Evaluating the potential application of automated FlowCam® technology for phytoplankton monitoring in the Swan Canning Estuary

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Murdoch University

Government of Western Australia
Department of Biodiversity, Conservation and Attractions

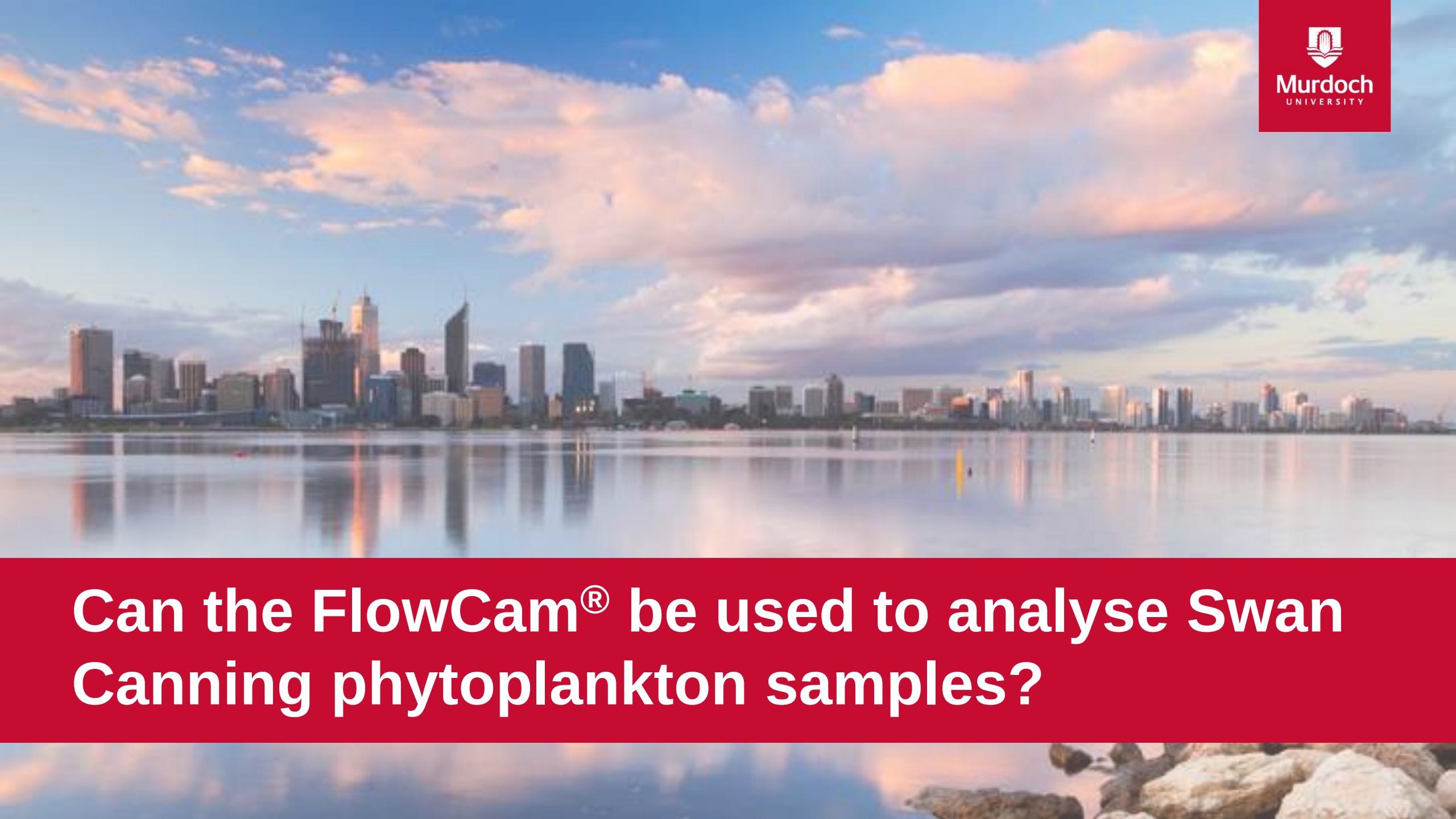
Government of Western Australia
Department of Water and Environmental Regulation

CSIRO

GOVERNMENT OF
WESTERN AUSTRALIA

Department of **Biodiversity, Conservation and Attractions**

Government of **Western Australia**
Department of **Water and Environmental Regulation**

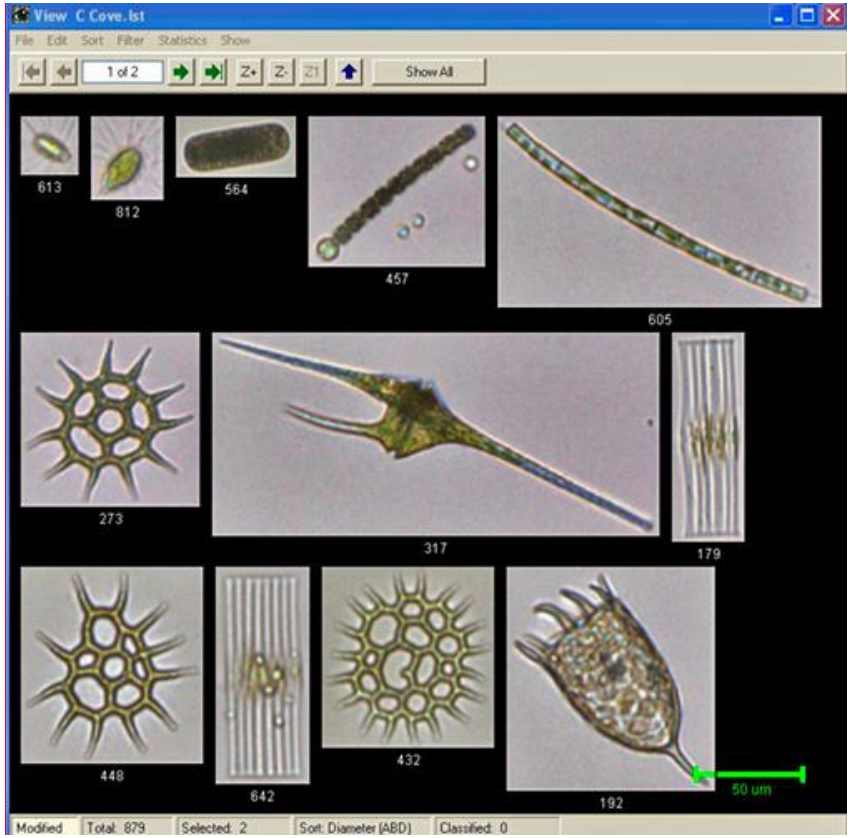


Can the FlowCam® be used to analyse Swan Canning phytoplankton samples?

FlowCam™

Murdoch UNIVERSITY

FLUID IMAGING TECHNOLOGIES, INC.



Possible FlowCam® benefits over microscopy

1. Faster sample turnaround time
2. Reduced taxonomist labour costs
3. Remove operator variability
4. Permanent record of samples
5. Add size class and biovolume data

FlowCam® for turbid and preserved samples

Portable FlowCam

Benchtop FlowCam

Can the FlowCam® be used to analyse Swan Canning phytoplankton samples?

- Sample preparation
- Run settings
- Image Libraries
- Auto-classification optimisation
- Comparison to microscopy

FlowCam®

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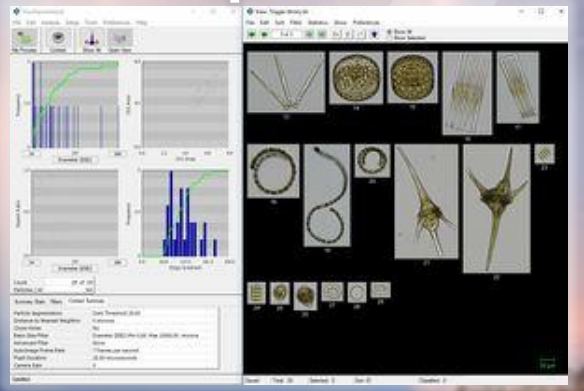
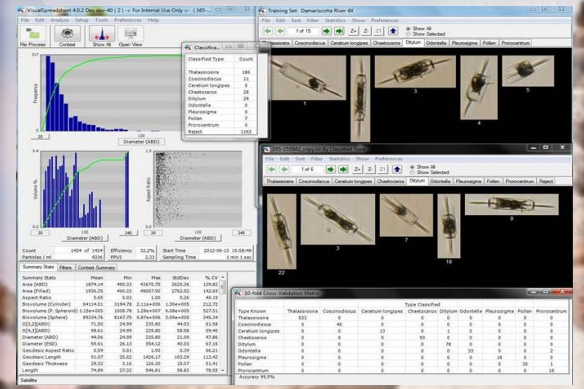
FlowCam®

