UFZ HELMHOLTZ Zentrum für Umweltforschung

Automated gating of flow cytometry data

Carmen Bruckmann

tbi Winterseminar 2023

Leipzig, February 16, 2023

Introduction to flow cytometry

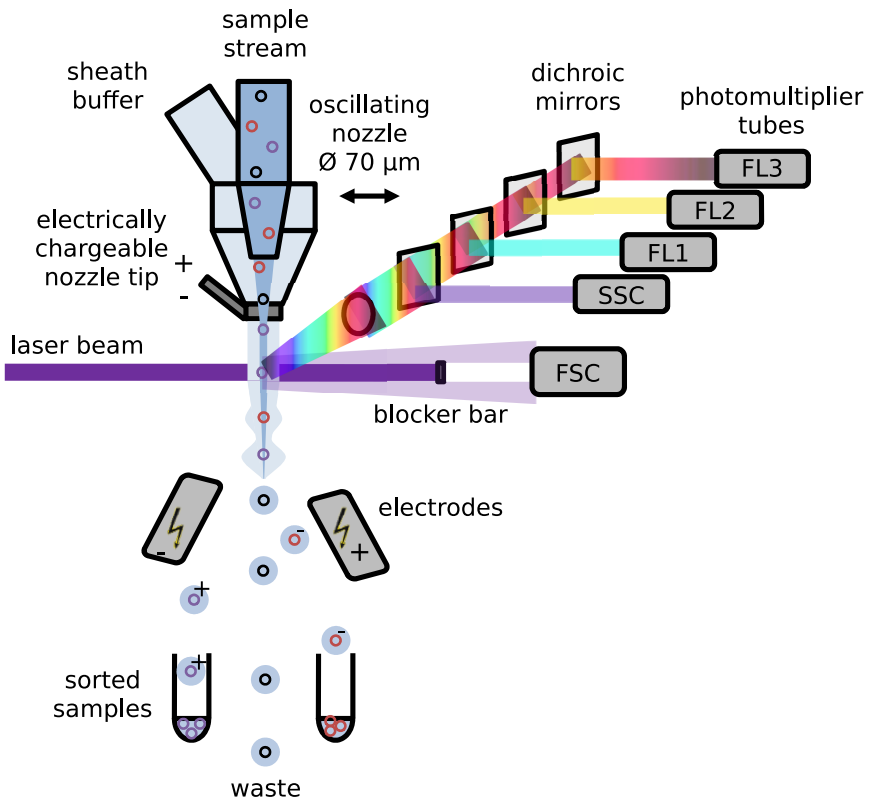

Figure: Adapted from: J. Lambrecht, **The community sensor - Monitoring and control of microbiome dynamics in anaerobic processes**, *PhD Dissertation, 2020*

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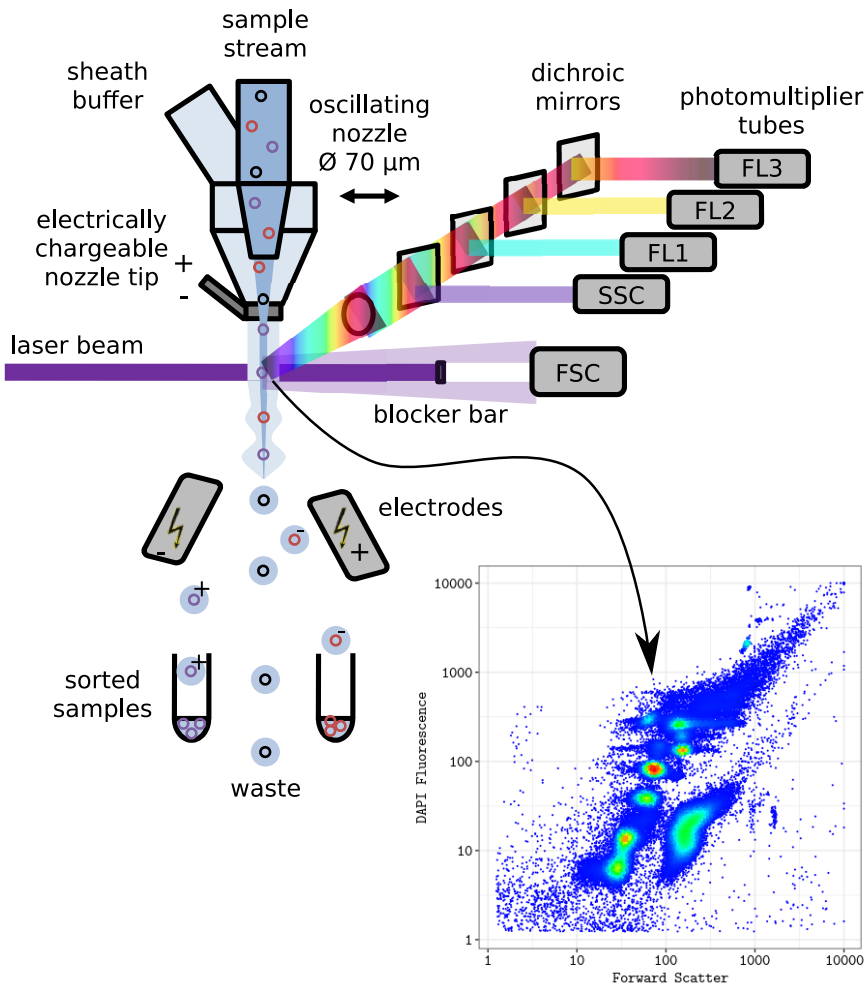

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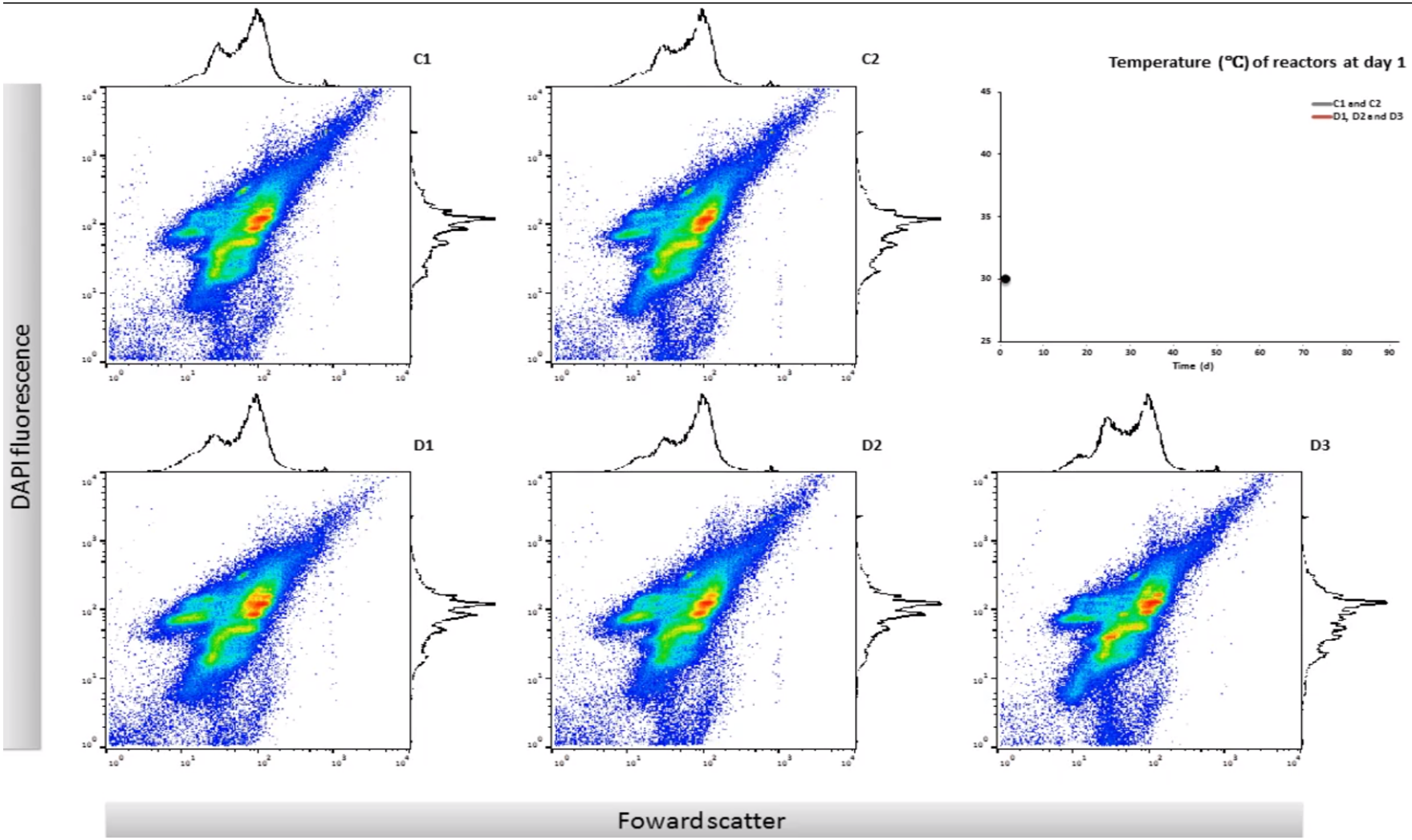
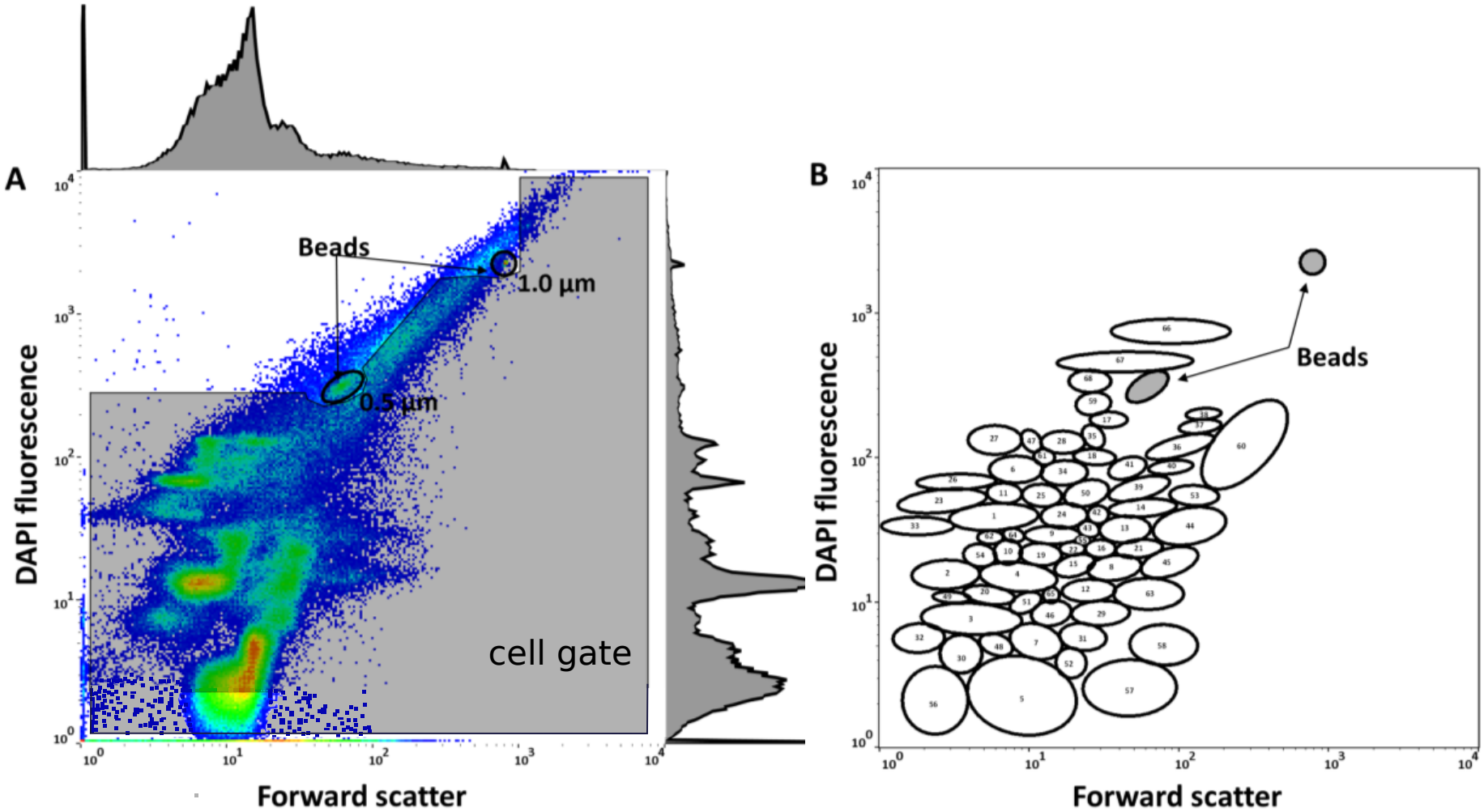


Liu et al., **Neutral mechanisms and niche differentiation in steady-state insular microbial communities revealed by single cell analysis**, *Environmental Microbiology, 2019*

Manual Gating



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Underlying ecological questions

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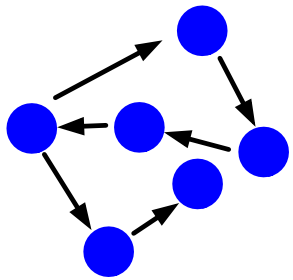

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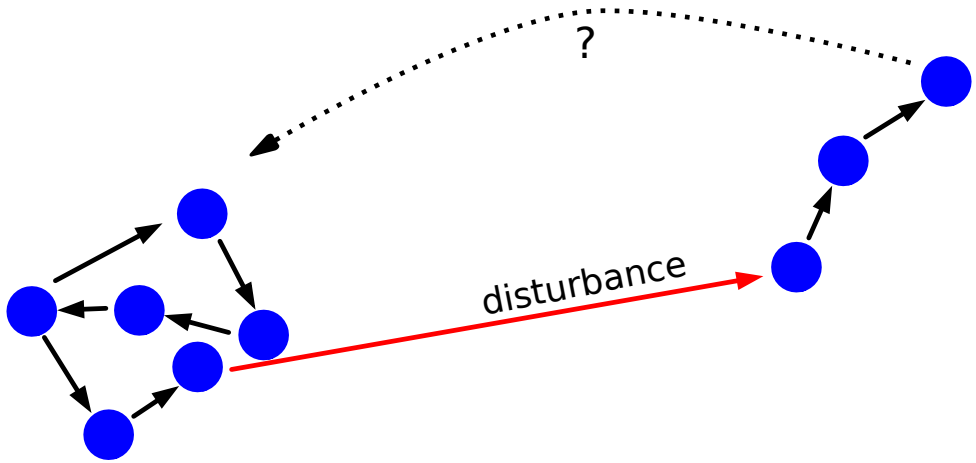

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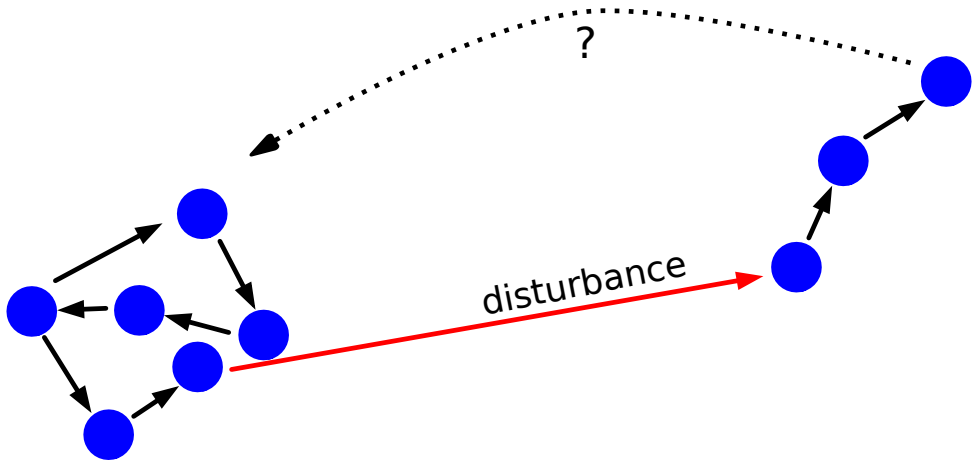
For all of this we need the cell numbers per subcommunity over time, and thus **gating**



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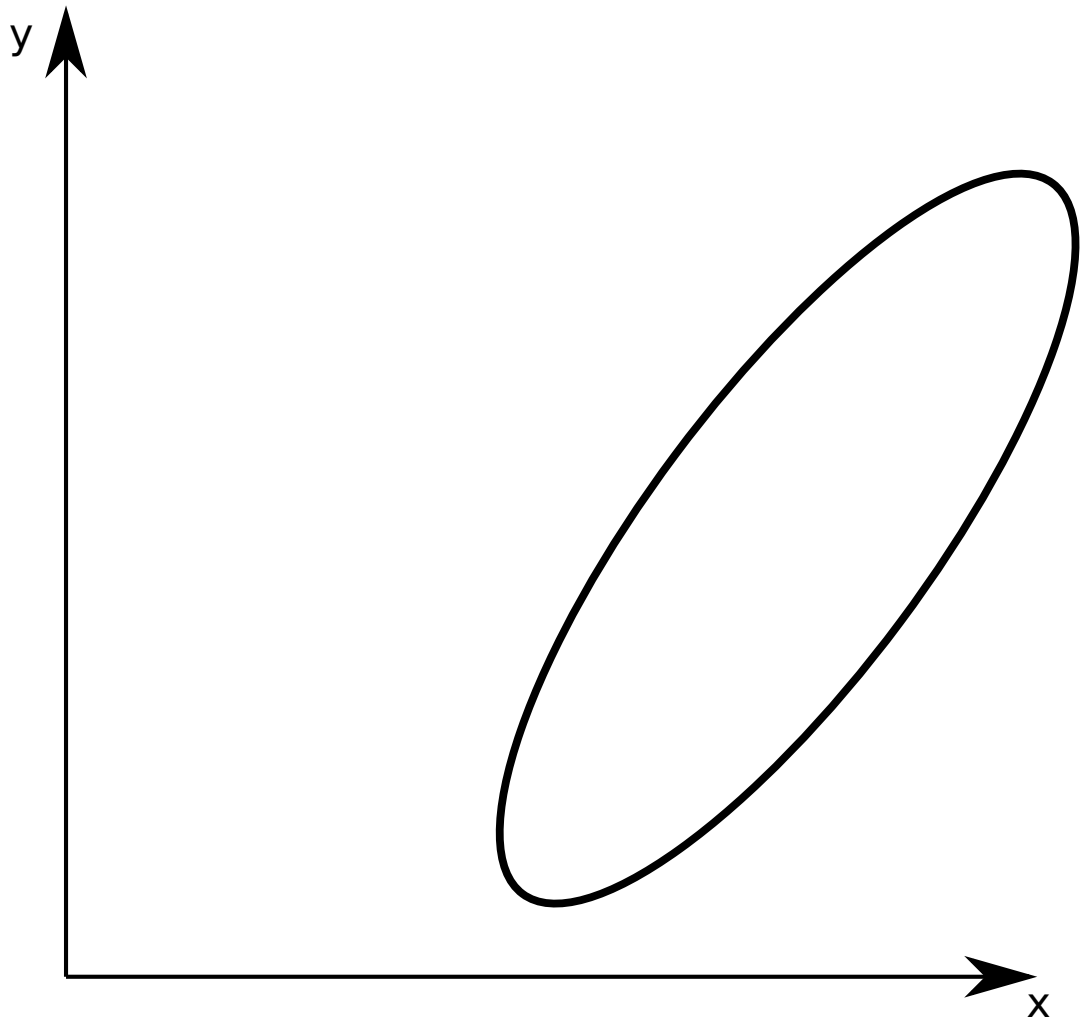
$\Rightarrow$ **Need of automated gating!**

Introduction

What is an ellipse?



