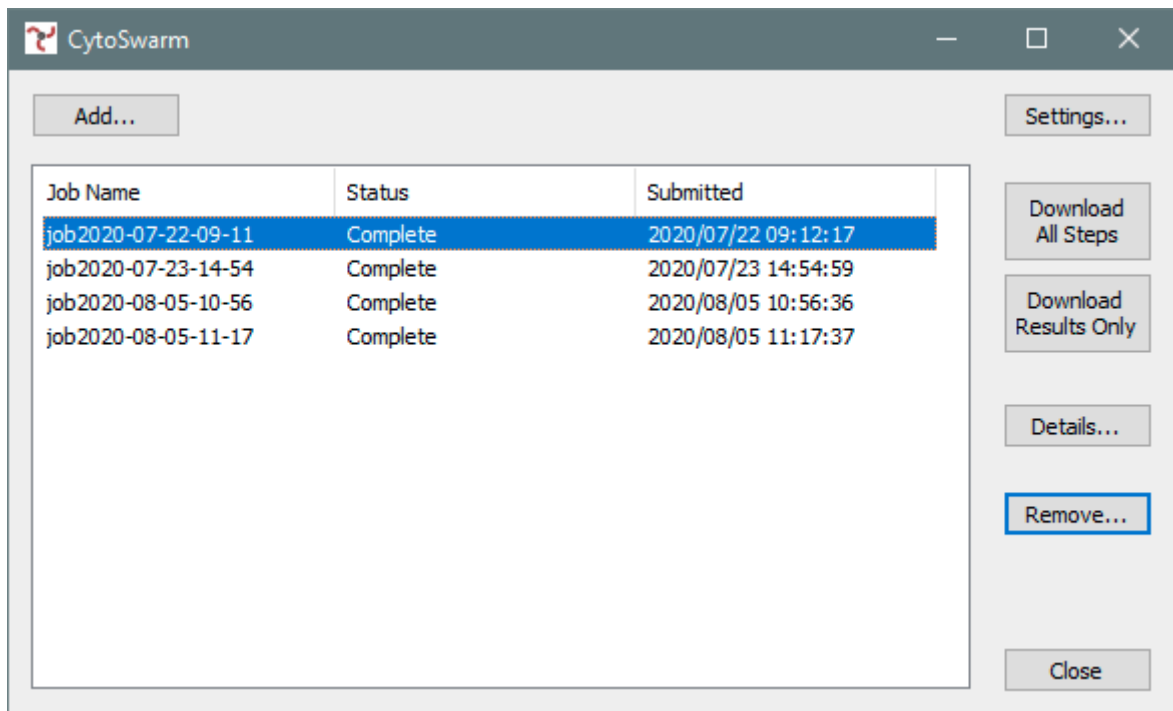


Please read the license agreement and click I accept to proceed.

If you do not accept the software will close.



After clicking the I accept button the main CytoSwarm program is displayed.



#### 4.1 Jobs List

This list displays the jobs on the server the status of each job and the date and time the job was submitted to the server for processing.

The following states are displayed

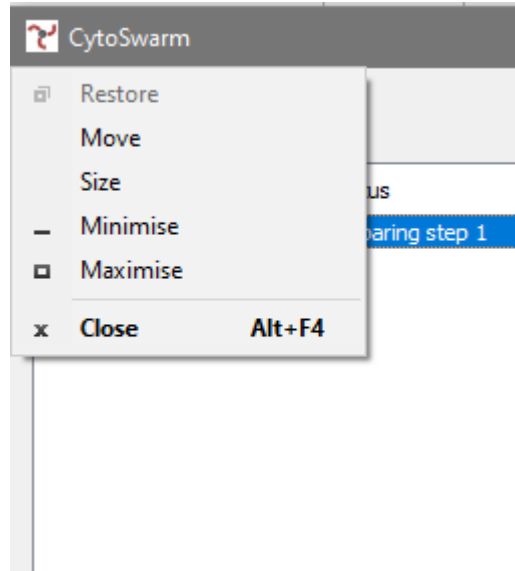
Preparing Step <step number> - repeated for each step, in order

Processing Step <Step Number> - repeated for each step, in order

Ready – All processing completed successfully

Failed - an error has occurred at one stage of the processing this may have halted processing of subsequent steps, select Details for the job to view details

## 4.2 CytoSwarm Application Menu



Clicking the CytoSwarm icon displays the CytoSwarm Application menu

### 4.2.1 Move and Resize Options

Use the Restore, Minimize, Maximize, Move and Size options to change the size or position of the application window,