VisualSpreadsheet®



Classifier Advanced



Sample preparation

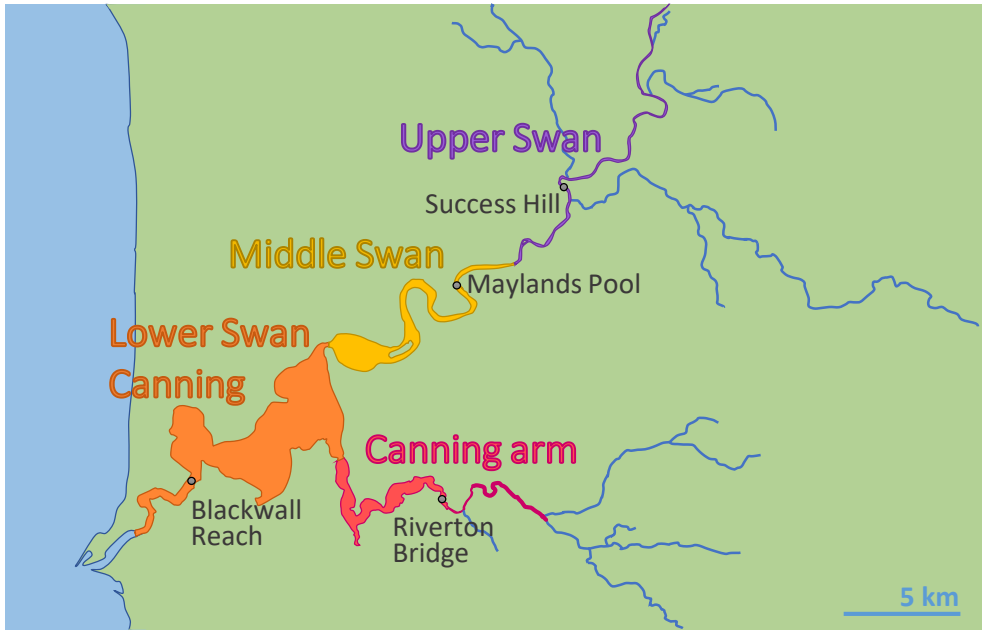
Run settings

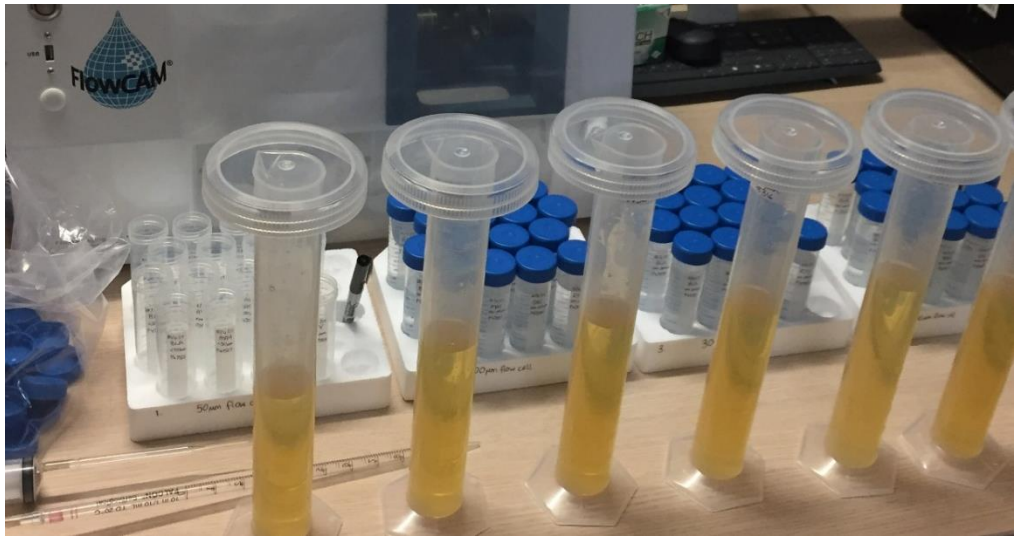
Image Libraries

Auto-classification optimisation

Comparison to microscopy

- 12 library samples
- 12 test samples
- 0-50 µm size fraction
- Concentrated





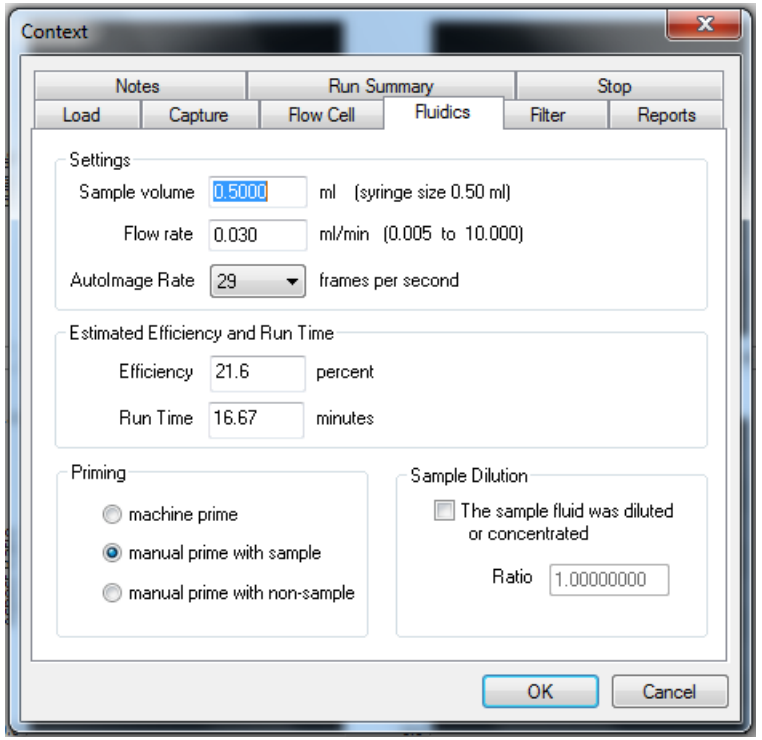
Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy



- FlowCam® VS I updated in 2013 (equivalent to VS IV)
- 50 µm flow cell and 20X objective

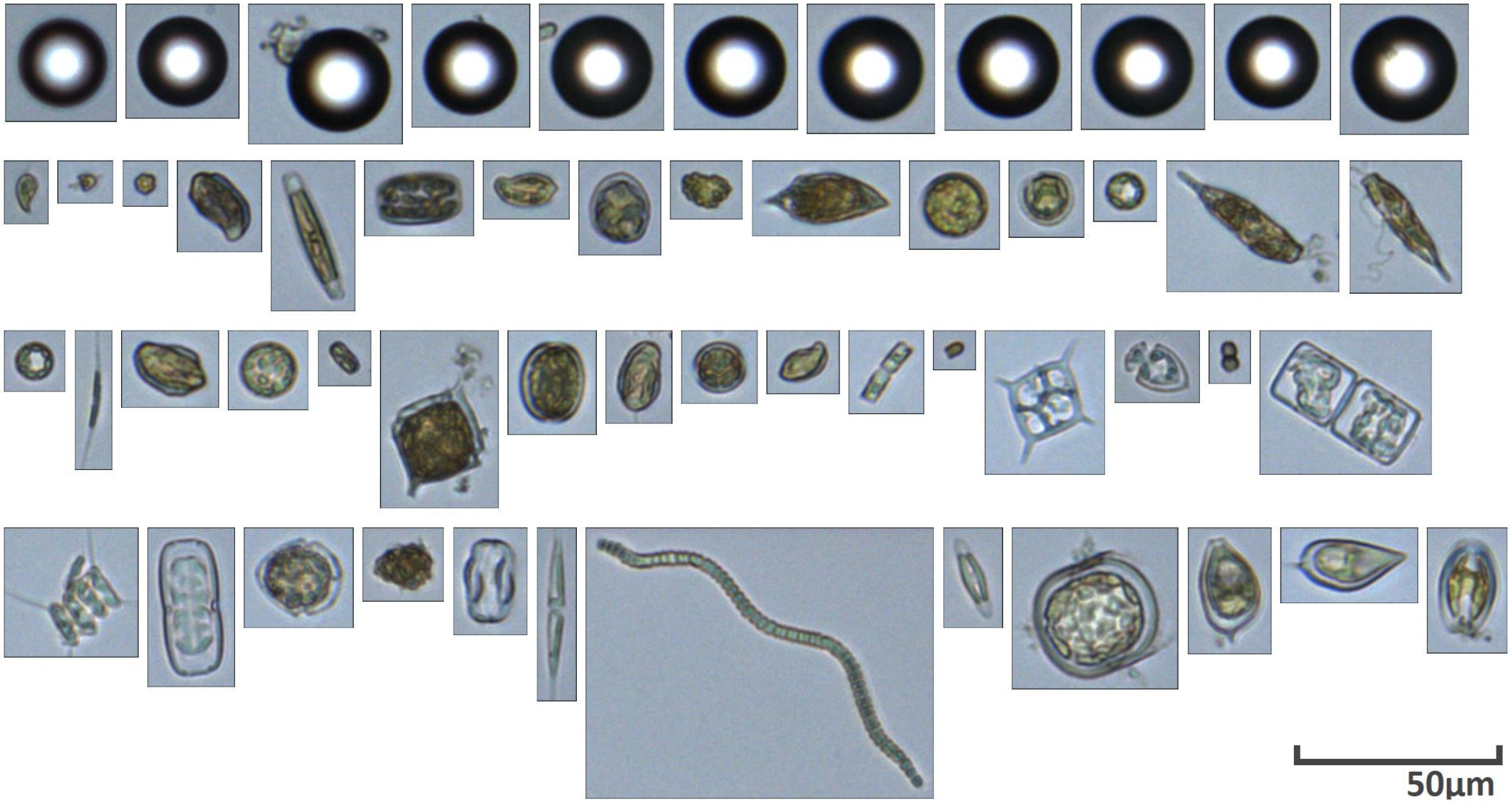
Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy



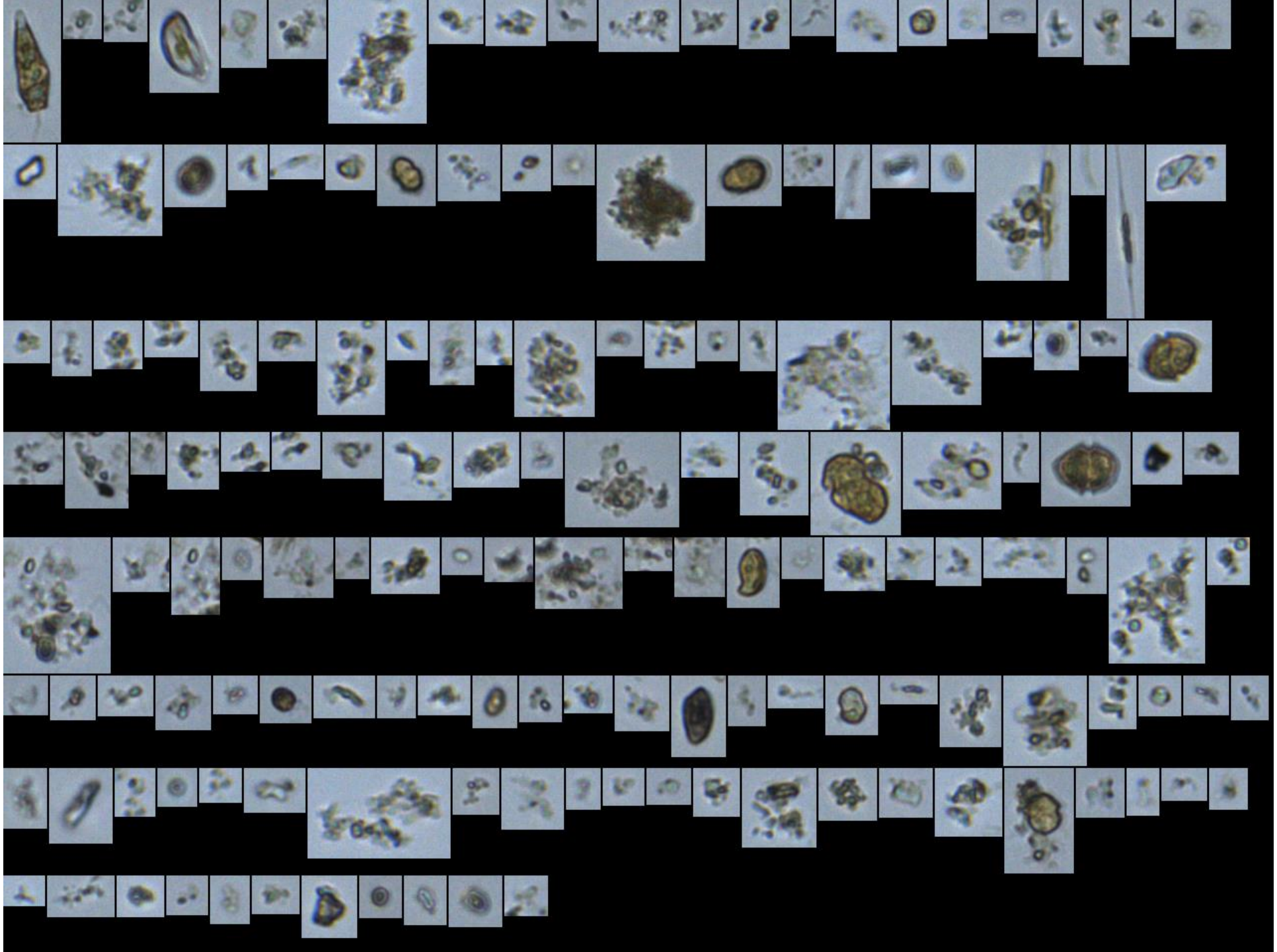
Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy



