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# Evaluating the potential application of automated FlowCam<sup>®</sup> technology for phytoplankton monitoring in the Swan Canning Estuary

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Department of Biodiversity,  
Conservation and Attractions



Government of Western Australia  
Department of Water and Environmental Regulation



CSIRO



Department of **Biodiversity,  
Conservation and Attractions**



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



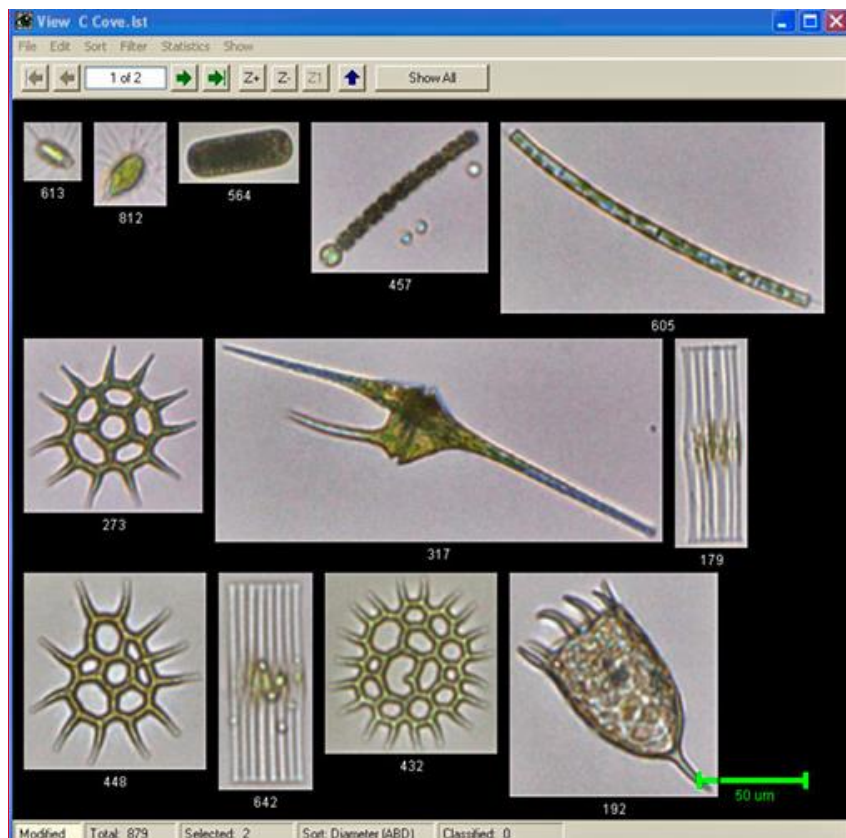


**Can the FlowCam<sup>®</sup> be used to analyse Swan  
Canning phytoplankton samples?**

# FlowCam™



FLUID IMAGING TECHNOLOGIES, INC.





# Possible FlowCam<sup>®</sup> 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<sup>®</sup> for turbid and preserved samples



Portable FlowCam



Benchtop FlowCam

# Can the FlowCam<sup>®</sup> be used to analyse Swan Canning phytoplankton samples?

Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy





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

Sample preparation

Run settings

Image Libraries

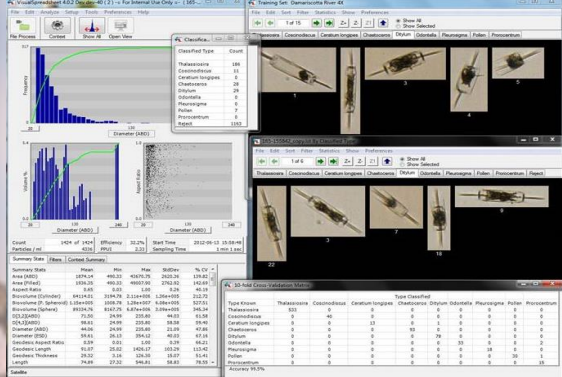
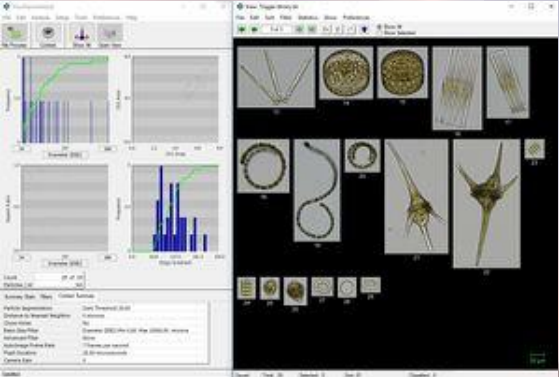
Auto-classification optimisation

Comparison to microscopy



VisualSpreadsheet®

Classifier Advanced



Sample  
preparation

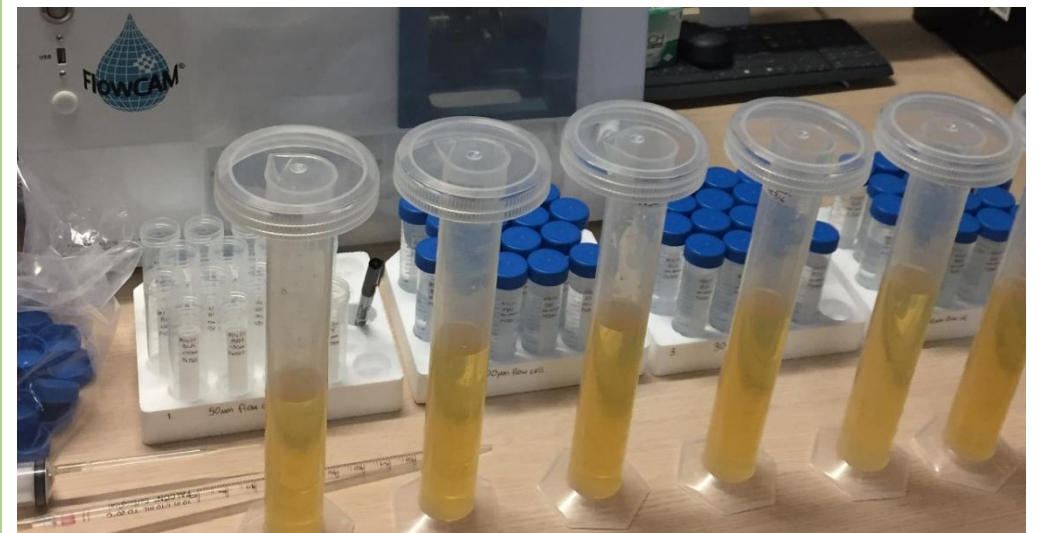
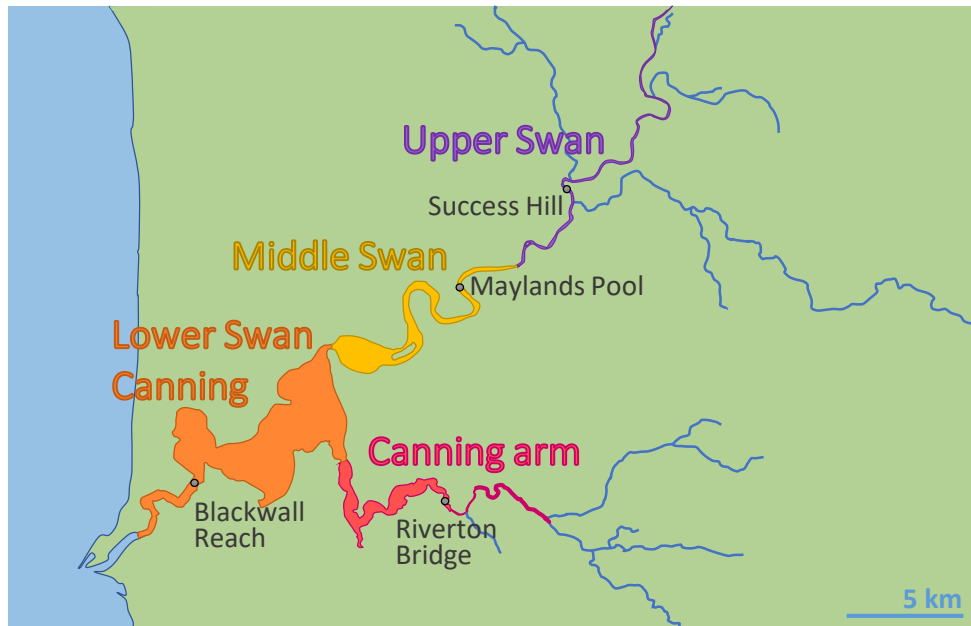
Run settings

Image  
Libraries

Auto-  
classification  
optimisation

Comparison  
to microscopy

- 12 library samples
- 12 test samples
- 0-50  $\mu\text{m}$  size fraction
- Concentrated



