FLOW CYTOMETRY & ONTOLOGIES

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FLOW CYTOMETRY IN THE ONTOLOGY FOR BIOMEDICAL INVESTIGATIONS (OBI)

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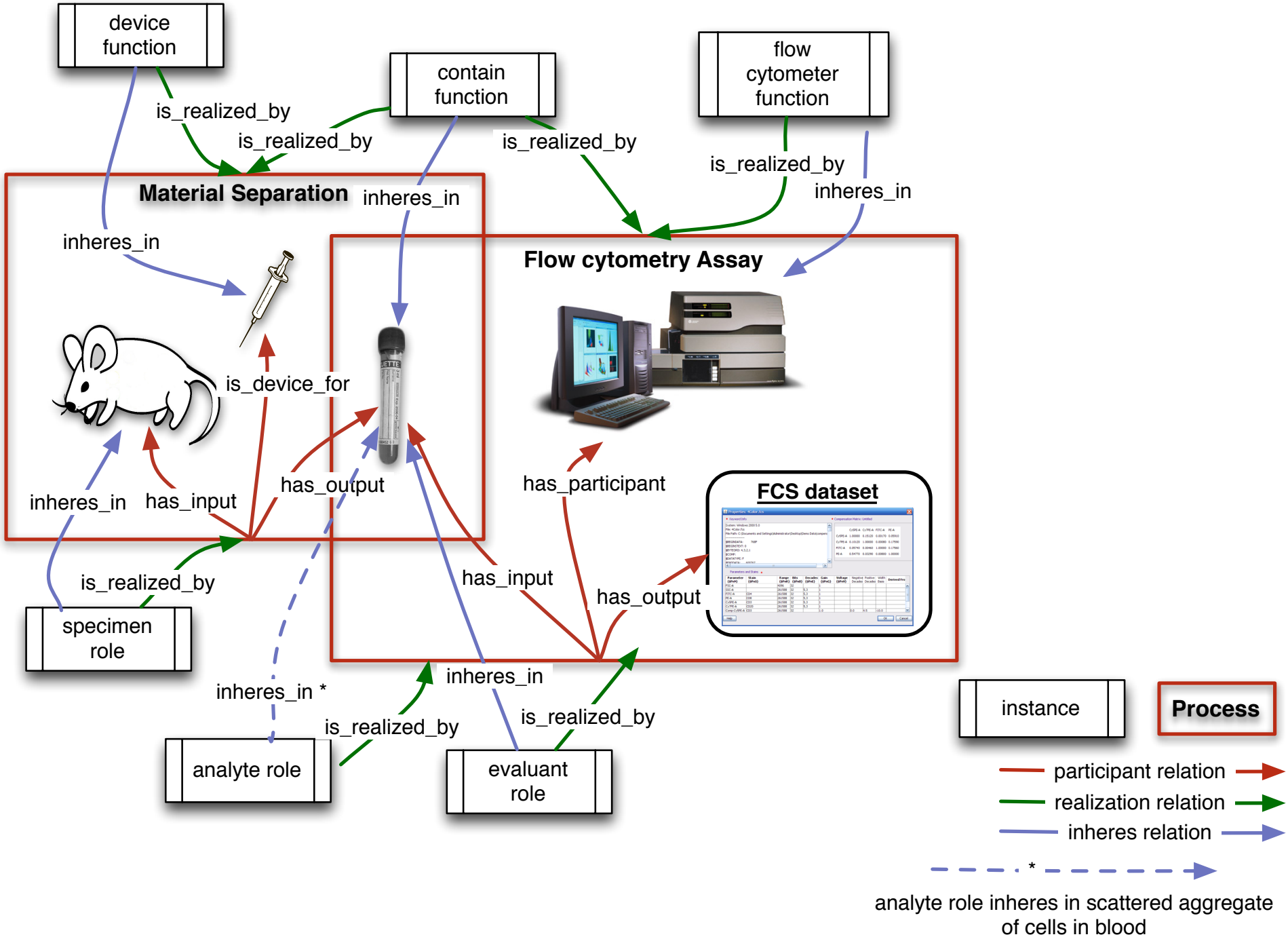
[1] BC Cancer Agency, Vancouver, Canada