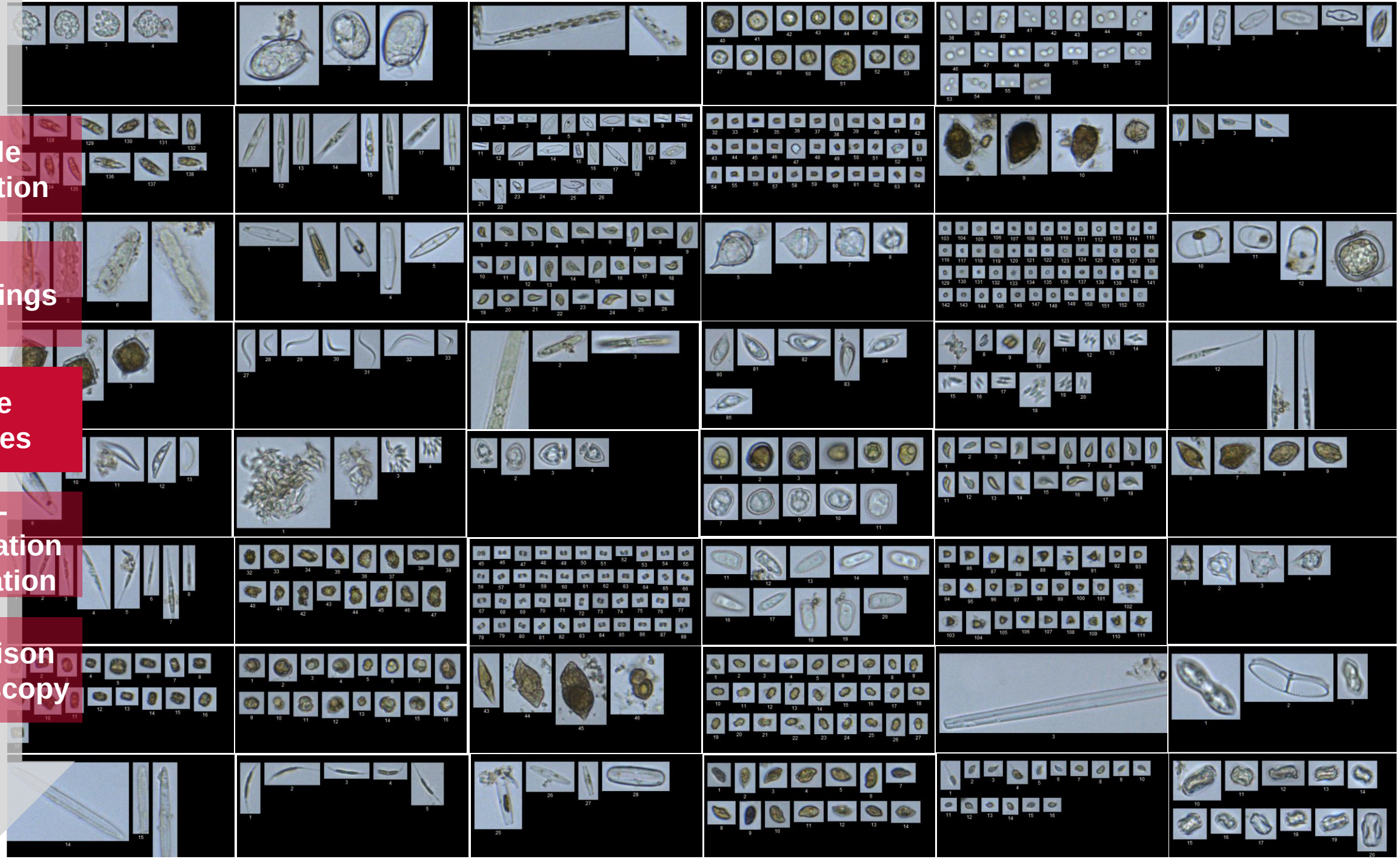
Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy



Sample preparation

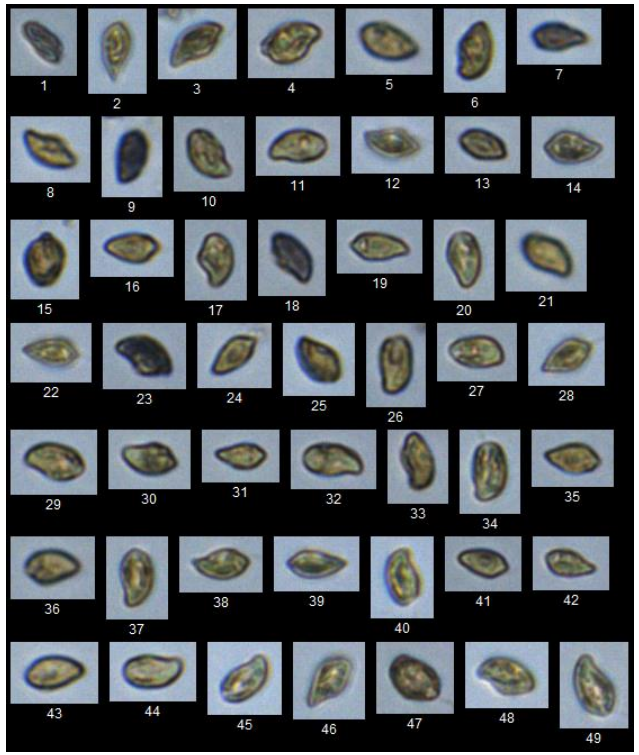
Run settings

Image Libraries

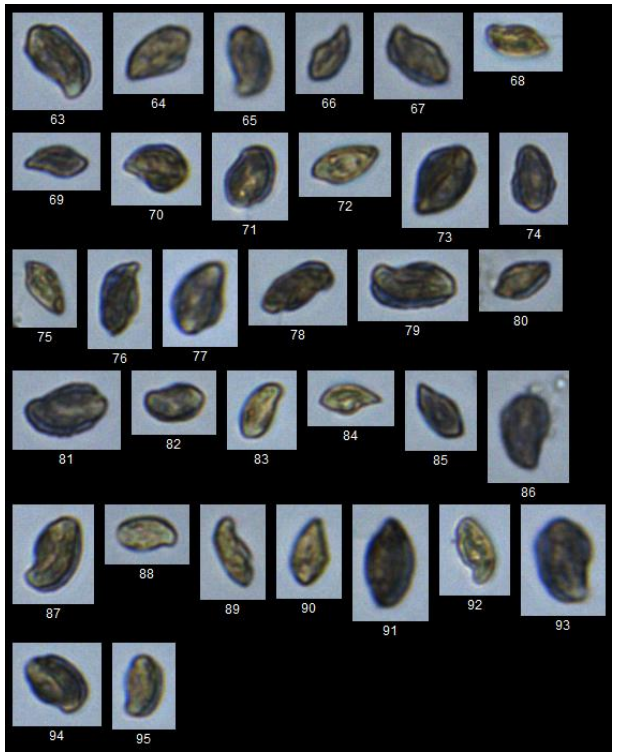
Auto-classification optimisation

Comparison to microscopy

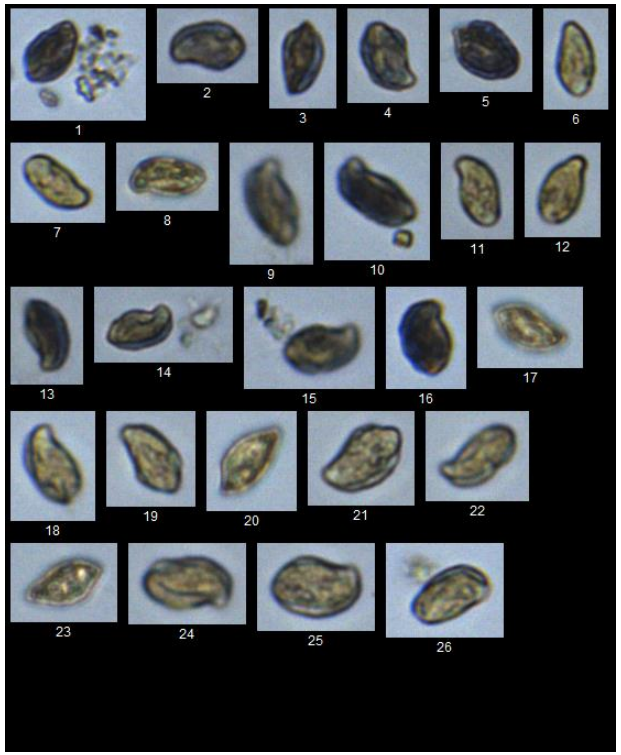
e.g. *Cryptomonas* spp. (847 total images)