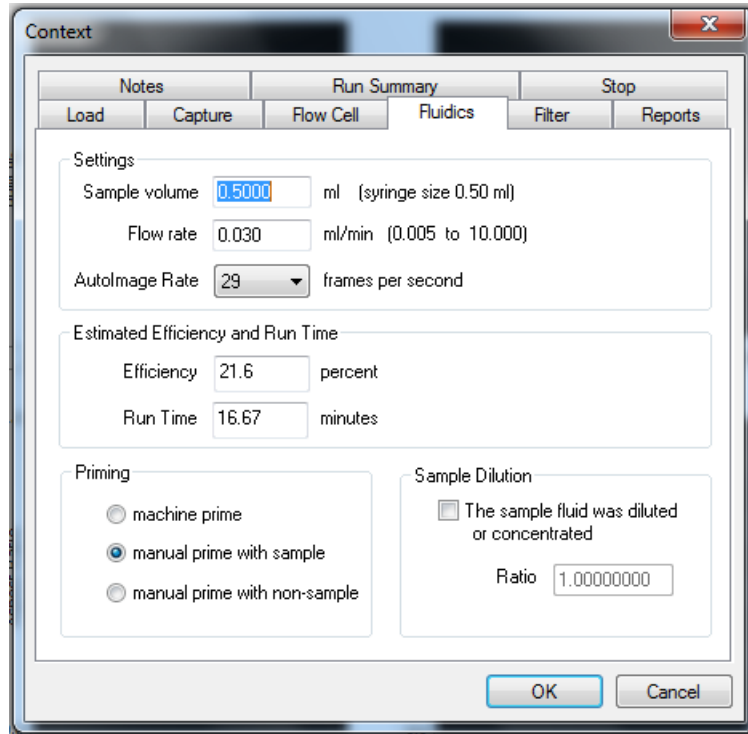
Sample  
preparation

Run settings

Image  
Libraries

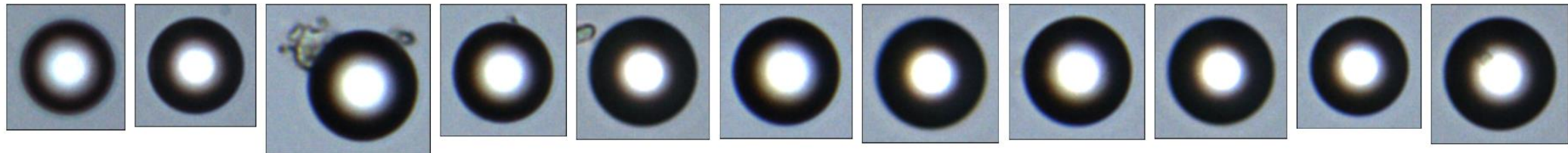
Auto-  
classification  
optimisation

Comparison  
to microscopy



- FlowCam<sup>®</sup> VS I updated in 2013 (equivalent to VS IV)
- 50  $\mu\text{m}$  flow cell and 20X objective

Sample  
preparation



Run settings

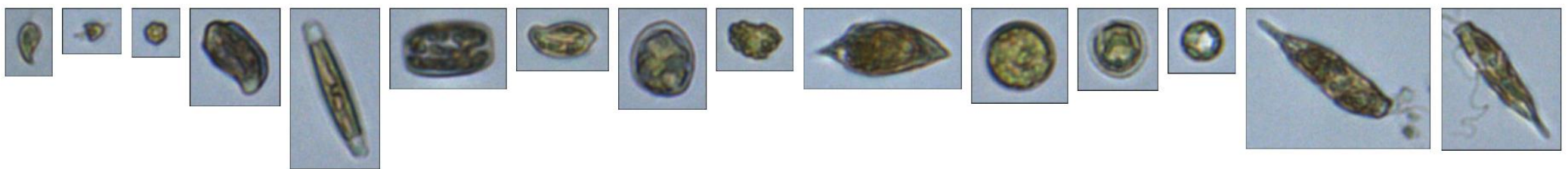
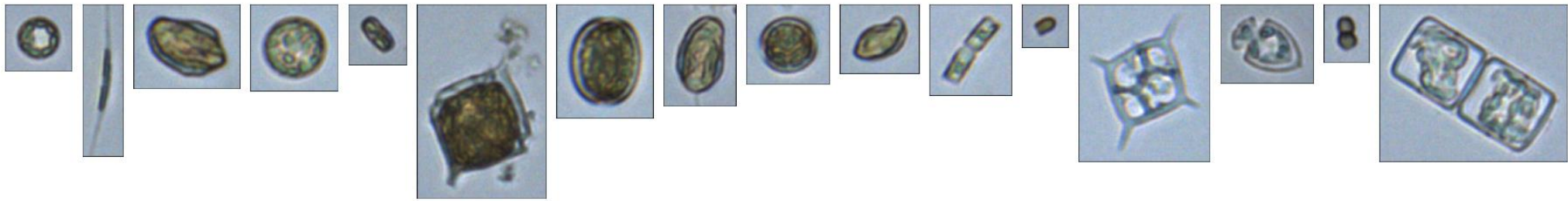