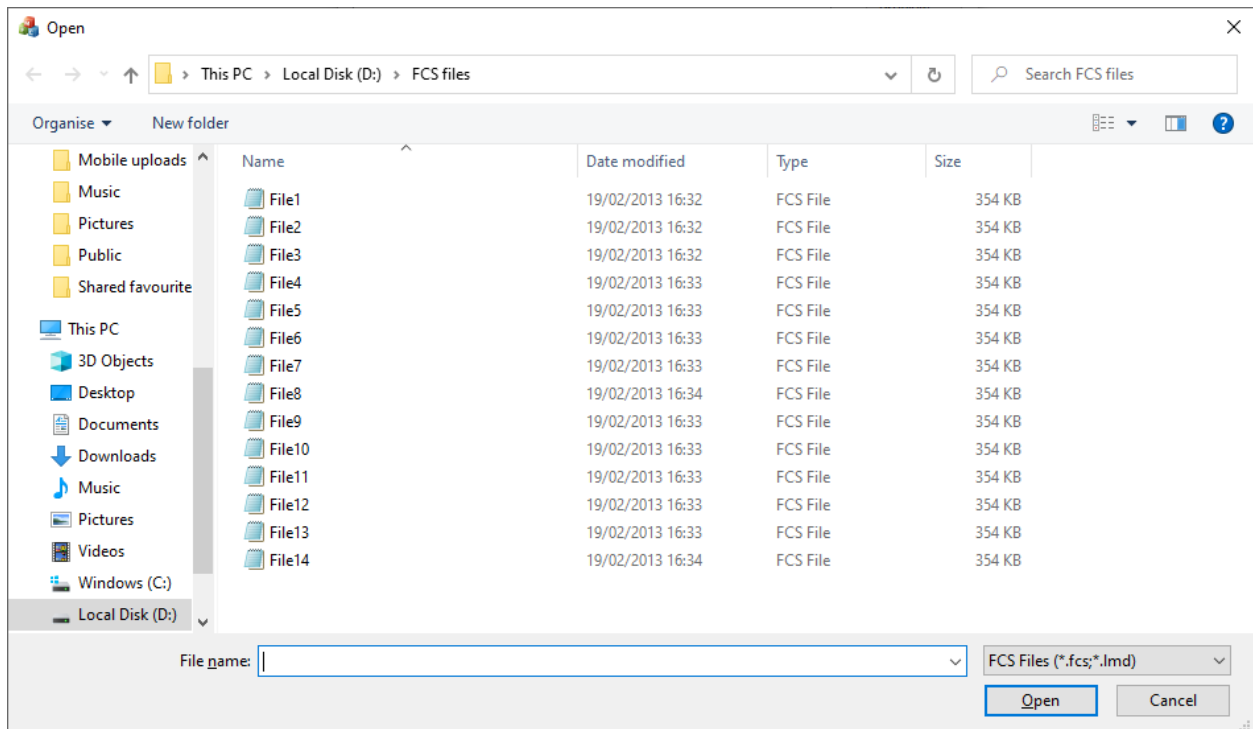
### 4.2.2 Close

Click the Close option to close the CytoSwarm software

## 4.3 Add Button

Clicking the Add button opens the file selection dialog, this is the first step in the process of file selection and algorithm selection and configuration.

### 4.3.1 File selection dialog



The file selection dialog is used to select the individual FCS files for processing, highlight the required files then click Open

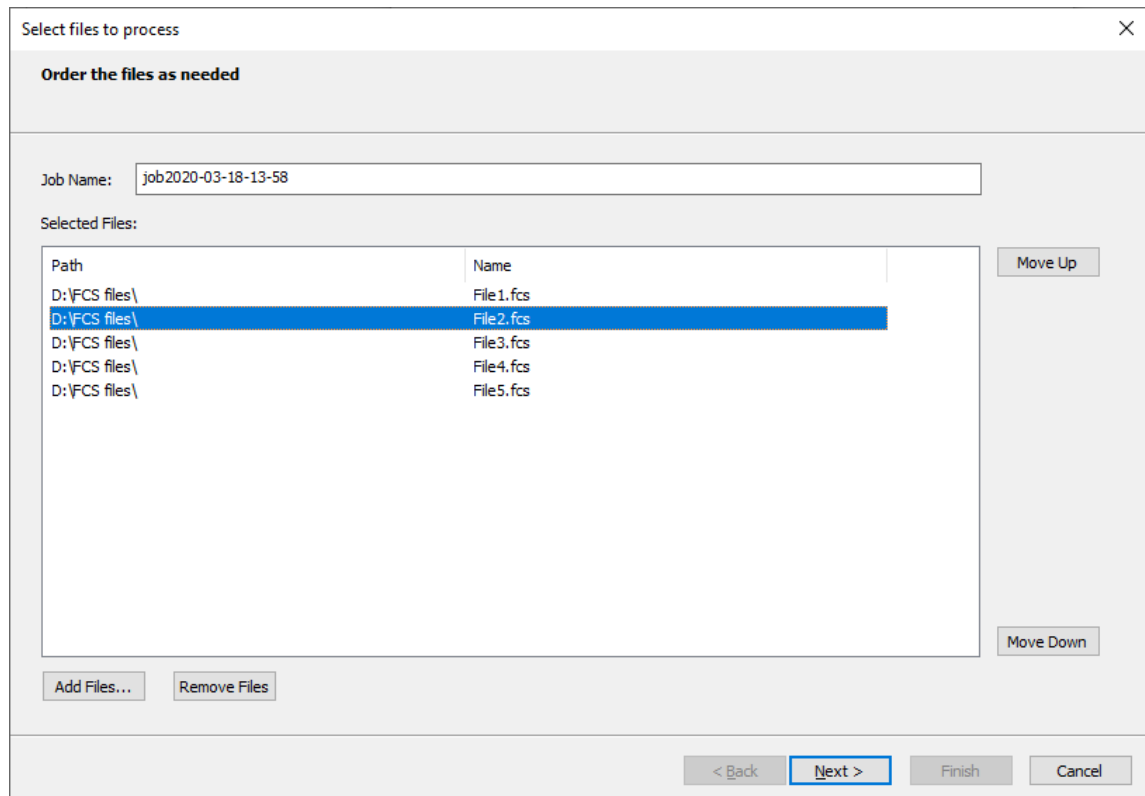
Clicking cancel closes the file selection dialog and returns you to the CytoSwarm program.