6-15 µm
(76 images)



15-25 µm
(744 images)



>25 µm
(27 images)

Sample preparation

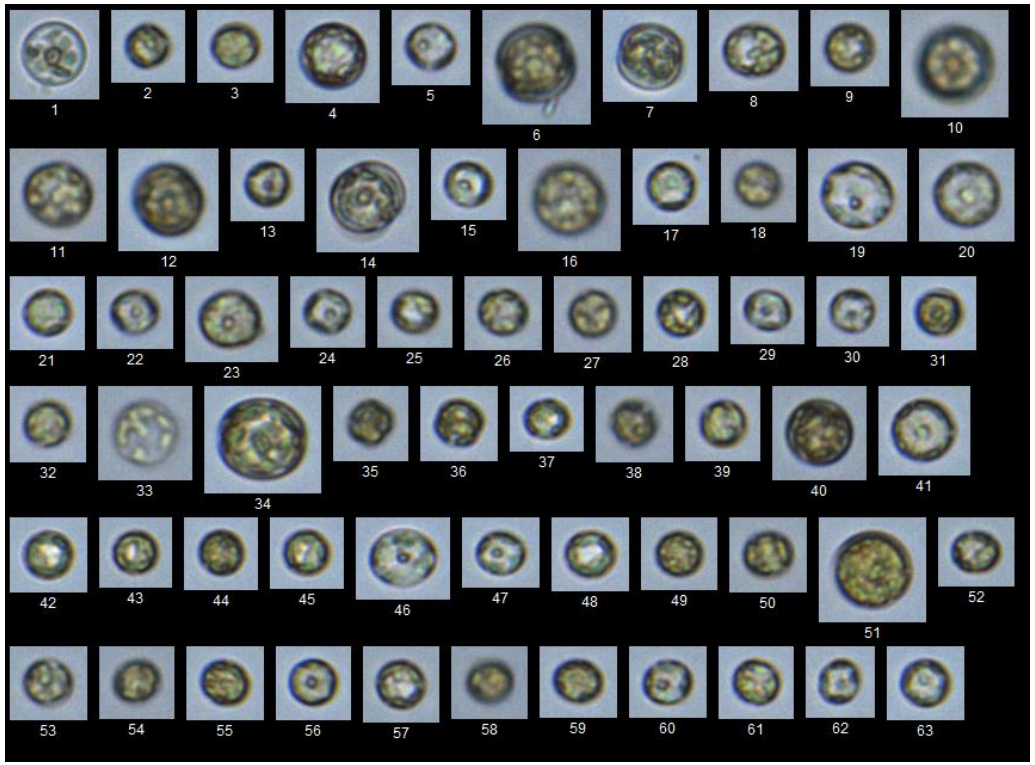
Run settings

Image Libraries

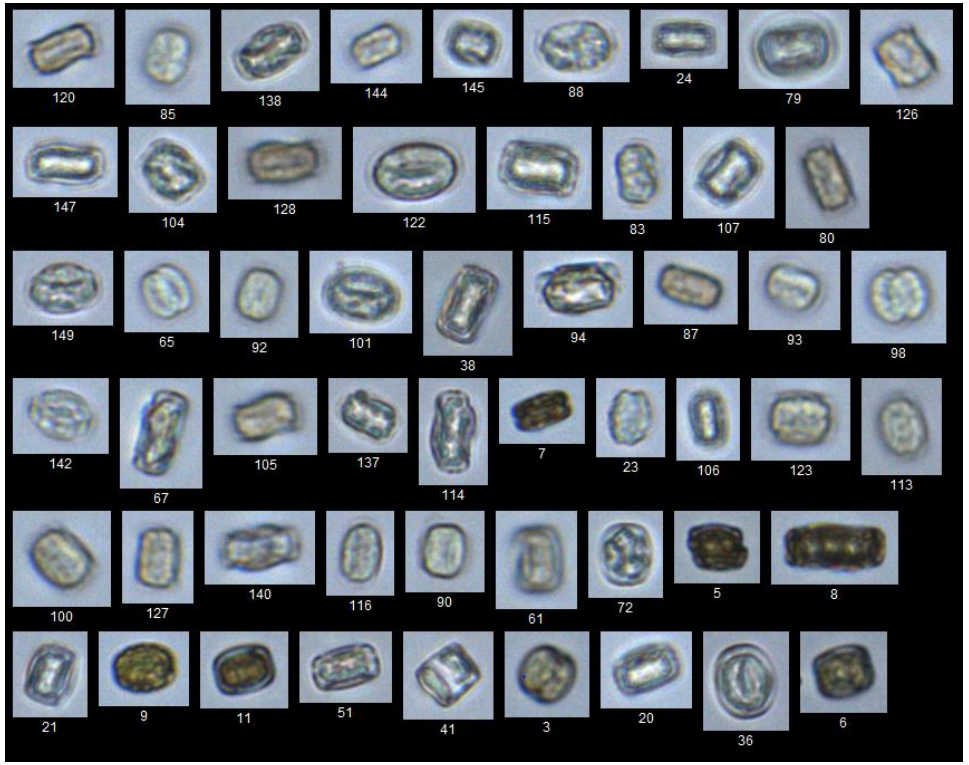
Auto-classification optimisation

Comparison to microscopy

e.g. *Cyclotella* or *Thalassiosira* spp. (616 total images)



Valve view
(444 images)



Girdle view
(153 images)

Sample preparation

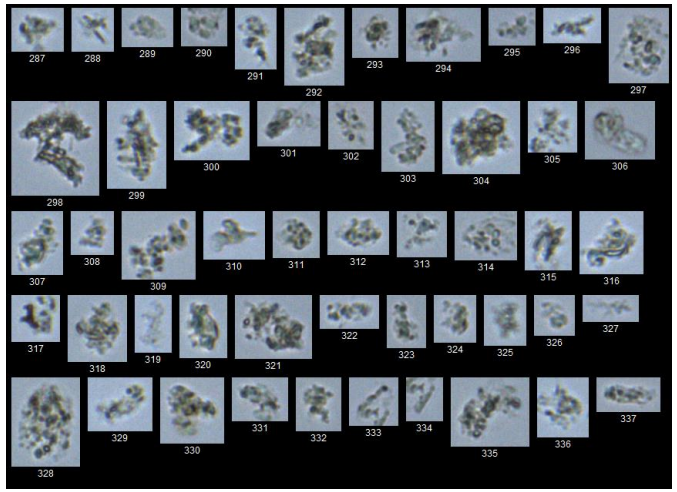
Run settings

Image Libraries

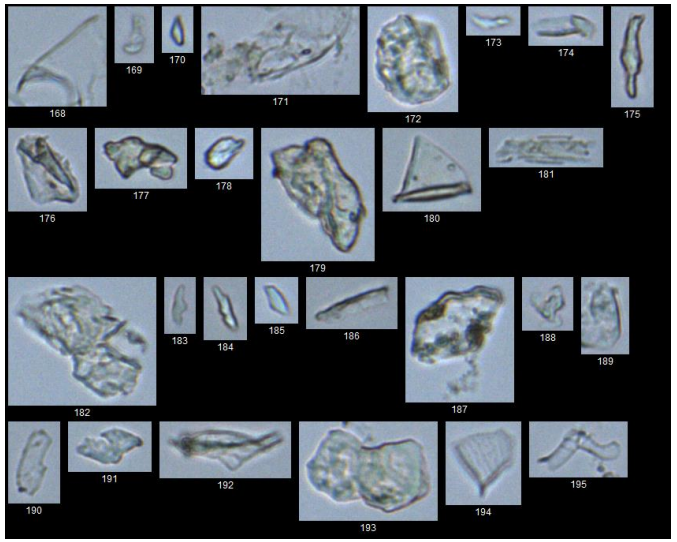
Auto-classification optimisation

Comparison to microscopy

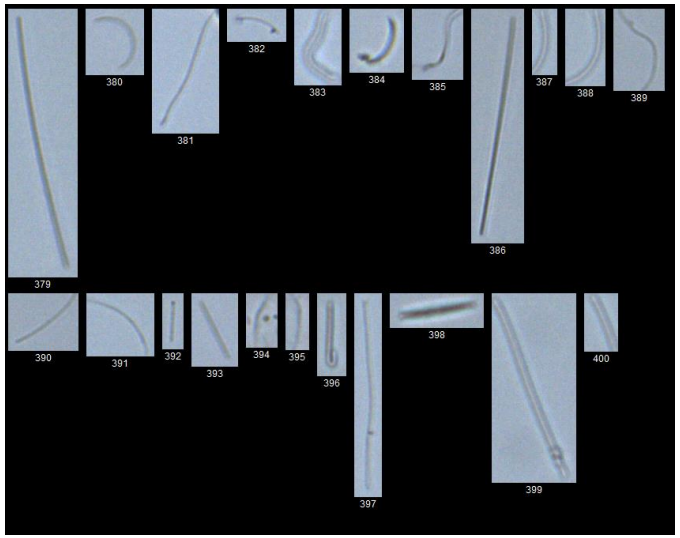
e.g. Detritus (10,952 total images)



Clusters of dots
(2,454 images)



Clear things
(450 images)



Long thin things
(582 images)

Sample preparation

Run settings

Image Libraries

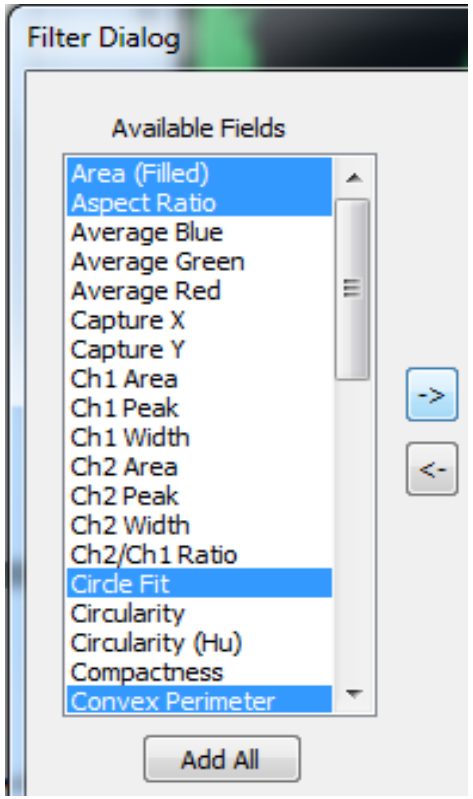
Auto-classification optimisation

Comparison to microscopy

VisualSpreadsheet® (version 4.12.3)

Classifier Advanced add-on

Selected particle properties:



Sample preparation

Run settings

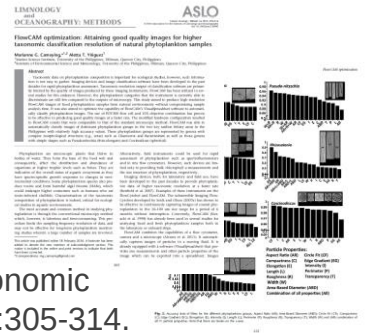
Image Libraries

Auto-classification optimisation

Comparison to microscopy

- One other study has assessed the auto-classification accuracy of VisualSpreadsheet®

$$\text{Filter Accuracy (FA)} = \frac{\text{True Positive (TP)} + \boxed{\text{True Negative (TN)}}}{\text{False Positive (FP)} + \text{False Negative (FN)} + \text{TP} + \boxed{\text{TN}}}$$



Camoying, MG & AT Yñiguez, 2016. FlowCAM optimization: Attaining good quality images for higher taxonomic classification resolution of natural phytoplankton samples. *Limnology and Oceanography: Methods* **14**(5):305-314.

Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy

$$\text{Recall (R)} = \%\ \text{of target images correctly identified}$$

$$\text{Precision (P)} = \%\ \text{of identifications as target that are correct}$$

$$\text{F1 Accuracy (F1)} = 2 \times \frac{\text{Precision (P)} \times \text{Recall (R)}}{\text{Precision (P)} + \text{Recall (R)}}$$

Sample preparation

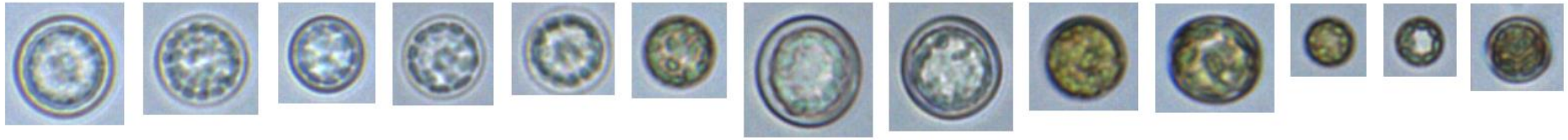
Run settings

Image Libraries

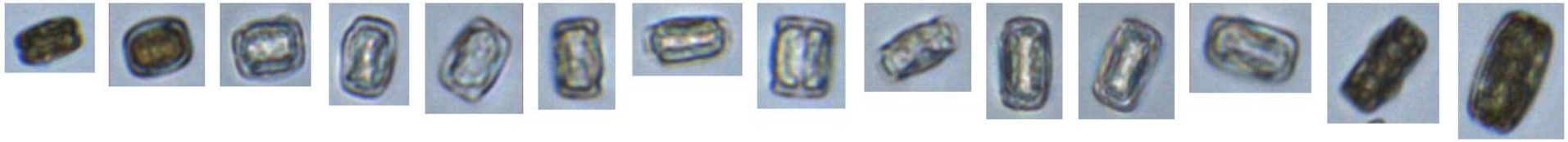
Auto-classification optimisation

Comparison to microscopy

Circle morphotype: *Cyclotella* spp. (valve)

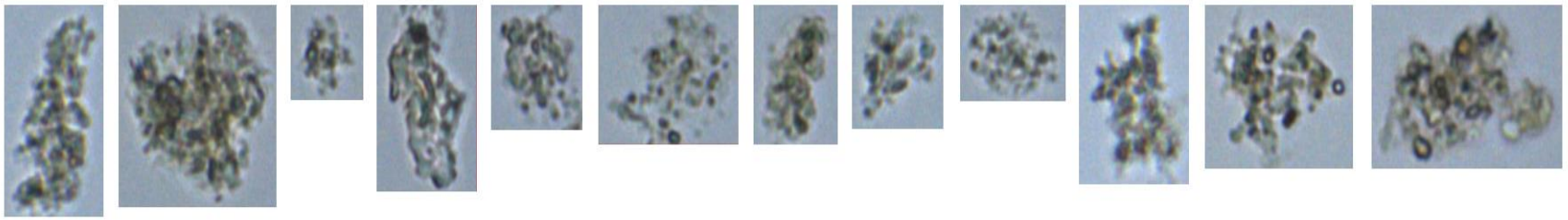


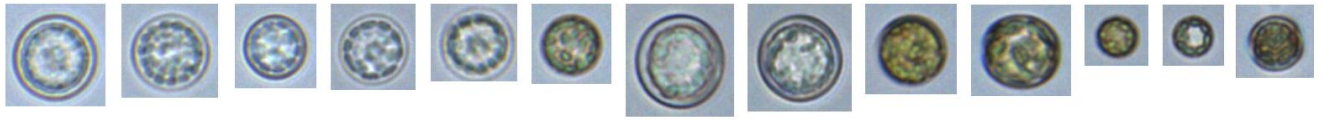
Rectangle morphotype: *Cyclotella* spp. (girdle)



Pointy morphotype: *Nitzschia* spp.

Irregular morphotype: Detritus (dots)





Sample preparation

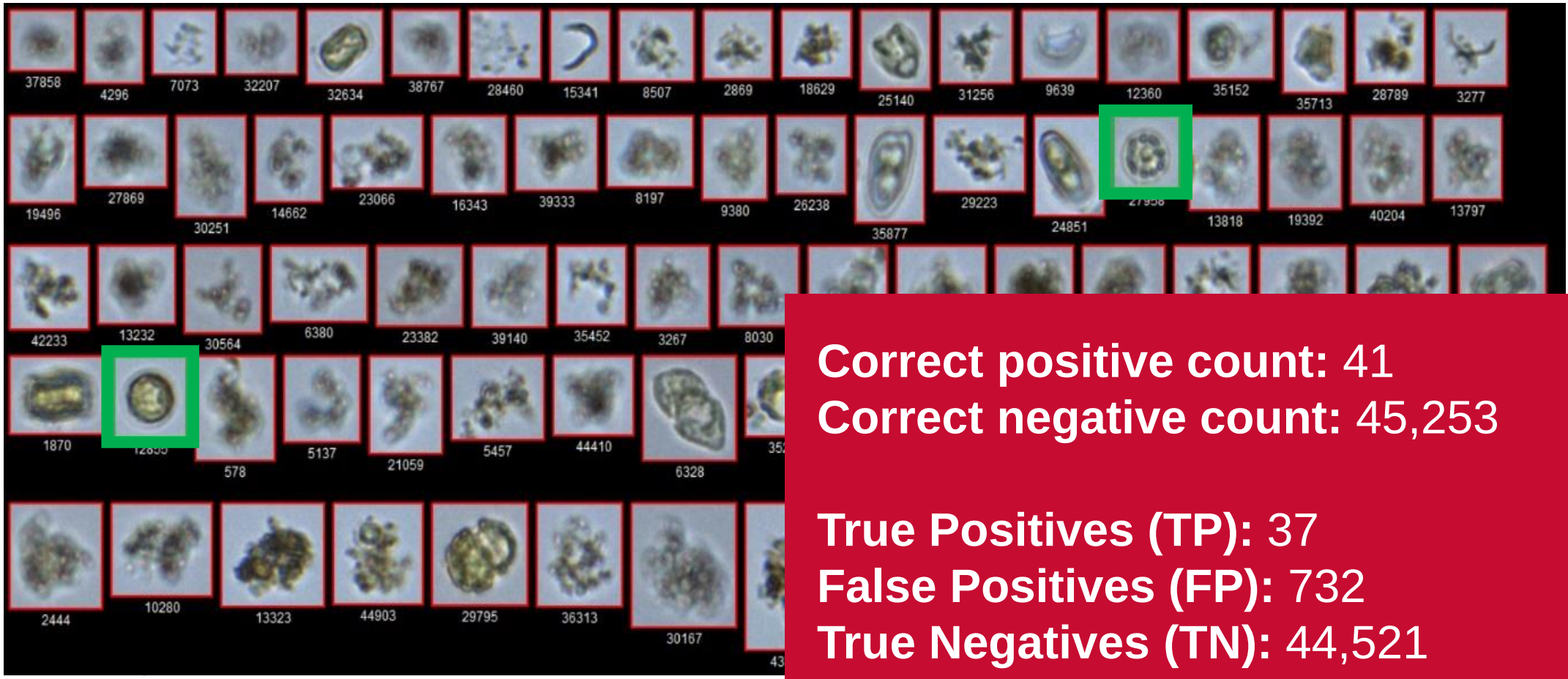
Run settings

Image Libraries

Auto-classification optimisation

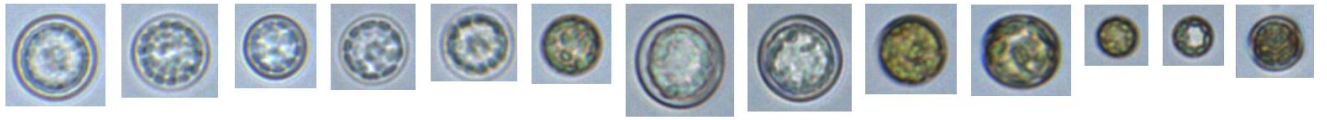
Comparison to microscopy

Circle morphotype, FA accuracy



Correct positive count: 41
Correct negative count: 45,253

True Positives (TP): 37
False Positives (FP): 732
True Negatives (TN): 44,521
False Negatives (FN): 4