Image  
Libraries



Auto-  
classification  
optimisation



Comparison  
to microscopy

50µm

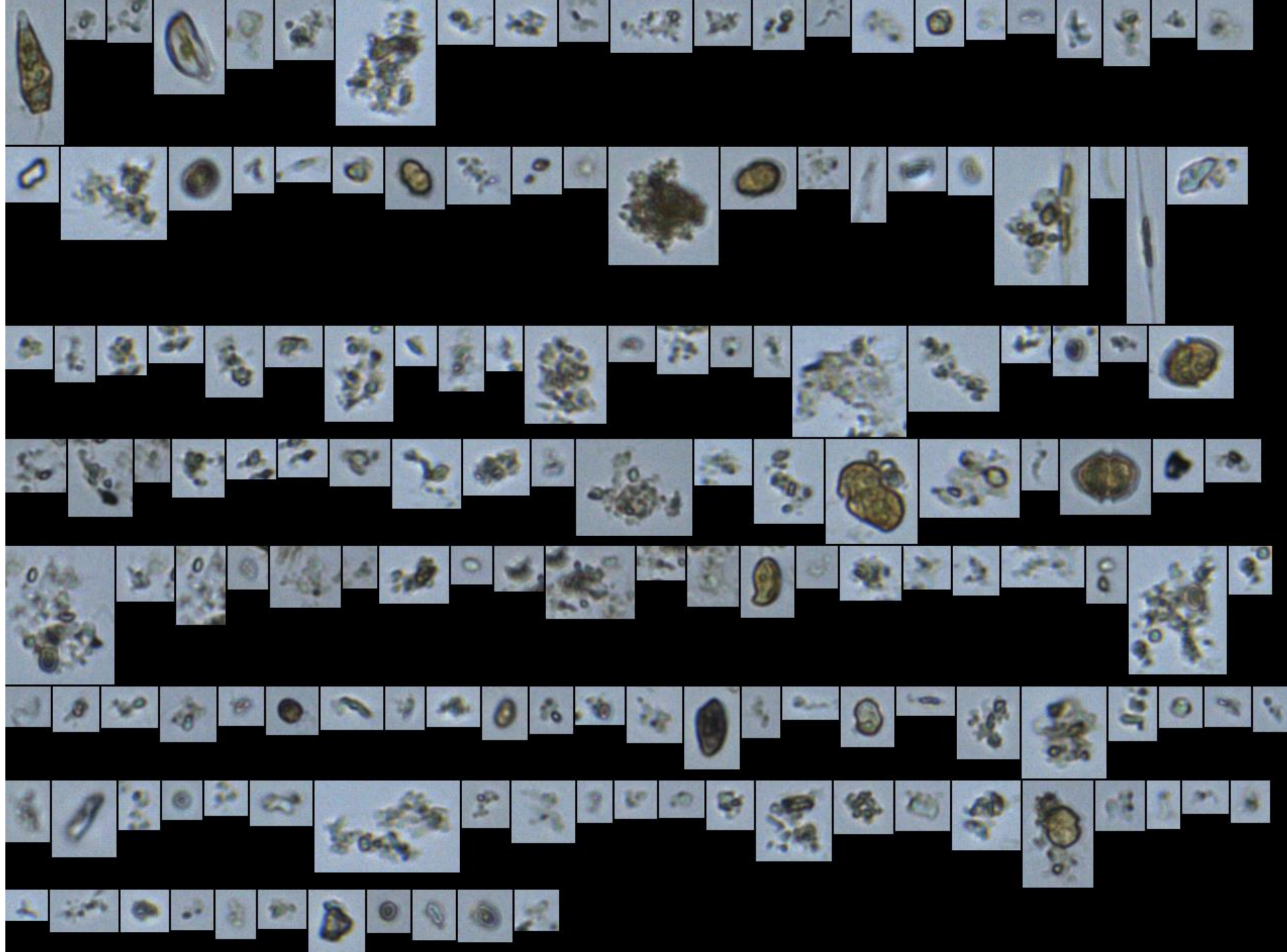
Sample preparation

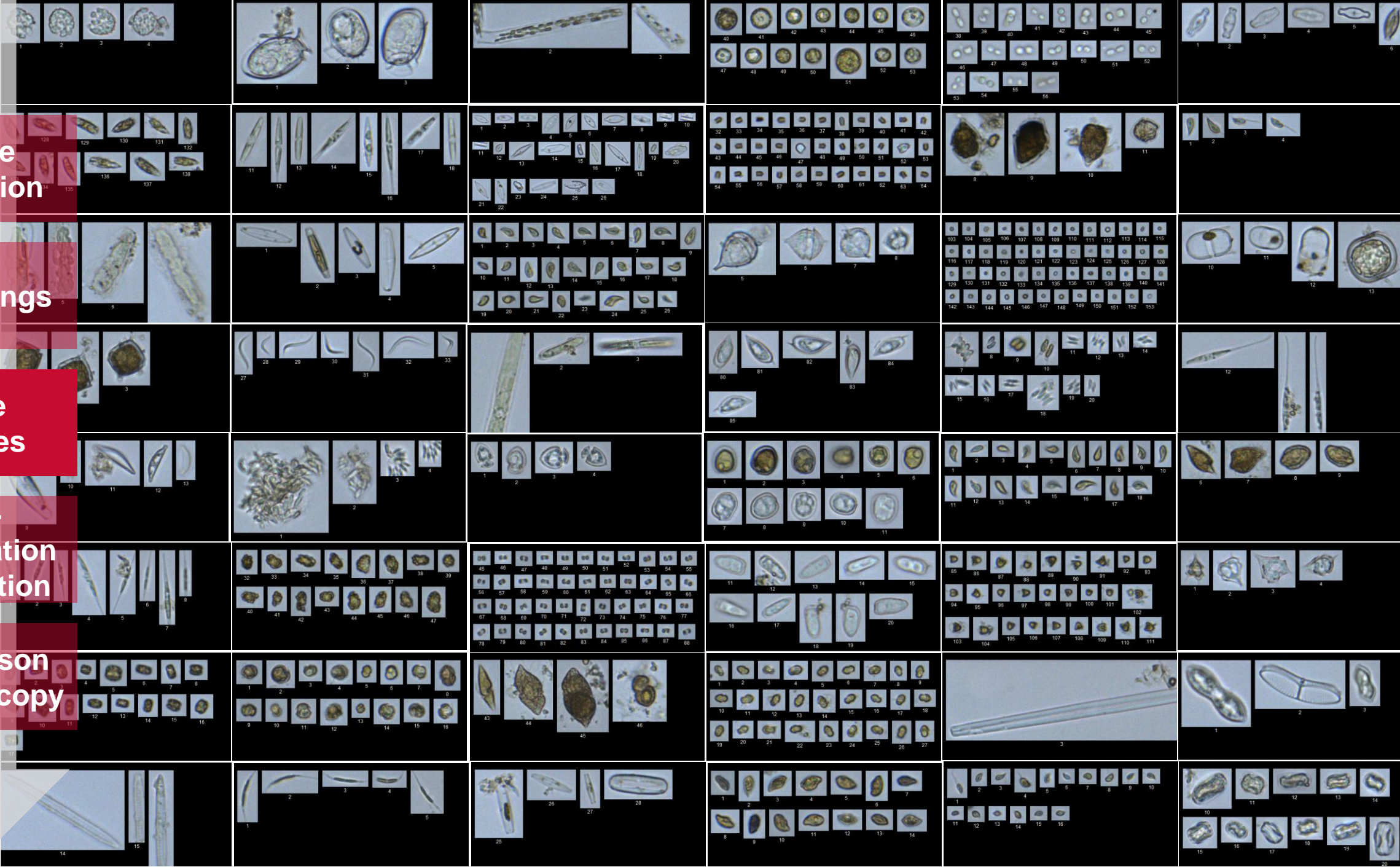
Run settings

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)

Sample  
preparation

Run settings

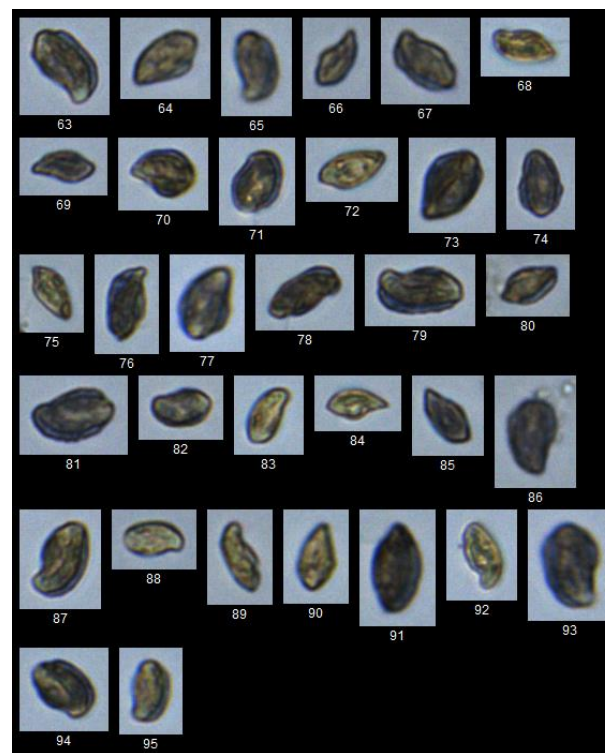
Image  
Libraries

Auto-  
classification  
optimisation

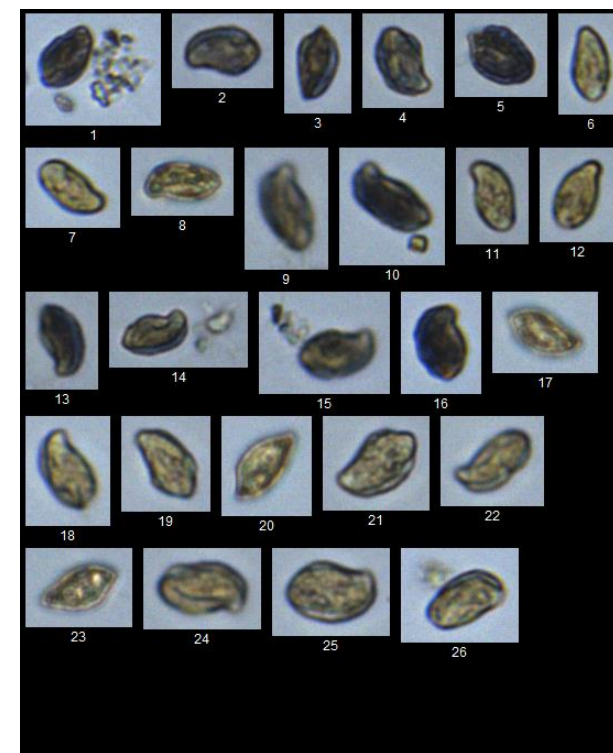
Comparison  
to microscopy



6-15  $\mu\text{m}$   
(76 images)

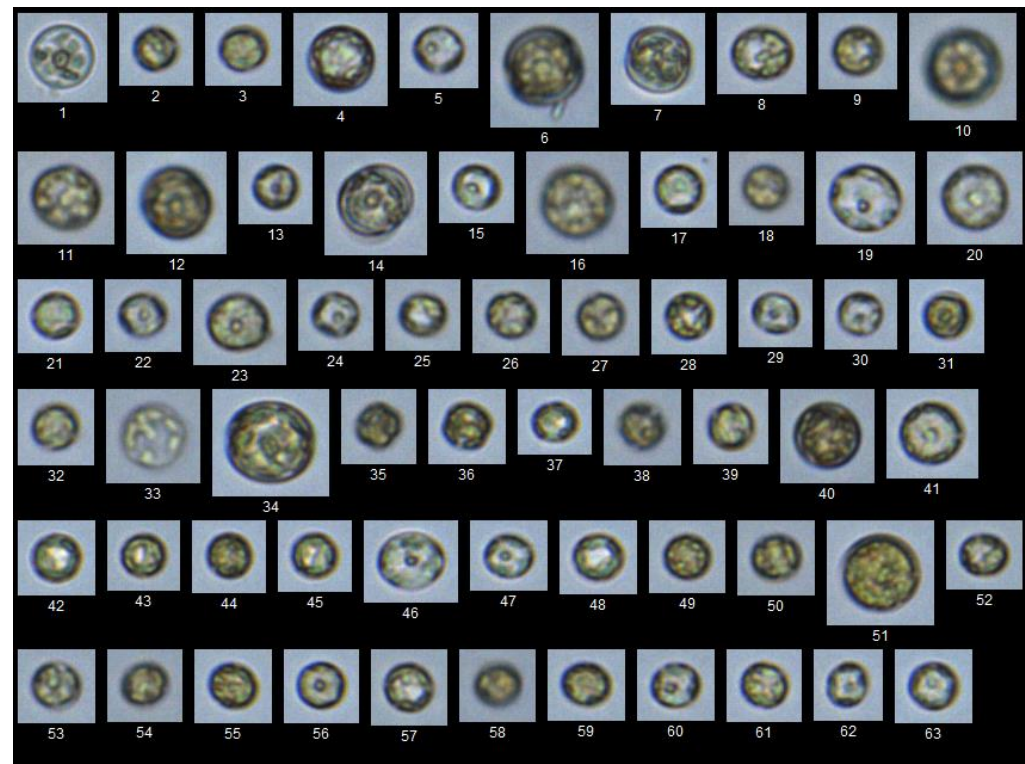


15-25  $\mu\text{m}$   
(744 images)

