Accelerating analysis of cytometry data sets leveraging a visualization tool built within CellEngine, a cloud-based cytometry analysis software

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SUMMARY

Cytometry panels have become a hallmark of clinical phase 1 and 2 trials as they provide crucial insights such as the presence of different cell subsets, activation and exhaustion states and expression of therapeutic targets. These panels can provide large sets of readouts and need to be combined with subject metadata (e.g., health data, demographics, timepoint, and dose). Several bioinformatics tools are available to interpret these complex datasets; however, these usually require significant expertise in data manipulation, which sometimes forces the person doing the data interpretation to outsource the data visualization and statistical analysis to a bioinformatics team. As a consequence, the scientist can be one step-removed from the "raw" data, critically preventing the interpretation of biomarker effects in the context of the raw staining profiles and associated quality controls (e.g. in-run controls). Here, we show how a new tool integrated with the CellEngine cytometry analysis software can accelerate data interpretation without removing the scientist from the raw data.

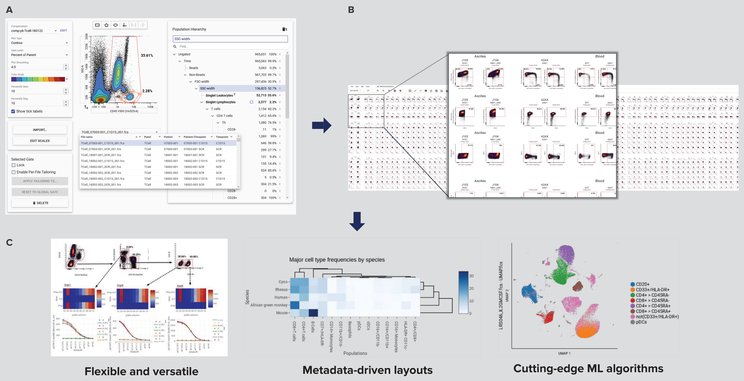


Figure 1. CellEngine is a cloud-based cytometry analysis software that is designed to analyze large data sets with a metadata-guided workflow and a global-first analysis for high level of rigor. A) Visual example of gating interface. B) CellEngine allows visualization of large data sets within its layout feature, allowing to easily and quickly verify large data sets and change the gating individually or in a group-manner. C) CellEngine offers a variety of visualization tools in addition to clustering algorithms.

METHODS

The visualization tool pulls live data from CellEngine, displaying both study- and cohort-level information in summary views as well as the sample-level staining profiles. As a live link, any changes made in CellEngine, such as ones to gating and compensation, or the addition of new samples during an ongoing study, are automatically reflected in the dashboard. This link also gives the ability to consult staining profiles and performance of controls directly within the tool without having to switch between applications. CellEngine is capable of analyzing 10,000s of samples at once, and thus can handle the largest of studies.

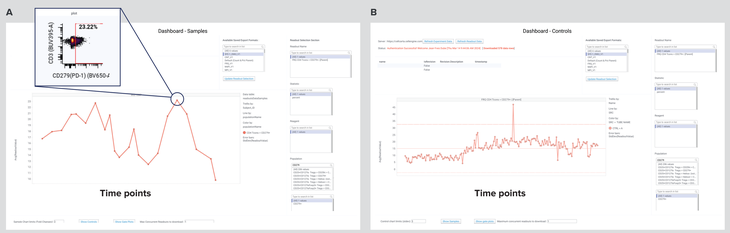
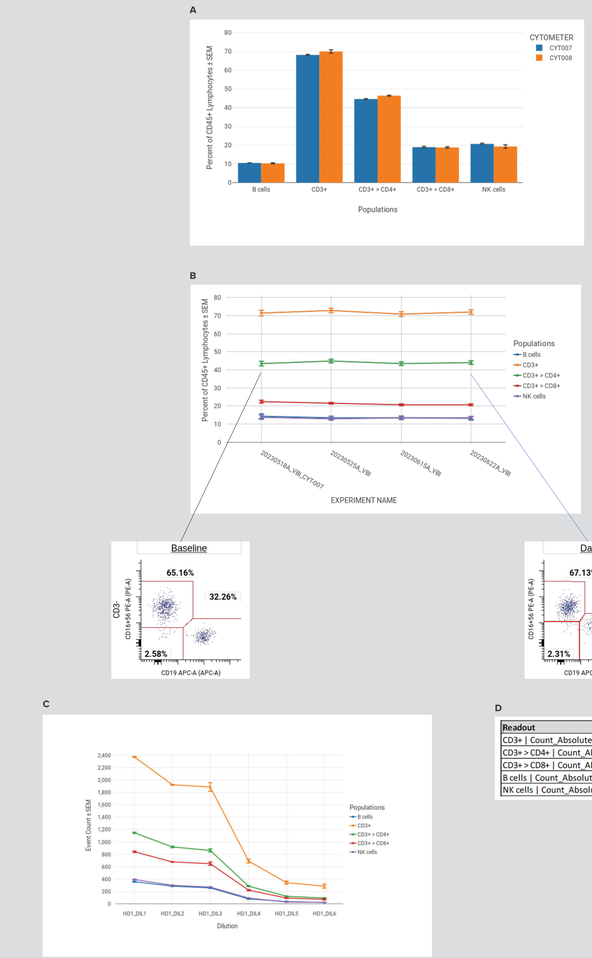


Figure 2. Example of visualization tool (Dashboard) interface. A) PD-1 expression shown in a subject across multiple timepoints with the ability to consult the PD-L1 dot plot with gating for each time point. B) Trending of the CD-Chex control associated with each run across the full length of the program, allowing easy appraisal of the performance of the different runs.