[2] http://purl.obolibrary.org/obo/obi

OBI terms

- Instruments and parts
  - Flow cytometers sorters, analyzers, light sources, filters….
- Fluorochromes (in the Chemical Entities of Biological Interest (ChEBI))
  - More than 400 have been added, each including formula, synonyms…
- Processes
  - Flow cytometry assays
  - Gating
- Processes objectives
  - Partitioning


device function
is_realized_by
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flow cytometer function
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Material Separation
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Flow cytometry Assay
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has_input
has_output
has_participant
FCS dataset
is_realized_by
specimen role
has_input
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Process
participant relation
realization relation
inheres relation
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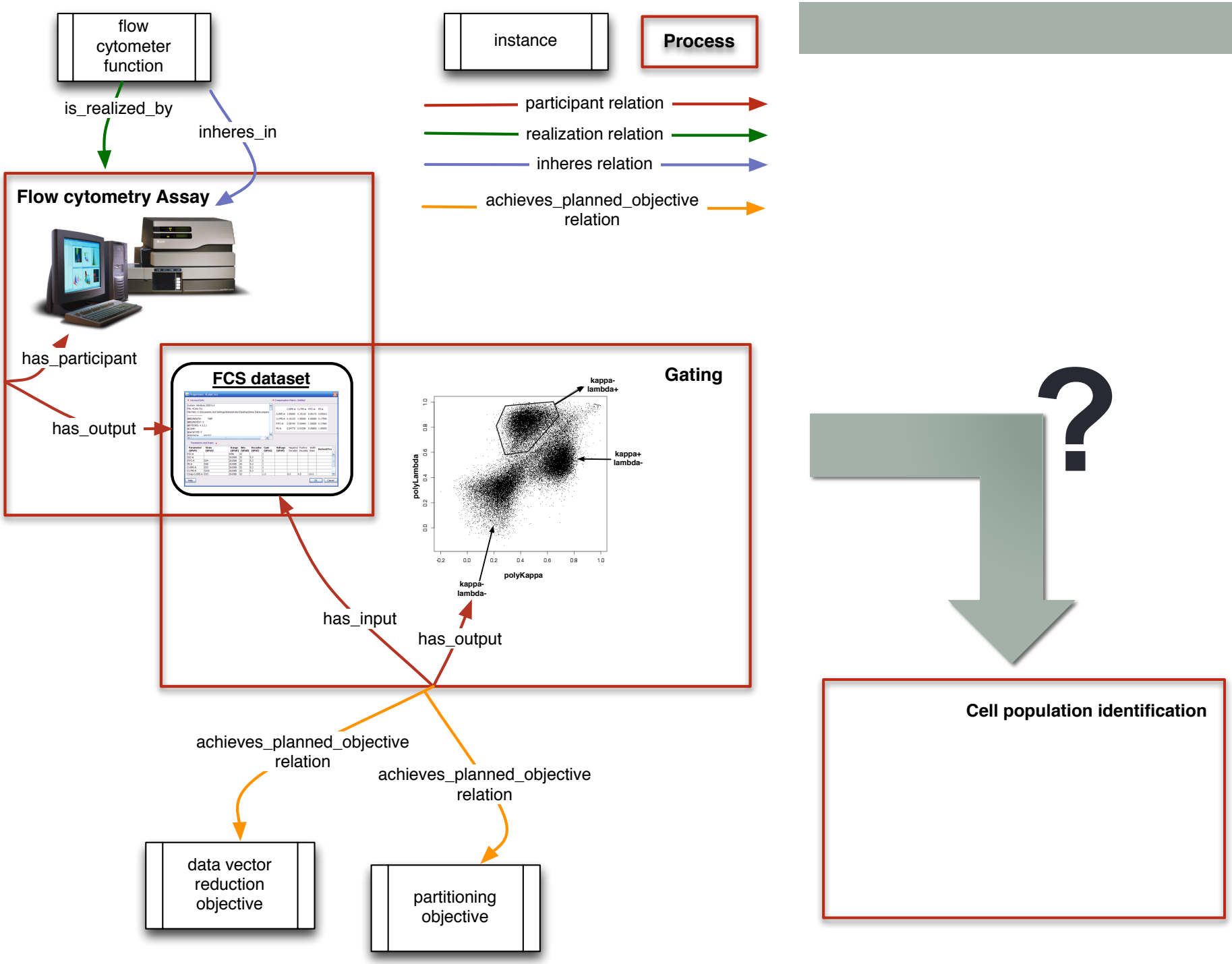
analyte role inheres in scattered aggregate of cells in blood


