Instructions for lymf.jar

Introduction

Lymf.jar is a software that allows for streamlined data analysis of CellProfilerTM files created by the GCCounter\_12.9(2) pipeline. Lymf.jar can be used when there are two groups that are to be analysed, for instance before and after vaccination.

Instructions

1. Analyse your images using GCCounter in CellProfilerTM.

2. Name folders produced by CellProfilerTM accordingly, C01L, C02L etc, these will end up in the left column of the merged document and C01R, C02R etc, these will end up in the right column. If you have several folders that belong to the same secondary lymphoid organ name them C01L, C01L(2), C01L(3) and lymf.jar will merge them for you.

3. Place your files in a desired folder, place lymf.jar and the folder containing the files on your desktop, open the terminal and go to your desktop and type : java –jar lymf-1.1jar /users/*nameofcomputer*/desktop/*nameoffolder*

4. Click enter and a report.csv file will be created in the designated folder. Open the file, select the leftmost column, go to data and hit text to columns, select comma and click ok.

All data is now in one excel file with desired calculations such as combined germinal center area normalized by tissue area.

Here follows some of the more important rows and columns:

The first rows under “Total” displays total numbers of the entire LN sections. Column B and C are area. D-G are cell numbers for either colour. And H-K are cell numbers normalized by LN area.

For all rows below, starting with “GC average”, the columns H/I display CD3 cell numbers (in blue), N/O display TFH cell numbers (green) and AD/AE display GC B cell numbers (red), AN/AO display GC area. GC average are total GC numbers (area or cell numbers) normalized by the number of GCs. GC sum is the sum of all cells or area in GCs. GC nom is GC sum divided by the LN area.