### 4.3.2 Page 1 - Files to process dialog

This dialog displays the chosen files and the order in which they will be processed.

Pressing Back, when enabled, will return you to the previous step.

Pressing Cancel on any of the following steps Cancels the process and returns to the CytoSwarm application.



#### 4.3.2.1 Job Name

This is the name used to identify this task on the server, the default name is the current date and time you can enter any name you require to identify this job.

#### 4.3.2.2 Selected Files

This list displays the currently selected files in the order they will be processed

#### 4.3.2.3 Move Up

Moves selected files up the list

#### 4.3.2.4 Move Down

Moves selected files down the list

#### 4.3.2.5 Add Files...

Opens the File selection dialog to add more files to the Selected Files list for processing

#### 4.3.2.6 Remove Files

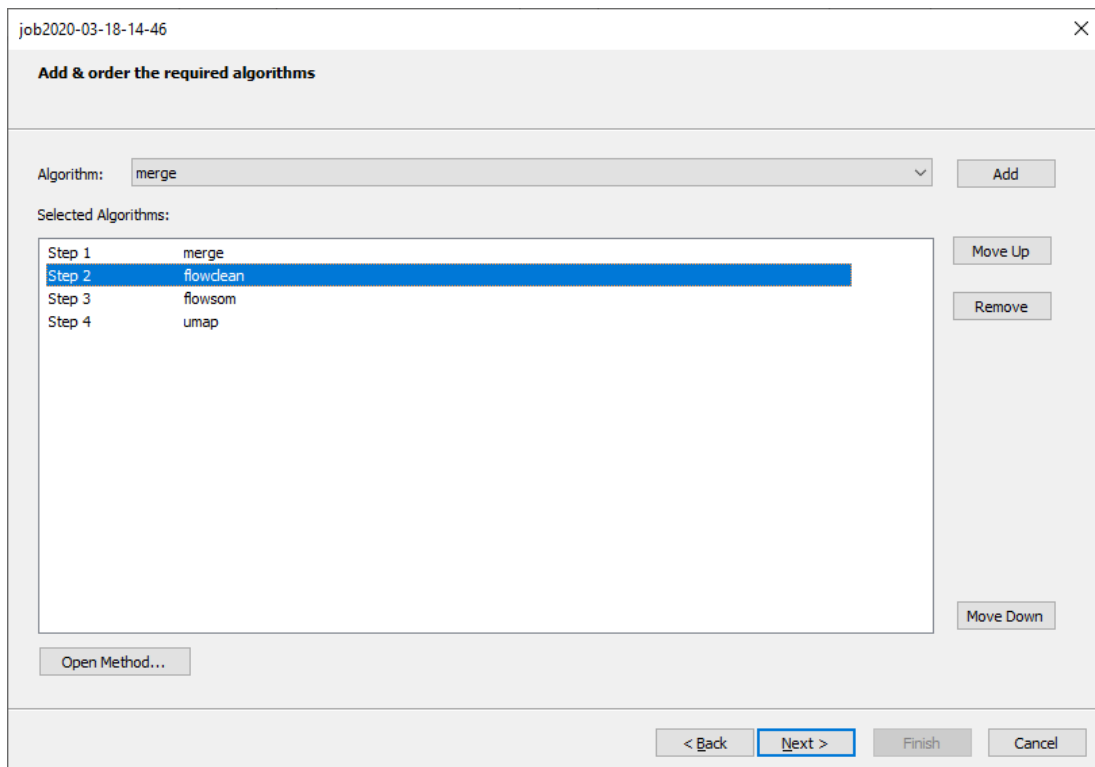
Removes selected files from the list

#### 4.3.2.7 Next

Moves on to the next step

### 4.3.3 Page 2 – Required Algorithms

This dialog allows selection of the algorithms to be performed and sets the order in which to perform them.



#### 4.3.3.1 Algorithm

This is a drop-down list of available algorithms, select an algorithm then press the **Add** button to add the selected algorithm to the list of selected algorithms.

#### 4.3.3.2 Add

Adds the selected Algorithm to the Selected Algorithm list

#### 4.3.3.3 Selected Algorithms

This list displays the currently selected algorithms in the order they will be processed

#### 4.3.3.4 Move Up

Moves selected algorithm up the list

#### 4.3.3.5 Remove

Removes selected algorithm from the list

#### 4.3.3.6 Move Down

Moves selected algorithm down the list

#### 4.3.3.7 Open Method

Pressing this button opens a file selection dialog allowing you to select a previously used Method (selected algorithms and algorithm options)

#### 4.3.3.8 Next

Moves on to the next step

### 4.3.4 Page 3 – Parameter Selection

This dialog allows selection of the parameters to be processed, this list will be used by all algorithms which allow parameters to be selected.

Individual algorithm options allow specific parameter selection if required

The screenshot shows a dialog box titled "Select default channels" with a close button (X) in the top right corner. The dialog contains a table with 18 rows, each representing a parameter. Each row has a checkbox in the first column, a "Name (\$PnV)" column, and a "Stain (\$PnS)" column. All checkboxes are checked. Below the table is an "All" checkbox, which is also checked. At the bottom right of the dialog are four buttons: "< Back", "Next >", "Finish", and "Cancel".

