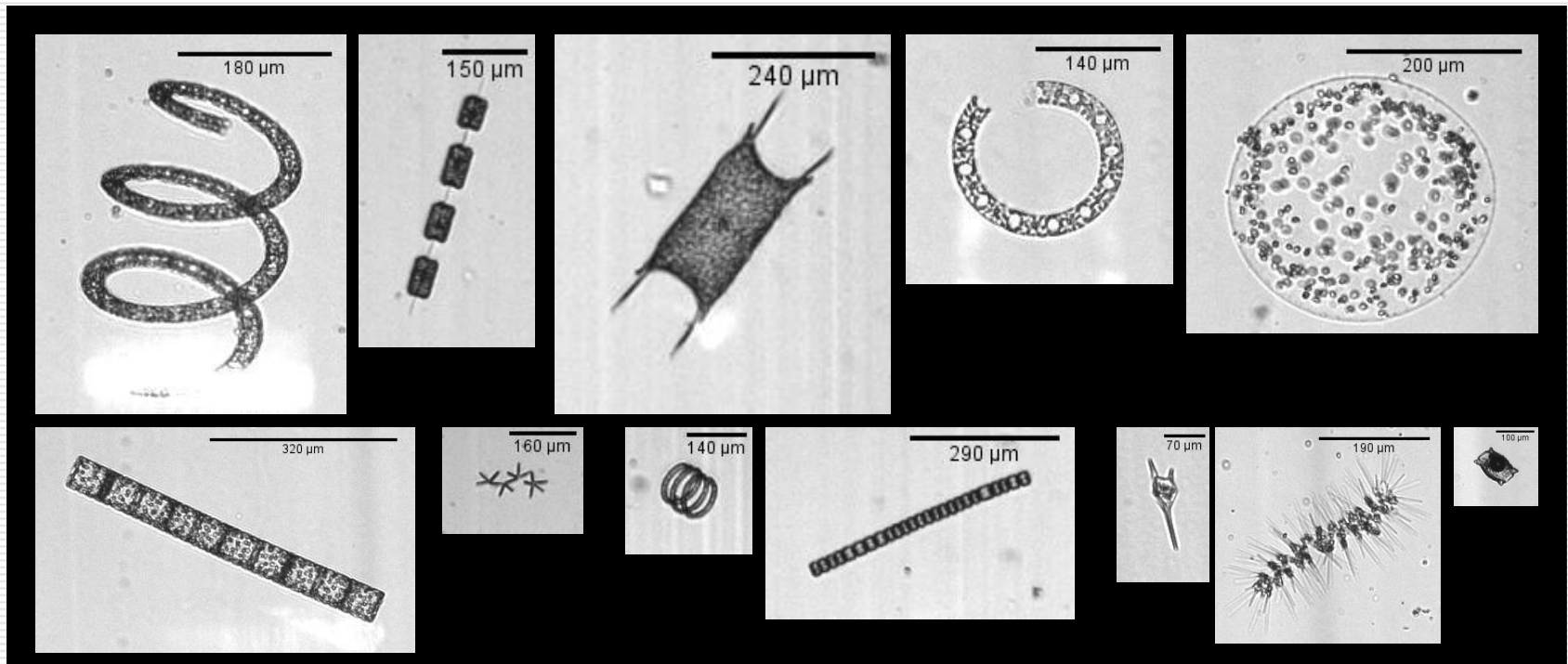
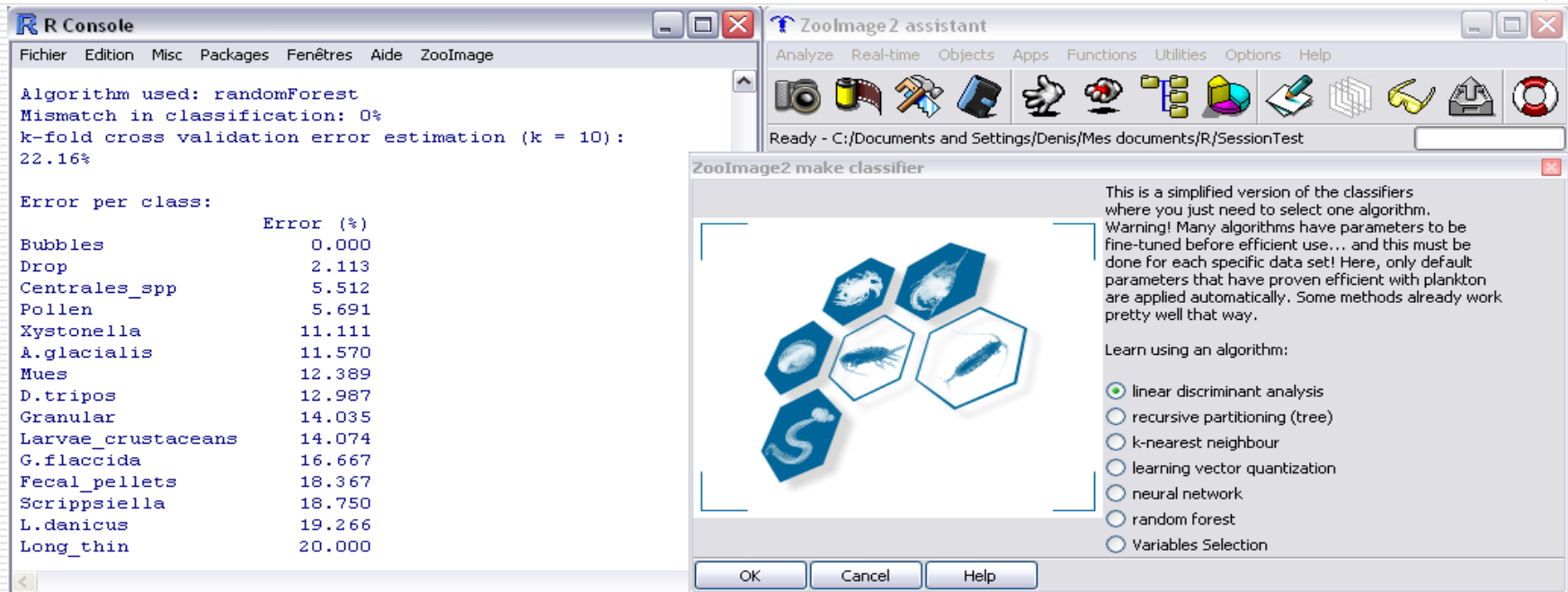