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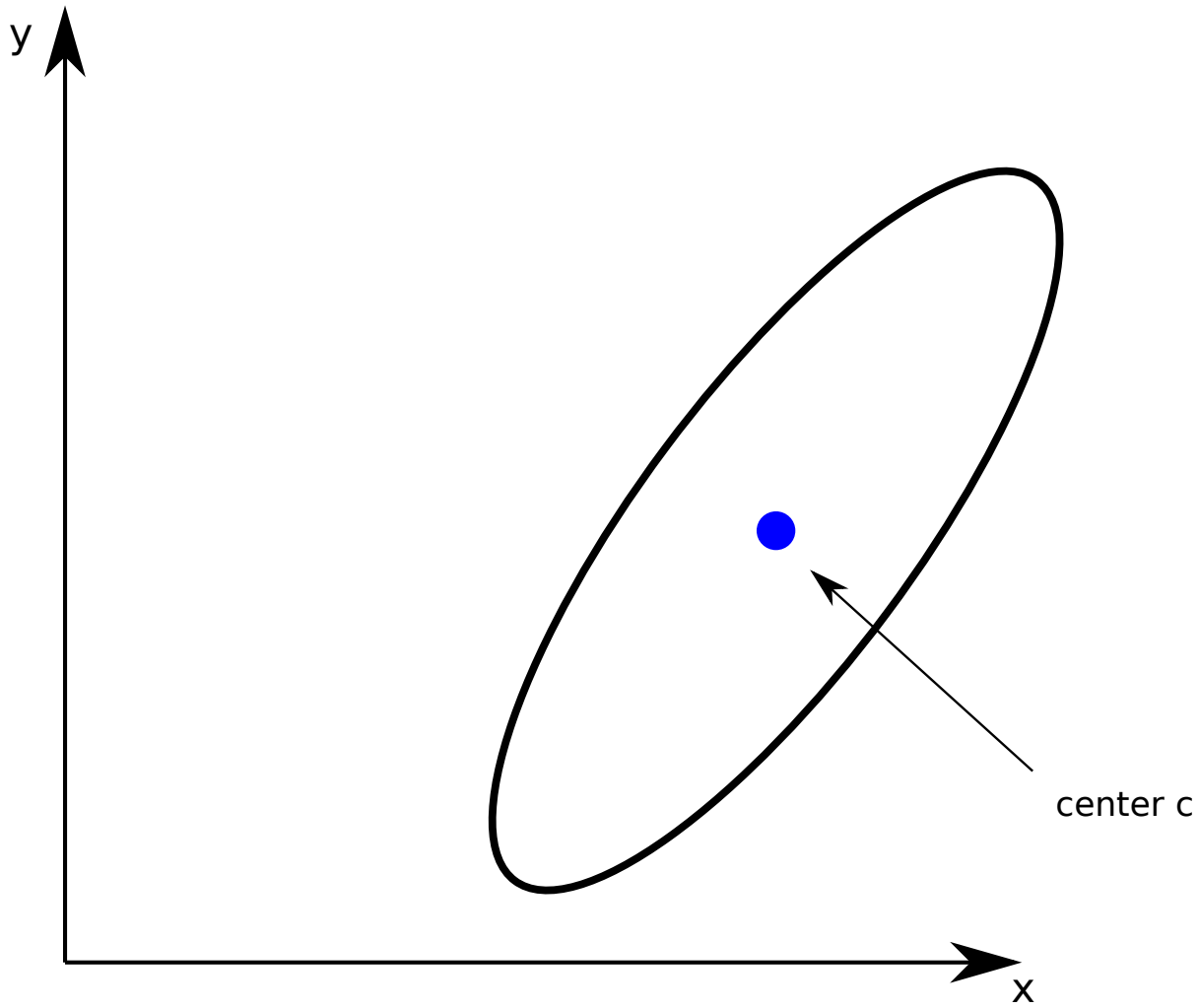

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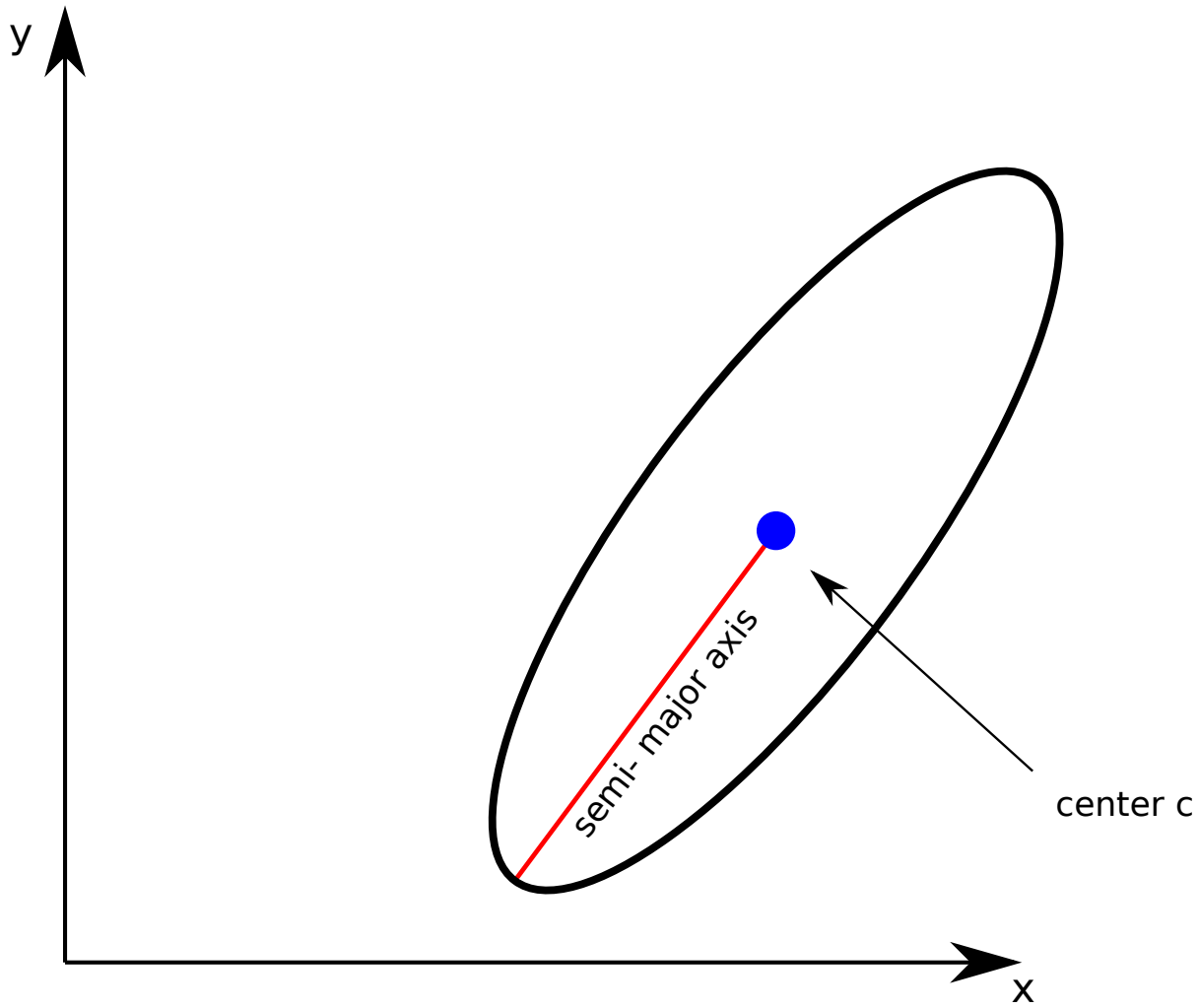

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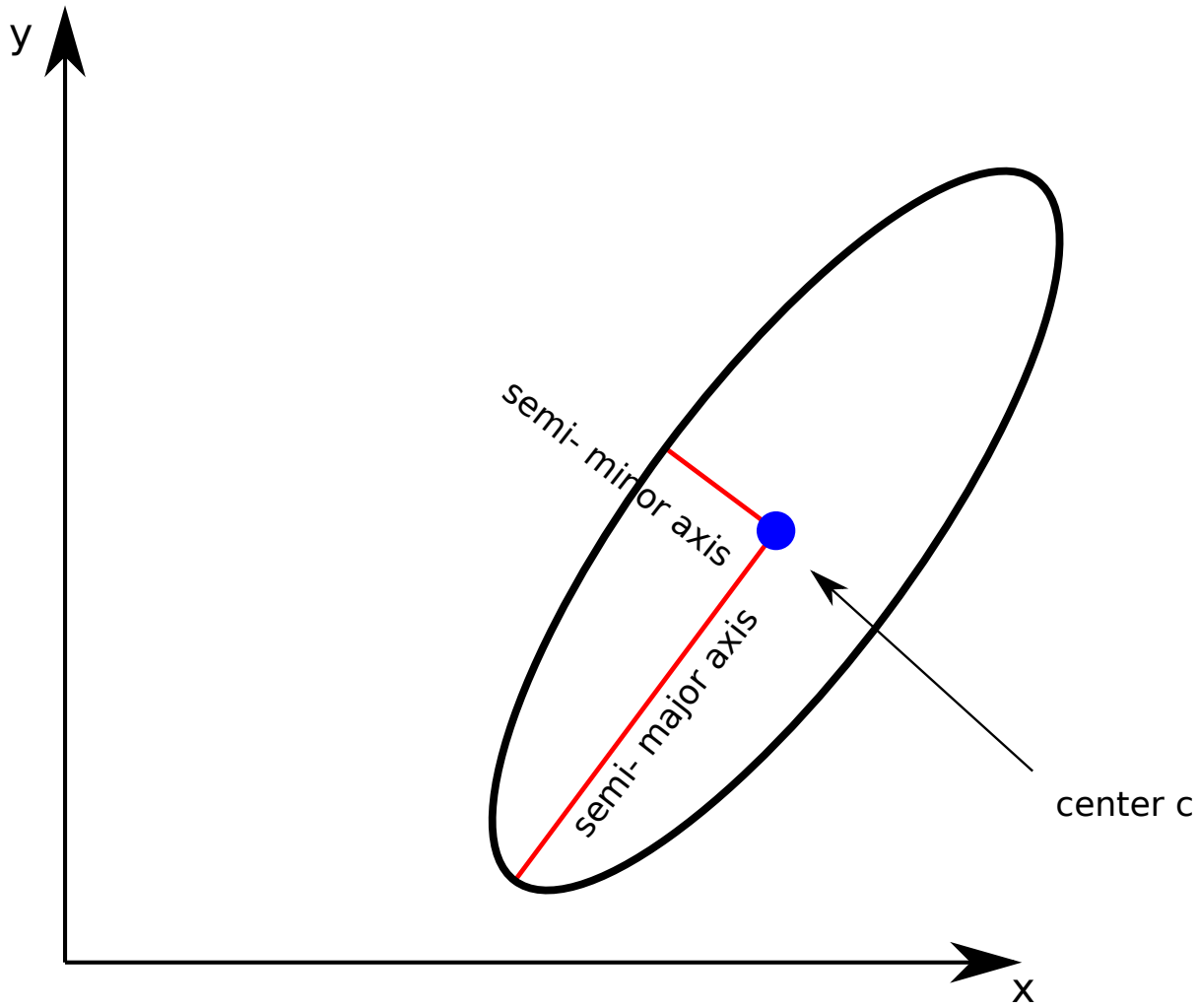

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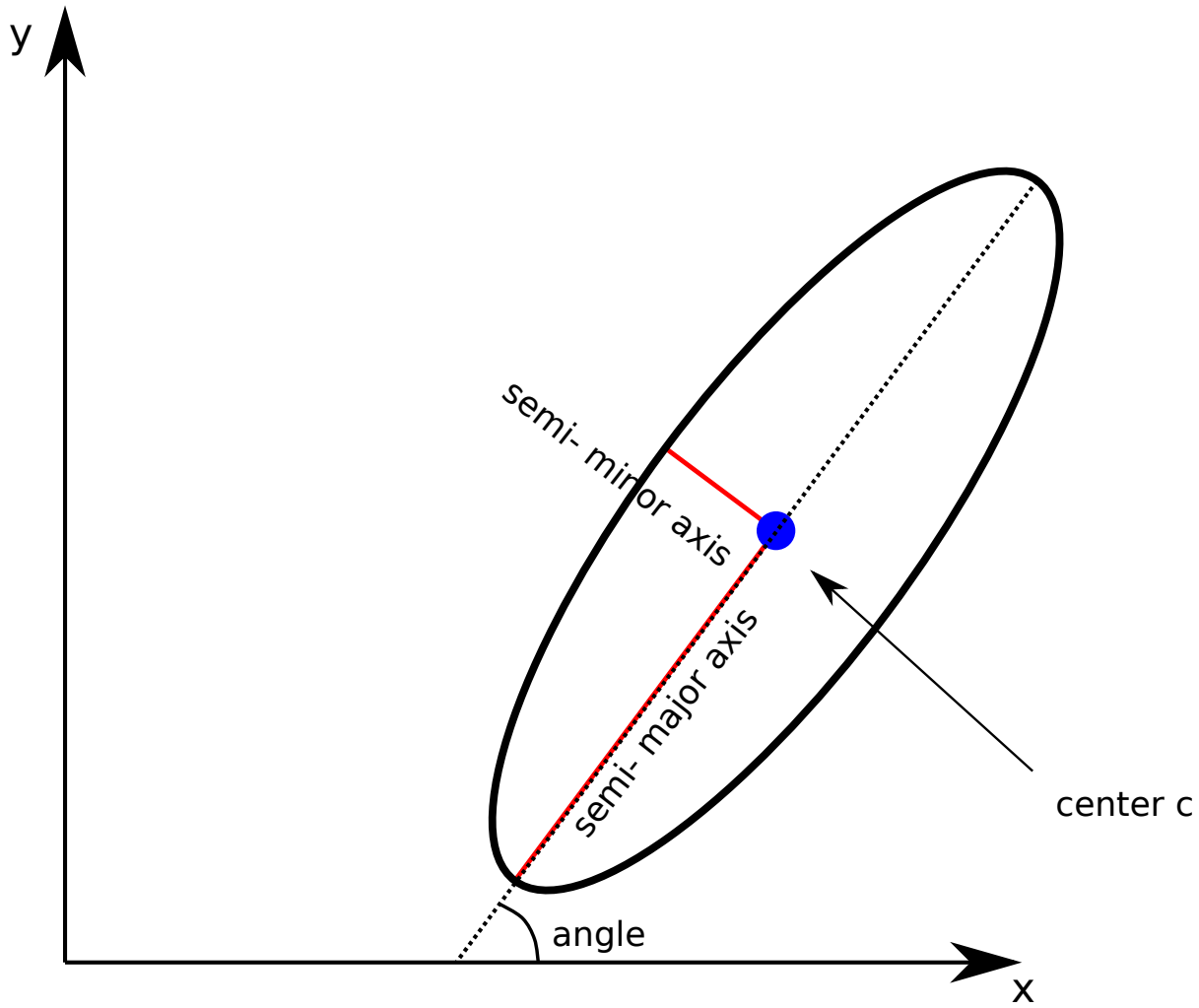

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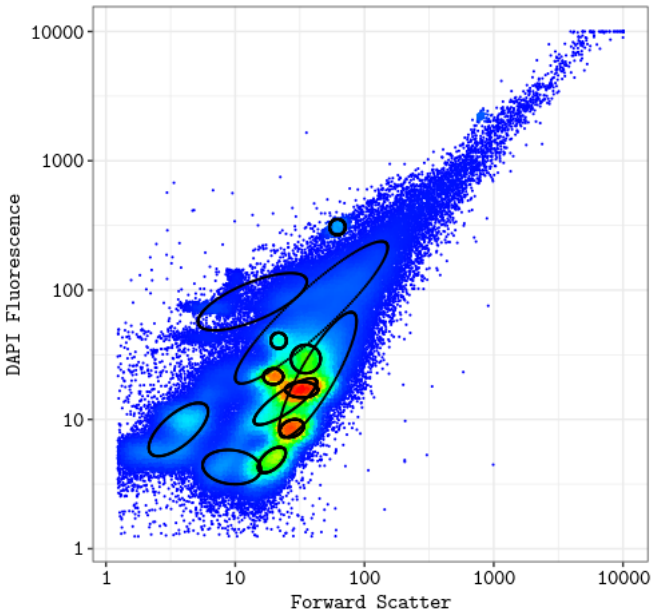
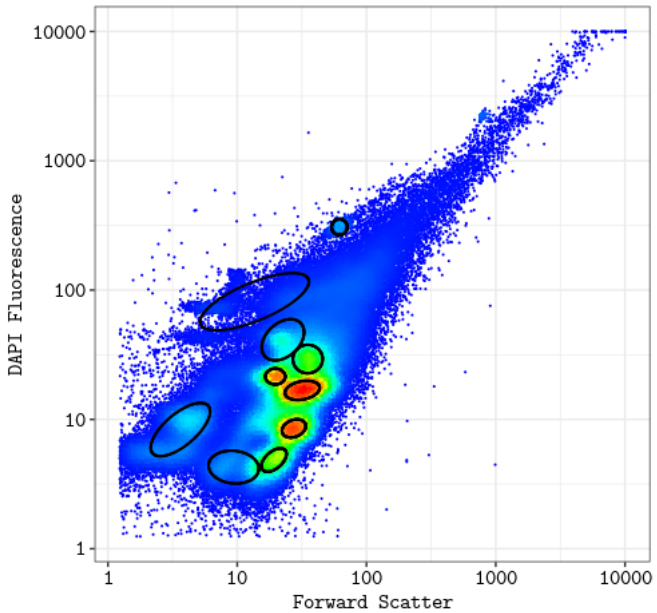


flowEMMi v1



flowEMMi v2

But how to remove the overlaps?

Merging

- Let E_1 and E_2 be two ellipses that overlap more than a given threshold,
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- In the end, the original ellipses E_1 and E_2 will be deleted and replaced by E_c, i.e. by μ_c and Σ_c.

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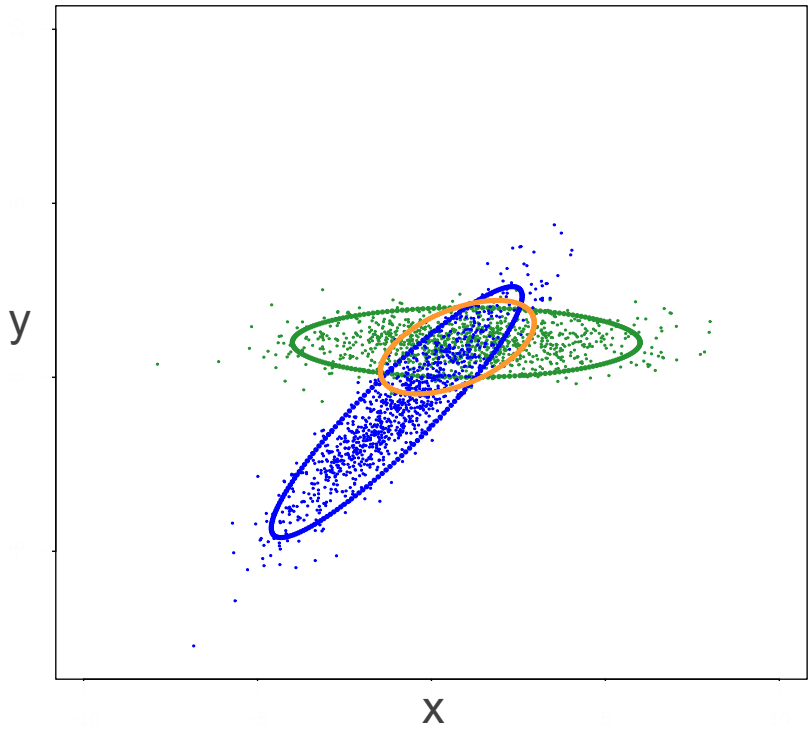

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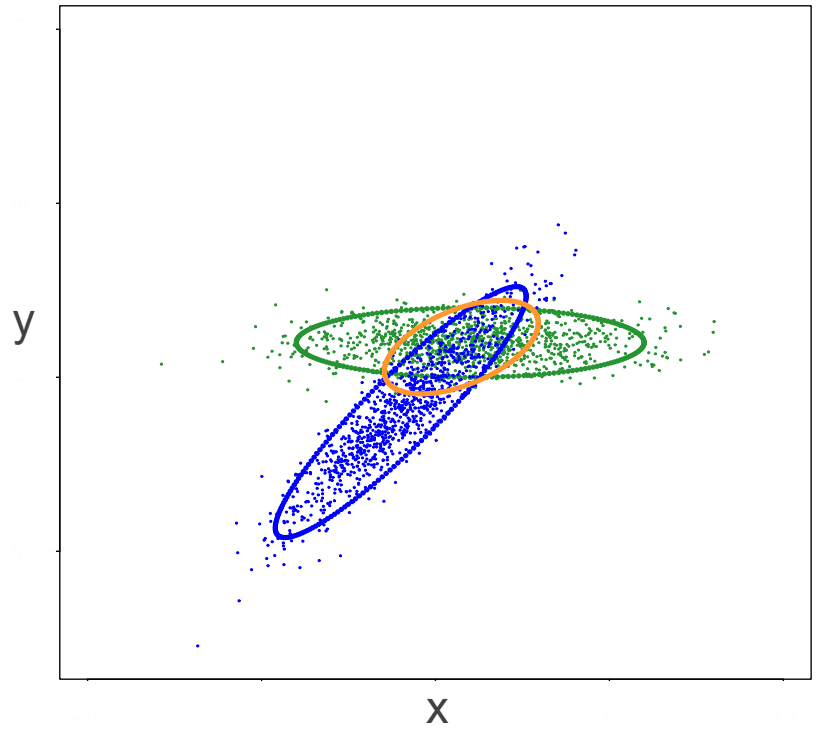

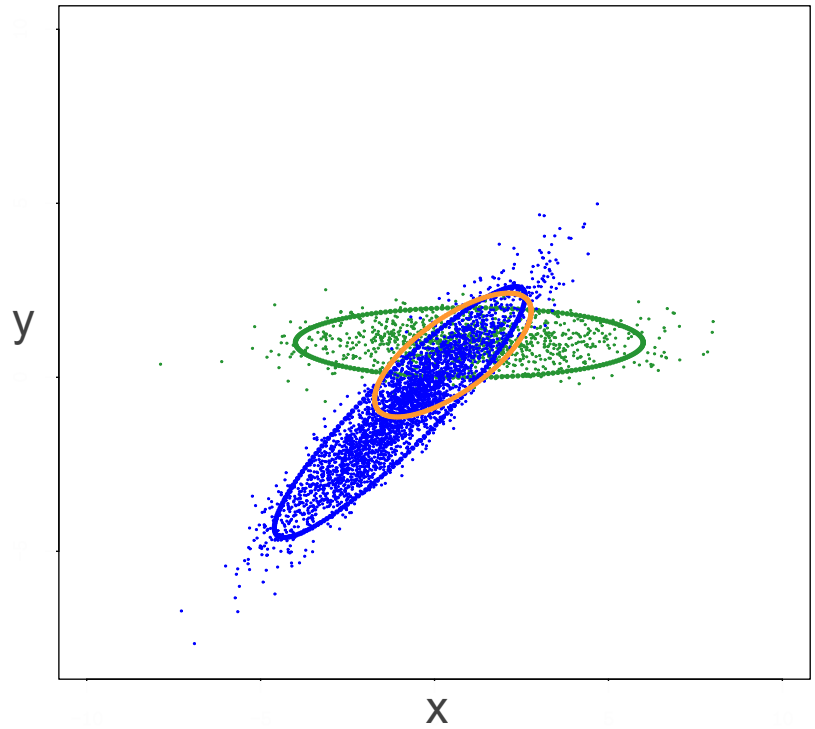

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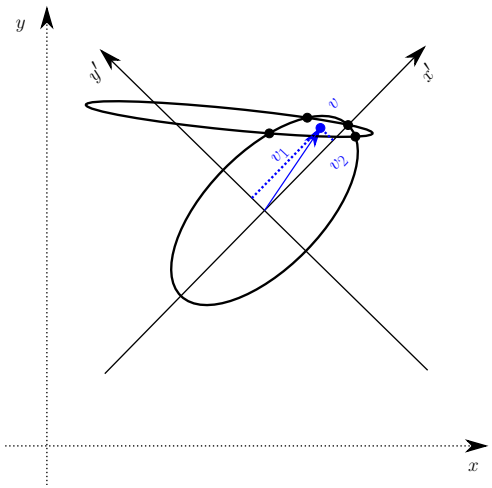