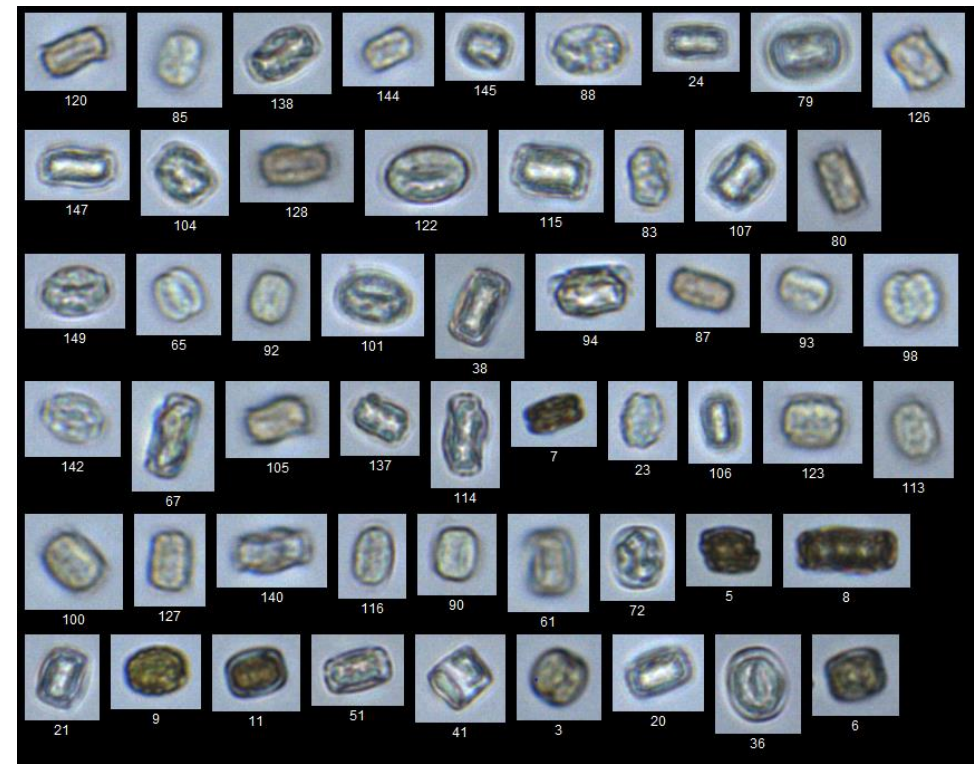


>25  $\mu\text{m}$   
(27 images)

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



Valve view  
(444 images)



Girdle view  
(153 images)

Sample preparation

Run settings

Image Libraries

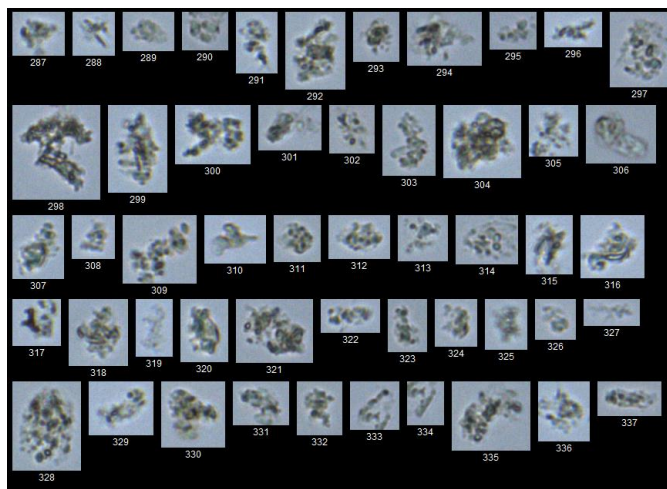
Auto-classification optimisation

Comparison to microscopy

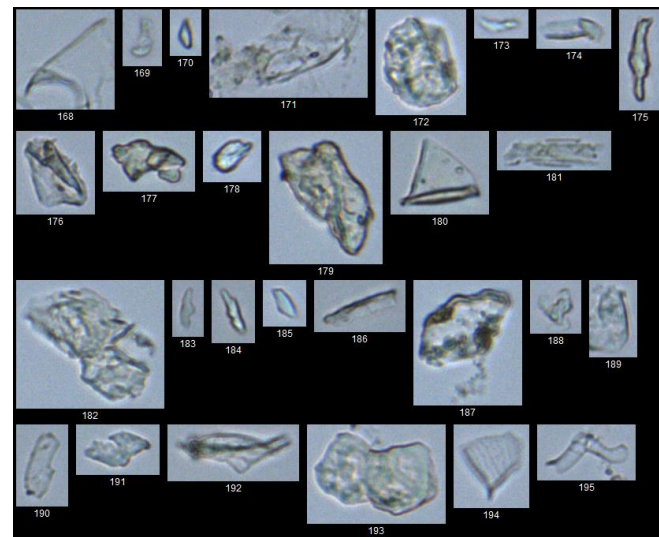




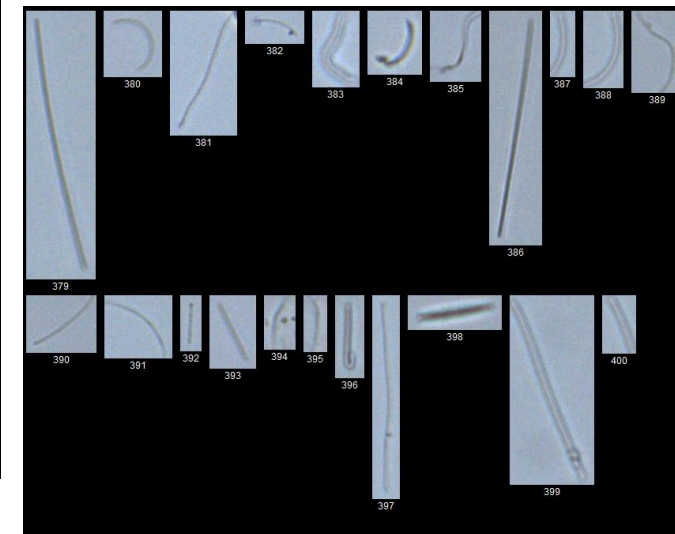
e.g. Detritus (10,952 total images)



Clusters of dots  
(2,454 images)



Clear things  
(450 images)



Long thin things  
(582 images)

Sample  
preparation

Run settings

Image  
Libraries

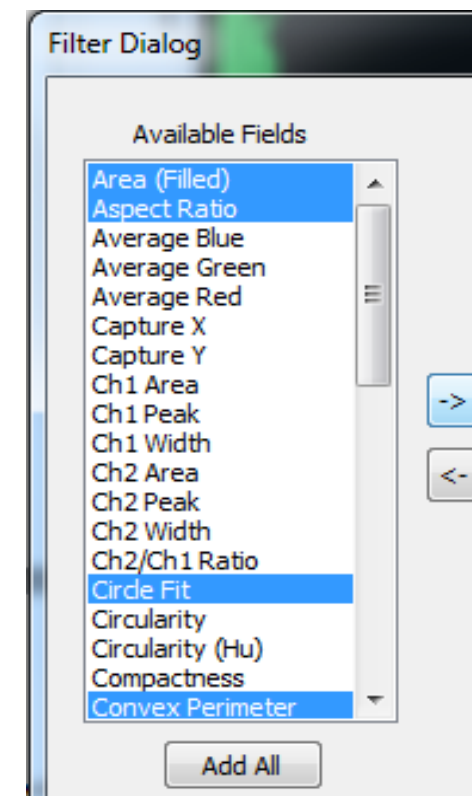
Auto-  
classification  
optimisation

Comparison  
to microscopy



# VisualSpreadsheet<sup>®</sup> (version 4.12.3) Classifier Advanced add-on

Selected particle properties:



Sample  
preparation

Run settings

Image  
Libraries

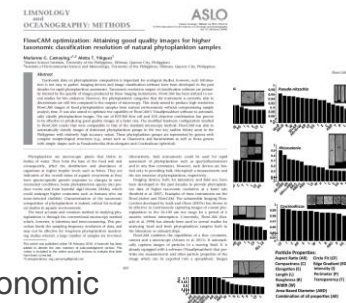
Auto-  
classification  
optimisation

Comparison  
to microscopy

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

Filter Accuracy (FA) =

$$\frac{\text{True Positive (TP)} + \text{True Negative (TN)}}{\text{False Positive (FP)} + \text{False Negative (FN)} + \text{TP} + \text{TN}}$$



Sample  
preparation

Recall (R) = % of target images correctly identified

Run settings

Image  
Libraries

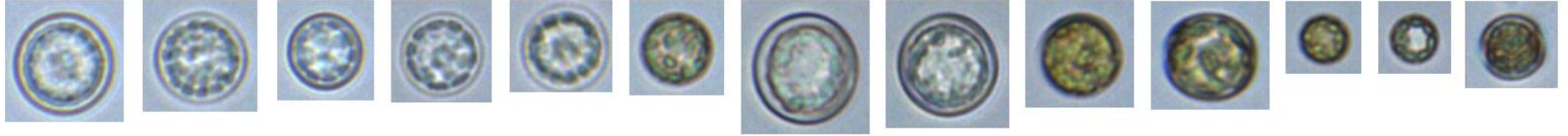
Precision (P) = % of identifications as target that are correct