# Semi-automatic analysis of FlowCAM phytoplankton data using Zoo/PhytoImage



Grosjean Ph., K. Denis, G. Wacquet, V. Rousseau, J.-Y. Parent, Ch. Lancelot, D. Hamad,  
F. Artigas, A. Lefebvre, N. Naud-Masson, D. Maurer, A. Tunin-Ley, F. Colas, C. Belin

# Zoo/PhytoImage



The screenshot shows two windows from the ZooImage2 software. The 'R Console' window displays the following output:

```
Algorithm used: randomForest
Mismatch in classification: 0%
k-fold cross validation error estimation (k = 10):
22.16%

Error per class:
```

	Error (%)
Bubbles	0.000
Drop	2.113
Centrales_spp	5.512
Pollen	5.691
Xystonella	11.111
A.glacialis	11.570
Mues	12.389
D.tripos	12.987
Granular	14.035
Larvae_crustaceans	14.074
G.flaccida	16.667
Fecal_pellets	18.367
Scrippsiella	18.750
L.danicus	19.266
Long_thin	20.000

The 'ZooImage2 assistant' window shows a 'ZooImage2 make classifier' dialog. It contains a preview of six plankton images and a list of algorithms to choose from:

- linear discriminant analysis
- recursive partitioning (tree)
- k-nearest neighbour
- learning vector quantization
- neural network
- random forest
- Variables Selection

- ✓ <https://cran.r-project.org/web/packages/zooimage/>
- ✓ **Free (open source) software** written in R and Java specialized to classify zoo- and phytoplankton digital images
- ✓ **Machine learning** (supervised) classification
- ✓ Adaptable to analyze *any* plankton image, e.g., images from the FlowCAM...