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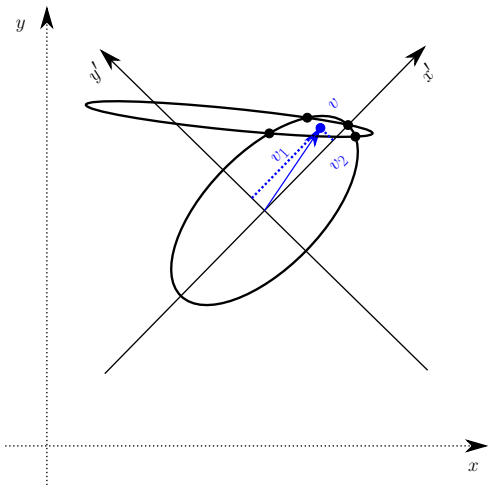

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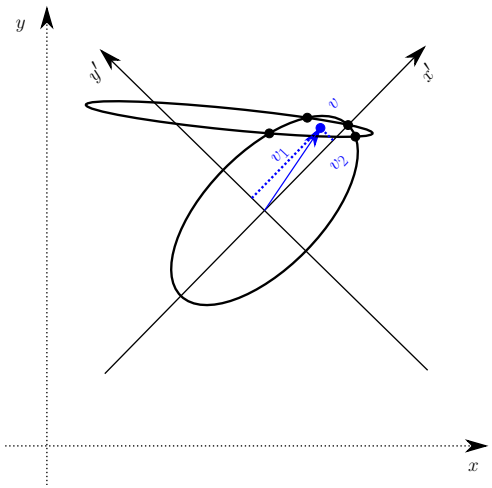

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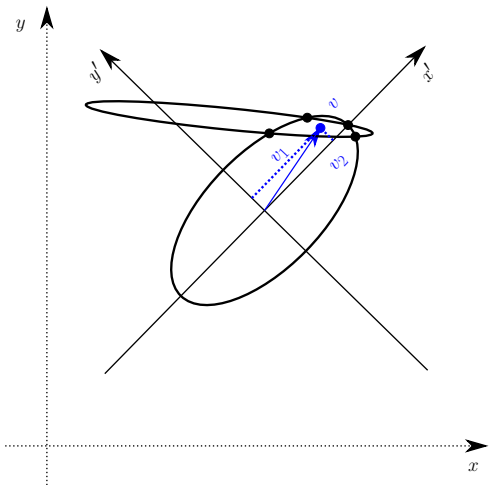

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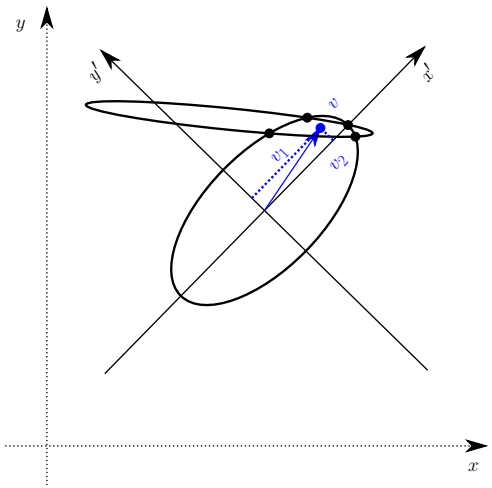

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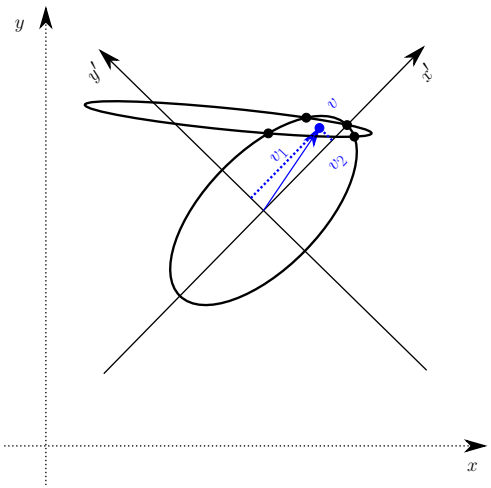

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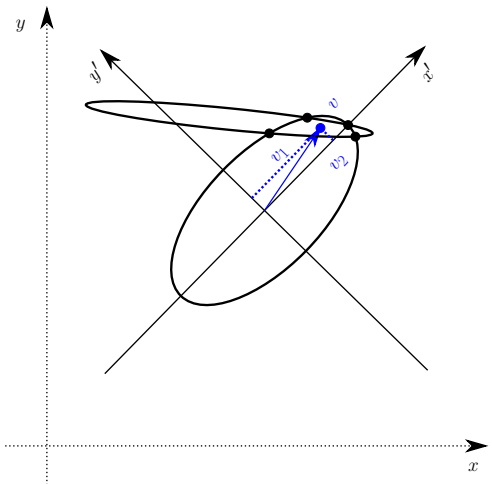

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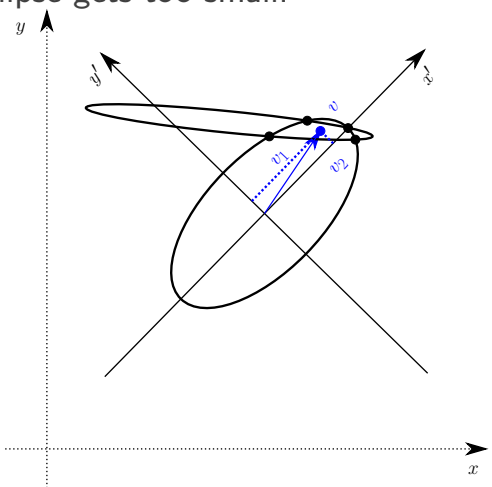
6. Recalculate E_2 in the same way.
7. Repeat steps 1. to 6. until E_1 and E_2 do not overlap anymore or until one ellipse gets too small.



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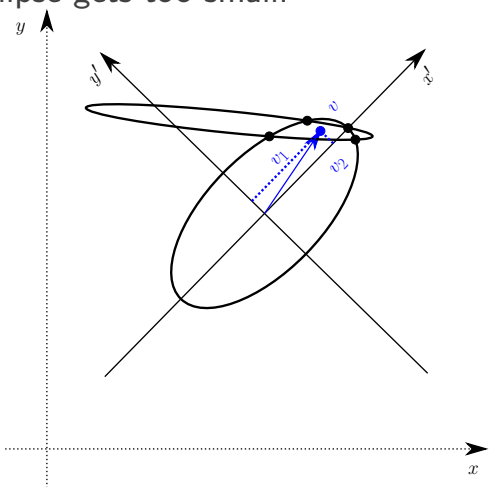
6. Recalculate E_2 in the same way.
7. Repeat steps 1. to 6. until E_1 and E_2 do not overlap anymore or until one ellipse gets too small.
8. If one ellipse gets too small, then merge the original E_1 and E_2 instead.



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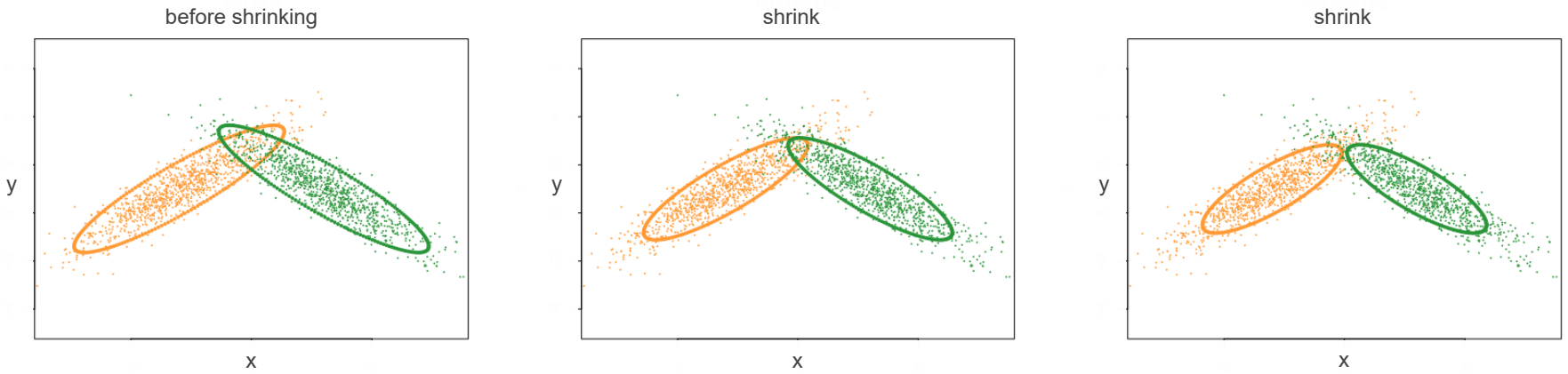

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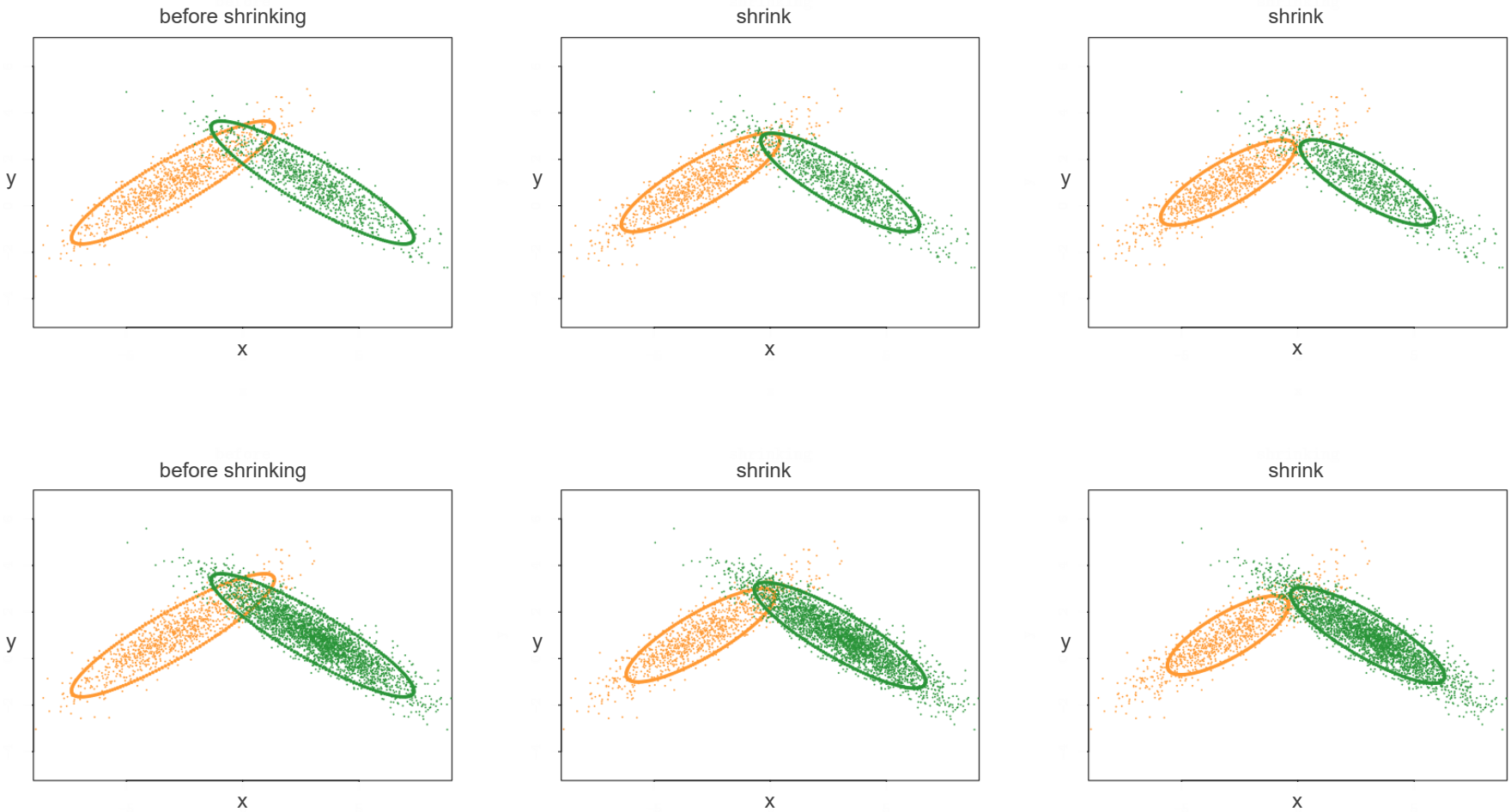

Workflow

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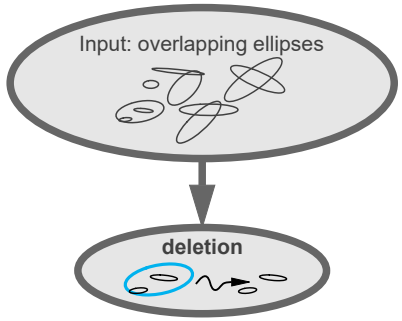

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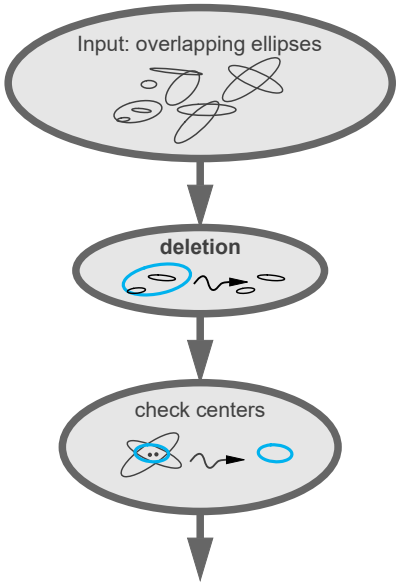

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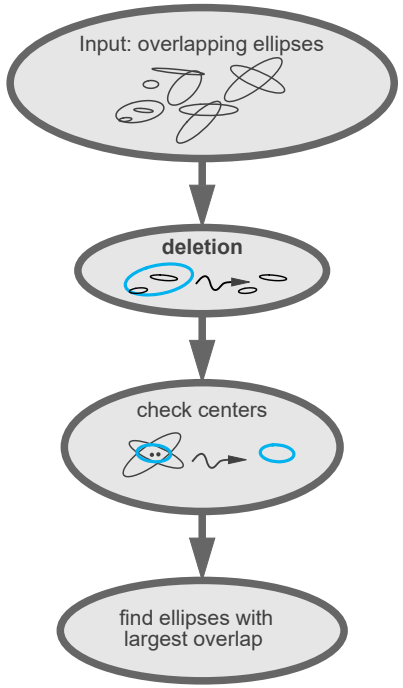
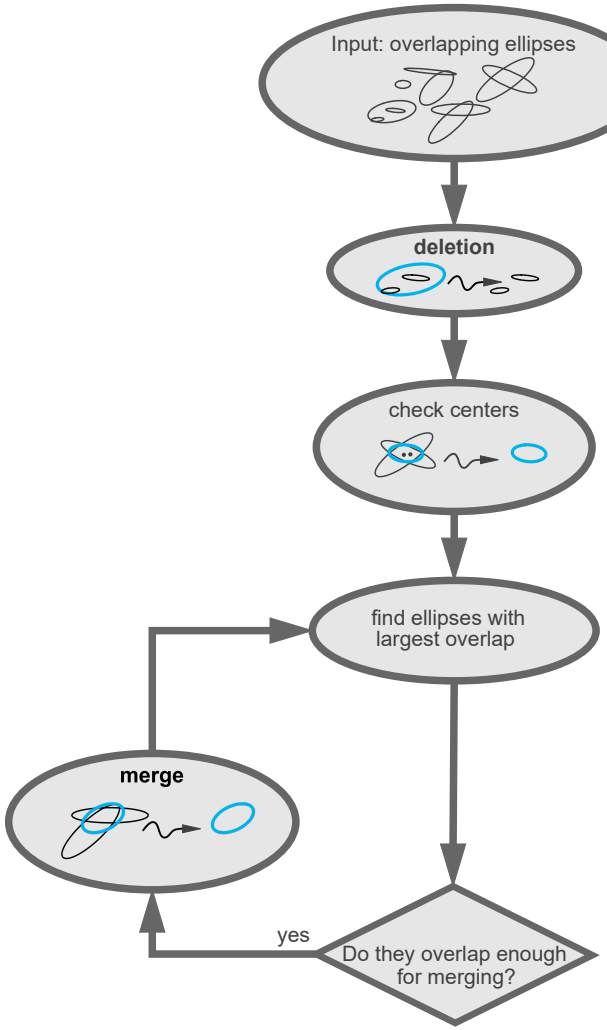
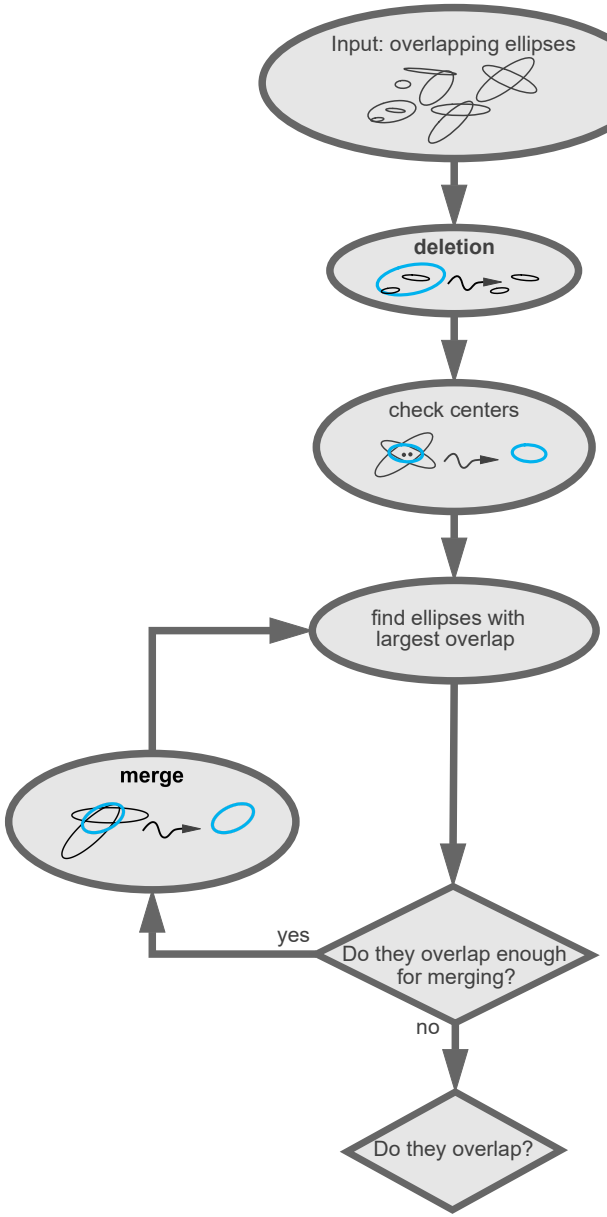

Input: overlapping ellipses
deletion
check centers
find ellipses with
largest overlap

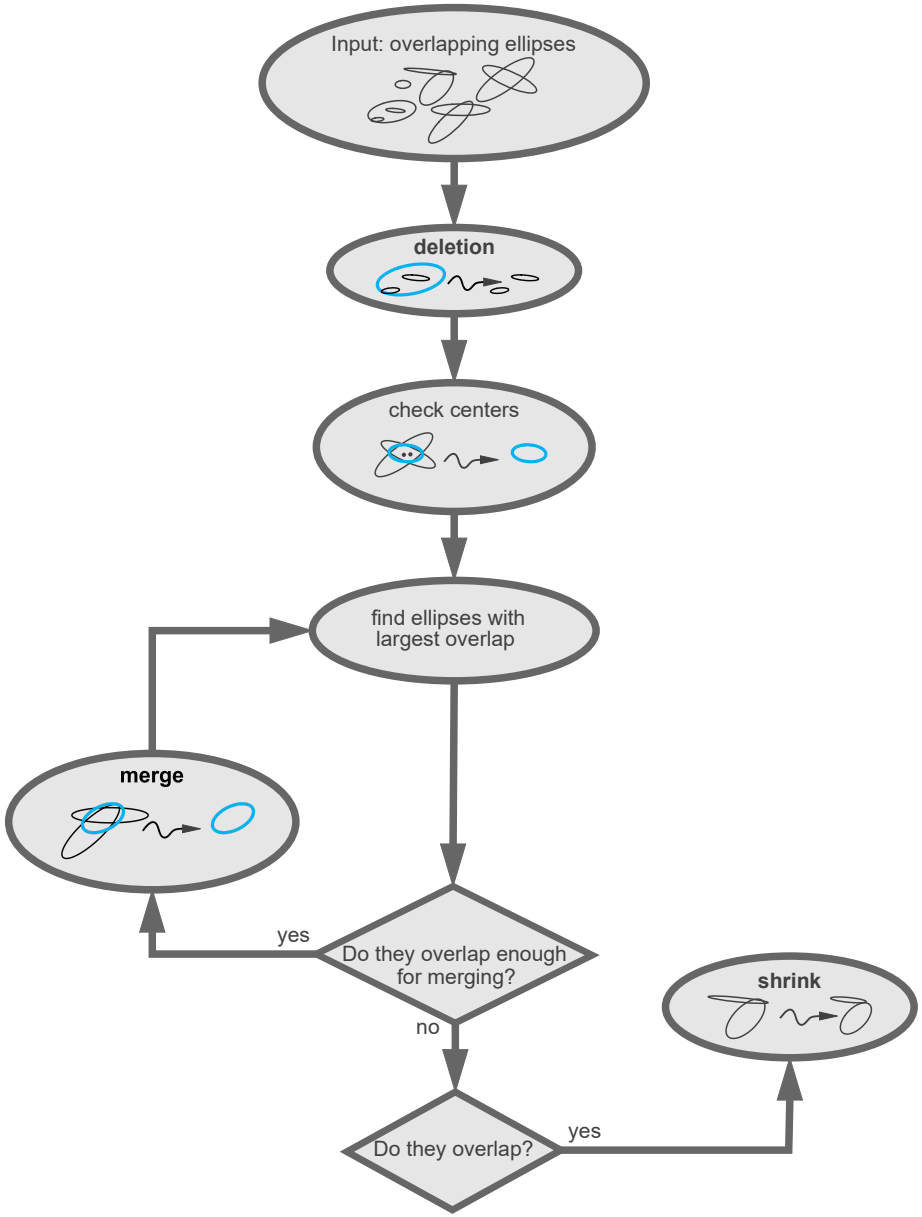
Workflow


Input: overlapping ellipses
deletion
check centers
find ellipses with largest overlap
merge
yes
Do they overlap enough for merging?