flow cytometer function
is_realized_by
inheres_in
instance
Process
participant relation
realization relation
inheres relation
achieves_planned_objective relation
Flow cytometry Assay
has_participant
has_output
FCS dataset
Gating
kappa- lambda+
kappa+ lambda-
kappa- lambda-
polyLambda
polyKappa
has_input
has_output
achieves_planned_objective relation
achieves_planned_objective relation
data vector reduction objective
partitioning objective
?
Cell population identification

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CONNECTING FCM ANALYSIS RESULTS WITH THE CELL ONTOLOGY IN AN AUTOMATED WAY

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Automated methods can't semantically label cell populations

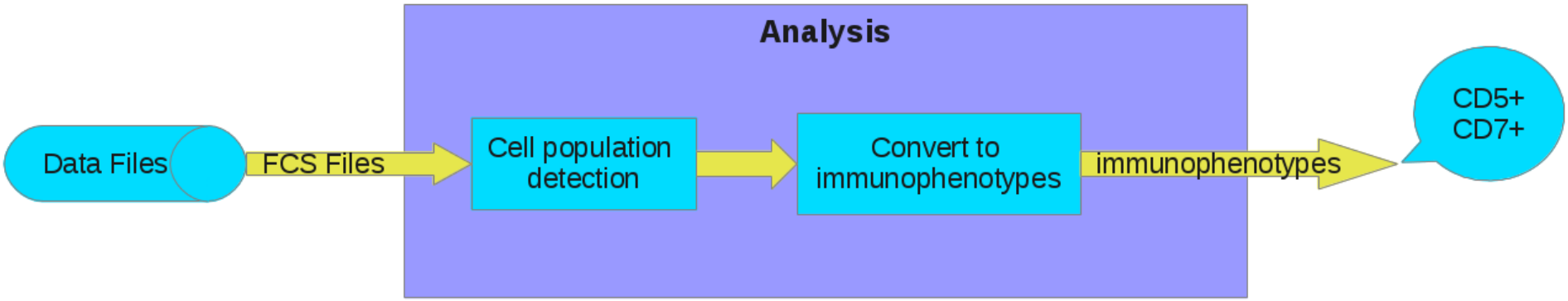
- Different researchers refer to cell populations types using different labels, depending on the experiment context
- Automated analysis label cell populations via their belonging to a cluster
- Cell groups can be identified via different immunophenotype, e.g., CD5+ or CD7+ to identify T-cells
- As a result, outputs from different sources can not be compared
- Labeling cell populations using common natural language will facilitate comparison and collaboration

SOLUTION

- A framework allowing to label immunophenotypes resulting from a Flow Cytometry (FCM) analysis (automated or manual) to a consensus label will allow researchers to unambiguously refer to a defined cell population
- Previous research on the same cell population and/or related will become accessible, even if different markers were used
- We call this **the Cell Population Labeler (CPL)**

FCM analysis

- Analysis outputs an immunophenotype (i.e., a set of markers, such as CD3+CD4+)
- Markers can be present/absent, or present at various levels such as low, intermediate and high
- Output fed to the CPL