# Zoo/PhytoImage version 3

Install latest R

(<http://cran.r-project.org>) then in R :

```
> install.packages("zooimage")
```

```
> library(zooimage)
```

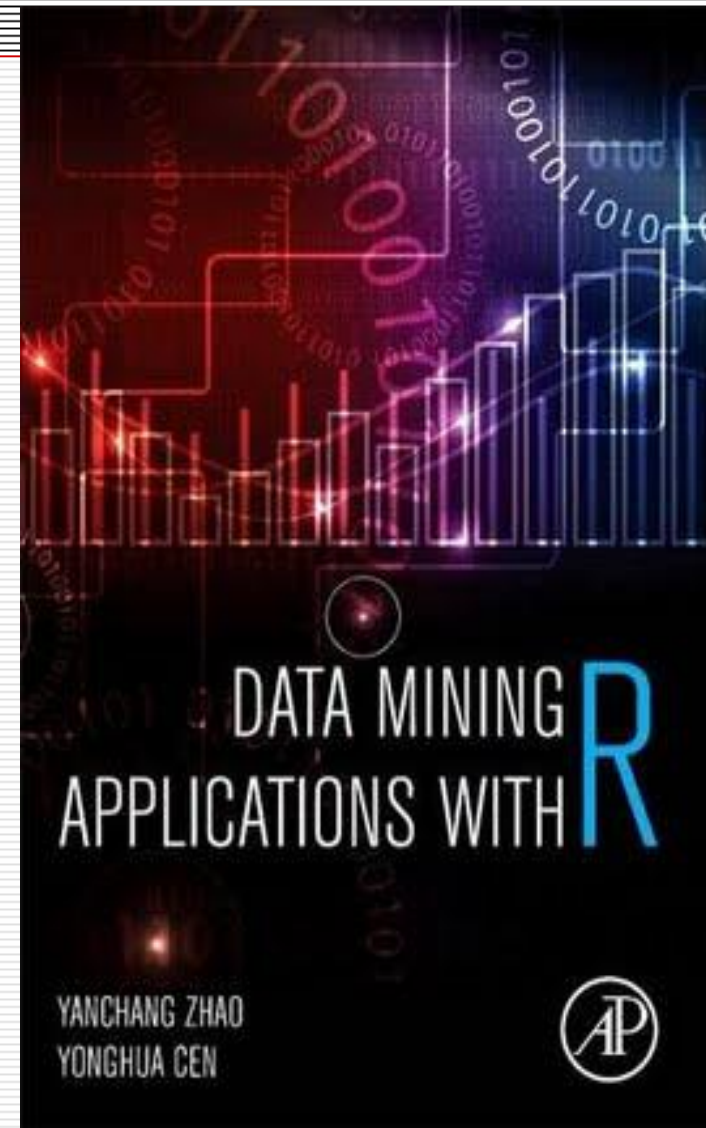
See : Data mining application with R

ISBN 978-0124115118,  
December 2013.  
Academic Press, Elsevier.

Chapter 12 is complete  
description and tutorial  
of Zoo/PhytoImage v.3.

Latest version 5 available from:

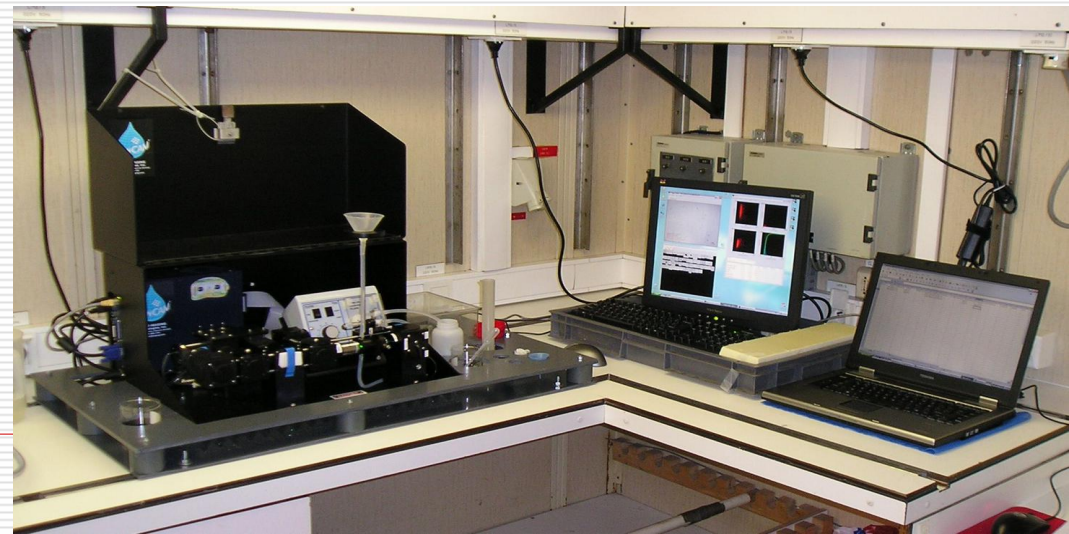
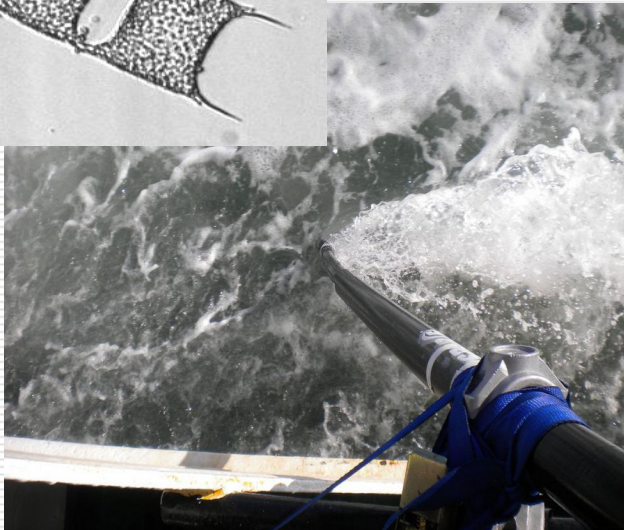
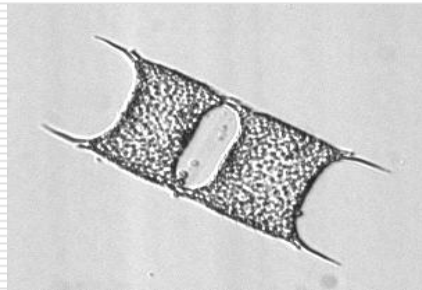
<https://r-forge.r-project.org/projects/zooimage/>