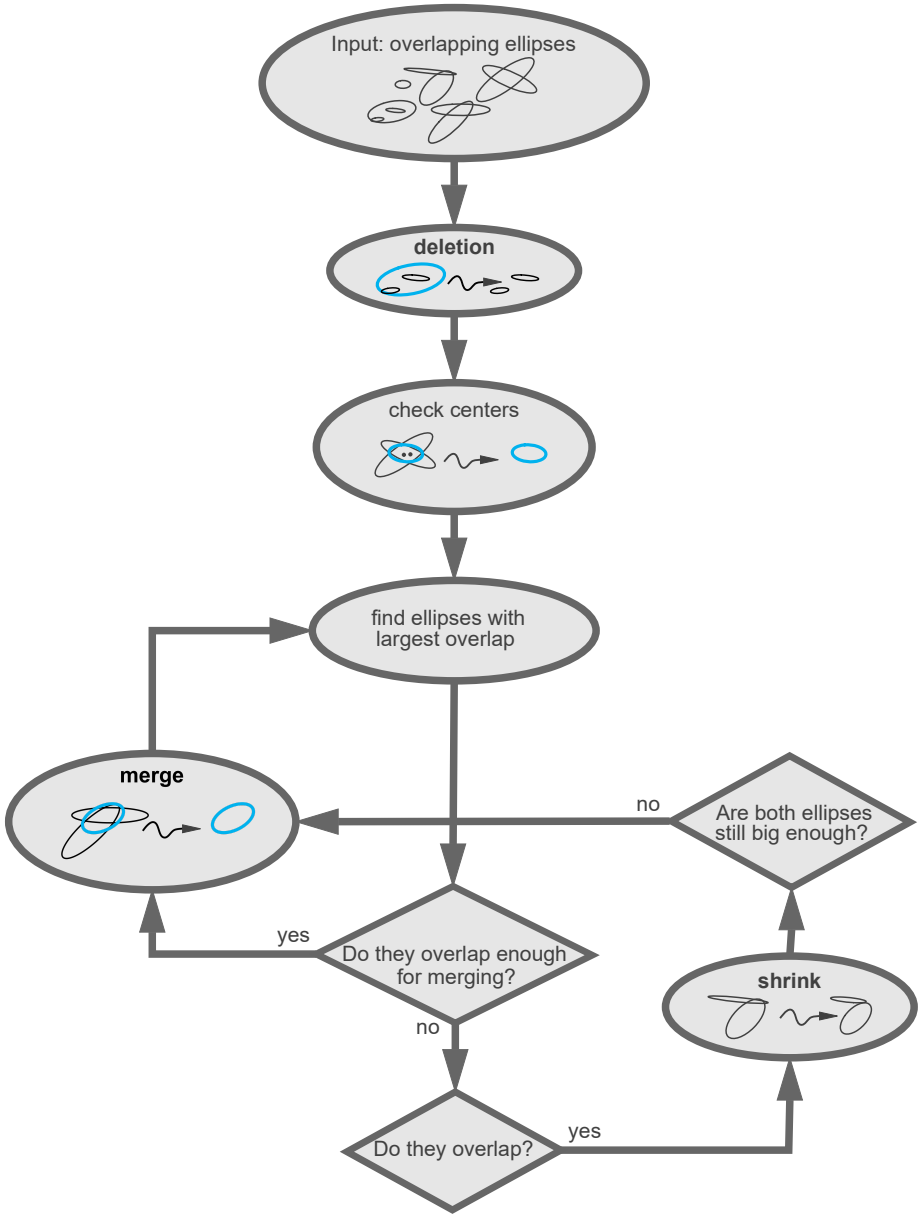
Workflow


Input: overlapping ellipses
deletion
check centers
find ellipses with largest overlap
merge
yes
Do they overlap enough for merging?
no
Do they overlap?

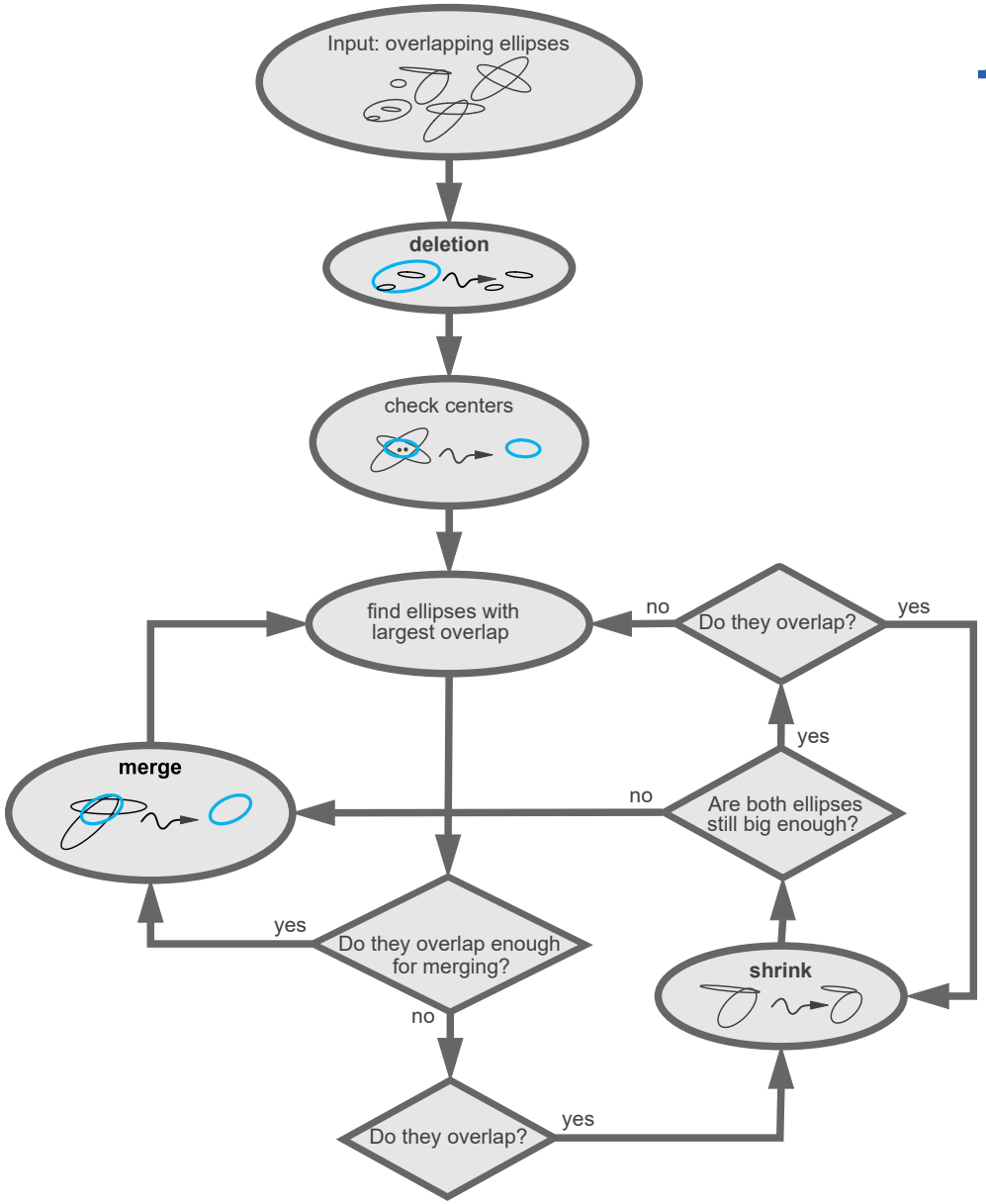
Workflow


Input: overlapping ellipses
deletion
check centers
find ellipses with largest overlap
merge
yes
Do they overlap enough for merging?
shrink
no
Do they overlap?
yes

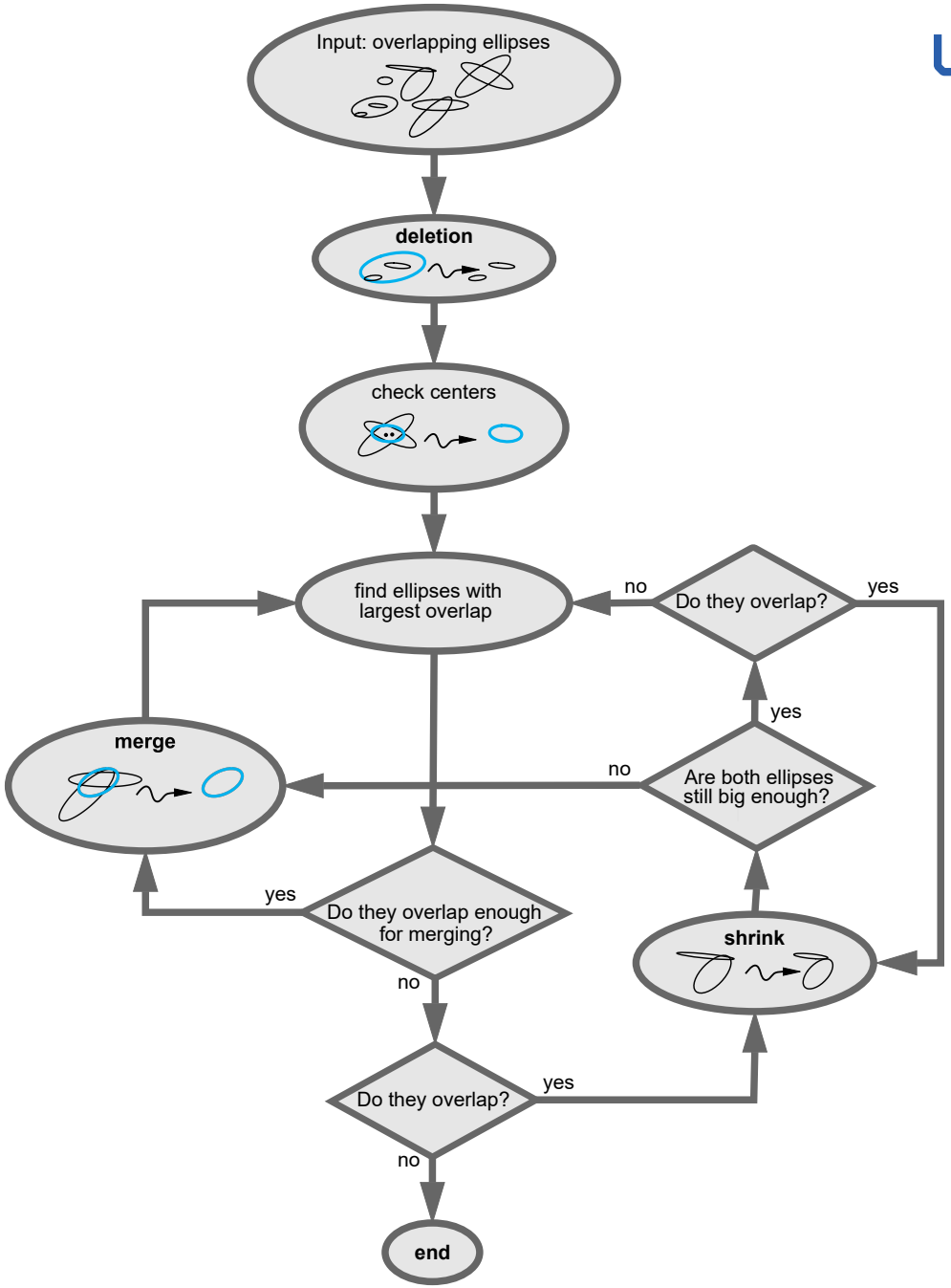
Workflow


Input: overlapping ellipses
deletion
check centers
find ellipses with largest overlap
merge
no
Are both ellipses still big enough?
yes
Do they overlap enough for merging?
shrink
no
Do they overlap?
yes

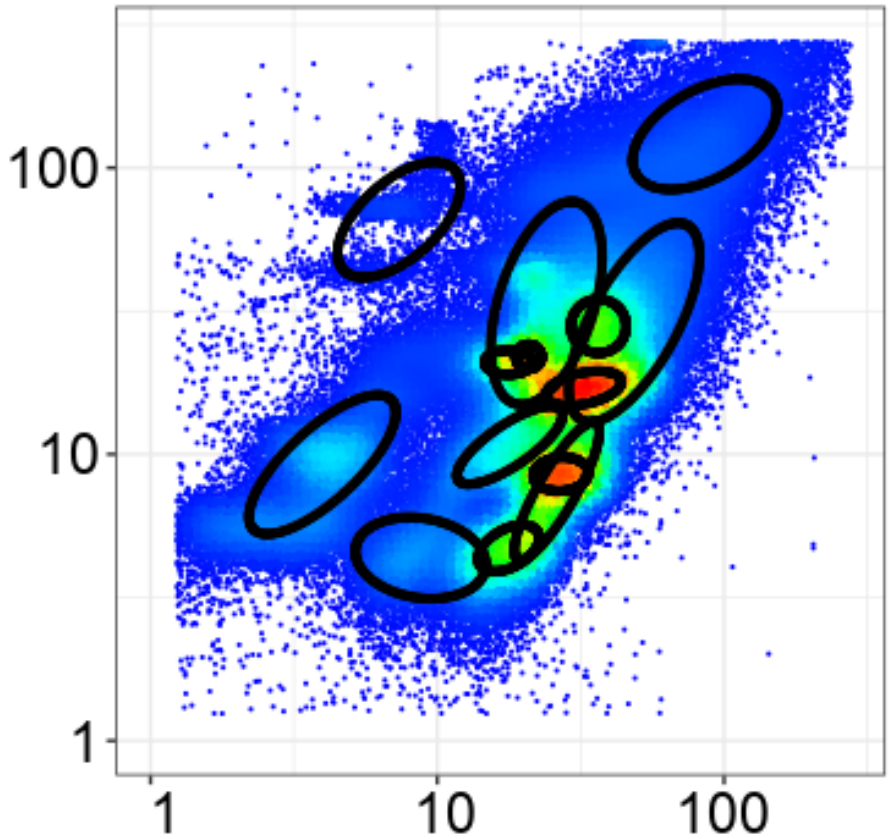
Workflow


Input: overlapping ellipses
deletion
check centers
find ellipses with largest overlap
Do they overlap?
no
yes
merge
Are both ellipses still big enough?
yes
no
Do they overlap enough for merging?
yes
shrink
no
Do they overlap?
yes

Workflow


Input: overlapping ellipses
deletion
check centers
find ellipses with largest overlap
Do they overlap?
no
yes
merge
Are both ellipses still big enough?
yes
no
Do they overlap enough for merging?
yes
shrink
no
Do they overlap?
yes
no
end

Example



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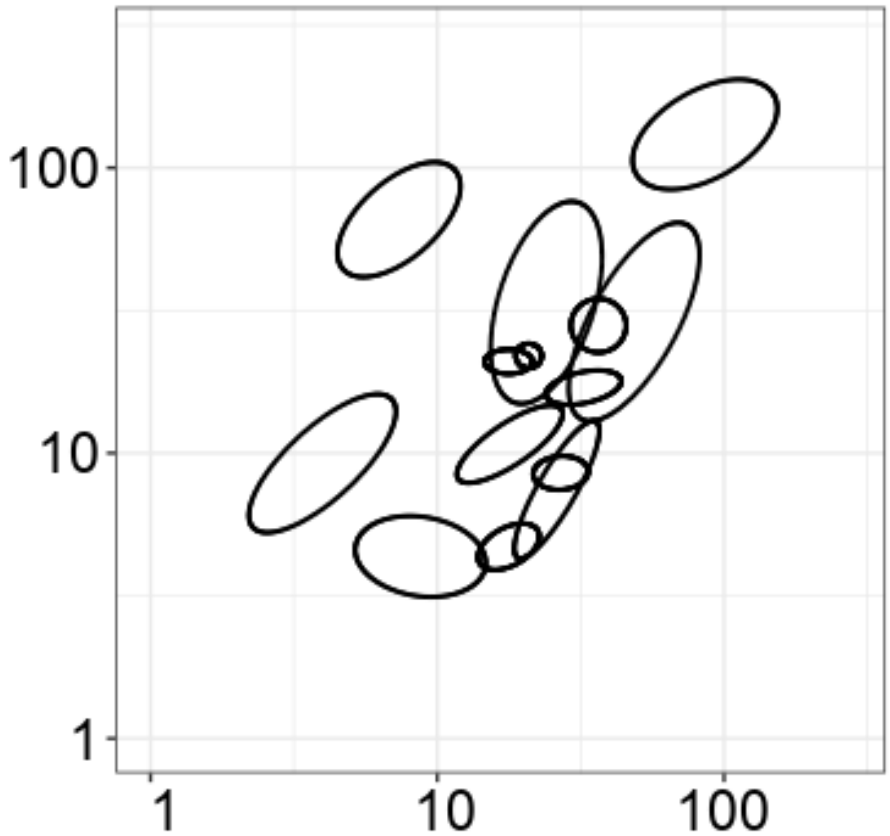

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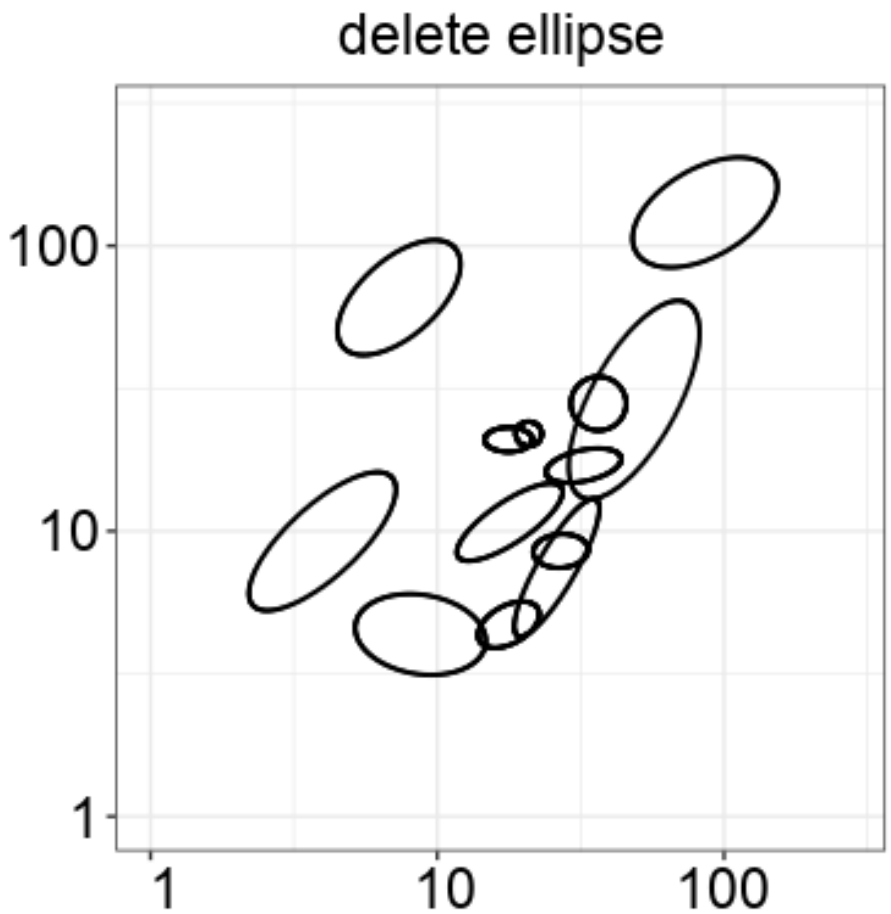
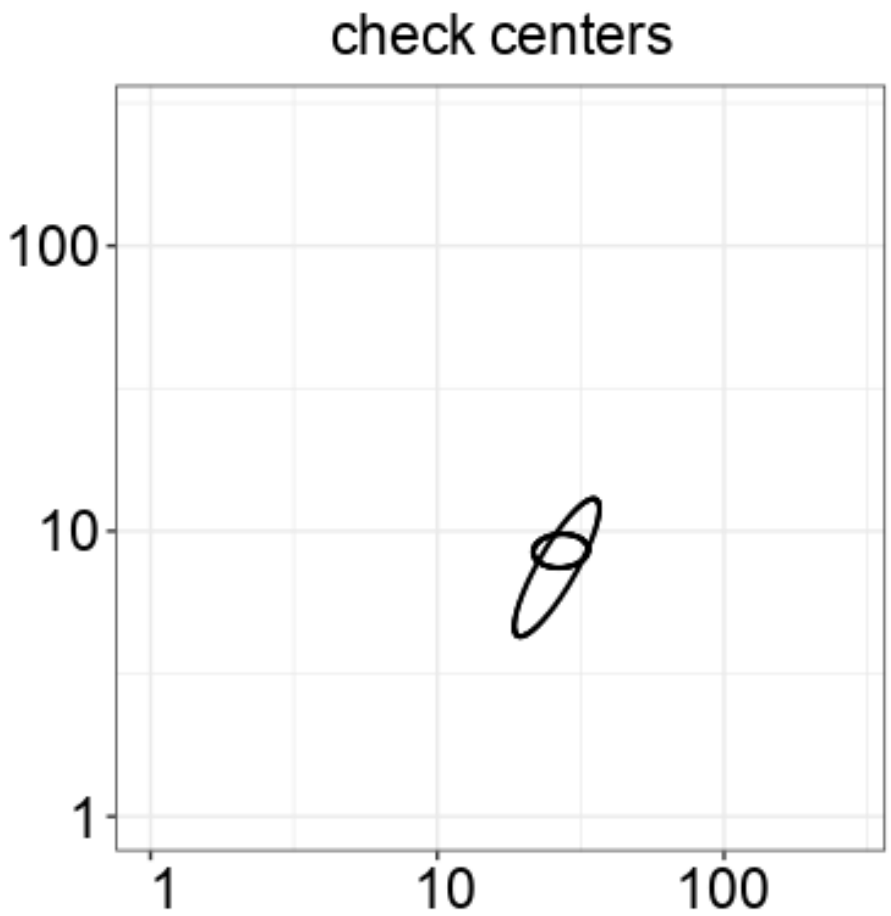
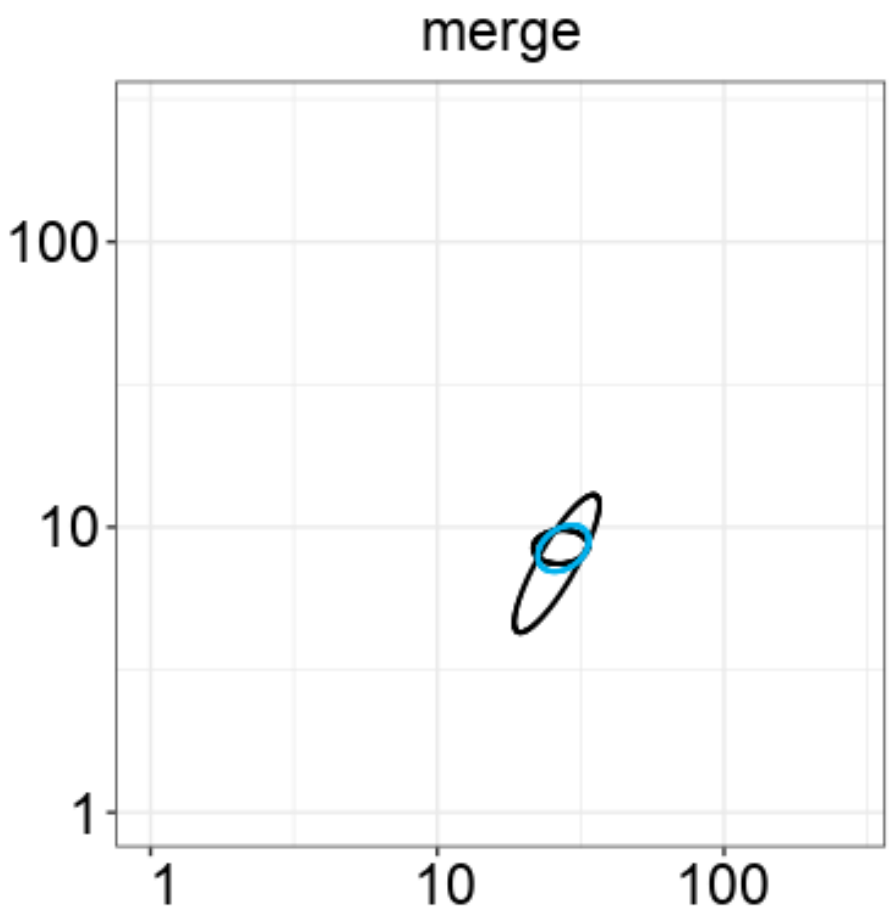