	Name (\$PnV)	Stain (\$PnS)
<input checked="" type="checkbox"/>	1 Event	Event
<input checked="" type="checkbox"/>	2 Time	Time
<input checked="" type="checkbox"/>	3 FSC-H	FSC-H
<input checked="" type="checkbox"/>	4 SSC-H	SSC-H
<input checked="" type="checkbox"/>	5 BL1-H	BL1-H
<input checked="" type="checkbox"/>	6 BL2-H	BL2-H
<input checked="" type="checkbox"/>	7 BL3-H	BL3-H
<input checked="" type="checkbox"/>	8 RL1-H	RL1-H
<input checked="" type="checkbox"/>	9 RL2-H	RL2-H
<input checked="" type="checkbox"/>	10 RL3-H	RL3-H
<input checked="" type="checkbox"/>	11 VL1-H	VL1-H
<input checked="" type="checkbox"/>	12 VL2-H	VL2-H
<input checked="" type="checkbox"/>	13 VL3-H	VL3-H
<input checked="" type="checkbox"/>	14 VL4-H	VL4-H
<input checked="" type="checkbox"/>	15 YL1-H	YL1-H
<input checked="" type="checkbox"/>	16 YL2-H	YL2-H
<input checked="" type="checkbox"/>	17 YL3-H	YL3-H
<input checked="" type="checkbox"/>	18 YL4-H	YL4-H

#### 4.3.4.1 Parameter List

This is the list of parameters present in the first file, select the checkbox for those parameters required for processing.

**! Note:** Errors will occur if the selected files do not all have the same number of parameters present

#### 4.3.4.2 All checkbox

Check or un-check this checkbox to select / deselect all parameters

#### 4.3.4.3 Selected Algorithms

This list displays the currently selected algorithms in the order they will be processed

#### 4.3.4.4 Next

Moves on to the next step

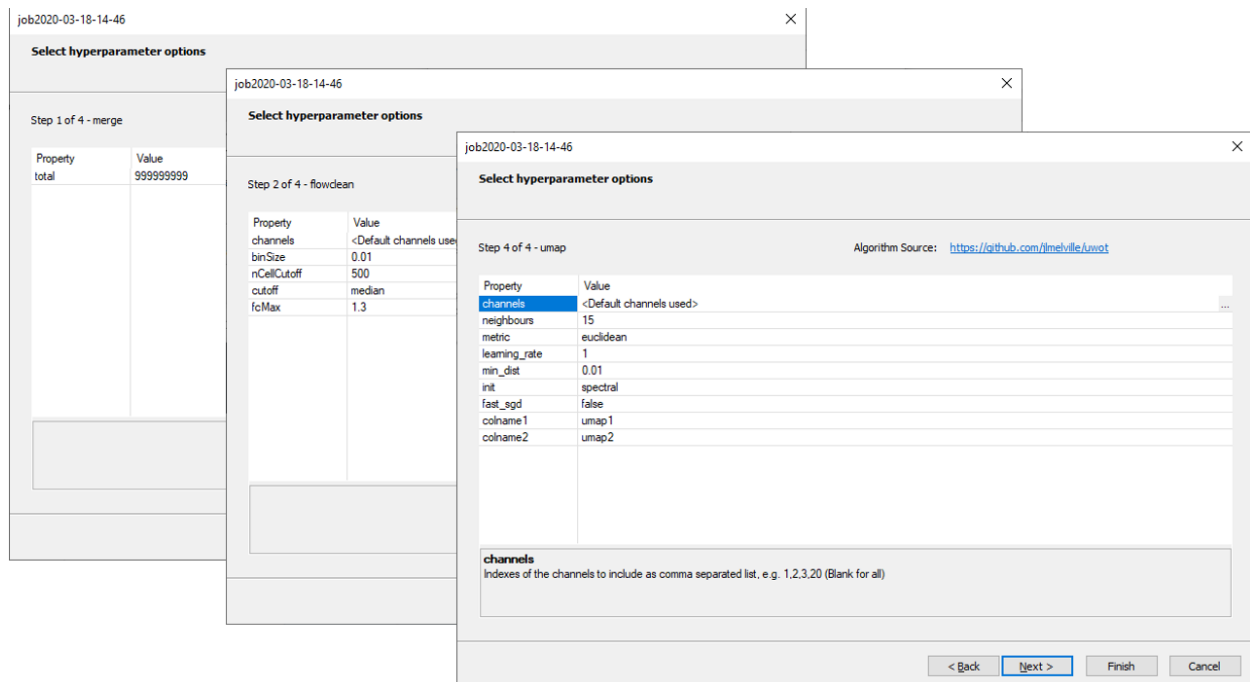
#### 4.3.4.5 Finish

Begin processing files with the selected algorithms.

### 4.3.5 Page 4 – Algorithm Hyper-parameter Selection

This series of dialogs allows selection of the algorithm specific options to be selected.

One page will be displayed for each selected algorithm showing the options available for that algorithm.



#### 4.3.5.1 Step Number – Algorithm Name

This displays the step number and the Algorithm Name. One page will be displayed for each selected algorithm

#### 4.3.5.2 Algorithm Source

Displays a selectable hyperlink to the source information for the current algorithm, this includes any license, user information and version information.