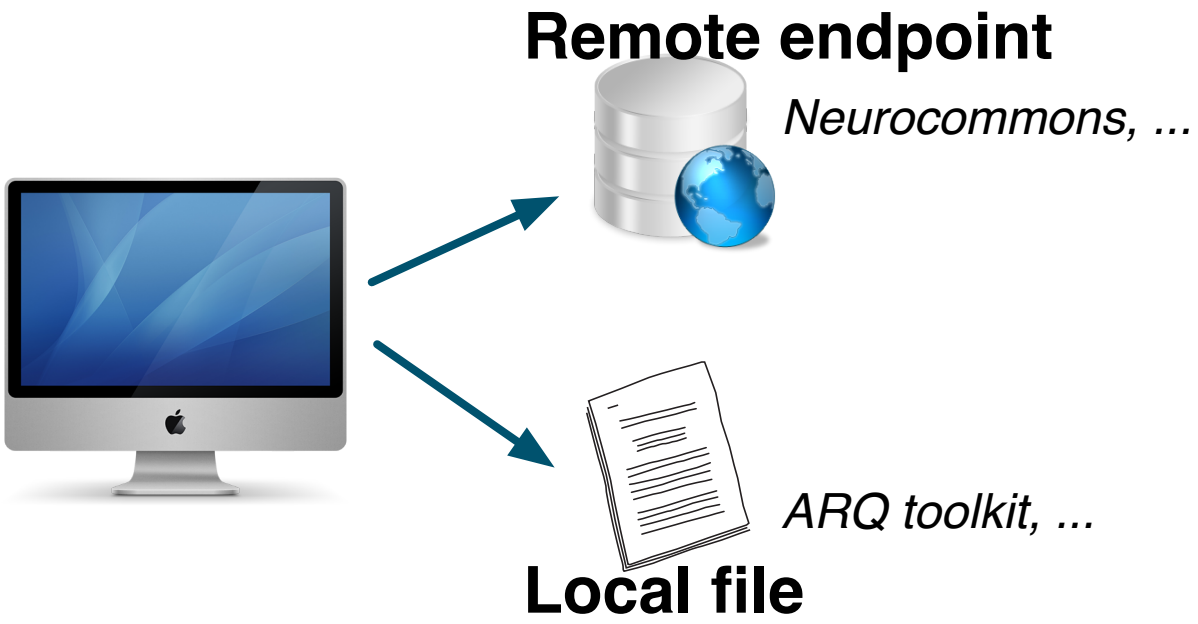
Cell Population Labeler (CPL)

- Identifies a subset of the Cell Ontology (CL) tree as corresponding to the given immunophenotype
- This subset can be a single node, empty, or a sub-tree
- With increase in the immunophenotype specificity, we expect the sub-tree (DAG) to get progressively pruned, and ideally to retrieve only a single node

Step 1: Access to the CL

- Use SPARQL to query the CL OWL

  Select ?celllabel where { ?x a owl:Class.?x rdfs:label ?celllabel.}



Problem: Available CL needs to be updated

Step 2: browse the content of the CL

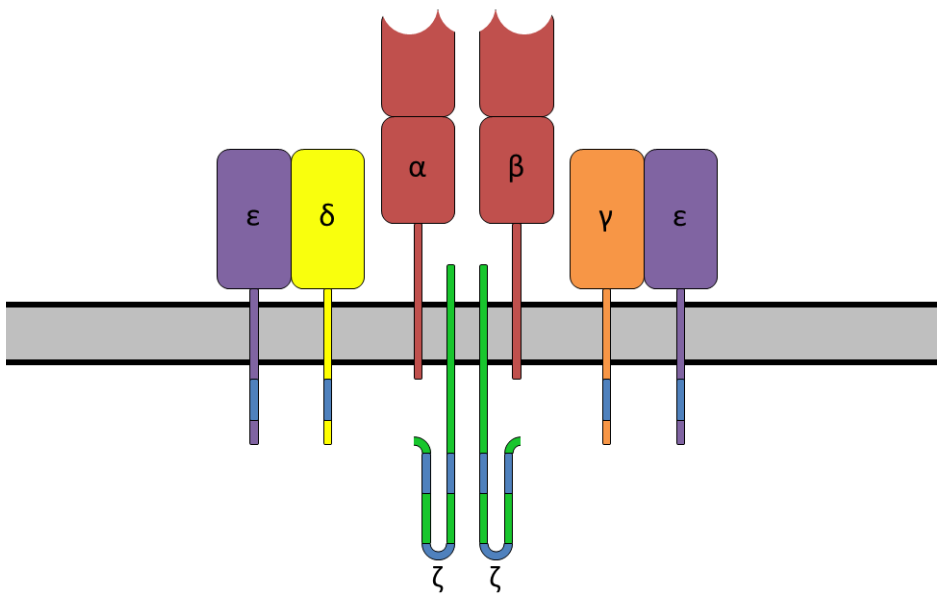
- Minor issues
  - Modelization issues, e.g., properties hierarchy
  - Missing terms
  - Release artifacts, e.g. duplicated relations