Auto-  
classification  
optimisation

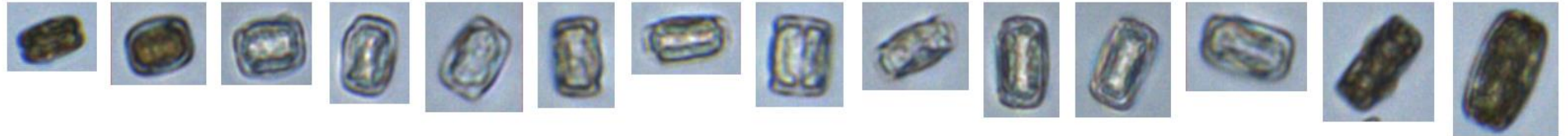
Comparison  
to microscopy

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

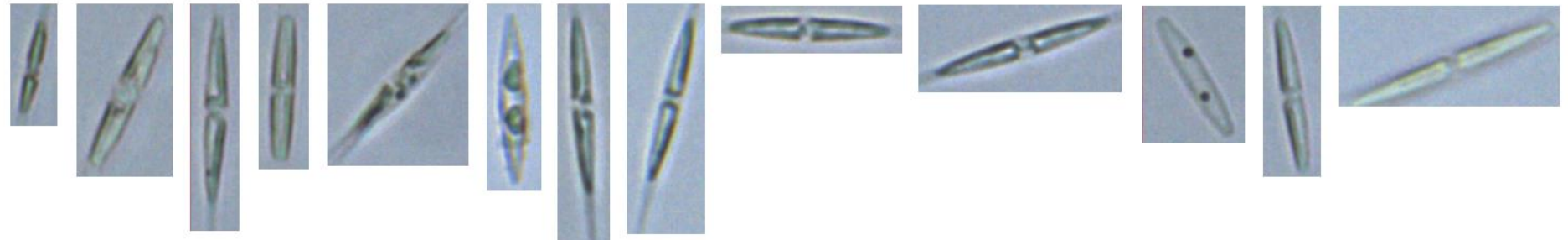
**Circle morphotype:** *Cyclotella* spp. (valve)



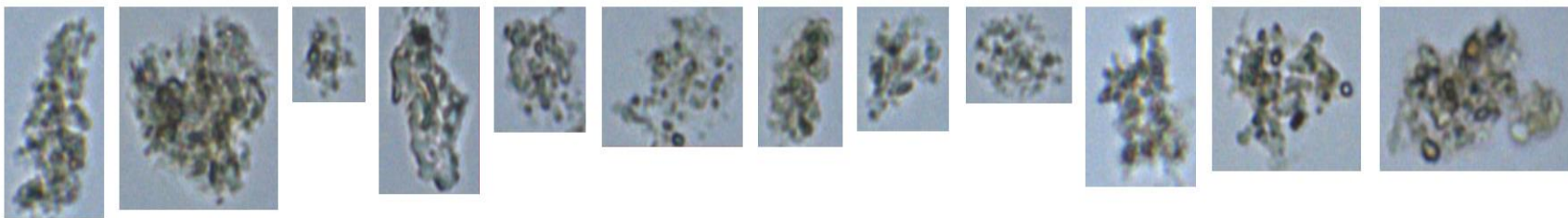
**Rectangle morphotype:** *Cyclotella* spp. (girdle)