#### 4.3.5.3 Selected Algorithm Properties / Value

Displays a list of the options available for each algorithm. Clicking on a property displays further information about the selected item

#### 4.3.5.4 Next

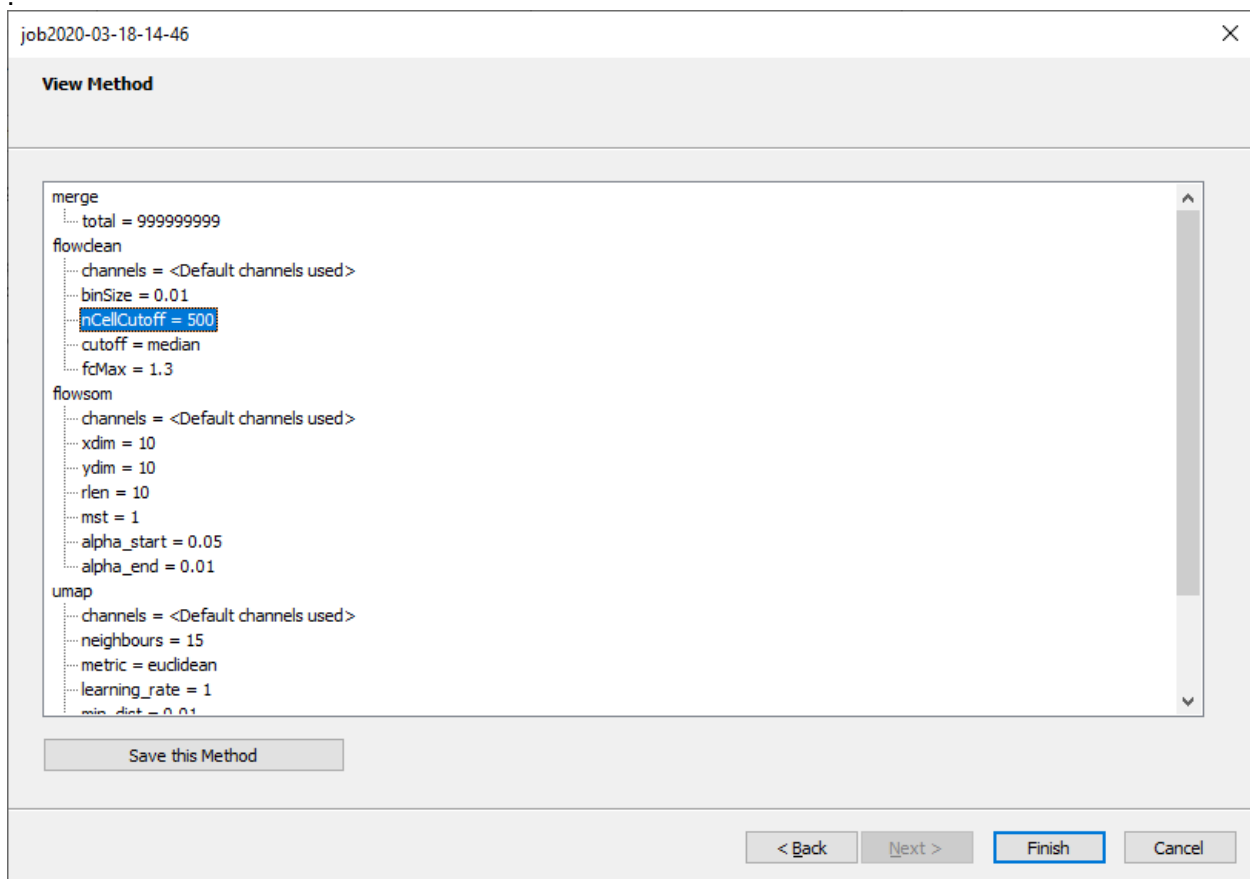
Moves on to the next step

#### 4.3.5.5 Finish

Begin processing files with the selected algorithms.

### 4.3.6 Page 5 – View Method

This page summarizes all the algorithms and the associated options that will be processed.



#### 4.3.6.1 Save This Method Button

Pressing this button opens a file save dialog allowing to save the selection of algorithms, the order of algorithms and the selected algorithm options.

#### 4.3.6.2 Finish

Begin processing files with the selected algorithms.



## 4.4 Settings Button

Pressing the settings button opens the settings dialog, this contains information on the defaults folders, licensing and version numbers

Settings

Default Folders

Source Folder: C:\Users\Public\Documents

Download Folder: C:\Users\Downloads

Licensing

To generate a licence key, we need a few pieces of information about your computer. Click the button to save this to a file that can then be emailed to us at [customersupport@appliedcytometry.com](mailto:customersupport@appliedcytometry.com)

We will only use your personal information as set out in our Privacy Policy available on our website, at <https://www.appliedcytometry.com/privacy/>

Email:

Voucher Code (if available):