# Application in real-time



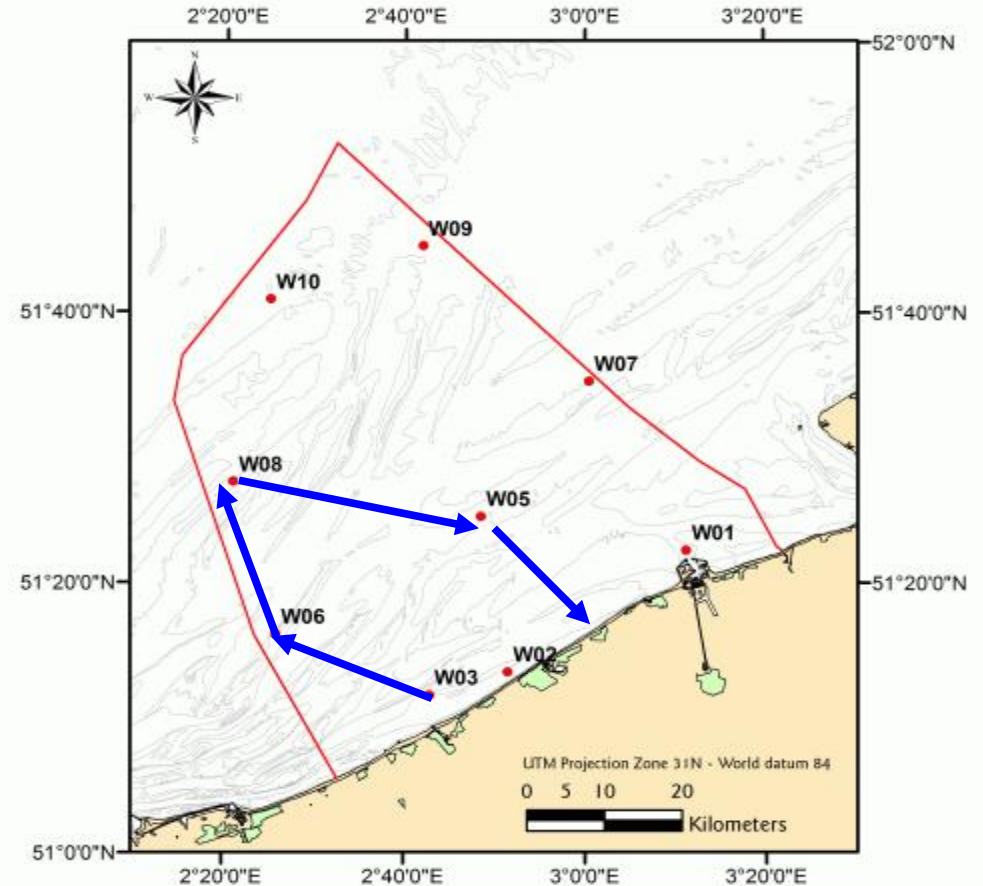
- Combination of the FlowCAM with Zoo/PhytoImage
- Aboard the 'Belgica' (Belgian oceanographic ship)
- 25 groups discriminated in real-time (incl. 18 phytoplankton groups)





