



**University of
Zurich^{UZH}**

High-dimensional cytometry data analysis

STA426 lecture materials

Lukas Weber

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Background

- Introduction: single-cell analysis, high-dimensional cytometry
 - see lecture slides from Week 10
- Differential discovery analyses
 - 10-50 protein markers per cell
 - *cell type* and *cell state* markers
 - cell populations (types) defined using clustering
 - testing for differential abundance (DA) of cell populations and differential states (DS) *within* cell populations

Outline

- Interactive demo
 - R-based differential discovery analysis workflow for high-dimensional cytometry data
 - we will follow a modified version of the CATALYST vignette (short version of workflow) and CyTOF workflow (long version)
- Links
 - Bioconductor: <http://bioconductor.org/>
 - CyTOF workflow: <http://bioconductor.org/packages/cytofWorkflow>
 - Paper describing CyTOF workflow in more detail: <https://f1000research.com/articles/6-748/v2>
 - CATALYST package: <http://bioconductor.org/packages/CATALYST>
 - CATALYST vignette: http://bioconductor.org/packages/release/bioc/vignettes/CATALYST/inst/doc/differential_analysis.html
 - diffcyt package: <http://bioconductor.org/packages/diffcyt>

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CyTOF workflow and F1000 paper

CyTOF workflow: differential discovery in high-throughput high-dimensional cytometry datasets

Malgorzata Nowicka^{1,2}, Carsten Krieg³, Lukas M. Weber^{1,2}, Felix J. Hartmann³, Silvia Guglietta⁴, Burkhard Becher³, Mitchell P. Levesque⁵ and Mark D. Robinson^{1,2*}

¹Institute for Molecular Life Sciences, University of Zurich, CH-8057 Zurich, Switzerland

²SIB Swiss Institute of Bioinformatics, University of Zurich, CH-8057 Zurich, Switzerland

³Institute of Experimental Immunology, University of Zurich, CH-8057 Zurich, Switzerland

⁴Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, I-20139 Milan, Italy

⁵Department of Dermatology, University Hospital Zurich, CH-8091 Zurich, Switzerland

* mark.robinson@imls.uzh.ch

F1000Research



METHOD ARTICLE

REVISED CyTOF workflow: differential discovery in high-throughput high-dimensional cytometry datasets [version 2; referees: 2 approved]

Malgorzata Nowicka^{1,2}, Carsten Krieg ³, Lukas M. Weber ^{1,2}, Felix J. Hartmann ³, Silvia Guglietta⁴, Burkhard Becher³, Mitchell P. Levesque⁵, Mark D. Robinson ^{1,2}

¹SIB Swiss Institute of Bioinformatics, University of Zurich, Zurich, 8057, Switzerland

²Institute for Molecular Life Sciences, University of Zurich, Zurich, 8057, Switzerland

³Institute of Experimental Immunology, University of Zurich, Zurich, 8057, Switzerland

⁴Department of Experimental Oncology, European Institute of Oncology, Via Adamello 16, Milan, I-20139, Italy

⁵Department of Dermatology, University Hospital Zurich, Zurich, CH-8091, Switzerland

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Open Peer Review

Referee Status:

CATALYST vignette

Differential analysis with CATALYST

Helena L Crowell^{1,2,3*} and Mark D Robinson^{1,2}

¹Institute for Molecular Life Sciences, University of Zurich, Switzerland

²SIB Swiss Institute of Bioinformatics, University of Zurich, Switzerland

³Department of Biosystems Science and Engineering ETH, ETH Zurich, Switzerland

*crowellh@student.ethz.ch

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Package

CATALYST 1.6.0

Contents

1 Example data

2 Data organization: The `daFrame` class

3 Diagnostic plots

3.1 `plotCounts` : Number of cells measured per sample

3.2 `plotMDS` : Multi-dimensional scaling plot

3.3 `plotExprHeatmap` : Heatmap of (scaled) median marker expressions

Exercise

- Perform a “null comparison” using code and data from the CATALYST vignette and/or CyTOF workflow
 - e.g. 4 vs. 4 comparison of “Reference” samples only (instead of comparing stimulated vs. reference samples)
 - note: the CATALYST vignette contains a subset of the full dataset; if you need more samples, try downloading the full data using code from the CyTOF workflow
 - re-run the analysis by modifying inputs to the plotting functions and differential testing functions
 - plot and interpret the results
 - what do the results show? what did you expect?