Generate License Request Enter License Licensed

Version

CytoSwarm Version: 1.0.0.0

Myriad Version: 1.0.0.0

OK Cancel

### 4.4.1 Default Folders

The default folders for source and downloads can be set here

Default Folders

Source Folder: D:\My Documents\

Download Folder: C:\Users\peternobes\Downloads

#### 4.4.1.1 Source Folder

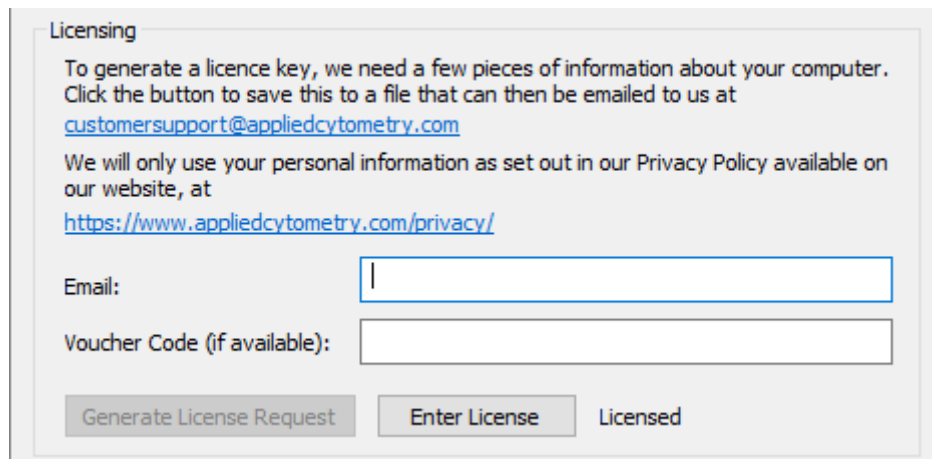
This is the folder that will be opened by default when selecting FCS files. Enter a location name or click the folder icon to select a folder.

#### **4.4.1.2 Download Folder**

This is the folder that will be opened by default when selecting a save location for downloaded files. Enter a location name or click the folder icon to select a folder.

## 4.4.2 Licensing

A license for CytoSwarm can be requested here and a license code entered



Licensing

To generate a licence key, we need a few pieces of information about your computer. Click the button to save this to a file that can then be emailed to us at [customersupport@appliedcytometry.com](mailto:customersupport@appliedcytometry.com)

We will only use your personal information as set out in our Privacy Policy available on our website, at <https://www.appliedcytometry.com/privacy/>

Email:

Voucher Code (if available):

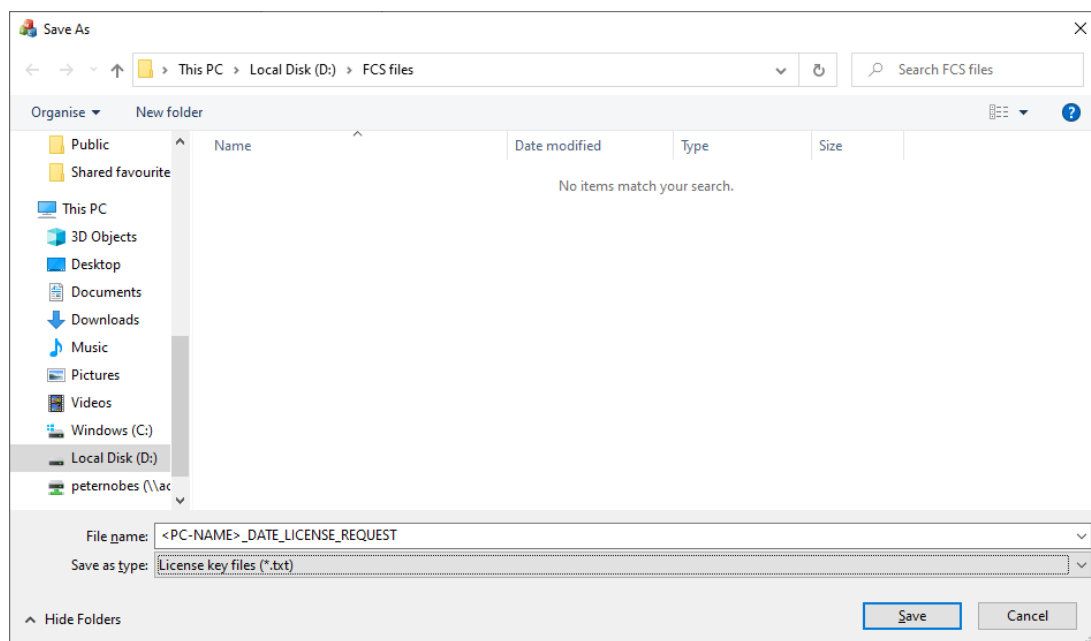
Generate License Request   Enter License   Licensed

Enter your email address and a voucher code if you have one.

Press the Generate License Request button to save a license request file. Email this request file to [customersupport@appliedcytometry.com](mailto:customersupport@appliedcytometry.com)

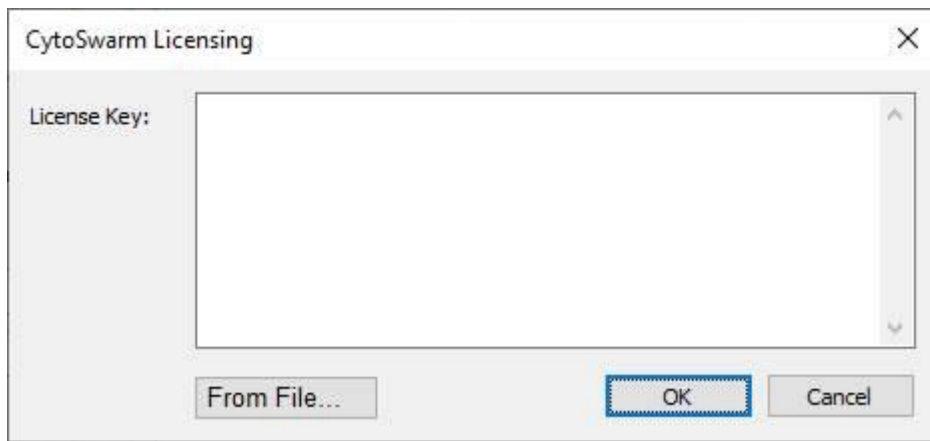
### 4.4.2.1 Generate License Request

Pressing the Generate License Request button opens a file save dialog allowing you to save a license



### 4.4.2.2 Enter License

Select the Enter License to enter the license key sent to you by Applied Cytometry.