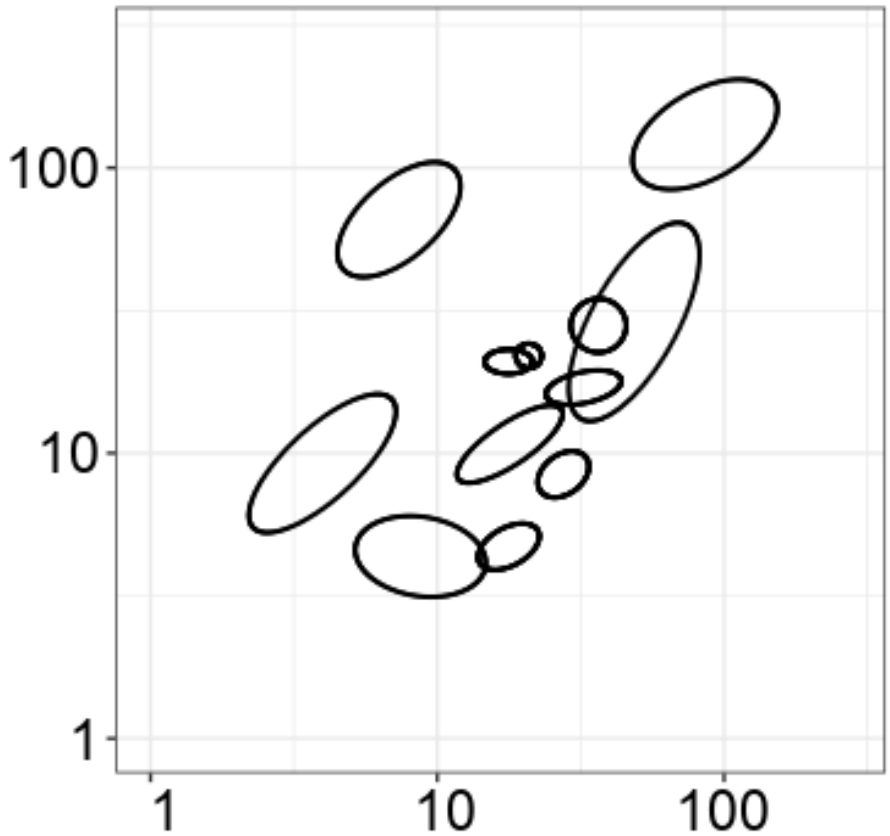
Example



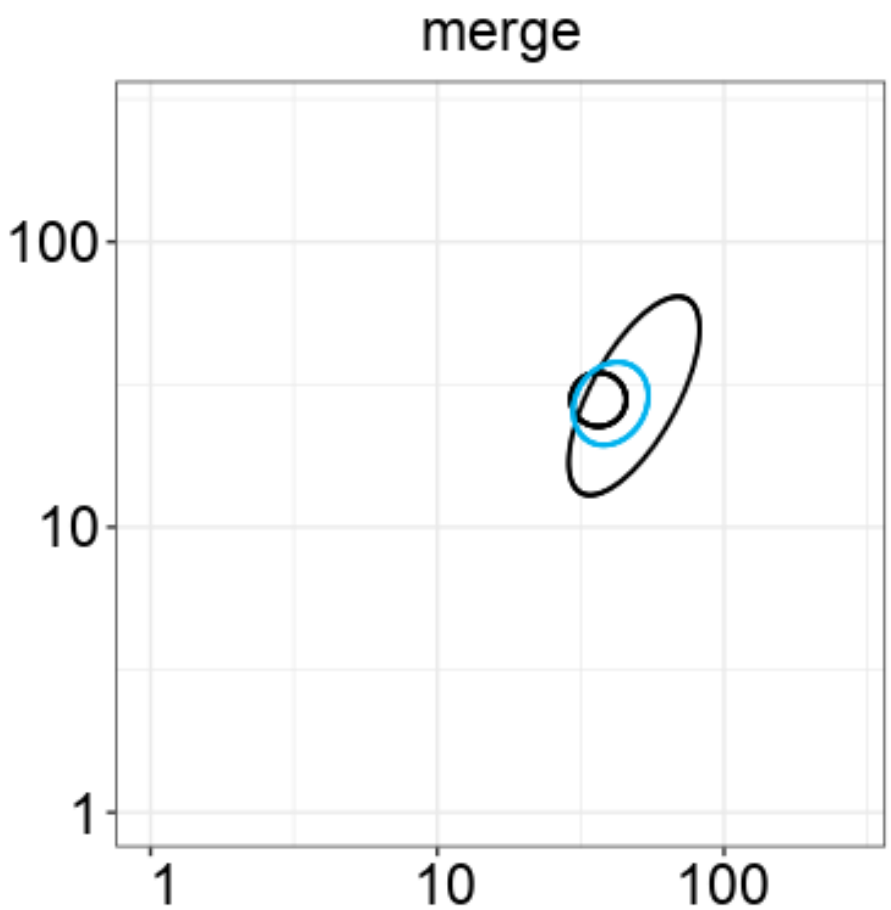
Example



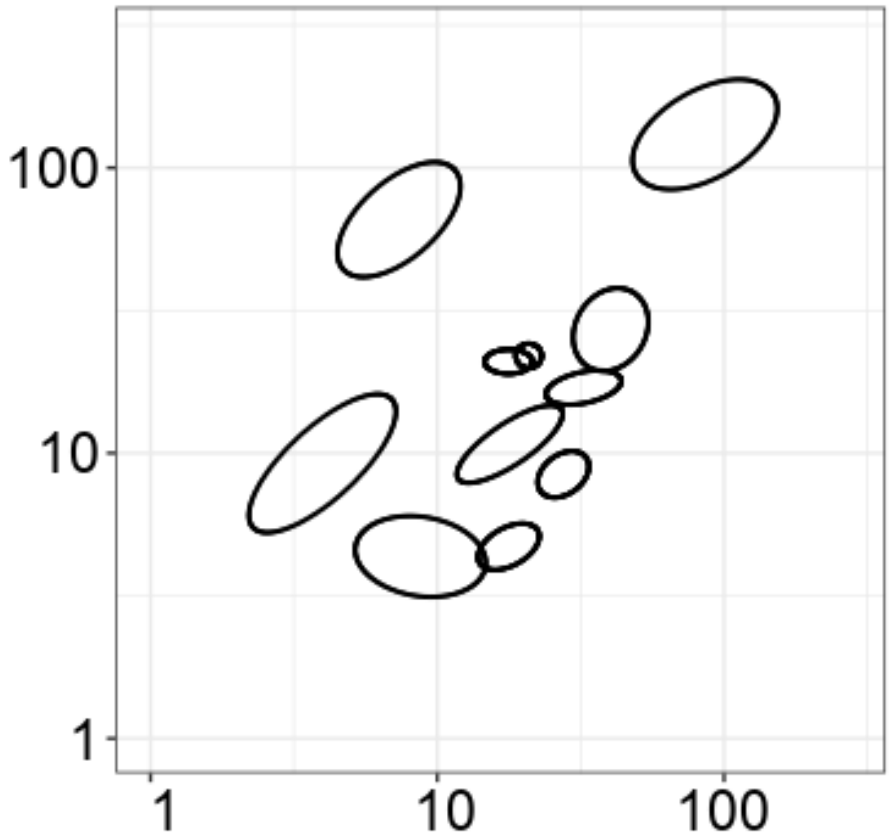
Example



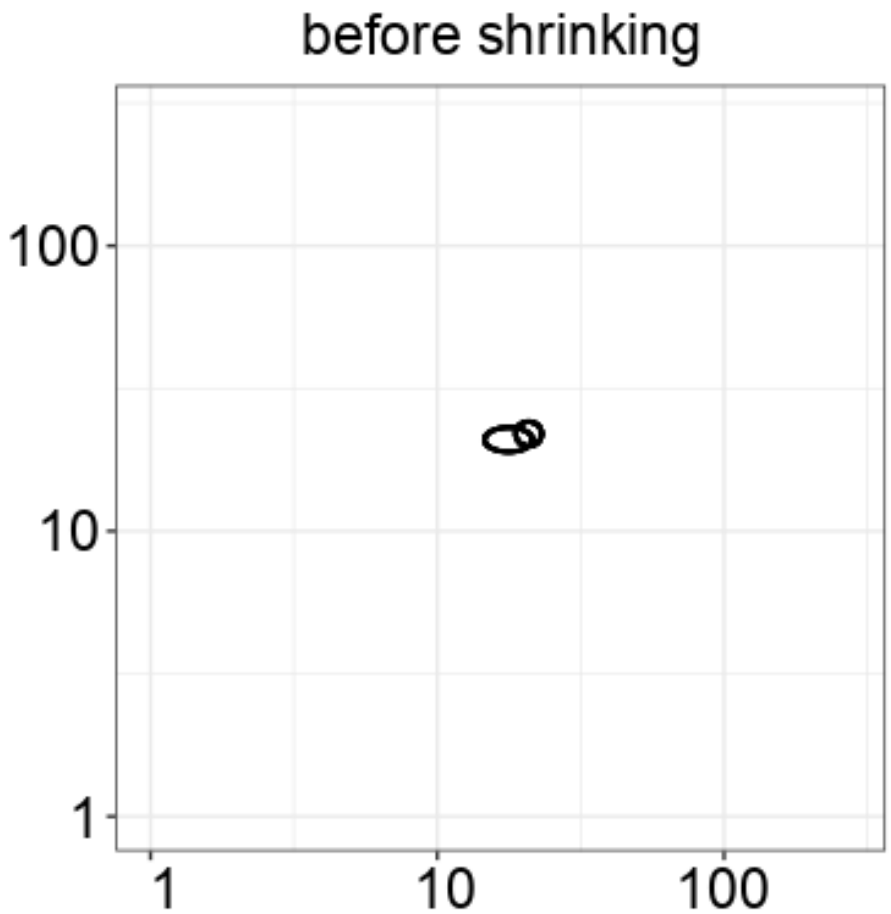
Example



Example



Example



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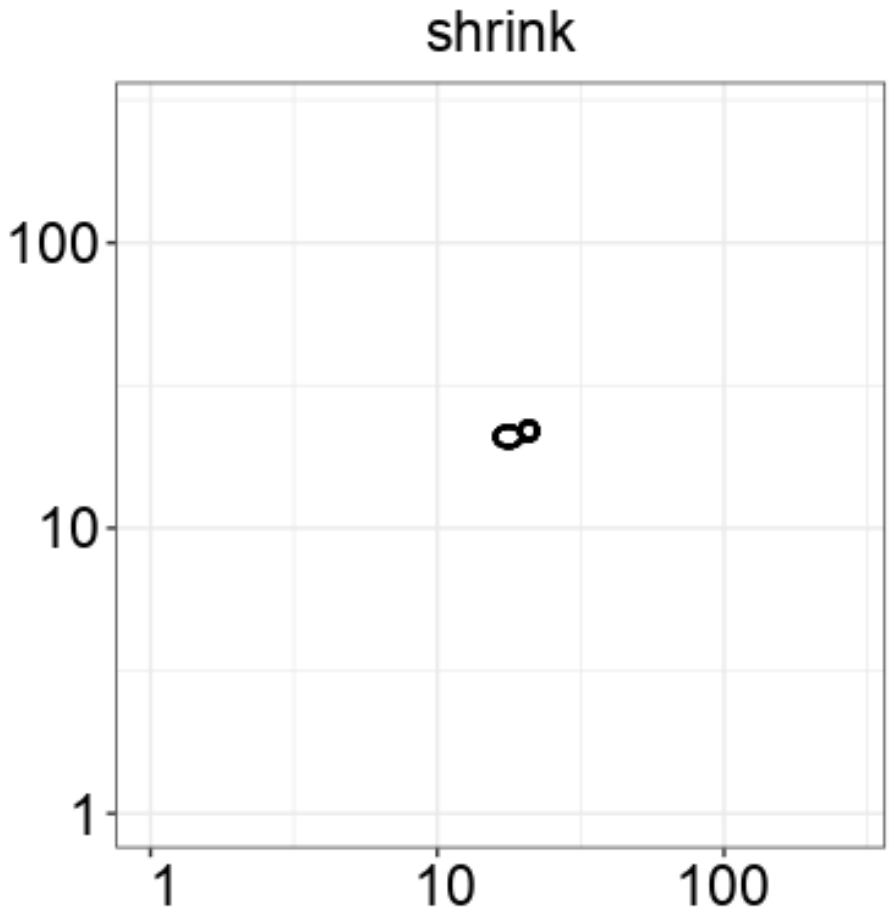
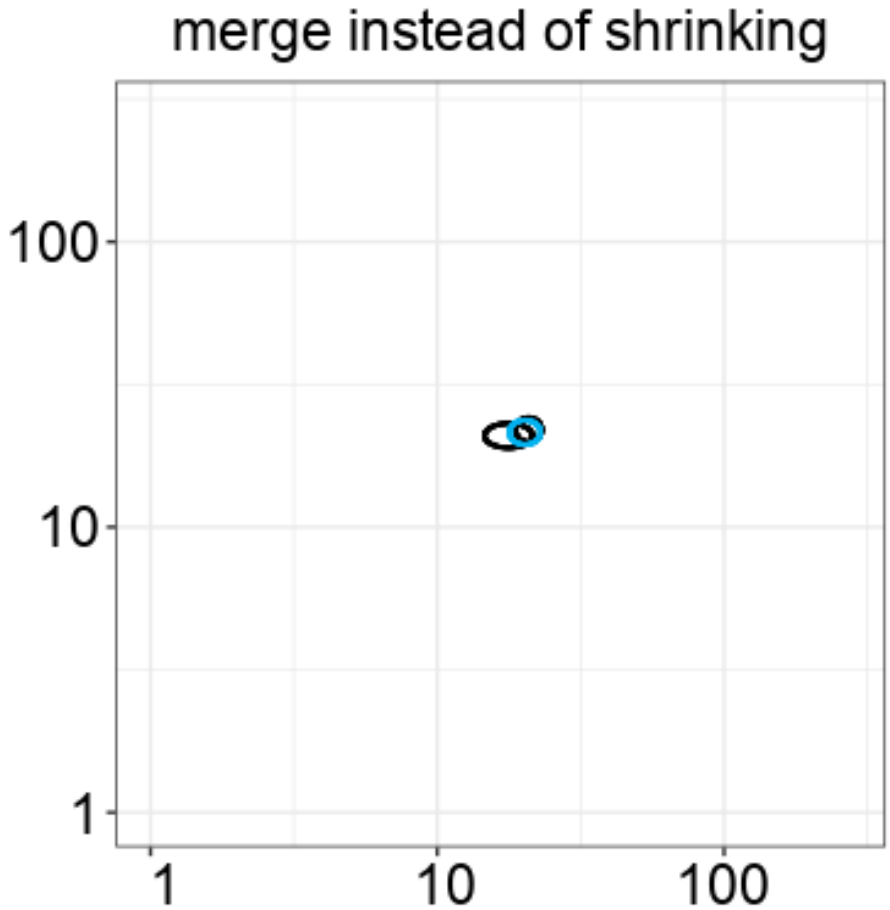
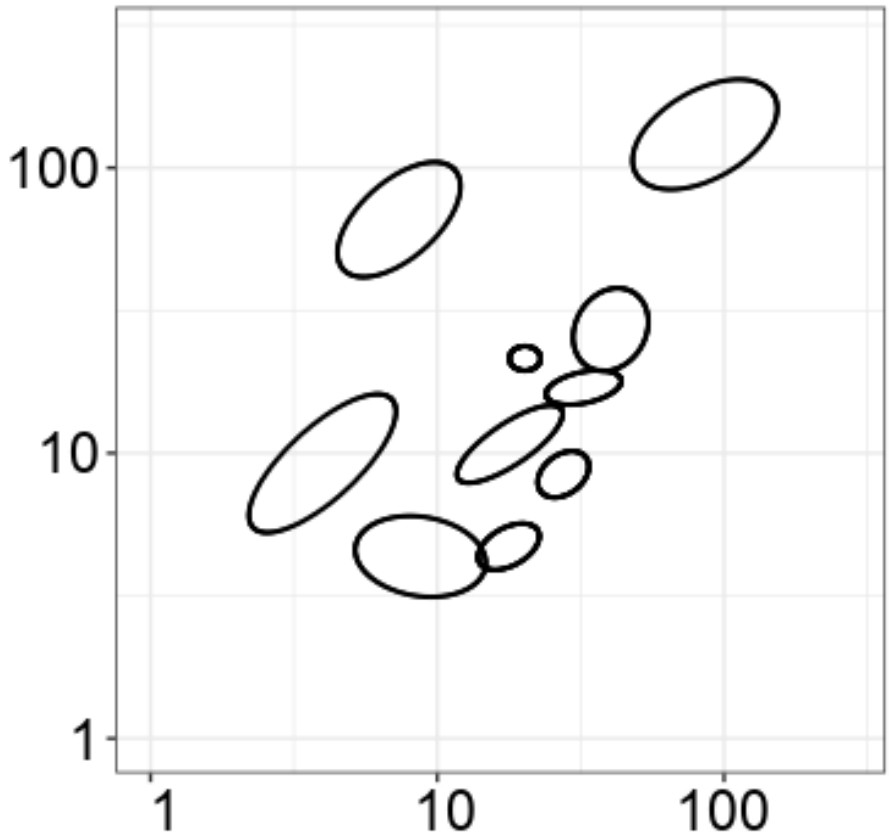