RESULTS

Using CellEngine and the visualization tool feature, validation data gathered from 6 healthy donors was illustrated to show performance of a TBNK assay. Inter-instrument precision, sample stability and lower limit of quantification were visualized. The CellEngine features allowed for quick and easy adjustment of the wanted visualizations, confirming that the TBNK assay offered similar performance on two different instruments, a sample stability of up to 35 days when shipped at either ambient or refrigerated temperature, and a lower limit of quantification of 1.1 cells/uL of whole blood for the B cell population. In addition to the visualization, as all features are fully integrated in CellEngine, dot plots of different samples were consulted at a click of a button to confirm quality of results and adjust gating if required.



Readout	Unit	LLOQ
CD3+ | Count_Absolute	cells/µL	5.2
CD3+ > CD4+ | Count_Absolute	cells/µL	2.2
CD3+ > CD8+ | Count_Absolute	cells/µL	1.1
B cells | Count_Absolute	cells/µL	1.1
NK cells | Count_Absolute	cells/µL	1.0

Figure 3. Results from a TBNK assay validation performed on 6 healthy donors were plotted using the visualization tool to assess the performance of the assay. A) Inter-instrument comparison for total T cells, CD4+ T cells, CD8+ T cells, B cells and NK cells. B) Whole blood stability assessment for all cell populations tested at baseline, 7 days, 28 days and 35 days post-draw. Dot plots of the B cell population are shown for baseline and 35 days timepoints. C) Effects of dilution of sample on the event count of the different monitored populations to assess lower limit of quantification. D) Table showing the lower limit of quantification (LLOQ) values for all populations.

In this example, we show how the visualization tool was used to monitor the effect of treatment using a TBNK assay in 7 subjects. B cell population was tracked longitudinally across multiple timepoints. The visualization tool was used to look at the performance of the two inter-run controls to easily identify which timepoints should not be considered during analysis. In addition, with the direct link to CellEngine, dot plots were monitored within the interface of the visualization tool to assess quality of staining profiles, allowing quick and in-depth interpretation of the flow cytometry data.

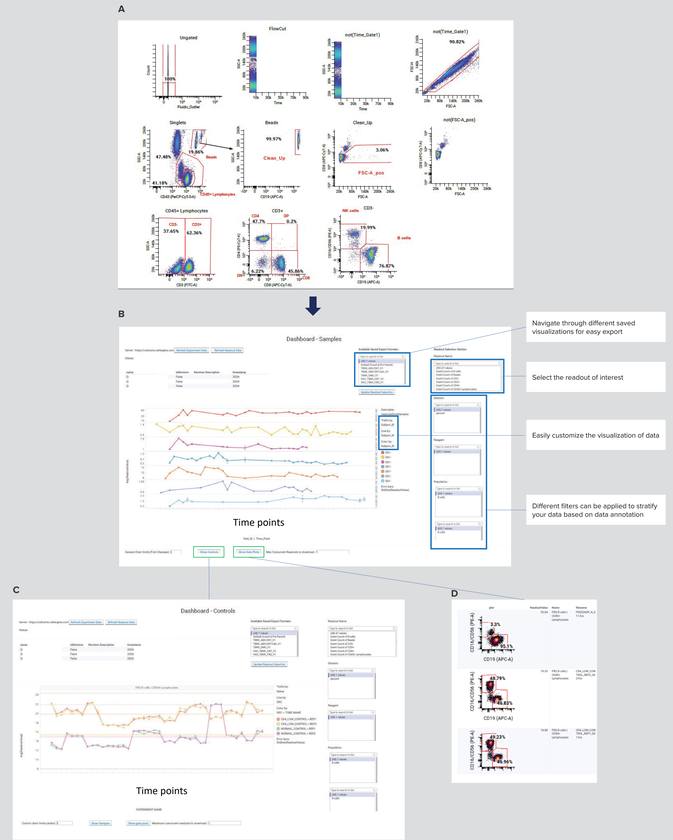


Figure 4. Results from a TBNK assay deployed in 7 subjects were plotted using the visualization tool to track the effect of treatment on B cells. A) Gating hierarchy performed in CellEngine. B) B cell absolute counts were tracked longitudinally across multiple timepoints using the visualization tool. C) Using the tool's "Show Controls" feature, the performance of the two inter-run controls was monitored across all runs. D) Using the tool's "Show Gate Plots" feature, the staining profiles of a particular timepoint were consulted for the subject's sample and the two inter-run controls.

CONCLUSIONS

This new tool can help maximize the utility of high-dimensional cytometry in clinical trial analysis while allowing to keep the crucial link between graphical representation of large number of samples and the data associated with each individual flow cytometry staining profile. The next evolution of this tool will allow the monitoring of results from different platforms to approach data in a true multi-omics fashion, this way of visualizing the data would allow maximization of the understanding of different data sets.

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