From the email containing your license key, save the license key file to a known location, return to the CytoSwarm licensing screen, and select Enter License. Select the From File button and point the software to the license key file.

Alternatively copy and paste the license key into the license key field and press OK.

The license status will update from **Unlicensed** to **Licensed**

#### 4.4.3 Version

The current CytoSwarm client and the server (Myriad) version numbers are displayed.



#### 4.5 Download Buttons

The Download buttons are enabled when one or more jobs are selected in the jobs list.

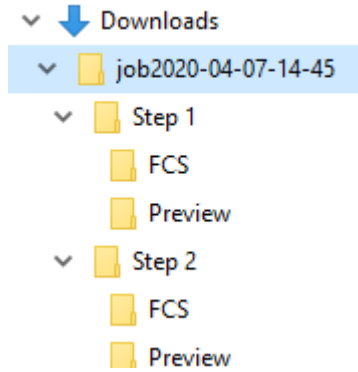
Select a download button to download files from the server, you can select to download all files from the server or just the output FCS files from final step.

If you click the “Download Results only” button, only the output file(s) from a successful job will be downloaded.

Downloaded files will be placed in a sub-folder within the selected folder, this folder will be created with the job name as the folder name.

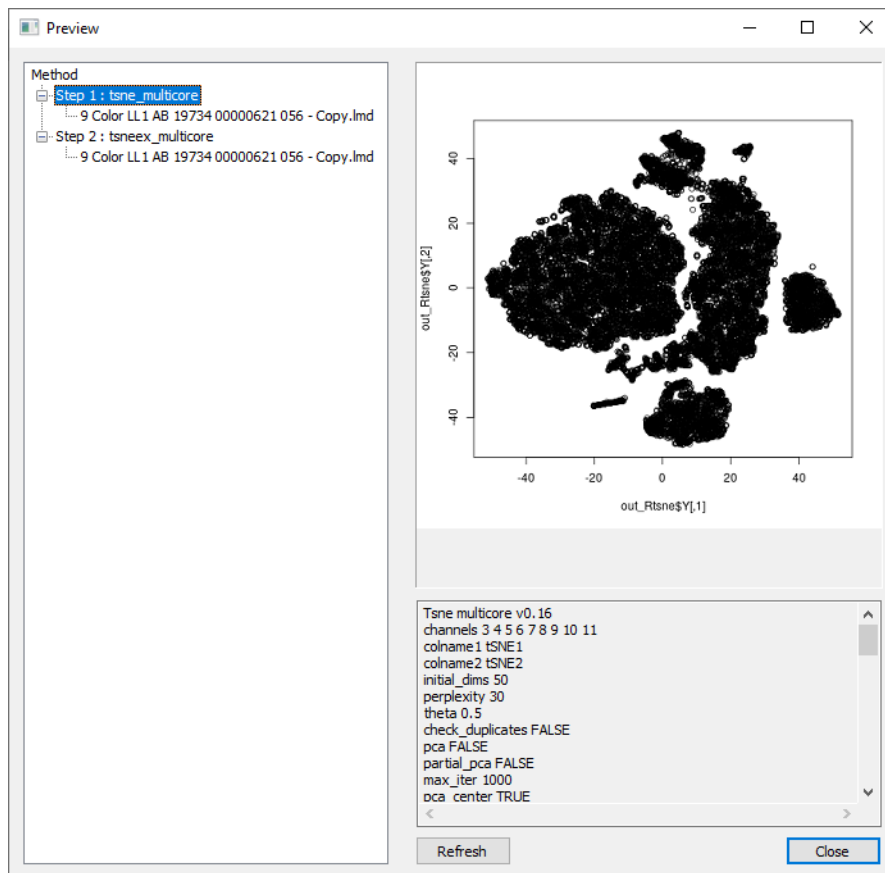
If you click the “Download All steps” button, the original file will be saved and additional folders will be created for each algorithm step successfully completed. Downloaded files will be placed in sub-folders within the selected folder, a new sub-folder will be created with the job name as the folder name.

In each ‘algorithm step’ folder an FCS folder will be created into which the output FCS file(s) is placed. If the algorithm generates a graphic output (e.g. pdf files) they will be placed in a preview folder under the relevant step.

