

FlowCAP - Flow Cytometry: Critical Assessment of Population Identification Methods*FlowCAP Summit 2010 Meeting Summary**NIH campus Sept 21 and 22, 2010 - Sponsorship from NIH/NIAID/DAIT*

The following observations were made in evaluating the experience from FlowCAP-I:

- Participation in FlowCAP-I was better than expected
 - 13 different groups, representing Industry and Academia from USA, Canada, and Australia submitted results from their algorithms.
 - The organizing committee is still receiving additional requests for participation in the challenges!
- 1st generation algorithms perform better than expected. Many of the methods showed a high level of concordance with the results from manual gating.
- Interest was better than expected with 67 people from an equally broad stakeholder community registering for the summit.
 - 8 Government agencies/divisions
 - 8 Companies from Industry
 - 16 Academia/Research Institutions
 - Computational Science/Bioinformatics experts
 - Statistics experts
 - Biologists (immunology, neurology, cancer research)
- Exuberant, interactive discussion by participants of the merits of FlowCAP-I
 - Easy to participate
 - Excellent responsiveness from the FlowCAP organizers
 - Critical components
 - “Standard” datasets
 - Objective evaluation criteria
- Great momentum and enthusiasm by participants and attendees for continuing this effort with FlowCAP-II, including the following recommended improvements:
 - Datasets that are more domain-representative:
 - Greater number of events per sample
 - Increased number of parameters (16 or more)
 - Uniform population identification for cross-sample comparison
 - Rare population titration
 - Cell stimulation together with unstimulated controls
 - Uncompensated data
 - Electronically mixed populations (perhaps from human/mouse titrations)
 - Biological replicates (data reproducibility, proficiency testing samples)
 - Bead data
 - Data formatting issues require better standardization of available cytometry file formats
 - Gold standard: Comparison against manual gating good choice for FlowCAP-I; but perhaps additional types of standards for FlowCAP-II.
 - Use manual gating for “selective” (directed) gating for classification of known cells.
 - Manual 2D gating can’t detect the multi-dimensionally defined populations
 - Use the ensemble of all algorithms as the gold standard for “exhaustive” gating (clustering) for discovery datasets.
 - Ability to detect biological outcome differences where known
 - Possibly more
 - Handling of “outliers”

- Outliers/noise should not be excluded from the analysis if “k” is given, unless they are filtered from the dataset
- One “k” (cluster) for outliers may not be sufficient if included
- Additional evaluation metrics, perhaps specific for each dataset.
 - Is F-measure a sufficient metric? Should we weigh recall and precision differently for different clusters, challenges or datasets?
 - Threshold of rare population detection
 - Biological outcome prediction
 - Separation of clusters within each sample
 - Uncertainty measurement of the clusters
 - Is computational time relevant?
- Additional suggested challenges –
 - Rare population detection threshold
 - Pre-processing of samples (QC)
 - Cross sample comparison (alignment and mapping of clusters across samples)
- Dataset descriptions should include
 - Metadata descriptions about the specimens and staining procedures compliant with the MIFlowCyt data standard
 - Biological outcome to determine if algorithms/computational methods also reach the same conclusion
 - Target populations for classification datasets (i.e. CD3+, CD4+)
- Increased time between release of datasets and deadline for results submission.

GOALS FOR FLOWCAP MOVING FORWARD

- Maintain the momentum of the project
- Obtain ongoing support for:
 - Collection and annotation of additional datasets
 - Determination of ideal evaluation metrics for each dataset and challenge
 - Increased public awareness of the project
 - Planning of FlowCAP-II challenge and summit
 - Analysis of algorithm results for FlowCAP-II
- Rapidly attain the goal of making computational algorithms an essential component of standard FCM data analysis
- Set of criteria to become FlowCAP compliant – as requested by participants

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- Fred Hutchinson Cancer Research Center
- University of Texas Southwestern Medical Center
- TreeStar Inc.
- University of British Columbia
- FlowCAP Organizing Committee:
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 - Raphael Gottardo, Fred Hutchinson Cancer Research Center
 - Richard H. Scheuermann, University of Texas Southwestern Medical Center
 - Jill Schoenfeld, TreeStar Inc.

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