**Pointy morphotype:** *Nitzschia* spp.



**Irregular morphotype:** Detritus (dots)



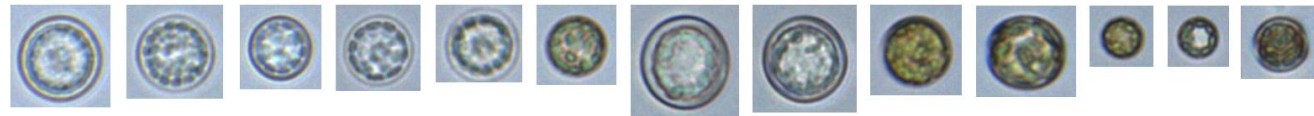
Sample preparation

Run settings

Image Libraries

Auto-classification optimisation

Comparison to microscopy



## Circle morphotype, FA accuracy

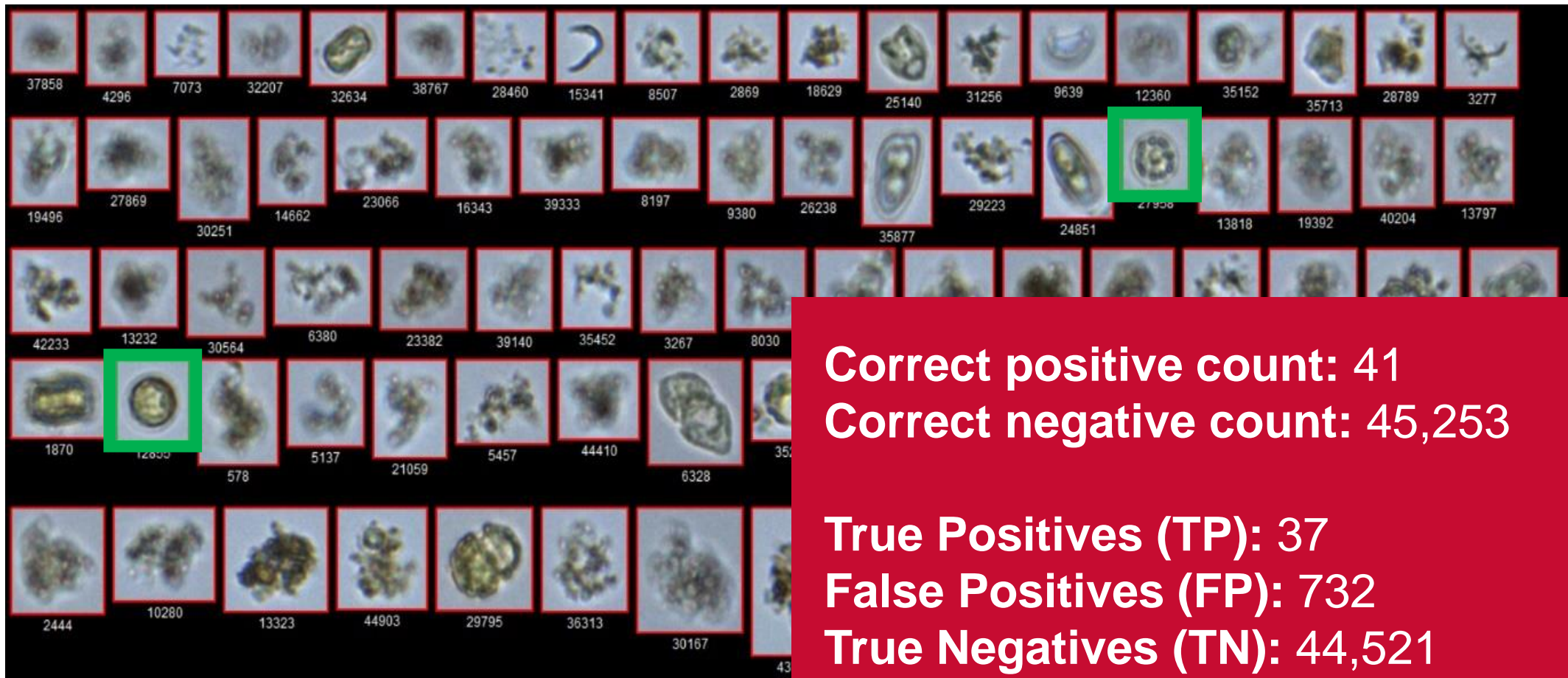
Sample preparation

Run settings

Image Libraries

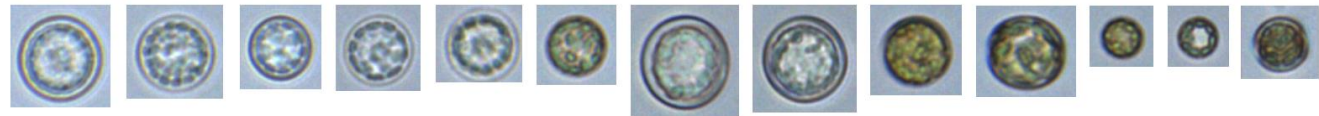
Auto-classification optimisation

Comparison to microscopy



**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**



# Circle morphotype, F1 accuracy

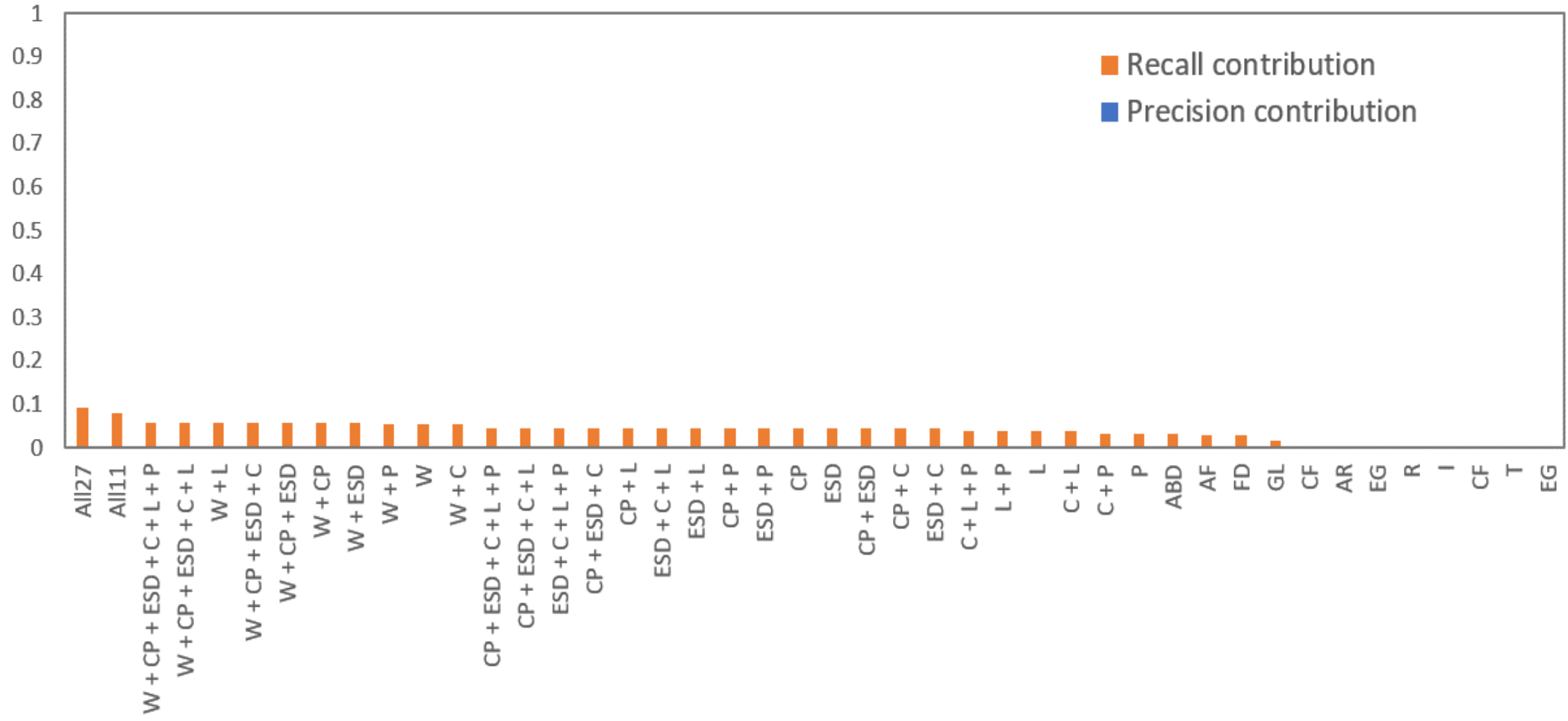
Sample preparation

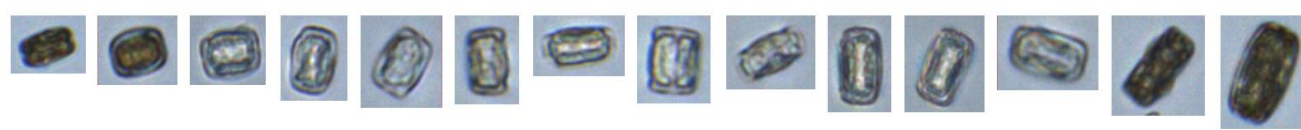
Run settings

Image Libraries

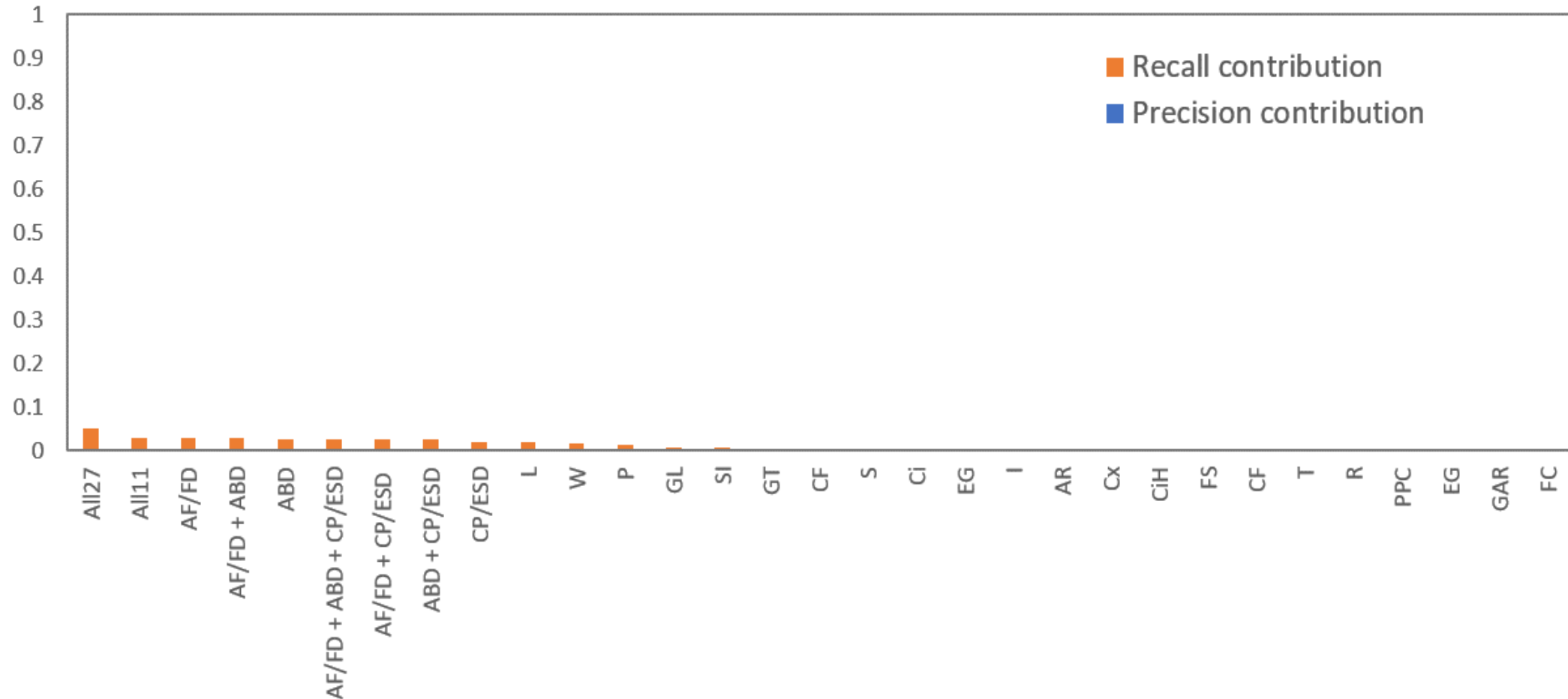
Auto-classification optimisation

Comparison to microscopy





## Rectangle morphotype, F1 accuracy



Sample preparation

Run settings

Image Libraries

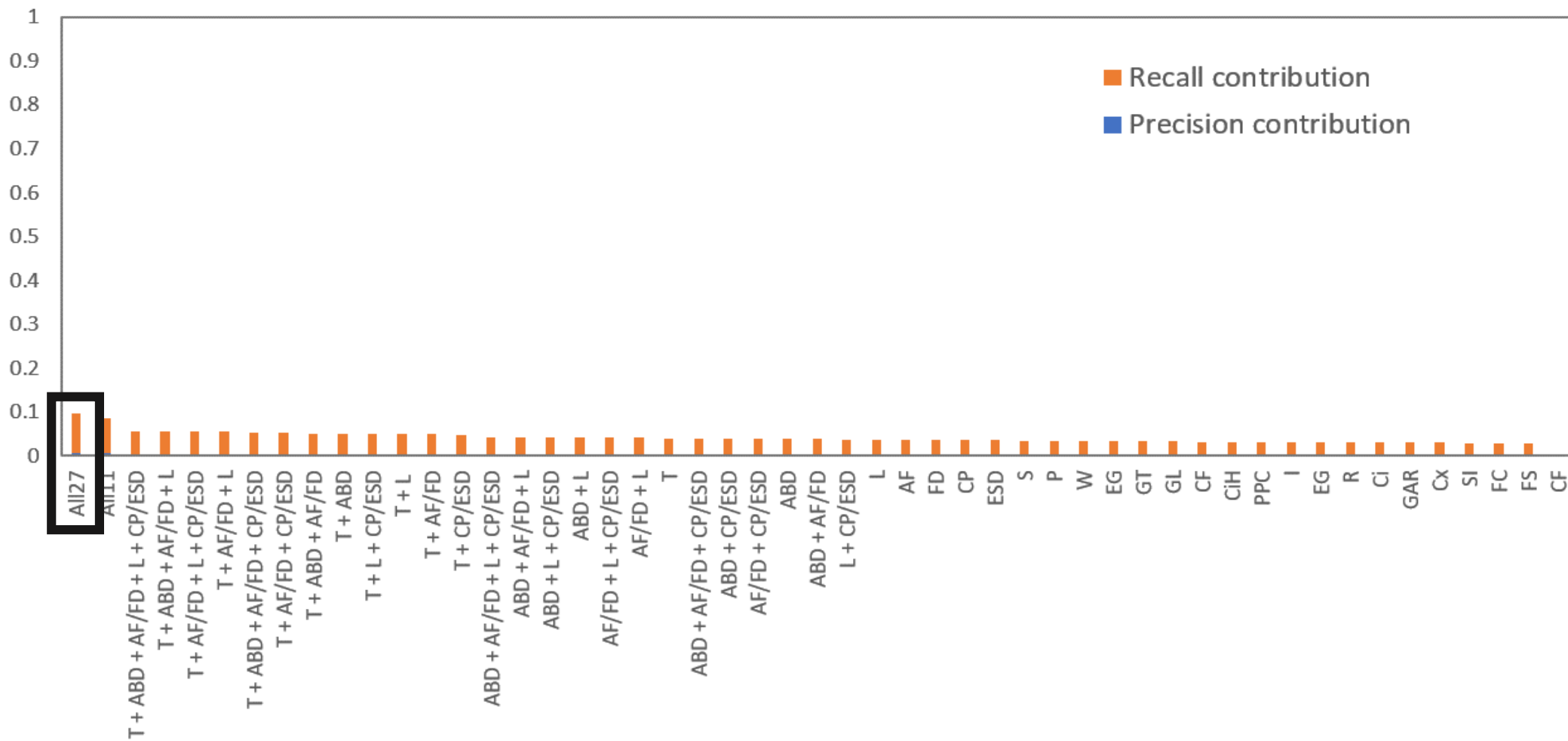
Auto-classification optimisation

Comparison to microscopy





## Pointy morphotype, F1 accuracy



Sample preparation

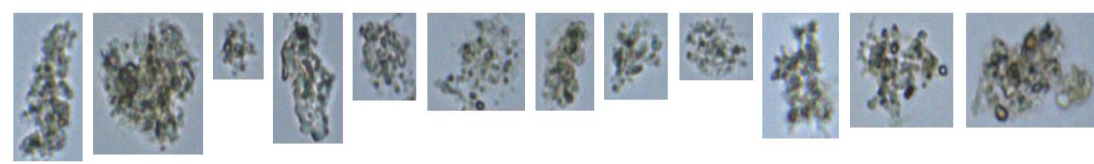
Run settings

Image Libraries

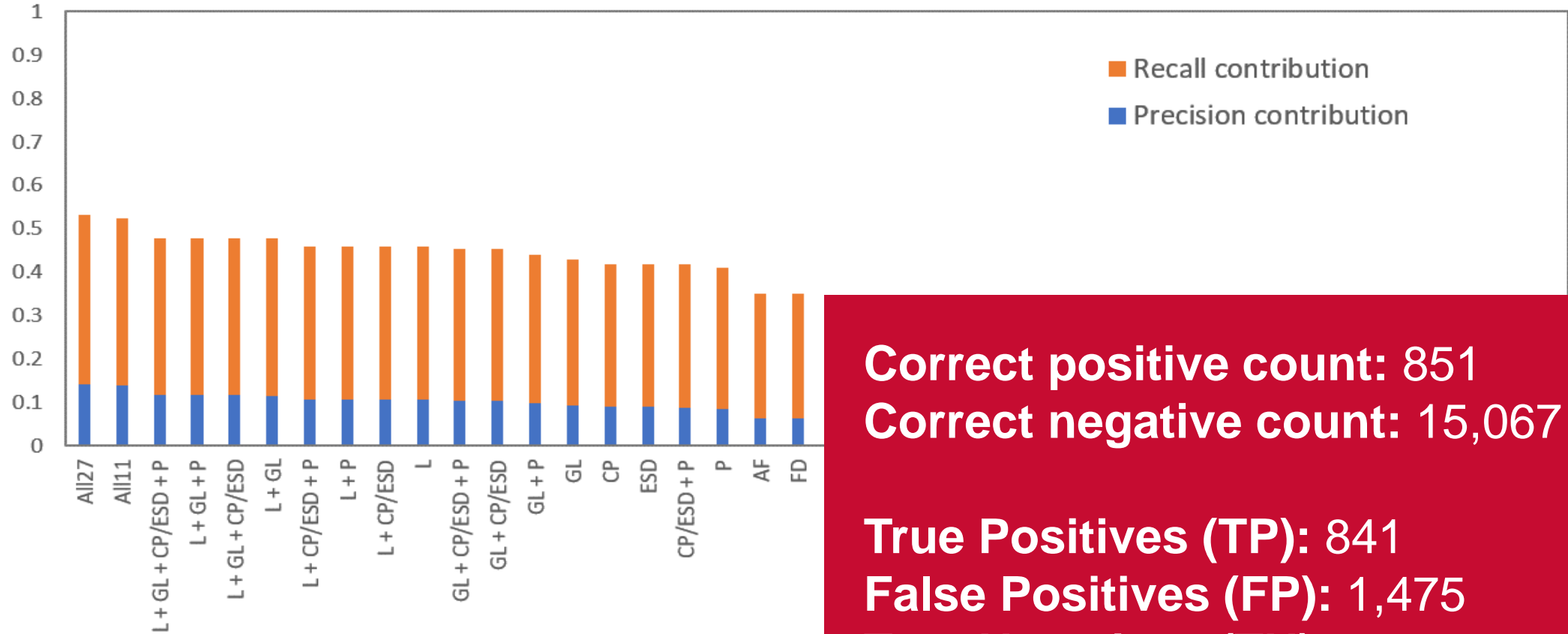