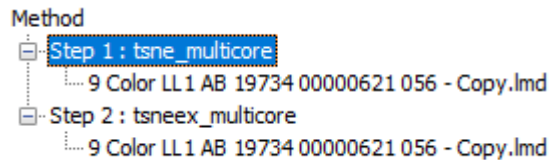


#### 4.6 Details Button

The details button is enabled when one job is selected in the Jobs list. Pressing the details button opens a dialog showing the progress of the selected job

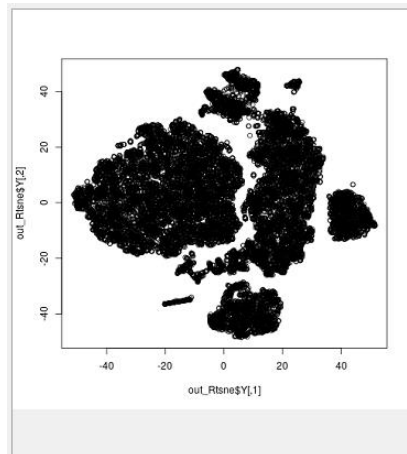


The Method area displays the files and selected algorithms for the current job

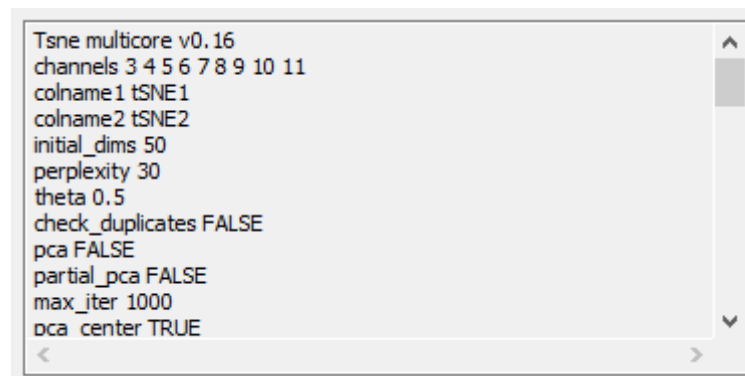


If a completed step in the method is selected, the Preview area shows the graphical output of the algorithm created at that step (if the algorithm creates a graphical output).

If the selected step is still in progress no graphic is displayed.



When an in process, or a completed step is selected, the Status area displays the current progress of the algorithm, the settings used and any error messages. Scroll the list to see later messages for the selected step.



## 4.7 Remove Button

The remove button is enabled if one or more jobs are selected from the Jobs list. Pressing remove will remove the selected jobs from the list and any associated files from the server.

## 5 Algorithm References

### 5.1 Merge

File concatenation routine © Appliedcytometry 2020

### 5.2 Flowcut

Description: Common technical complications such as clogging can result in spurious events and fluorescence intensity shifting, flowCut is designed to detect and remove technical artifacts from your data by removing segments that show statistical differences from other segments.

Github reference = <https://github.com/jmeskas/flowCut>

Author: Justin Meskas, Sherrie Wang

License: Artistic-2.0

Citation (from R)

Meskas J, Wang S (2020). *flowCut: Precise and Accurate Automated Removal of Outlier Events and Flagging of Files Based on Time Versus Fluorescence Analysis*. R package version 0.99.27.

### 5.3 Flowsom

Github reference = <https://github.com/saeyslab/FlowSOM>

License: GPL2 - <https://github.com/saeyslab/FlowSOM/blob/master/DESCRIPTION>

References

[1] Sofie Van Gassen, Britt Callebaut, Mary J. Van Helden, Bart N. Lambrecht, Piet Demeester, Tom Dhaene and Yvan Saey. FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. *Cytometry A* 2015, volume 87.7 (p. 636-645)

### 5.4 Tsne\_multicore

Github reference = <https://github.com/jkrijthe/Rtsne>

License: <https://github.com/jkrijthe/Rtsne/blob/master/LICENSE>

References

[1] L.J.P. van der Maaten and G.E. Hinton. "Visualizing High-Dimensional Data Using t-SNE." *Journal of Machine Learning Research* 9(Nov):2579-2605, 2008.

[2] L.J.P van der Maaten. "Accelerating t-SNE using tree-based algorithms." *Journal of Machine Learning Research* 15.1:3221-3245, 2014.

[3] Jesse H. Krijthe (2015). *Rtsne: T-Distributed Stochastic Neighbor Embedding using a Barnes-Hut Implementation*, URL: <https://github.com/jkrijthe/Rtsne>

[5] For Multicore-TSNE: Ulyanov, Dmitry, *Multicore-TSNE*, 2016, <https://github.com/DmitryUlyanov/Multicore-TSNE>,

## 5.5 Tsneex\_multicore

Github reference = <https://github.com/omiq-ai/Multicore-opt-SNE>

License: <https://github.com/omiq-ai/Multicore-opt-SNE/blob/master/LICENSE.txt>

### References

[1] Opt-sne

Anna C. Belkina, Christopher O. Ciccolella, Rina Anno, Josef Spidlen, Richard Halpert, Jennifer Snyder-Cappione. **Automated optimal parameters for T-distributed stochastic neighbor embedding improve visualization and allow analysis of large datasets (2018)** bioRxiv 451690; doi: <https://doi.org/10.1101/451690>

## 5.6 umap

Github reference = <https://github.com/jlmeville/umap>

License: <https://github.com/jlmeville/umap/blob/master/LICENSE>

Reference

[1] McInnes, L. & Healy, J. UMAP: uniform manifold approximation and projection for dimension reduction. Preprint at <https://arxiv.org/abs/1802.03426>

## 5.7 split

File de-concatenation routine © Appliedcytometry 2020

September 2020