Example



Example



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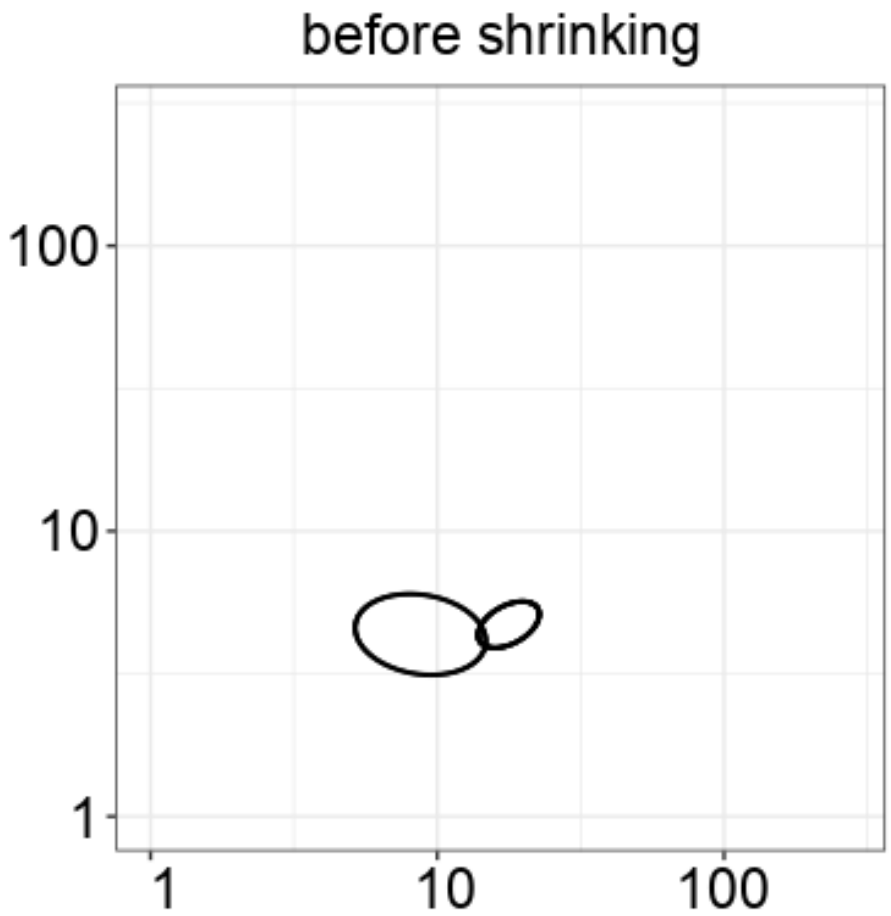

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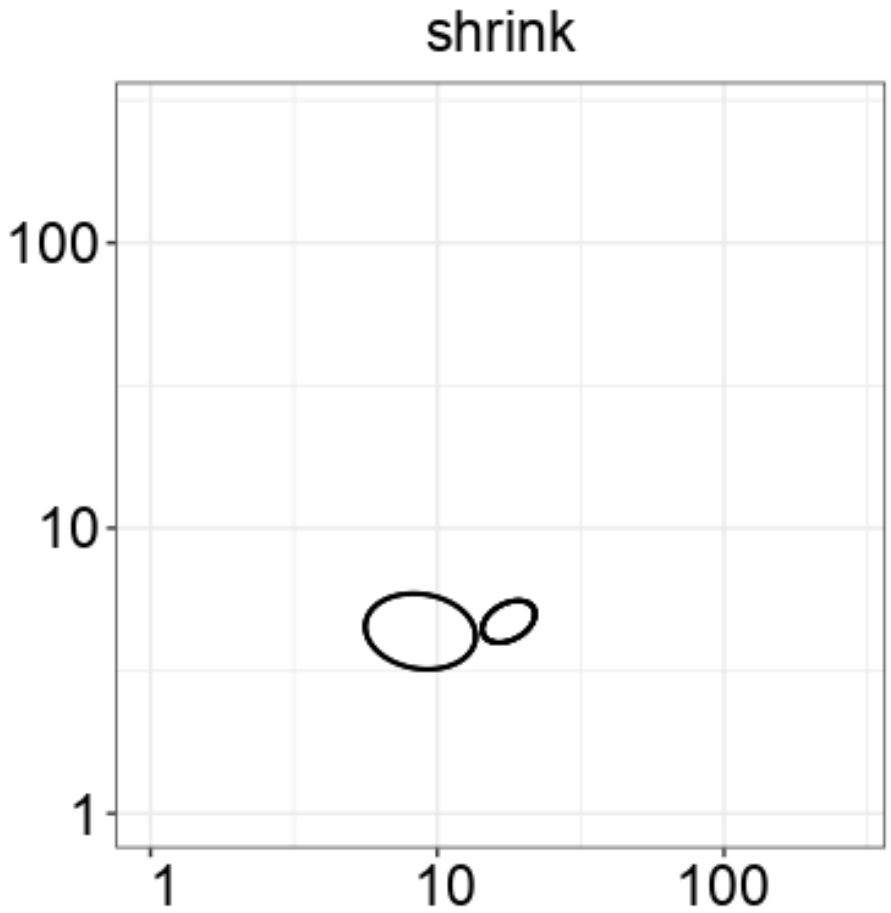

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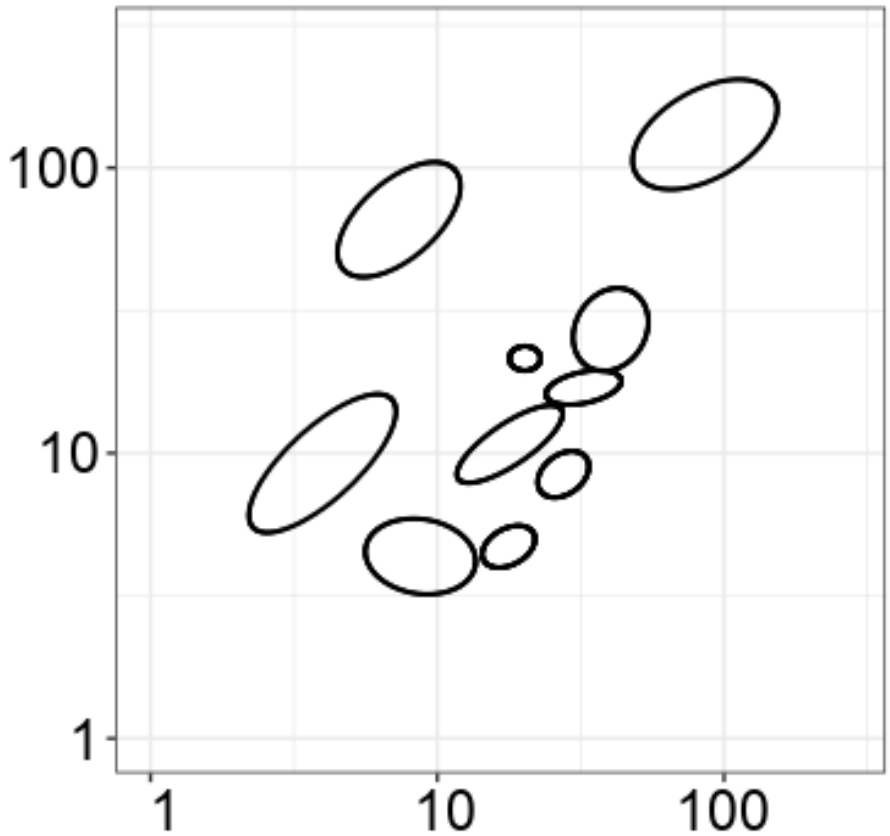

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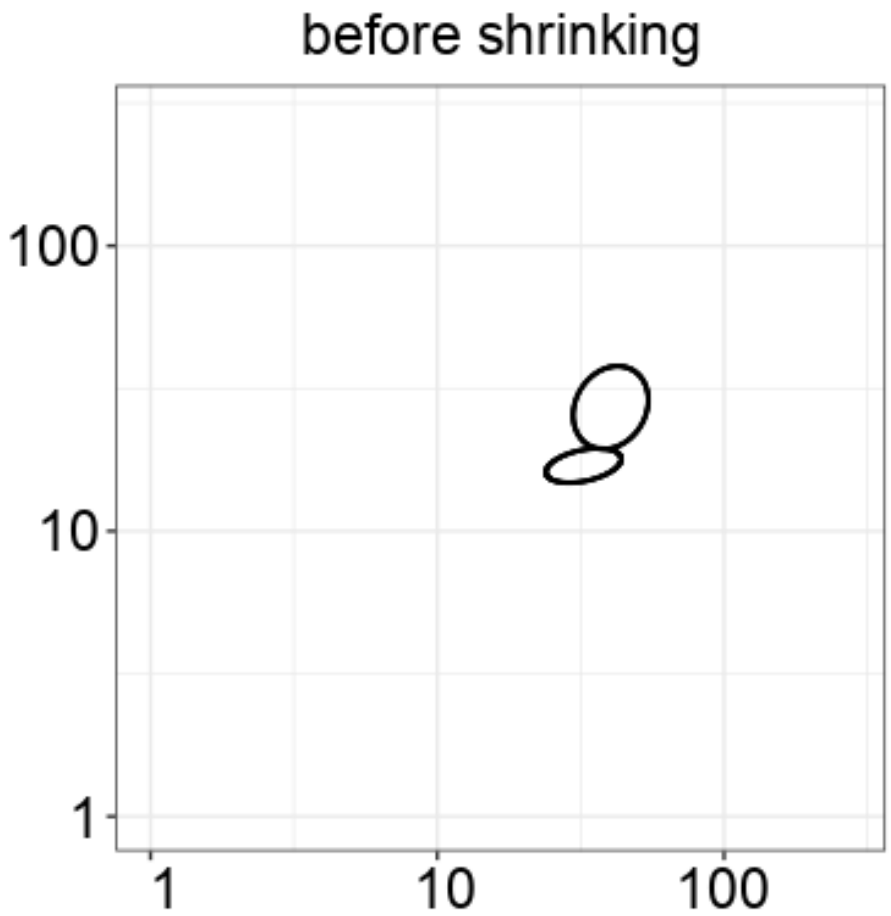

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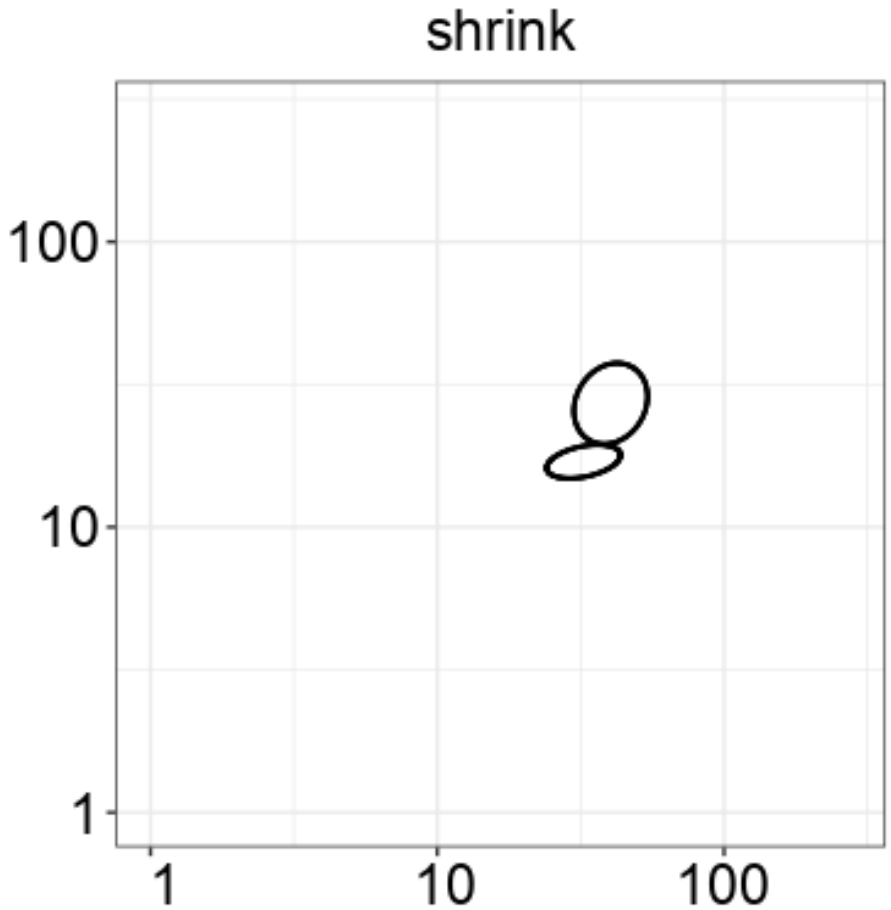

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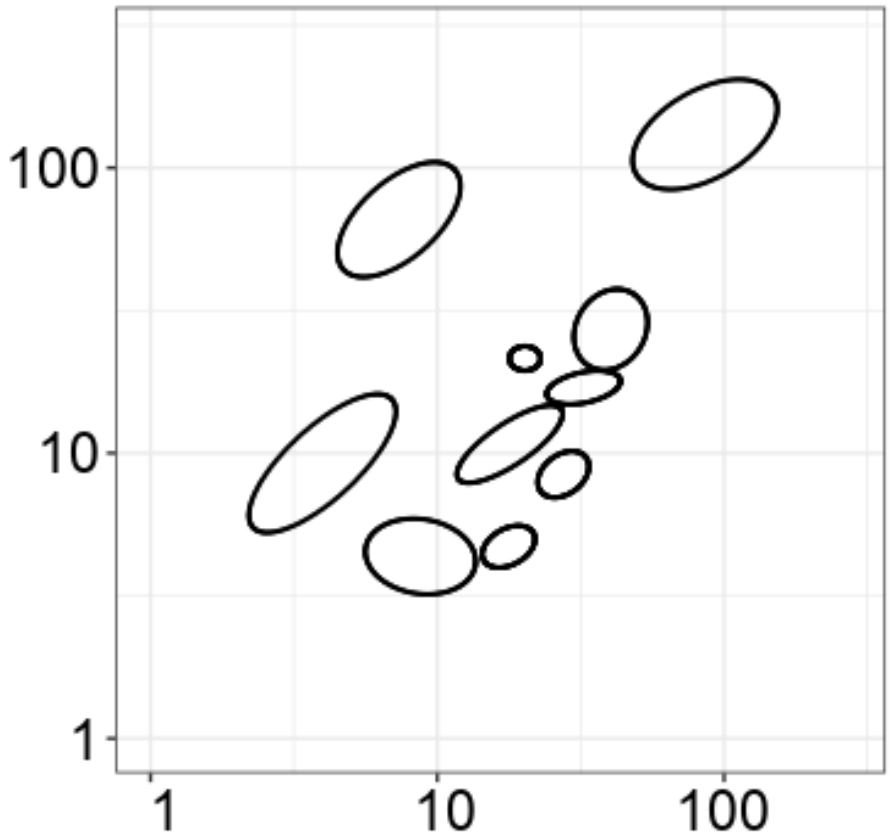
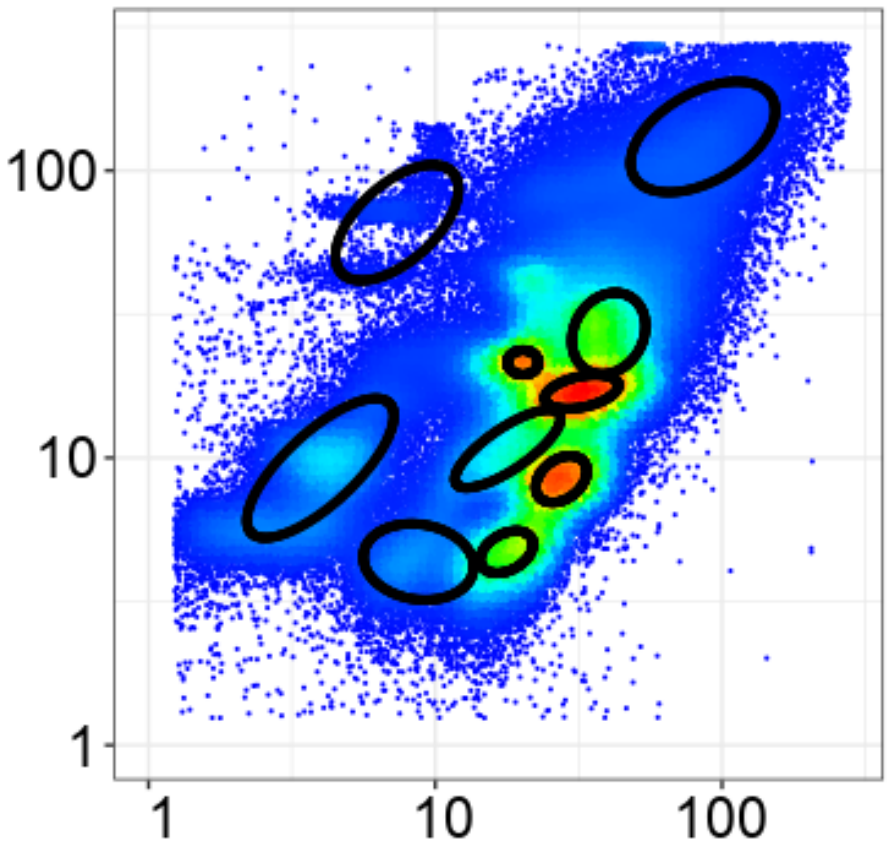
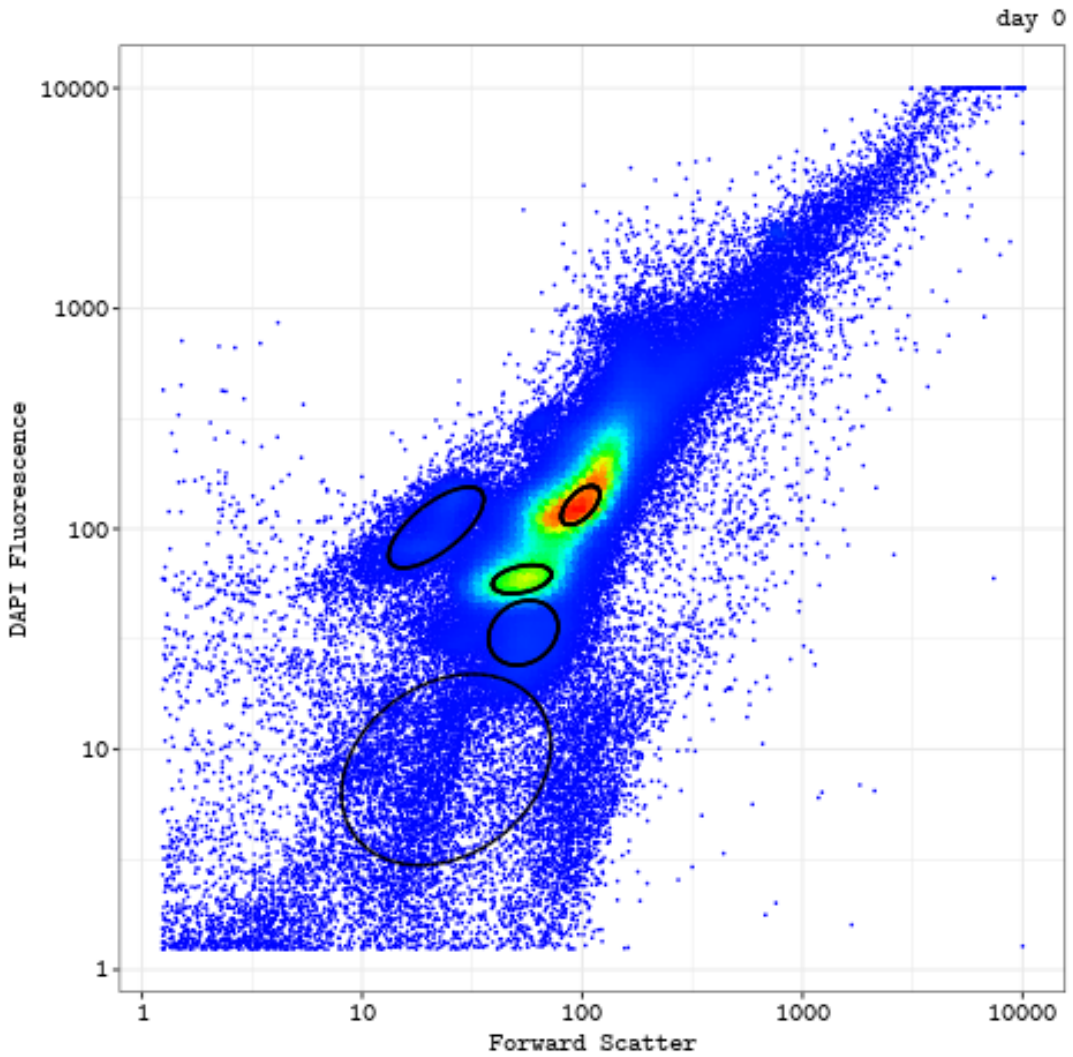