Auto-classification optimisation

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

**Correct count**

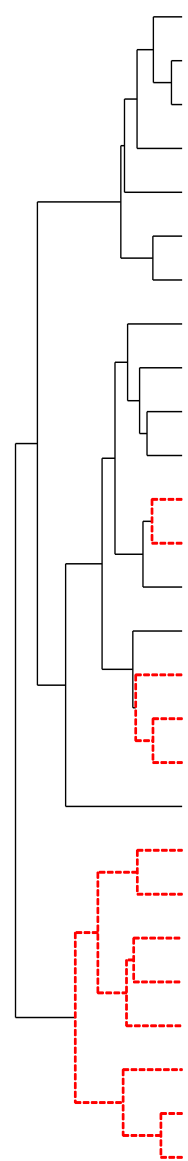
**Classifier Advanced**

Output 1

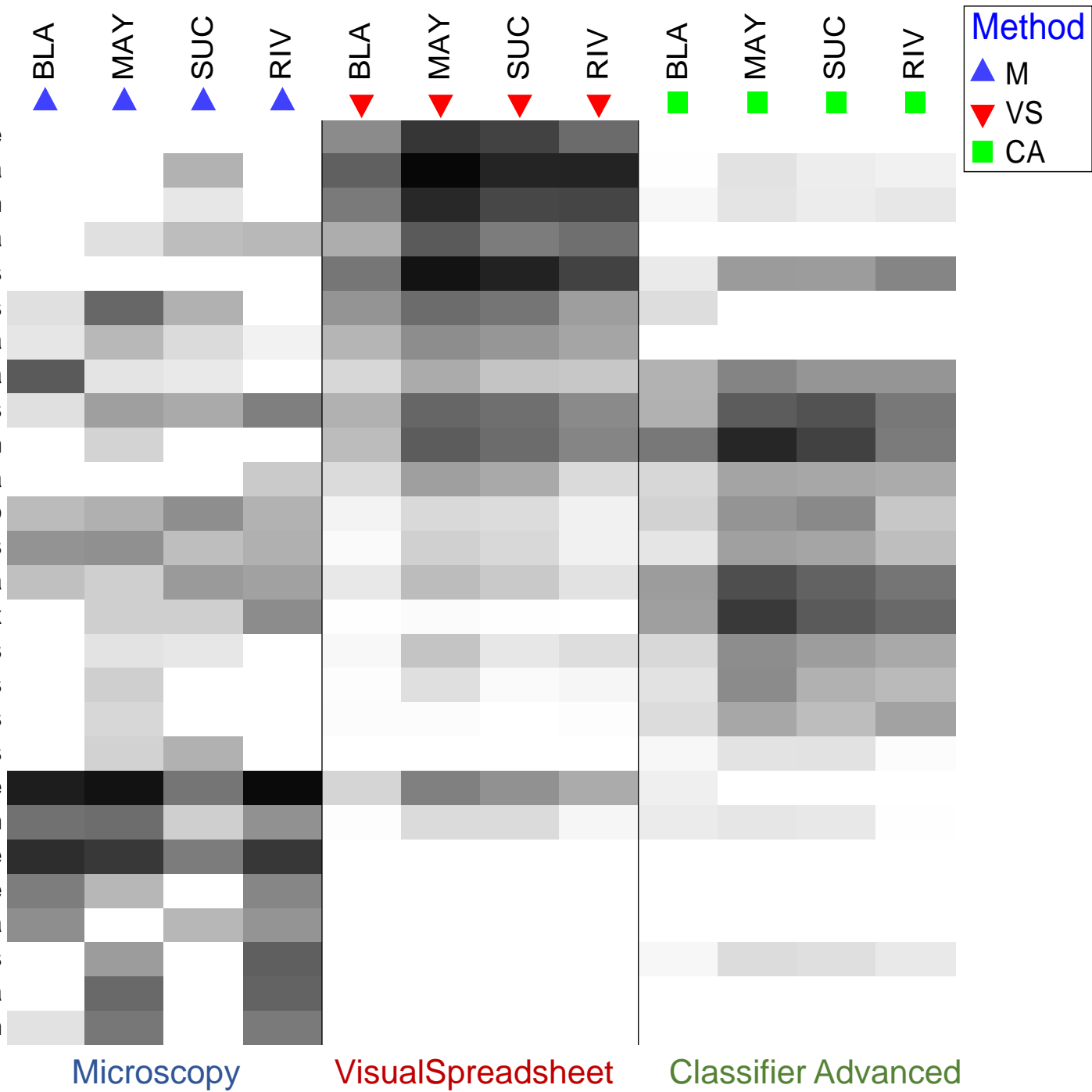
Output 2

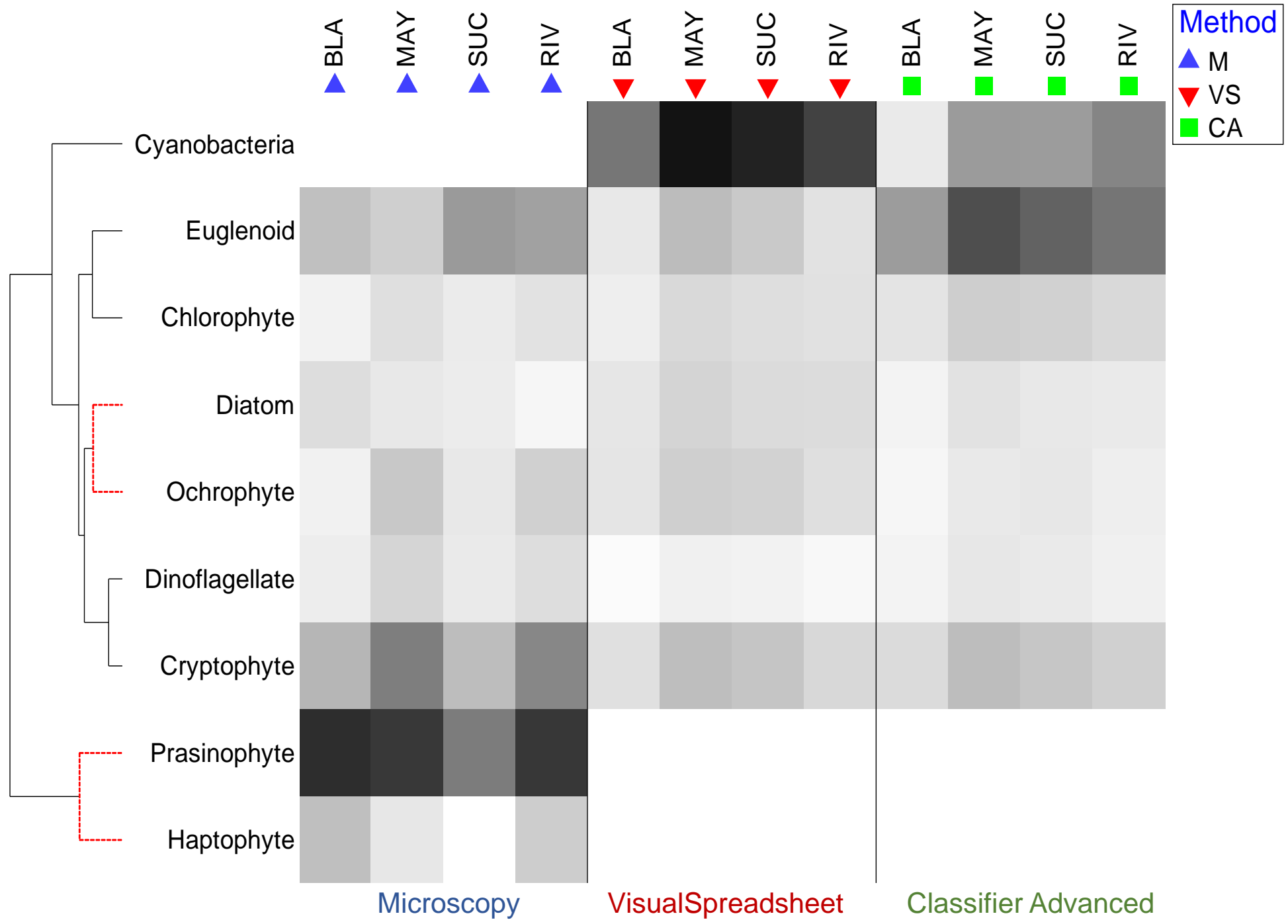
Output 3

	Correct count	Output 1	Output 2	Output 3
Baccilaria spp.				
Chroococcus spp.				
Cryptomonas spp.				
Cryptophyte spp.				
Cyclotella spp.				
Cylindrotheca spp.				
Dinophysis acuminata				
Entomeneis spp.				
Eutreptiella spp.				
Gymnodinium spp.				
Gyrodinium spp.				
Heterocapsa rotundata				
Heterosigma ashikiwo				
Hippodonta capitata				
Leucocryptos spp.				
Monoraphidium contortum				
Navicula spp.				
Nitzschia spp.				
Oxyrrhis marina				
Pedinellaceae spp.				
Pinnularia spp.				
Plagioselmis spp.				
Probiscia alata				
Prorocentrum dentatum				
Prorocentrum minimum				
Prorocentrum triestinum				
Protoperidinium spp.				
Pyramimonas spp.				
Scenedesmus spp.				
Scripsiella spp.				
Selanestrum spp.				
Skeletonema spp.				
Synedra spp.				
Teleaulax spp.				
Tryblionella spp.				
Unknown cells				
Non-target particles				

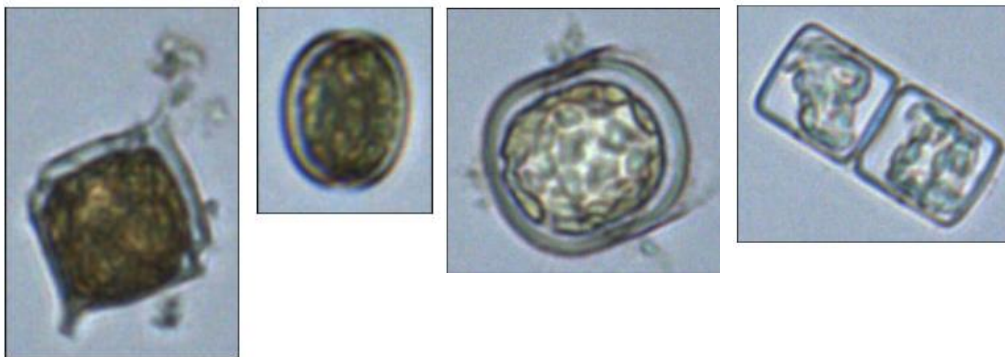


Pedinellaceae  
 Amphora  