- Feedback from users to improve the current resource.
- CL tracker available and CL team very responsive.

http://sourceforge.net/tracker/?group_id=76834&atid=925065

Step 2: Browse the content of the CL

- Missing information, such as parthood relationship between receptor and subunits. Need coordination between different resources (e.g., Gene Ontology, Protein Ontology)



The T-cell receptor complex with TCR-α and TCR-β chains (top), ζ-chain accessory molecules (bottom) and CD3 (represented byCD3γ, CD3δ and two CD3ε).

Source: wikipedia

Step 2: browse the content of the CL

- Scope of the CL

- CL aims at identify those markers that are necessary and sufficient to define a cell type.
- Some work in Richard's group to list extra marker expression characteristics for hematopoeitic cell types.

Step 3: Implementation of an automated pipeline

- Pipeline in R
- R is a free, open source, robust statistical programming environment for Windows, Mac & Linux that offers a wide range of statistical and visualization methods
- BioConductor provides R software modules for biological and clinical data analysis
- Integrates with other software tools via open data standards
- Use SPARQL from R
  - Several libraries available, need investigation/testing

28+ R packages for Flow Analysis (all since 2007)

- **Data processing & Visualization**
  - flowCore: Read/write & process flow data
  - plateCore: Analyze multiwell plates
  - flowUtils: Import gates, transformation and compensation
  - flowStats: Advanced statistical methods and functions
  - ncdfFlow: Advanced methods for large dataset processing
  - flowQ: Quality control of ungated data
  - QUALIFIER: Quality control and assessment of gated data
  - flowViz: Visualization (e.g., histograms, dot plots, density plots)
  - flowPlots: Graphical displays with statistical tests
  - flowWorkspace: Importing FlowJo workspaces
  - iFlow: GUI for exploratory analysis and visualization
  - flowTrans: Estimates parameters for data transformation
- **Gating**
  - flowClust: Clustering using t-mixture model with Box-Cox transformation
  - flowMerge: flowClust + entropy-based merging
  - flowMeans: k-means clustering and merging using the Mahalanobis distance
  - SamSpectral: Efficient spectral clustering using density-based down-sampling
  - flowPeaks: Unsupervised clustering using k-means \& mixture model
  - flowFP: Fingerprint generation
  - flowPhyto: Analysis of marine biology data
  - flowQB: Q&B analysis
  - FLAME: Multivariate finite mixtures of skew and heavy-tailed distributions
  - flowKoh: Self-organizing maps
  - NMF-curvHDR: Density-based clustering and non-negative matrix factorization
  - flowCore-flowStats: Sequential gating and normalization and a Beta-Binomial model
  - PRAMS: 2D Clustering and logistic regression
  - SPADE: Density-based sampling, k-means clustering, and minimum spanning trees
- **Discovery**
  - flowType: Automated phenotyping using 1D gates extrapolated to multiple dimensions
  - RchyOptimyx: Cellular hierarchies correlated with outcome of interest

