CellEngine's automatic gate adjustment tool quickly and accurately recapitulates manual gating: assessment in a COVID longitudinal clinical study

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INTRODUCTION

Gating is the backbone of cytometry analysis. Intersample variability can require adjusting gate position and size on a file-by-file basis. This process can be time-consuming, particularly when multiple gates need to be adjusted in large datasets. Various algorithms have been developed for supervised gating, but they are challenging to use, slow, and/or perform poorly, especially on rare populations. CellCarta developed a machine-learning algorithm that uses a manually gated subset of samples to quickly and consistently gate the remaining dataset, following the user's defined hierarchy, and incorporated it into the flow cytometry analysis software CellEngine™.

In order to explore the quality and time savings of using the automatic gate tailoring, we examined two markers with high variability in a large clinical study of COVID patients. We found that the algorithm saved a significant amount of time and produced populations highly similar to the populations gated manually.

METHODS

Materials

PBMCs were collected from COVID-positive patients by researchers at Aarhus University, Denmark, and University of Toronto, Canada, and stained with a 17-marker phenotyping panel. A total of 1,991 files were examined for this study.

Analysis

All gating and review was performed with CellEngine™ (www.cellengine.com, CellCarta). The gate positions used for the training data were the same ones set for the manually gated populations. Default settings were used for the algorithm variables (minimum improvement = 0.02, minimum events = 200, smoothing variance = 3).

Time and correlation plots were produced with Mathematica (Wolfram) and Excel (Microsoft).

RESULTS

Time Savings

After a review of the 17 markers used to stain the samples, CCR5 and perforin were selected to test autogating based on high variability in marker expression between samples. Samples were first gated manually for perforin (n=1991) and CCR5 (n=769). Then, representative files were chosen from the manually gated data and used as references for automatic gate tailoring. The perforin data had significant differences in marker distribution between the two sites where data was collected, necessitating the selection of a separate set of training data for each site. The number of files selected for the training data was 3 (CCR5), 6 (perforin on samples from Aarhus), or 9 (perforin on samples from Toronto). An overview of the autogating process is shown in Figure 1.

During the gating process, the time required was measured in 15-minute increments. Time was categorized as gating (manual positioning of gates), adjustment (choosing a training set for the algorithmic analysis), or review (verifying placement of each gate).

Use of the algorithm reduced total time by 69.3% for perforin and 88.5% for CCR5 (Figure 2). The majority of the time recorded for the automatically adjusted gates was spent performing a complete, manual review of all gate positions. This could have been reduced further by only reviewing a representative sampling of files.

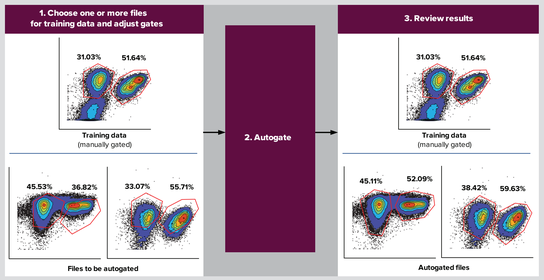
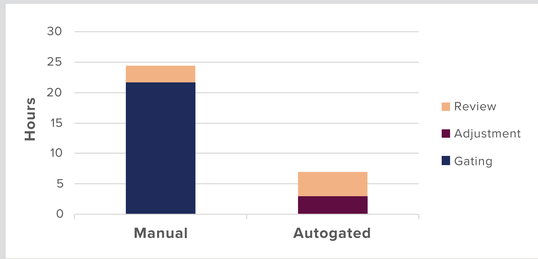


Figure 1. CellEngine's autogating algorithm uses one or more manually gated files as training data for the machine-learning algorithm, which then adjusts gates for other selected files.



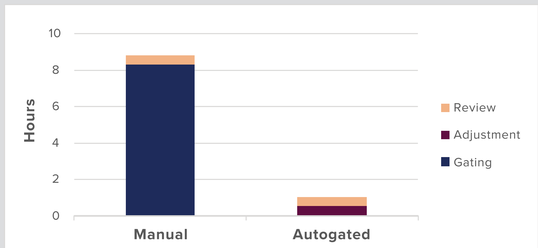


Figure 2. Use of automatic gate adjustment algorithm reduces time spent gating for perforin (top) and CCR5 (bottom).

Accuracy

During the gating review step, gate positions and shapes were inspected for placement and consistency (see representative images in Figure 3).

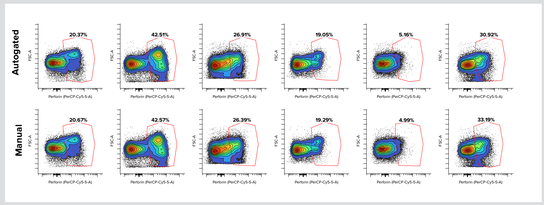


Figure 3. Despite the distribution variability, the autogated (top) and manually gated (bottom) populations show high correspondence.

To further determine accuracy of the algorithm, we compared the number of events in autogated and manual populations for each FCS file. Values were similar for both markers, with the slopes of best-fit lines of 0.991 and 0.989 for CCR5 and perforin respectively, and R2 values of 0.998 for both markers (Figure 4).

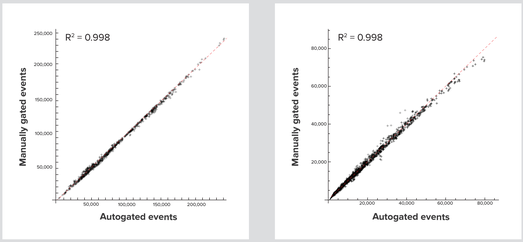


Figure 4. The number of events in autogated populations correlated closely with the number in manually gated populations in CCR5 (left) and perforin (right).

CONCLUSIONS

This study supports the ability of CellEngine's automatic gate adjustment tool to save time and produce results similar to manual gating for highly variable populations. Even in cases where the algorithm benefitted from collection site-specific training datasets, gating was substantially faster than manual gating. More broadly, this work suggests that machine-learning algorithms can reduce analysis workload while still using a researcher's judgement during the analysis process, overcoming some of the challenges seen with other algorithmic gating approaches.

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