Sample preparation

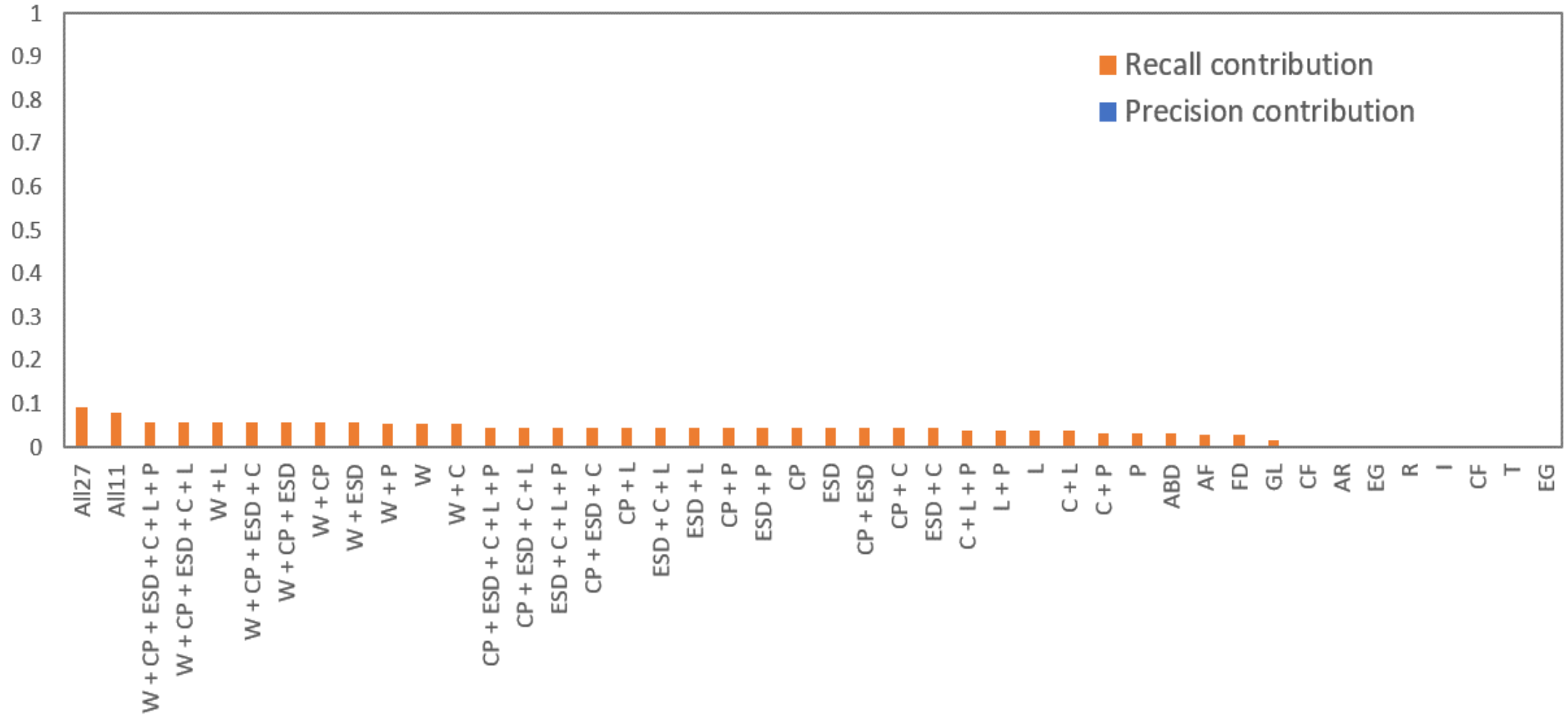
Run settings

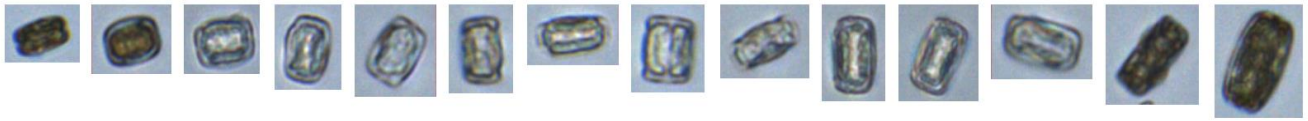
Image Libraries

Auto-classification optimisation

Comparison to microscopy

Circle morphotype, F1 accuracy





Sample preparation

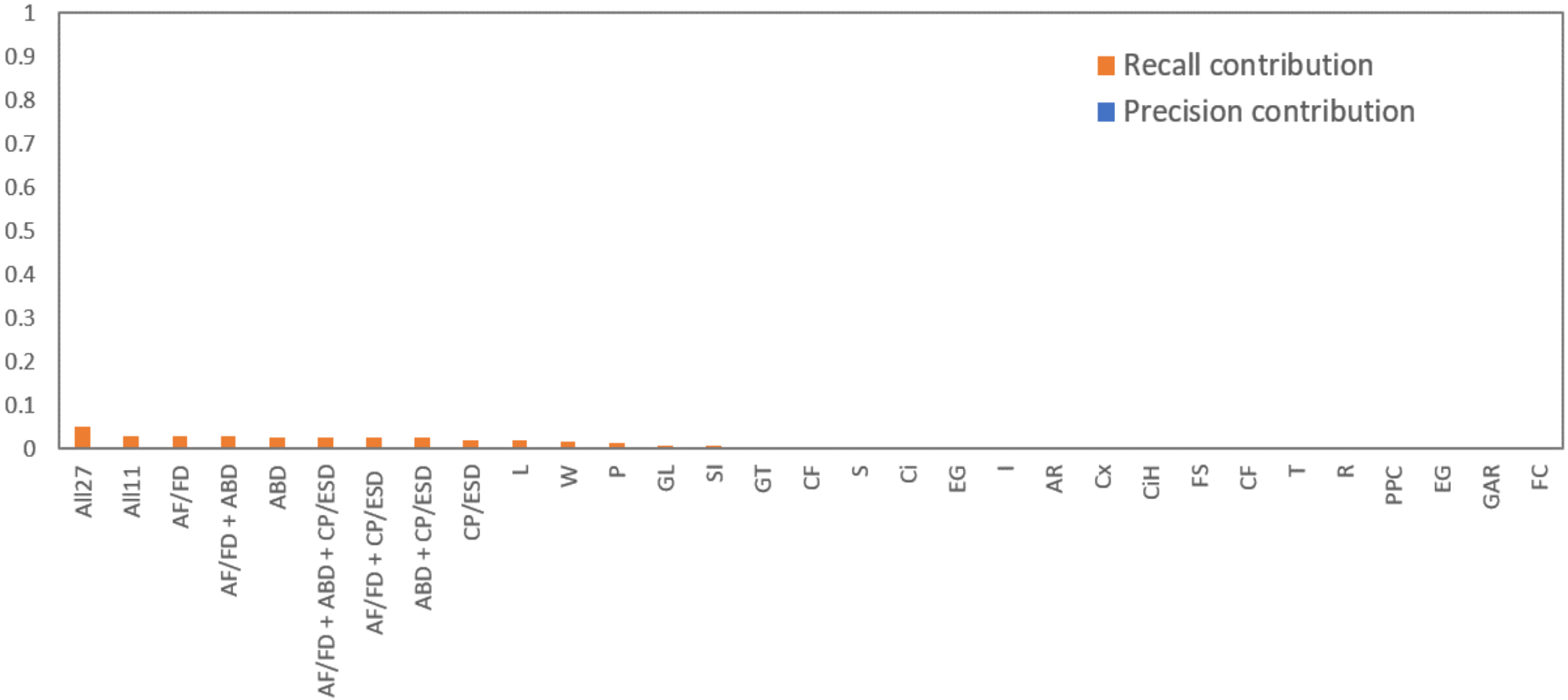
Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy

Rectangle morphotype, F1 accuracy



Sample preparation

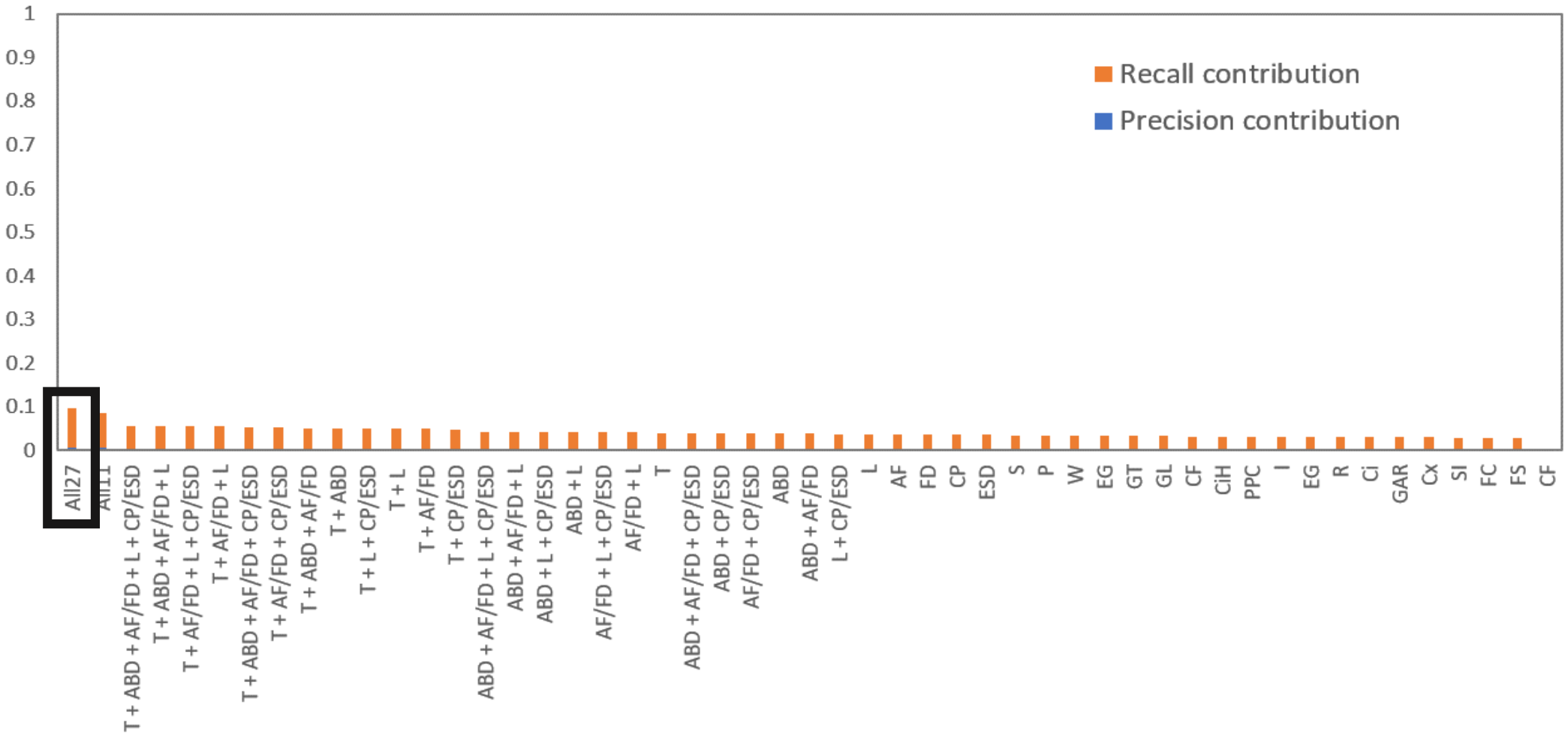
Run settings

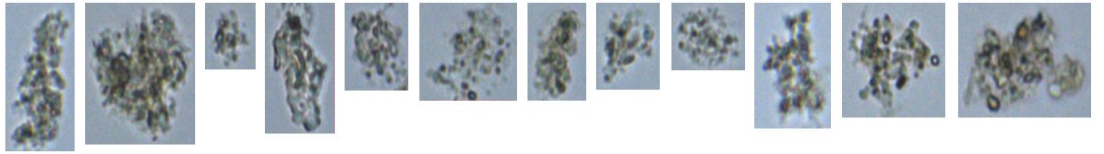
Image Libraries

Auto-classification optimisation

Comparison to microscopy

Pointy morphotype, F1 accuracy





Sample preparation

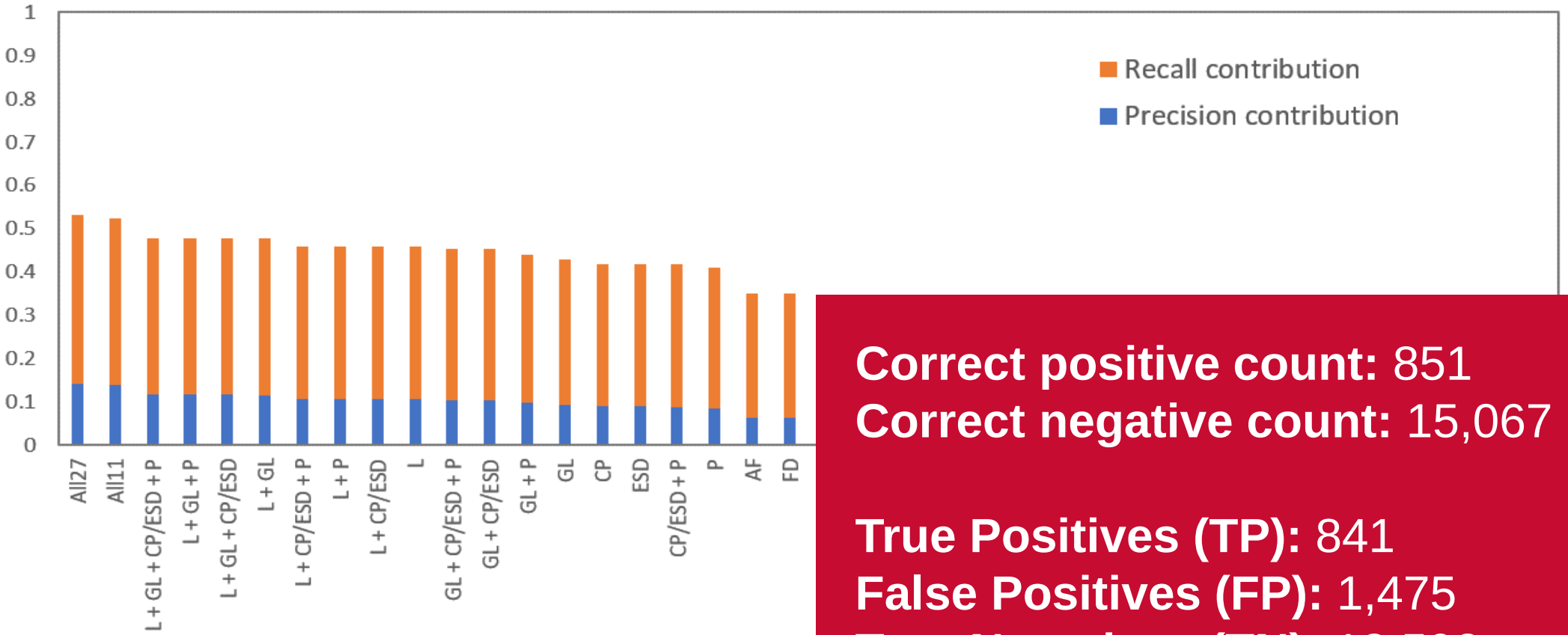
Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy

Irregular morphotype, F1 accuracy



Correct positive count: 851
Correct negative count: 15,067

True Positives (TP): 841
False Positives (FP): 1,475
True Negatives (TN): 13,592
False Negatives (FN): 10

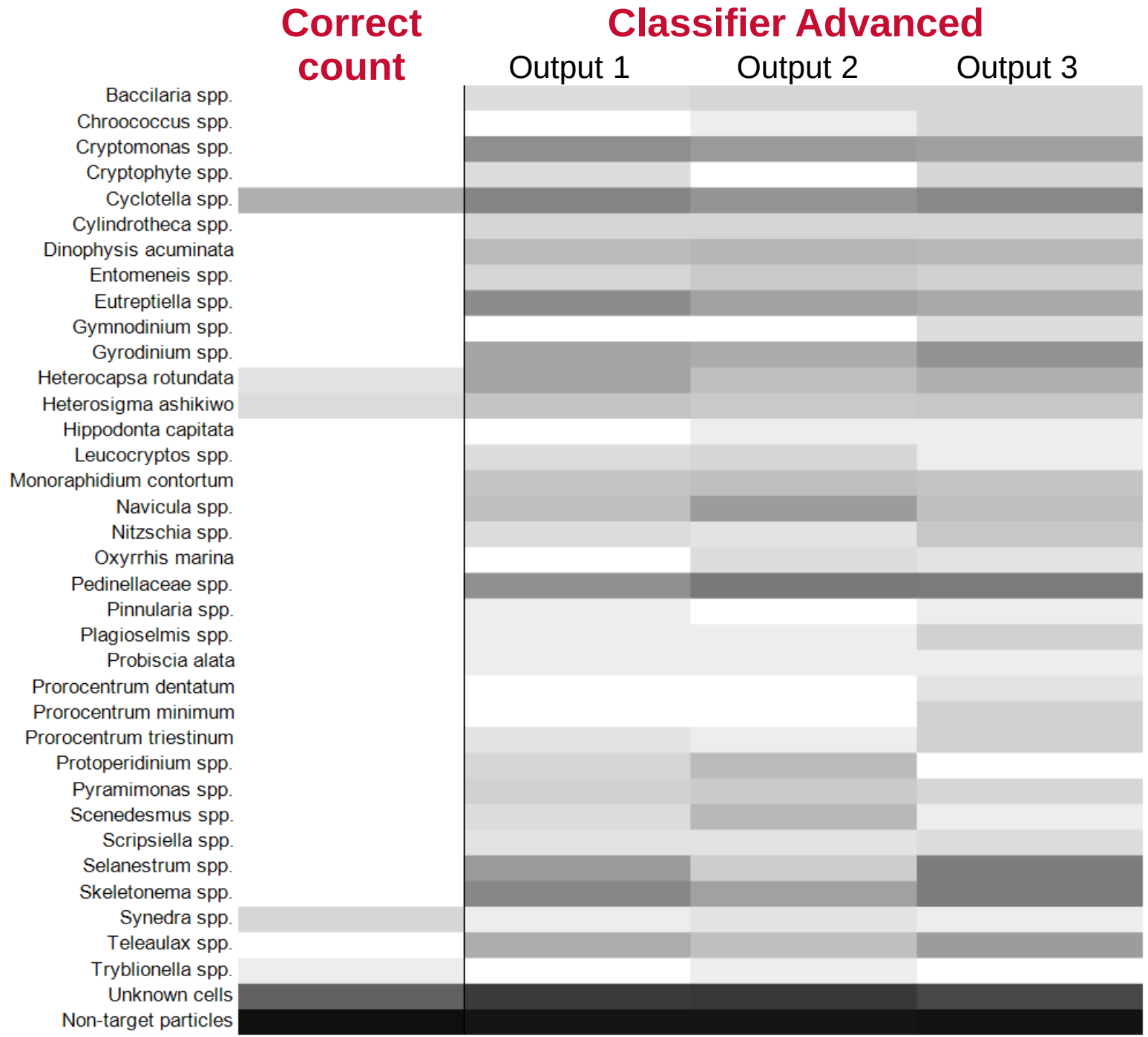
Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy



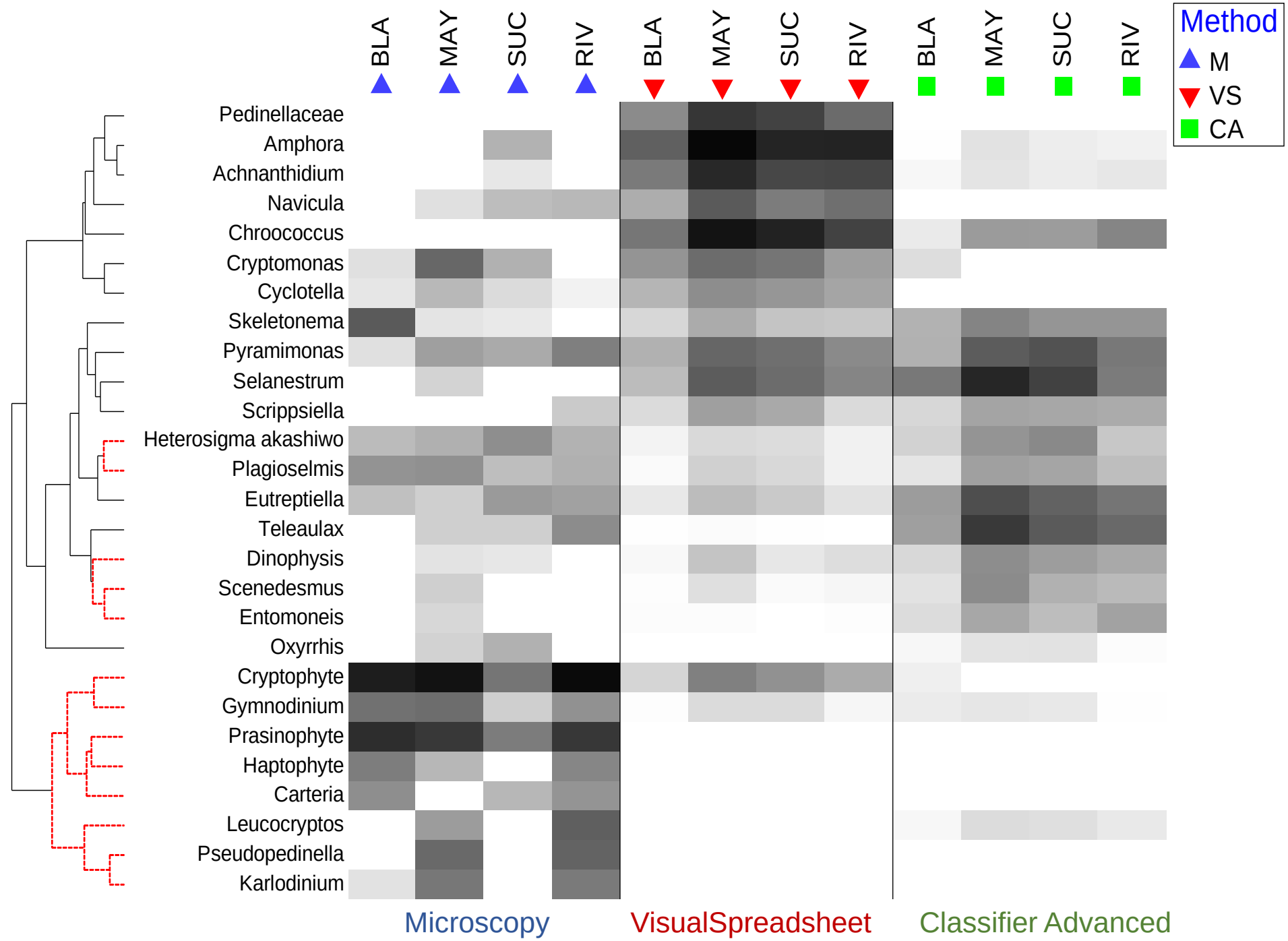
Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy



Sample preparation

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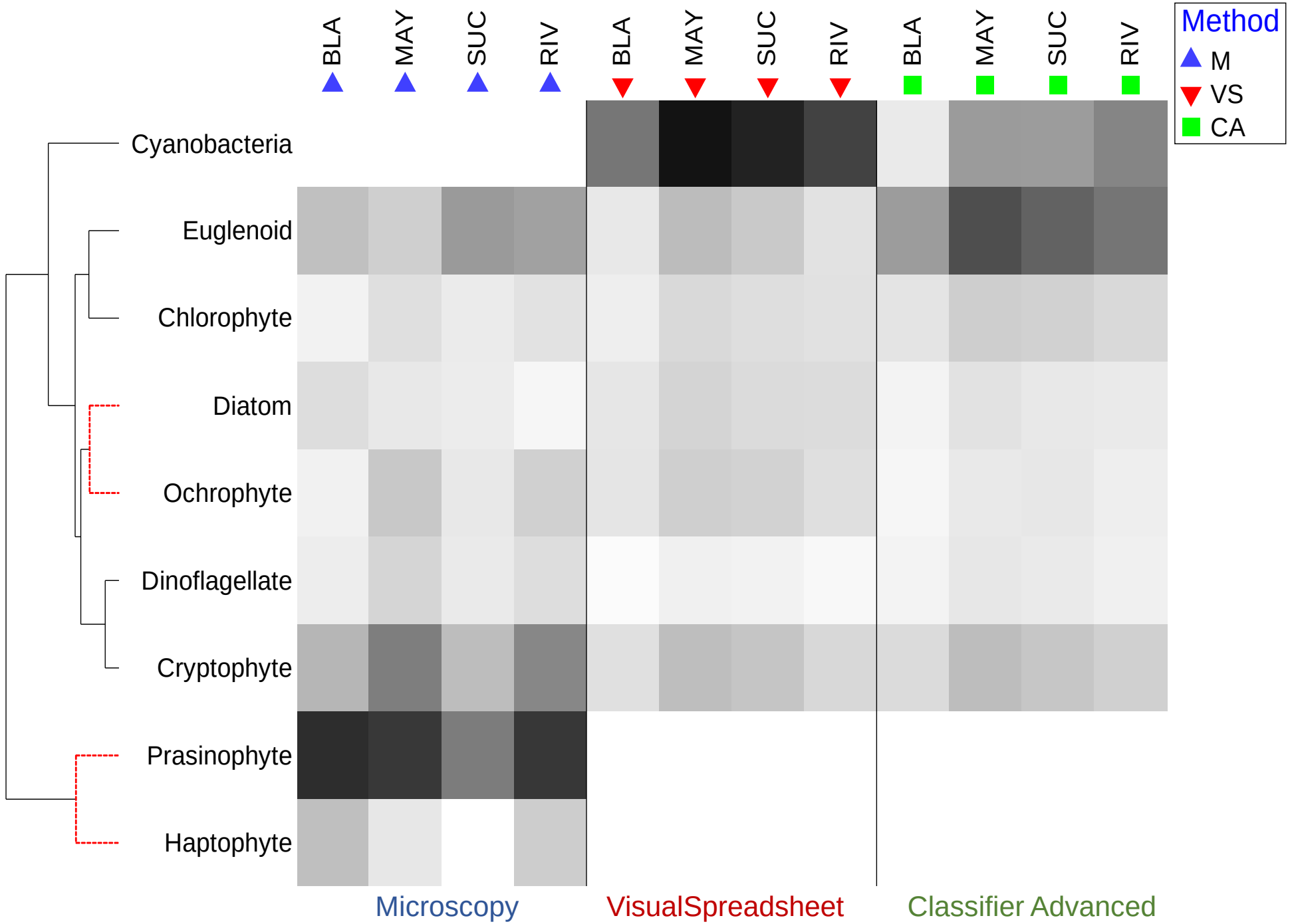
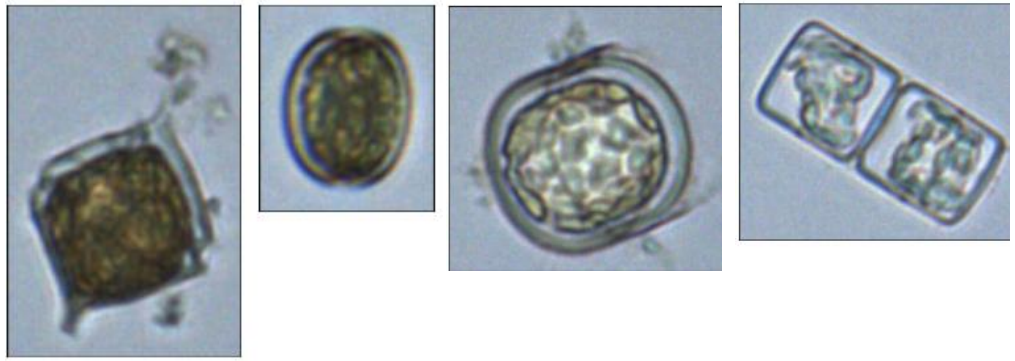
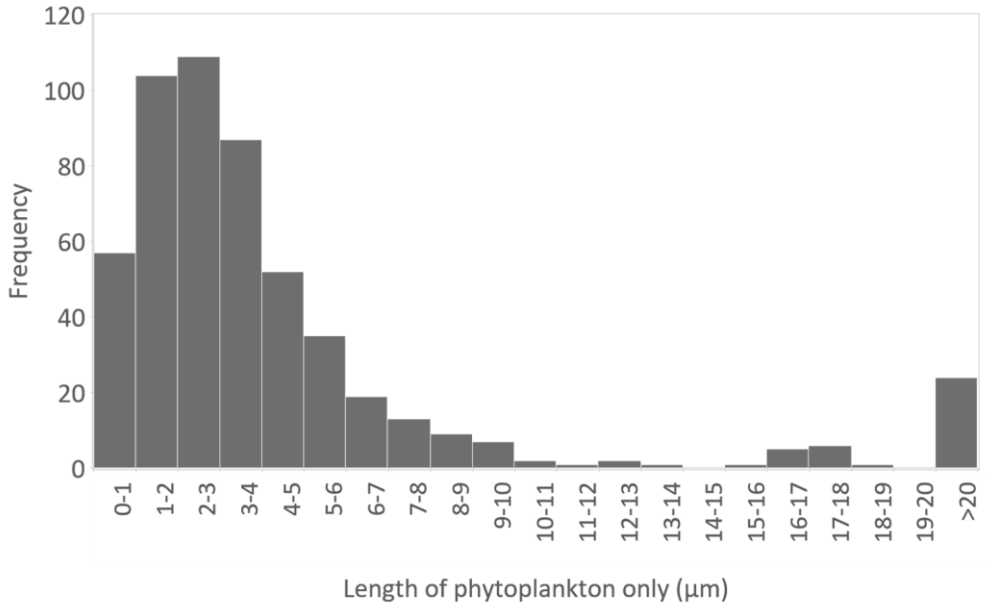
Auto-classification optimisation

Comparison to microscopy



What can the FlowCam® do for the Swan Canning?

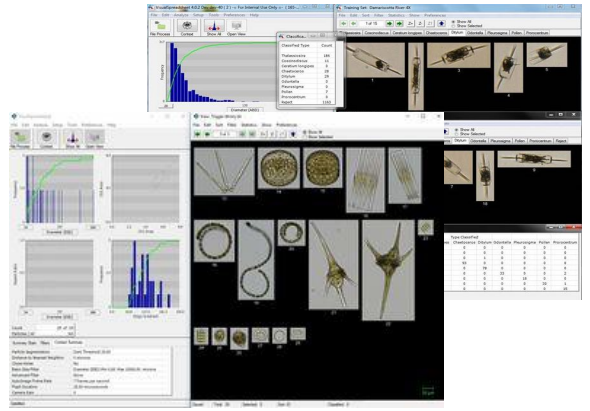




1. Clear digital images of phytoplankton
2. Measurements of phytoplankton

Issues with the FlowCam® for the Swan Canning

1. Time consuming
2. VisualSpreadsheet® and Classifier Advanced auto-classifications are not sufficient:
   - Very low accuracy
   - Inconsistency between and within software
   - No correlation with microscopy results



Can try alternative software packages to analyse the images collected with the FlowCam®

Can the FlowCam® be used to analyse Swan Canning phytoplankton samples?

FlowCam® software auto-classification accuracies are not currently sufficient for preserved turbid samples

Do you have any questions?

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