Example



Example 2

Automated gating of single samples



Gate Templates

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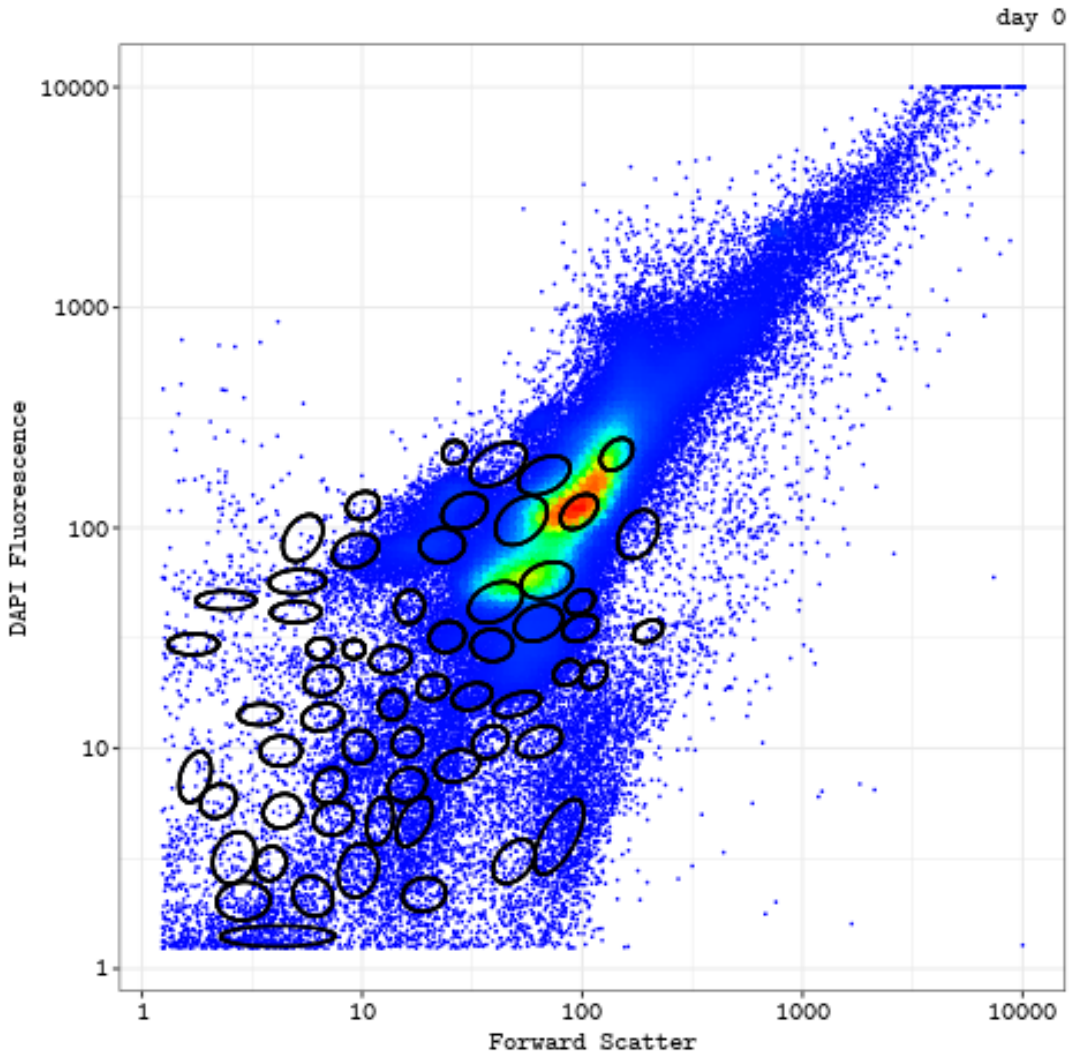
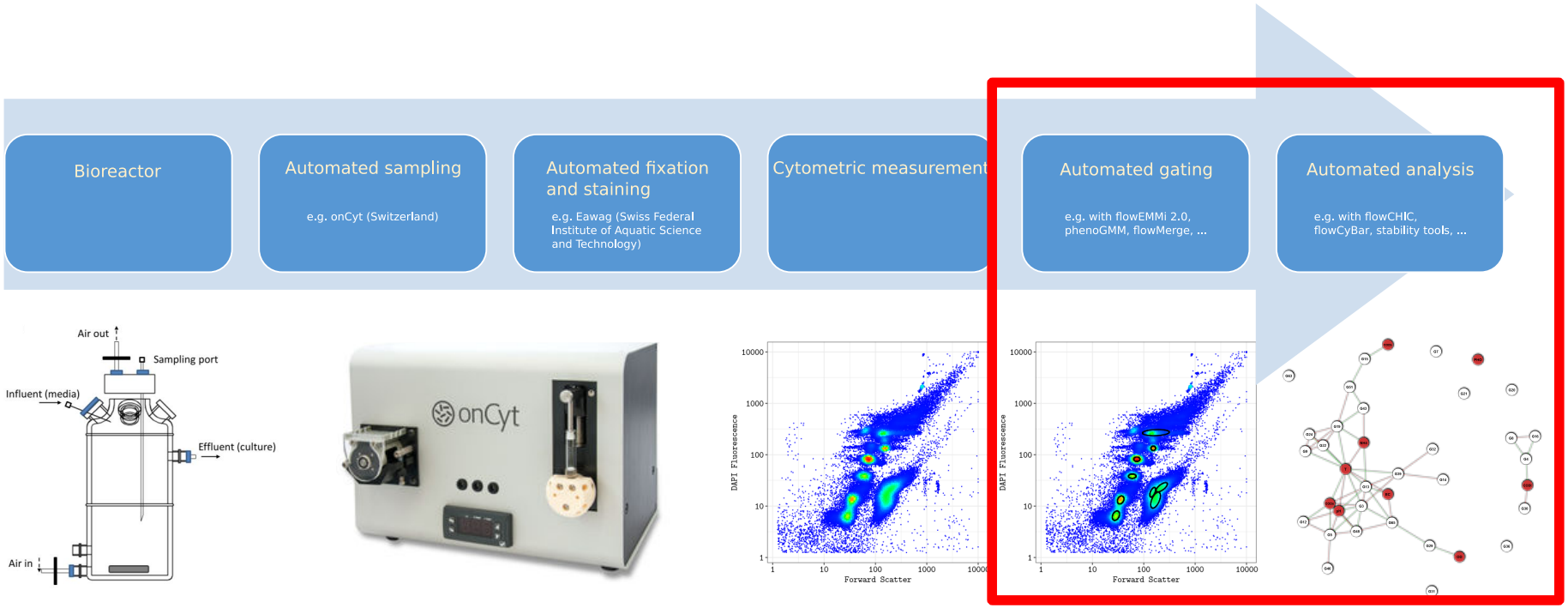
- For computational reasons this is done recursively
- With the gate template we can calculate *cell numbers per gate and sample*

Example 3

Automatically generated gate template



Summary/Outlook



Liu et al., **Neutral mechanisms and niche differentiation in steady-state insular microbial communities revealed by single cell analysis**, *Environmental Microbiology, 2019*

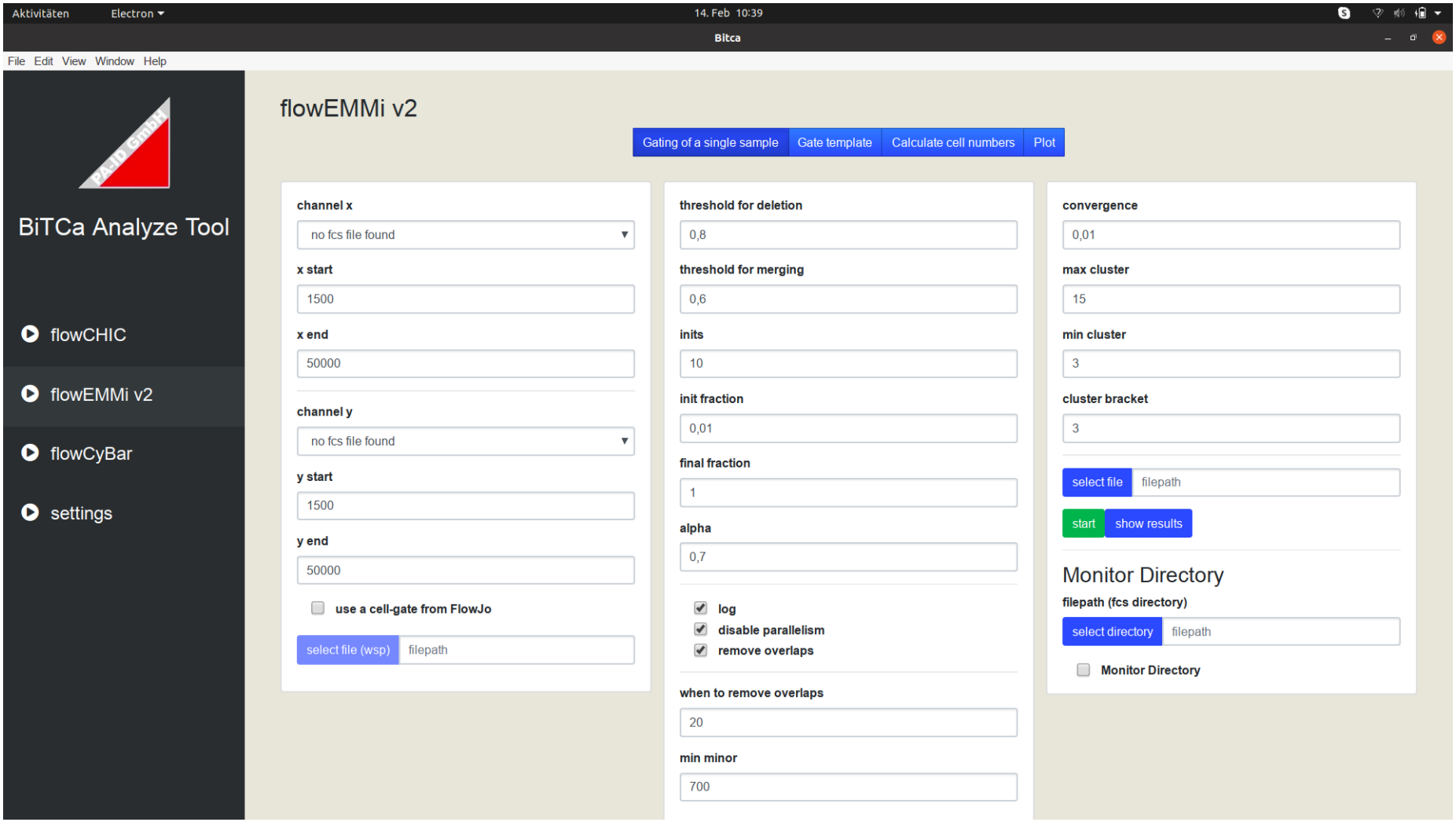
onCyt, *https://www.eawag.ch/de/news-agenda/news-plattform/newsarchiv/archiv-detail/online-durchflusszytometrie-sensor-fuer-bakterienkonzentrationen/*

Cichocki et al., **Bacterial mock communities as standards for reproducible cytometric microbiome analysis**, *Nature Protocols, 2020*

Ludwig et al., **flowEMMi: an automated model-based clustering tool for microbial cytometric data**, *BMC Bioinformatics, 2019*

Koch et al., **flowCyBar: Analyze flow cytometric data using gate information**, *R package version 1.30.0, 2019*

Summary/Outlook



Acknowledgements

UFZ HELMHOLTZ Zentrum für Umweltforschung

PA-ID GmbH

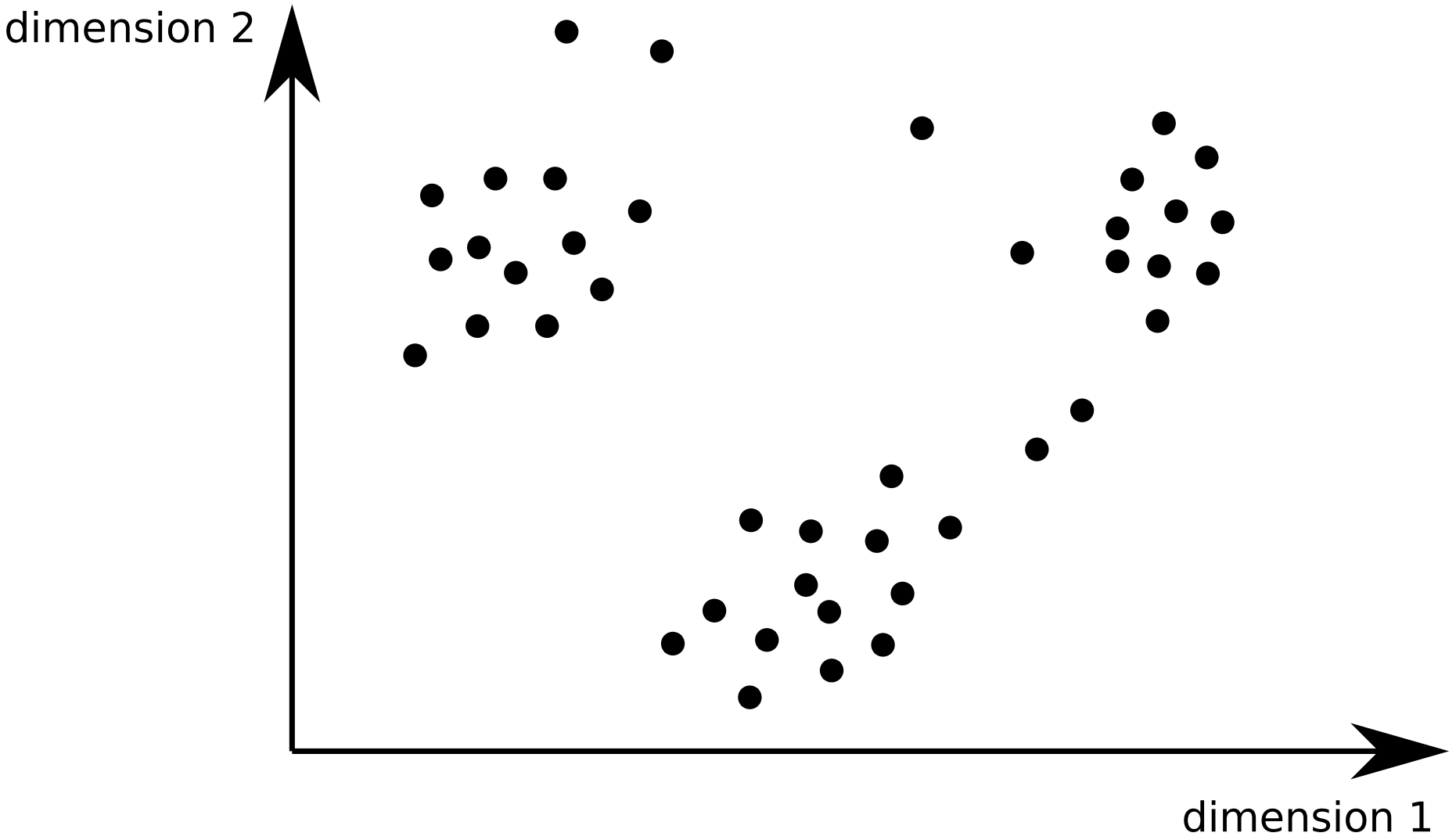
Bundesministerium für Wirtschaft und Energie

Bier Informatik est. 2002

ScaDS.AI DRESDEN LEIPZIG

Thank you for your attention and your support!

Appendix: The EM-algorithm



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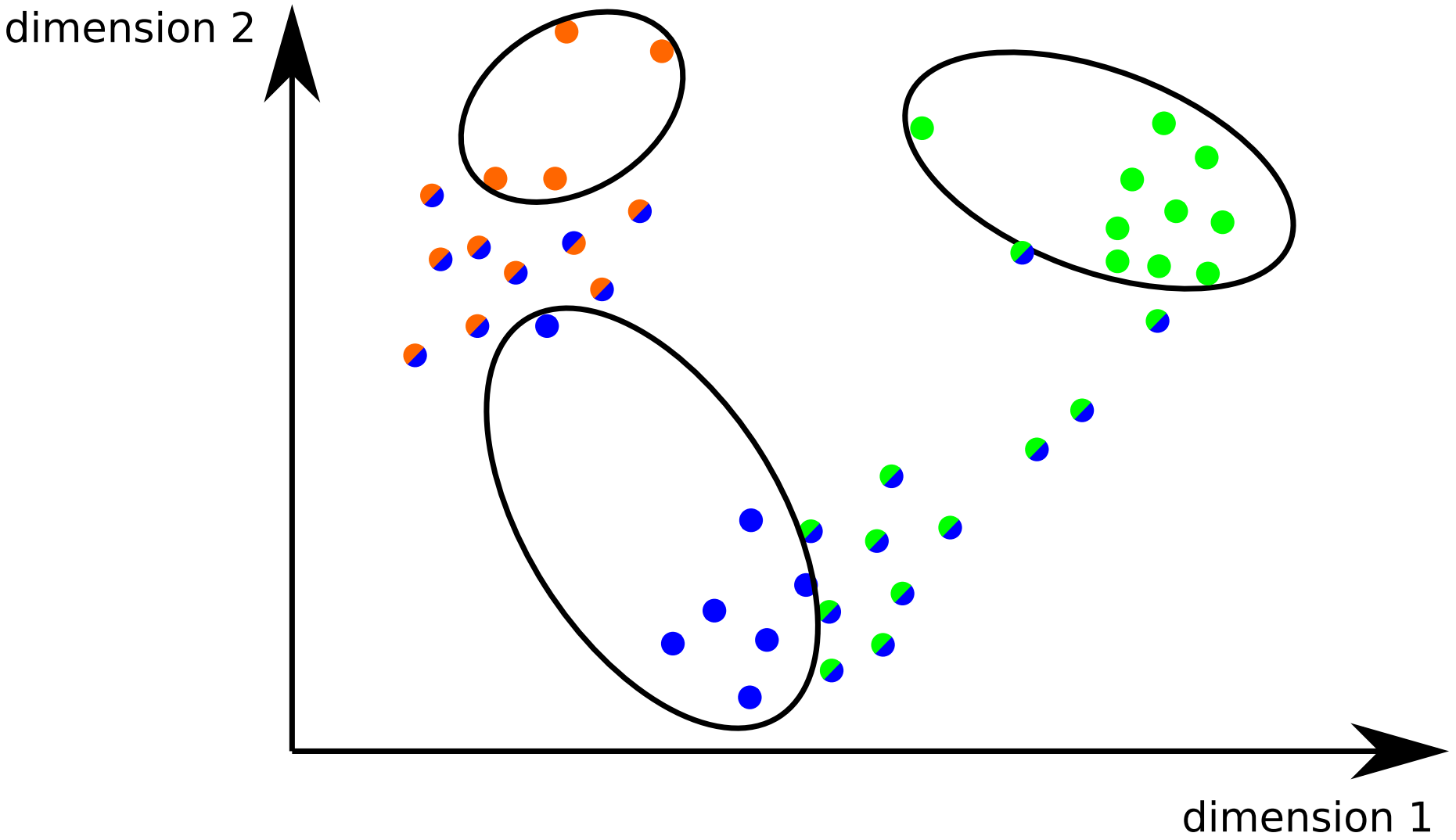

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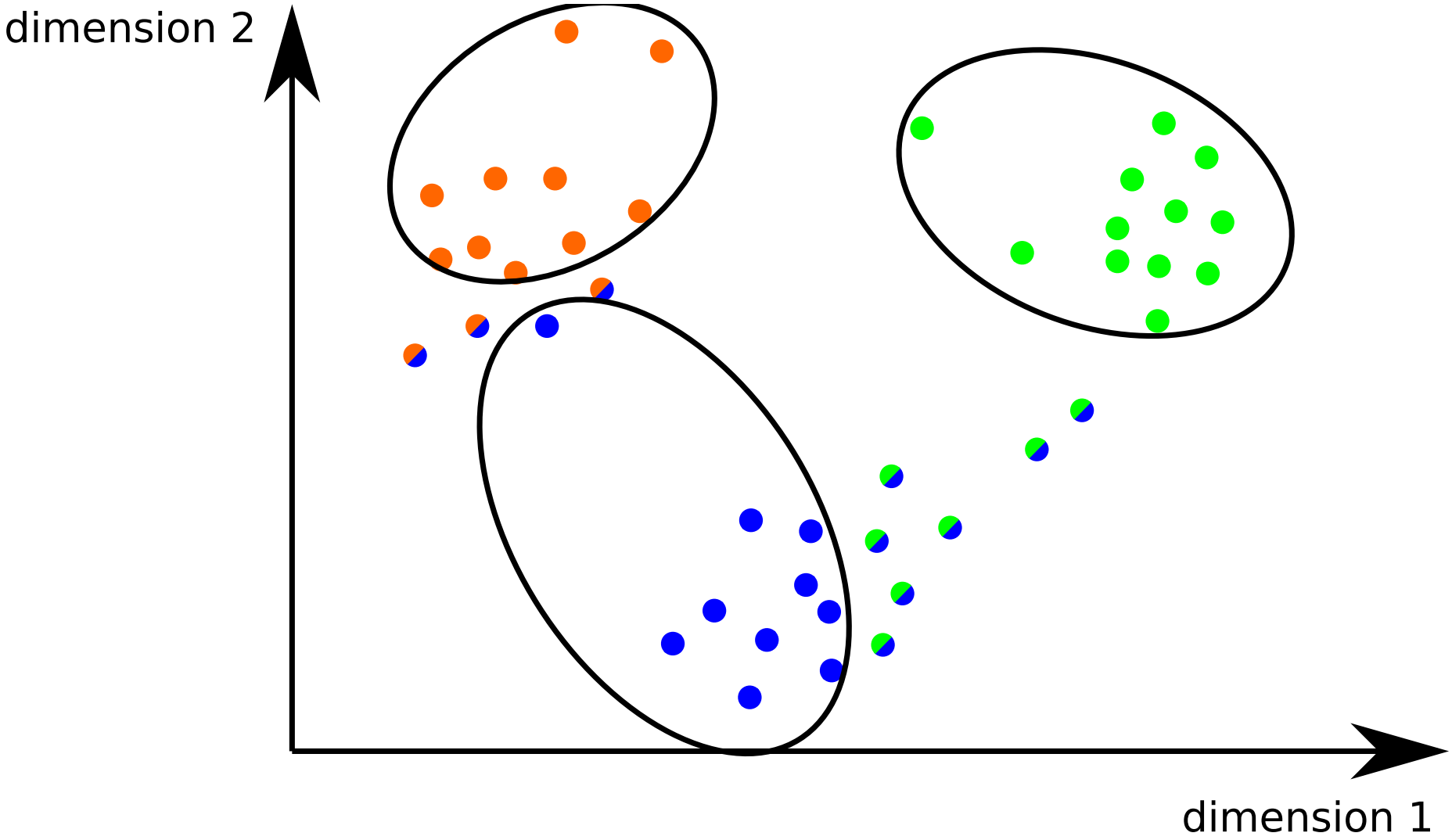