Known limitations

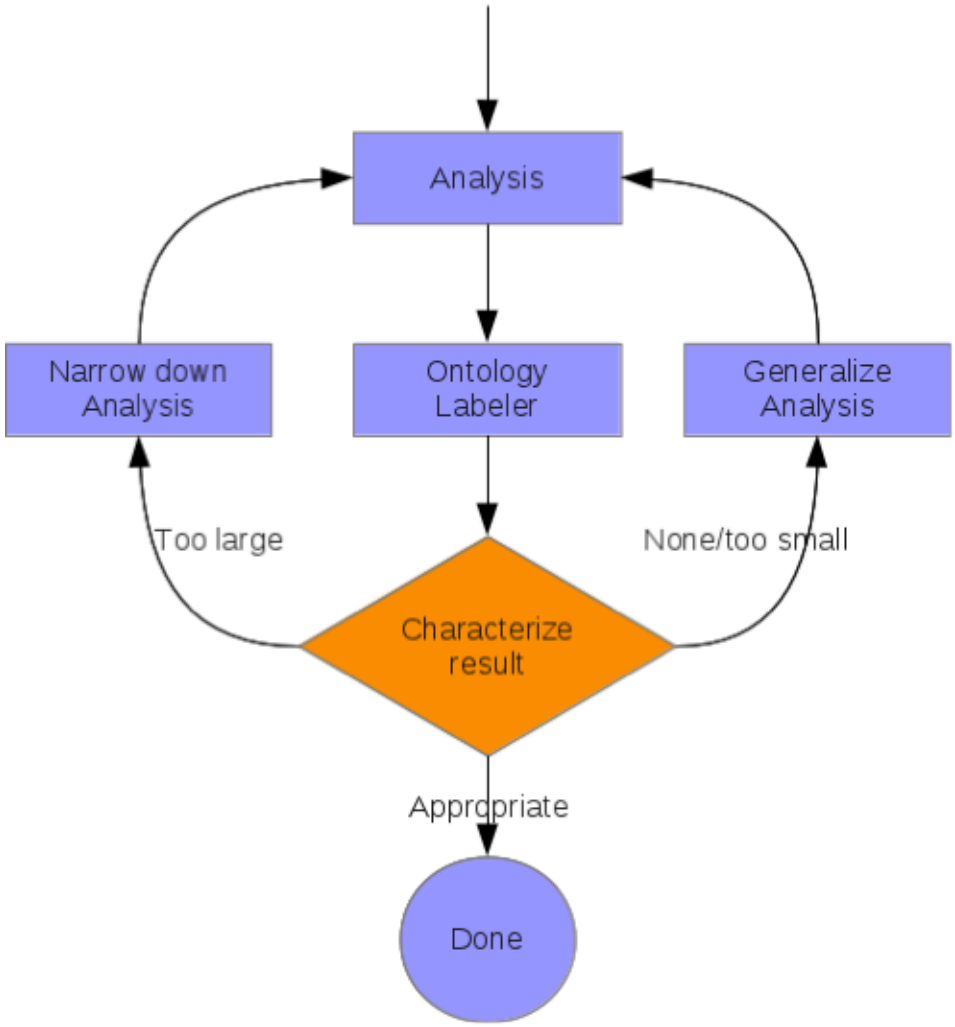
- Uses string matching between output immunophenotypes and CL markers
- It will be challenging to account for all lexical variants
  - CD45RO+ <-> has_plasma_membrane_part some 'receptor-type tyrosine-protein phosphatase C isoform CD45RO'
  - Relations: lacks_plasma_membrane_part, has_low_plasma_membrane_amount…
  - Synonyms: T cell, T-cell…

Proposal - flowCL

- A small extension to the CL
- Build specifically to address our use case
- Would allow for flexibility in development
- As it would import CL, it could easily be incorporated if desired, or distributed as distinct extension

Result

- Returned result can be refined with addition of additional markers
- Ideal case: single node



Additional features

- If multiple phenotypes, increase the degree of confidence of the result with the number of returned result sets it belongs too
- Based on the analysis output (e.g., if we have a DAG) we can exploit this hierarchy to favor results matching more specific immunophenotypes.
  - Prefer the value T helper lymphocytes over T cell lymphocytes when the immunophenotype is CD3+CD4+

Summary – current issues

- **String matching** between immunophenotypes and cell populations
- How to deal with **relative abundance** of markers (dim/bright)
- On the analysis side, how to identify population based on **previous knowledge** (e.g., kappa-lambda+)
- **Access** to the CL: remote, local, both?
- **Tooling** evaluation
- **CL content**: ensure action items are ported to release. Coordination with other efforts. Scope: cell knowledgebase?

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