 Achnantheidium  
 Navicula  
 Chroococcus  
 Cryptomonas  
 Cyclotella  
 Skeletonema  
 Pyramimonas  
 Selanestrum  
 Scripsiella  
 Heterosigma akashiwo  
 Plagioselmis  
 Eutreptiella  
 Teleaulax  
 Dinophysis  
 Scenedesmus  
 Entomoneis  
 Oxyrrhis  
 Cryptophyte  
 Gymnodinium  
 Prasinophyte  
 Haptophyte  
 Carteria  
 Leucocryptos  
 Pseudopedinella  
 Karlodinium

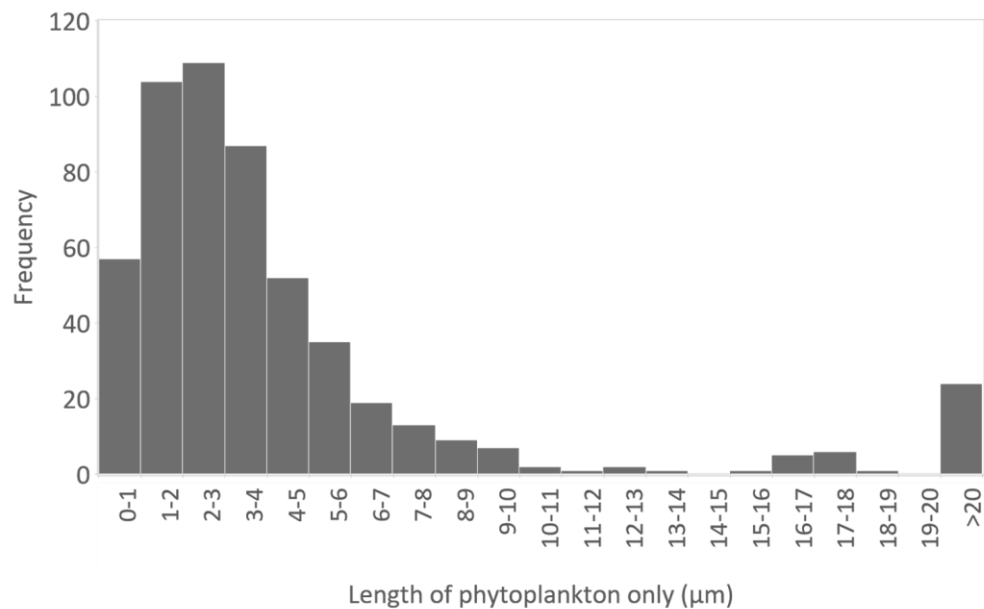




# What can the FlowCam<sup>®</sup> do for the Swan Canning?

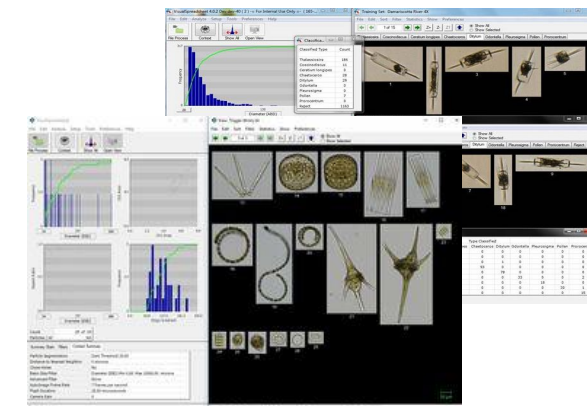


1. Clear digital images of phytoplankton
2. Measurements of phytoplankton



# Issues with the FlowCam<sup>®</sup> for the Swan Canning

1. Time consuming
2. VisualSpreadsheet<sup>®</sup> 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<sup>®</sup>

# Can the FlowCam<sup>®</sup> be used to analyse Swan Canning phytoplankton samples?

**FlowCam<sup>®</sup> software auto-classification accuracies are not currently sufficient for preserved turbid samples**



# Do you have any questions?

**Bianca Owen**

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## Acknowledgements



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Simon Rembold