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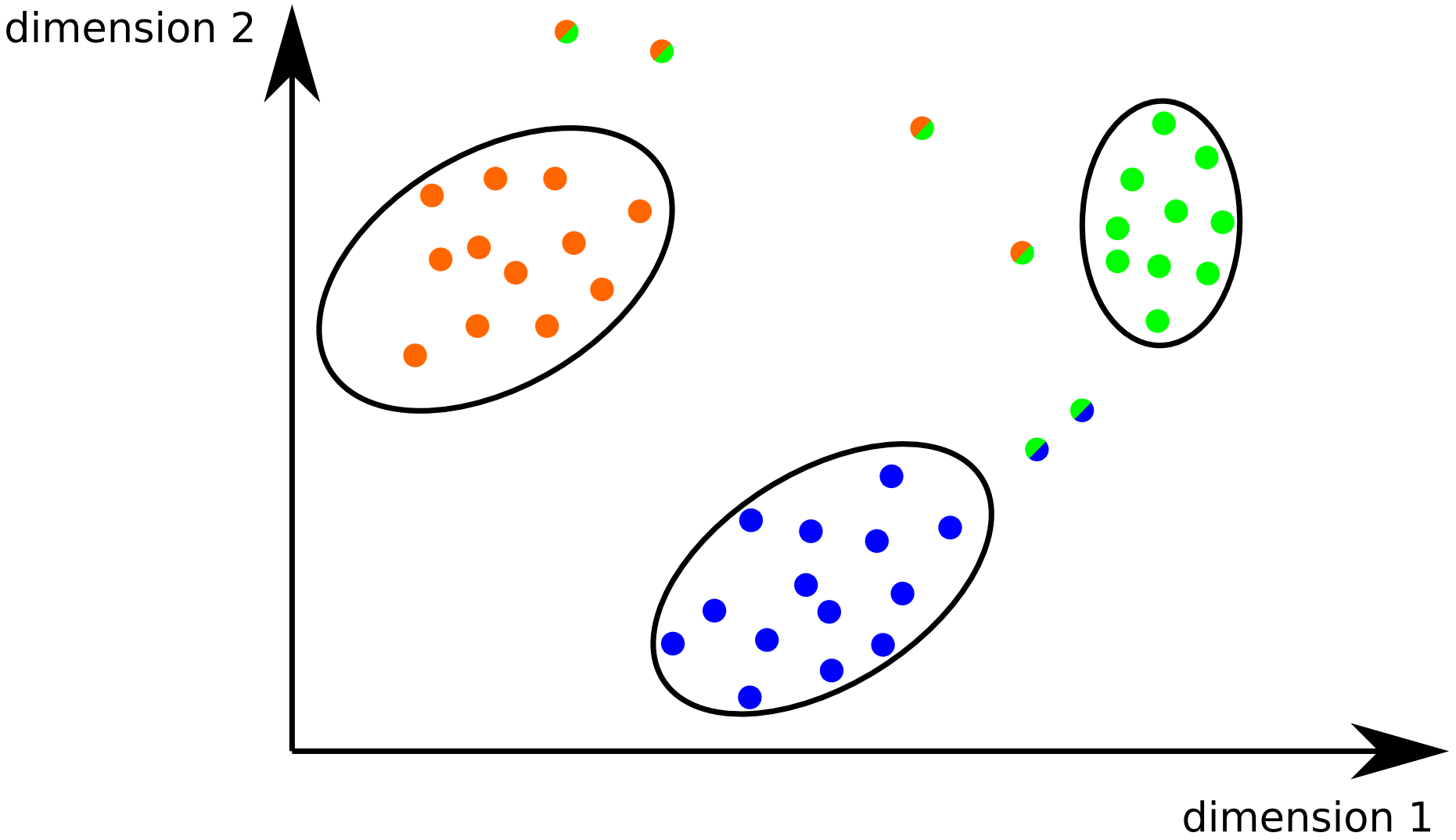
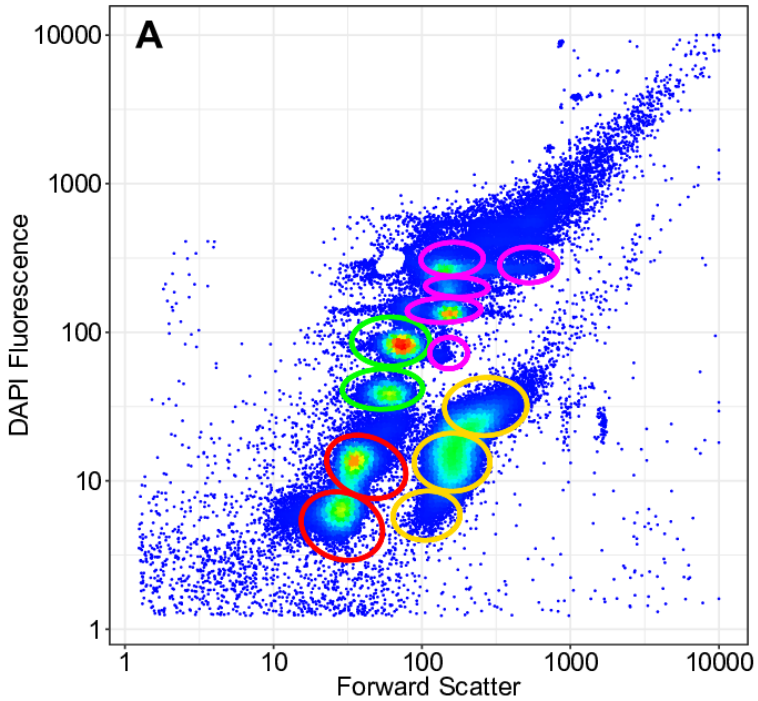
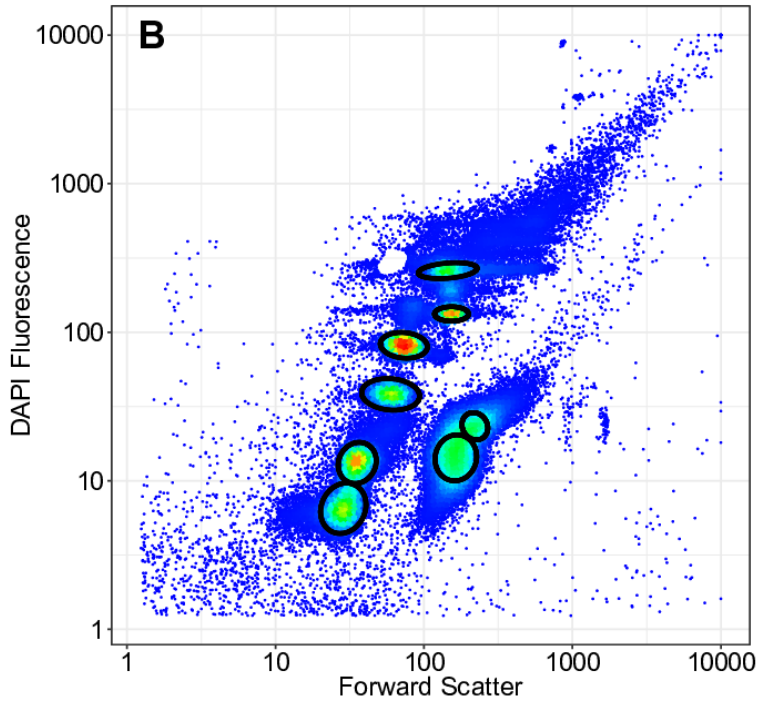


mock communities

manual gating



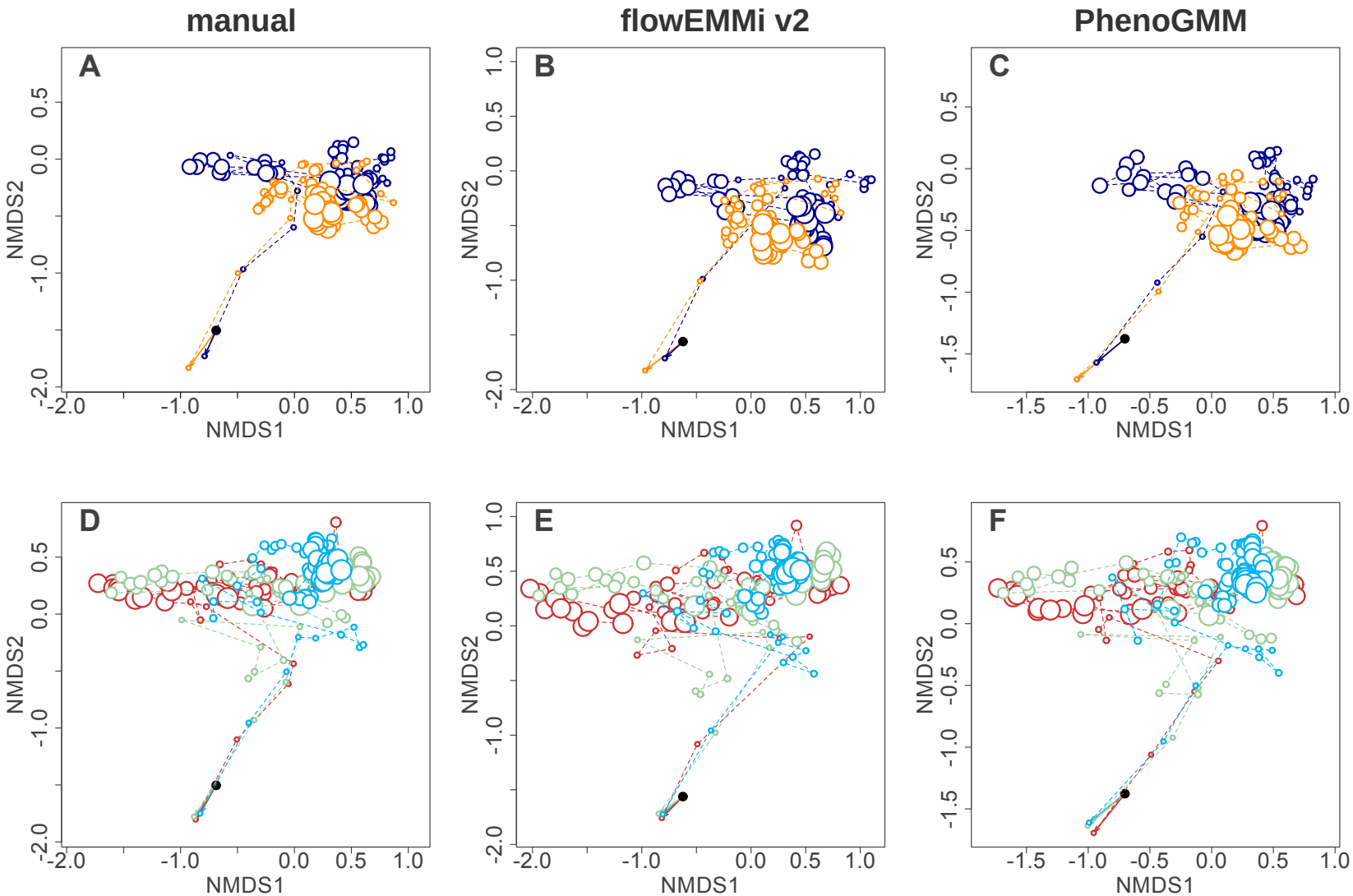
flowEMMi v2



- *Stenotrophomonas rhizophila*
- *Escherichia coli*
- *Kocuria rhizophila*
- *Paenibacillus polymyxa*

Cichocki et al., **Bacterial mock communities as standards for reproducible cytometric microbiome analysis**, *Nature Protocols, 2013*

Appendix: Community dynamics



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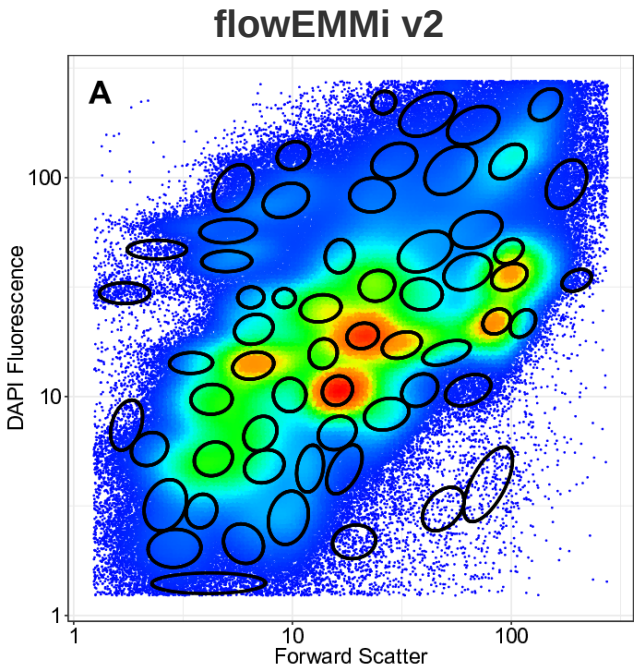
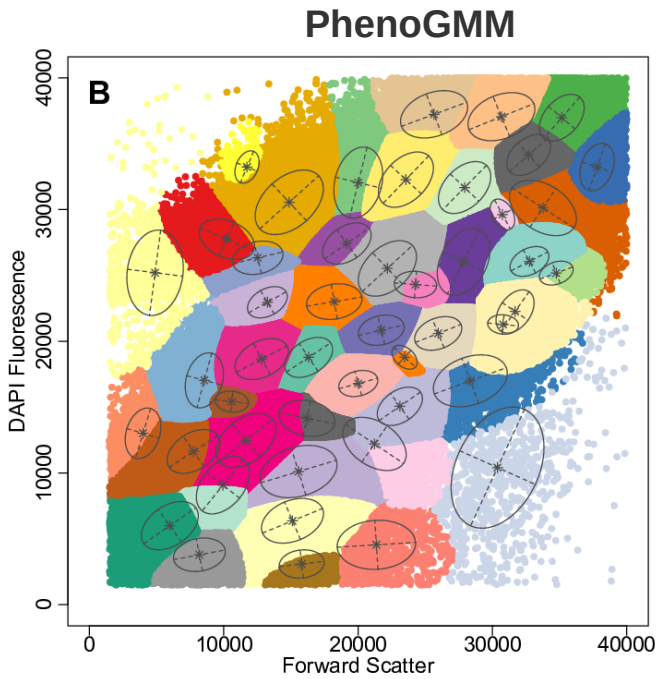
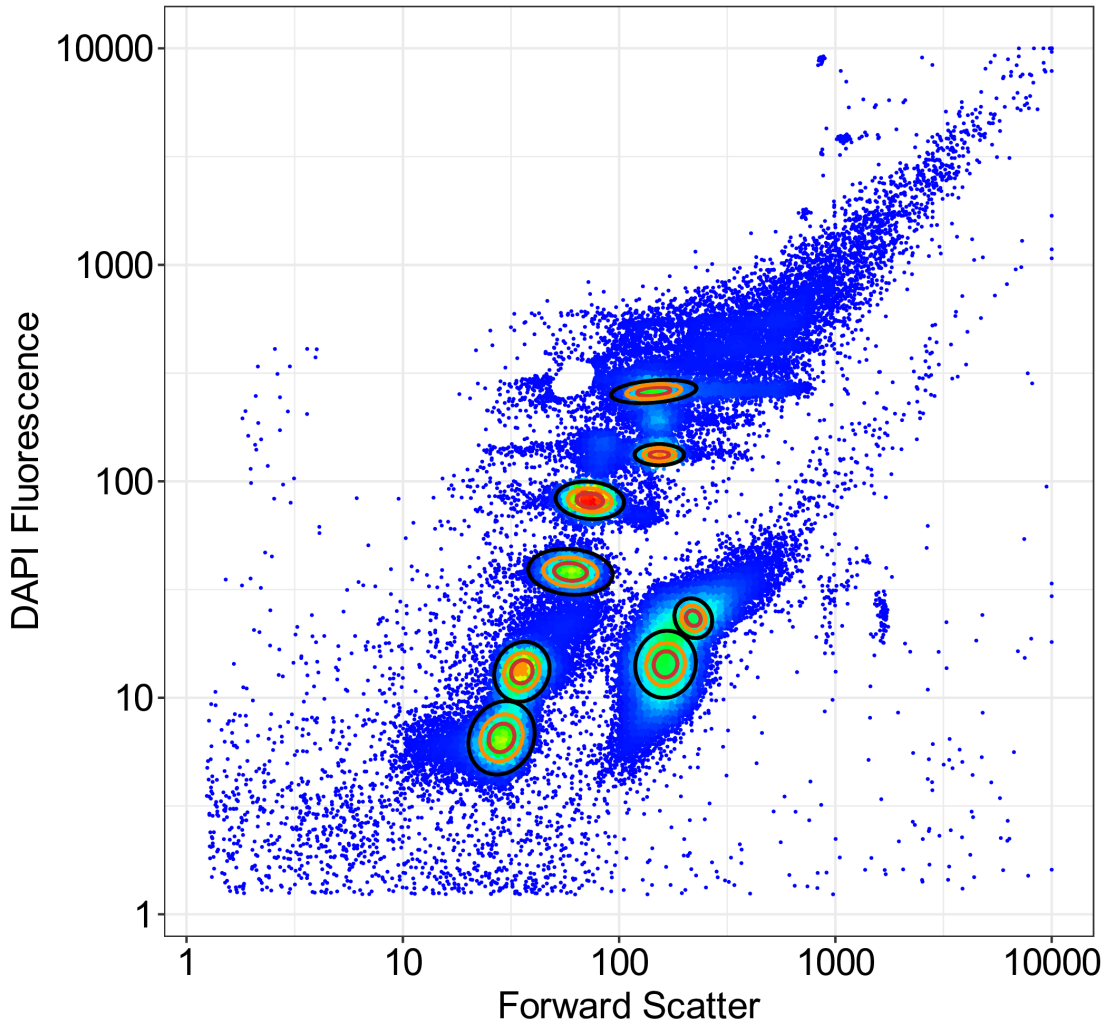
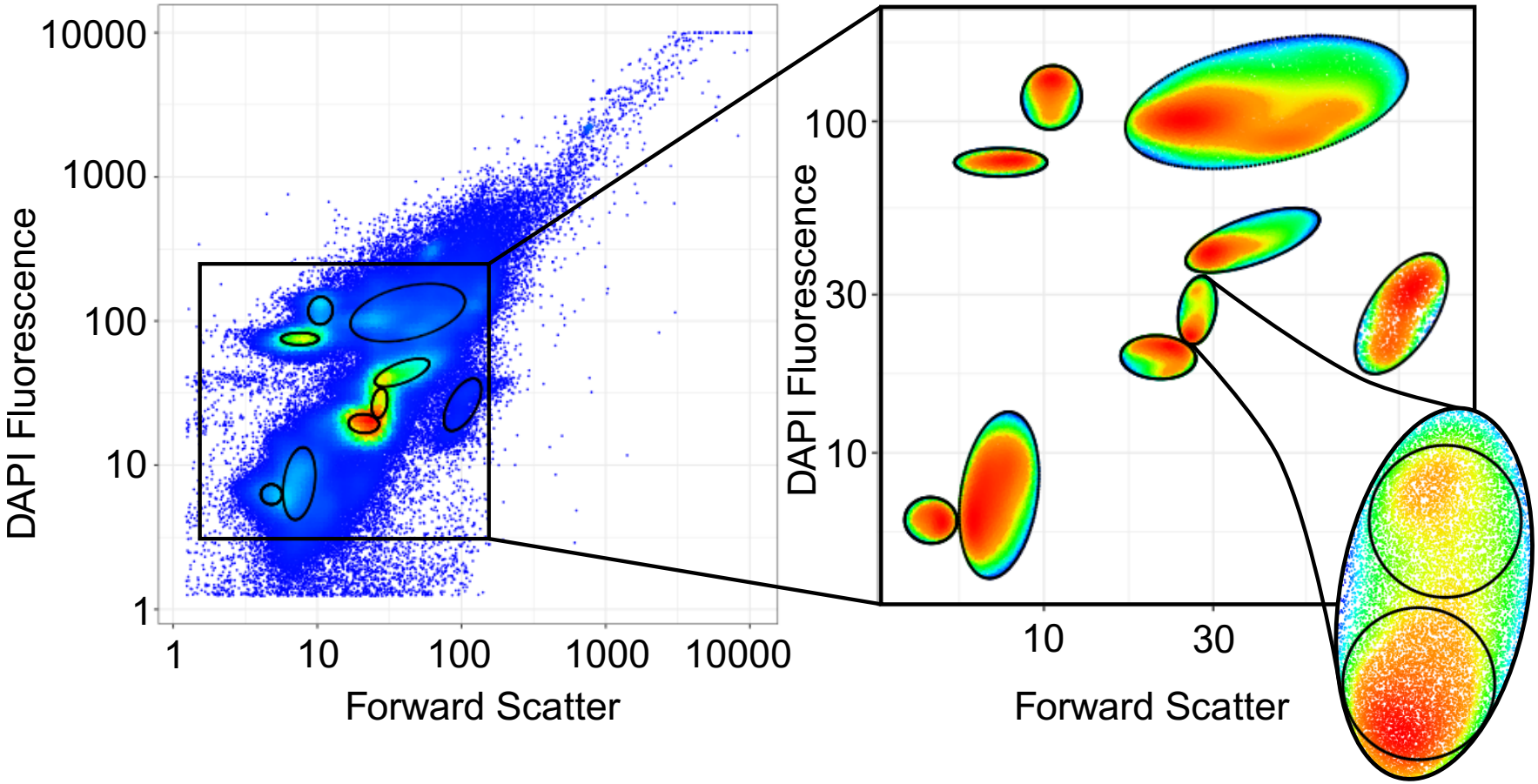




Appendix: Ellipse sizes



Appendix: Hierarchical Gating



Merging

Merging: The mathematical details

- Σ_1 and Σ_2 of E_1 and E_2 are both symmetric and positive definite $\Rightarrow$ use the Cholesky decomposition and re-scale them:

$$\Sigma_i = L_i \cdot D_i \cdot L_i^T \quad \text{for } i = 1, 2 \tag{1}$$

$$\Sigma_i' = L_i \cdot \frac{1}{w_i} \cdot D_i \cdot L_i^T \quad \text{for } i = 1, 2 \tag{2}$$

- Then, the new μ_c and Σ_c' of E_c', which will be re-scaled to E_c later, can be derived as the product of two Gaussians:

$$\mu_c = \left(\Sigma_1'^{-1} + \Sigma_2'^{-1}\right) \cdot \left(\Sigma_1'^{-1} \cdot \mu_1 + \Sigma_2'^{-1} \cdot \mu_2\right) \tag{3}$$

$$\Sigma_c' = \left(\Sigma_1'^{-1} + \Sigma_2'^{-1}\right)^{-1} = \Sigma_1' \cdot \left(\Sigma_1' + \Sigma_2'\right)^{-1} \cdot \Sigma_2' \tag{4}$$

- For the re-adjustment of the weights, we again need to decompose Σ_c' and multiply the diagonal elements by the sum of the weights.

$$\Sigma_c' = L_c' \cdot D_c' \cdot L_c'^T \tag{5}$$

$$\Sigma_c = L_c' \cdot (w_1 + w_2) \cdot D_c' \cdot L_c'^T \tag{6}$$