# Real-time monitoring of North Sea phytoplankton

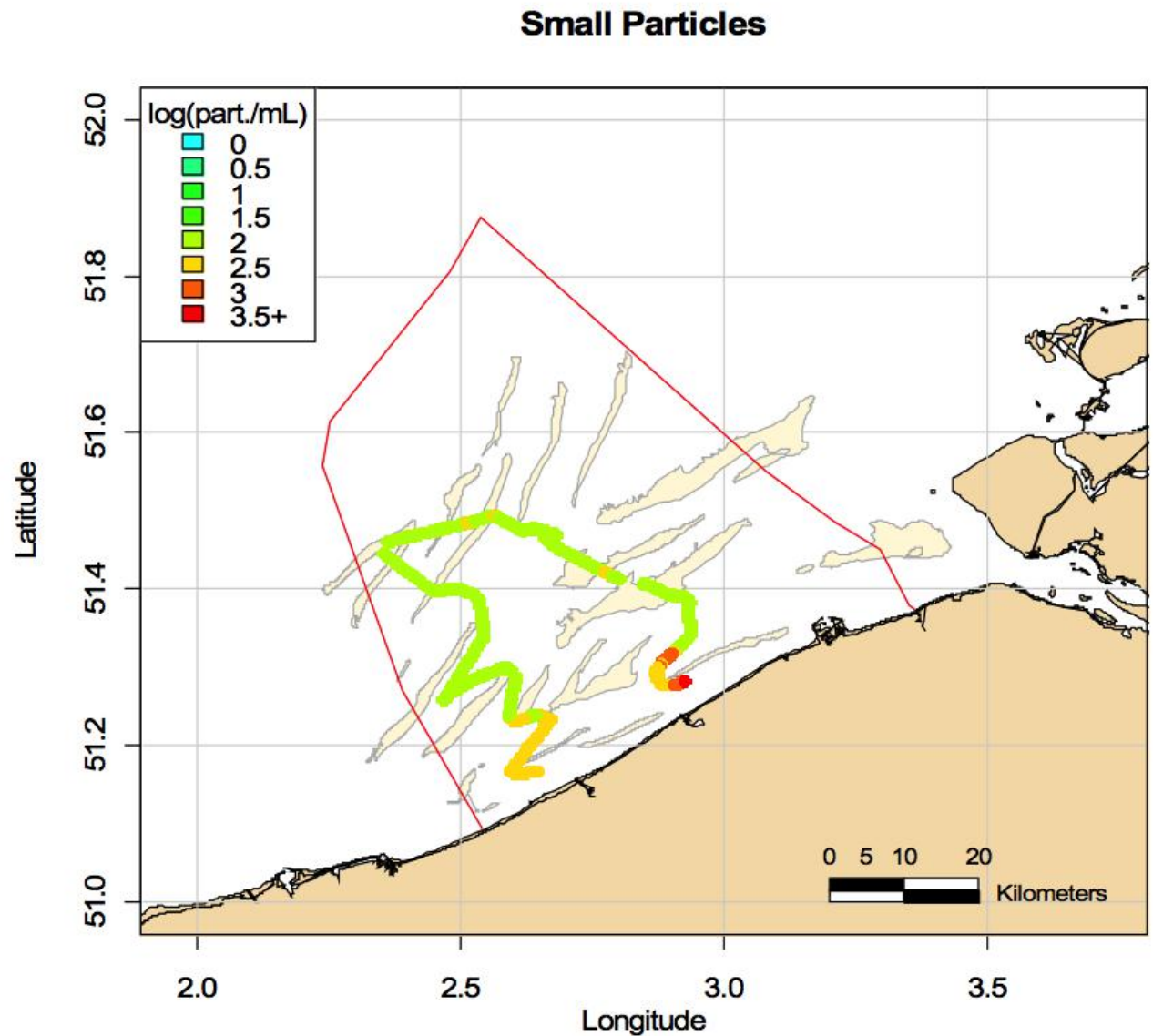
Application example: a one-day cruise in the Belgian Coastal Zone (BCZ).



# Real-time monitoring - results

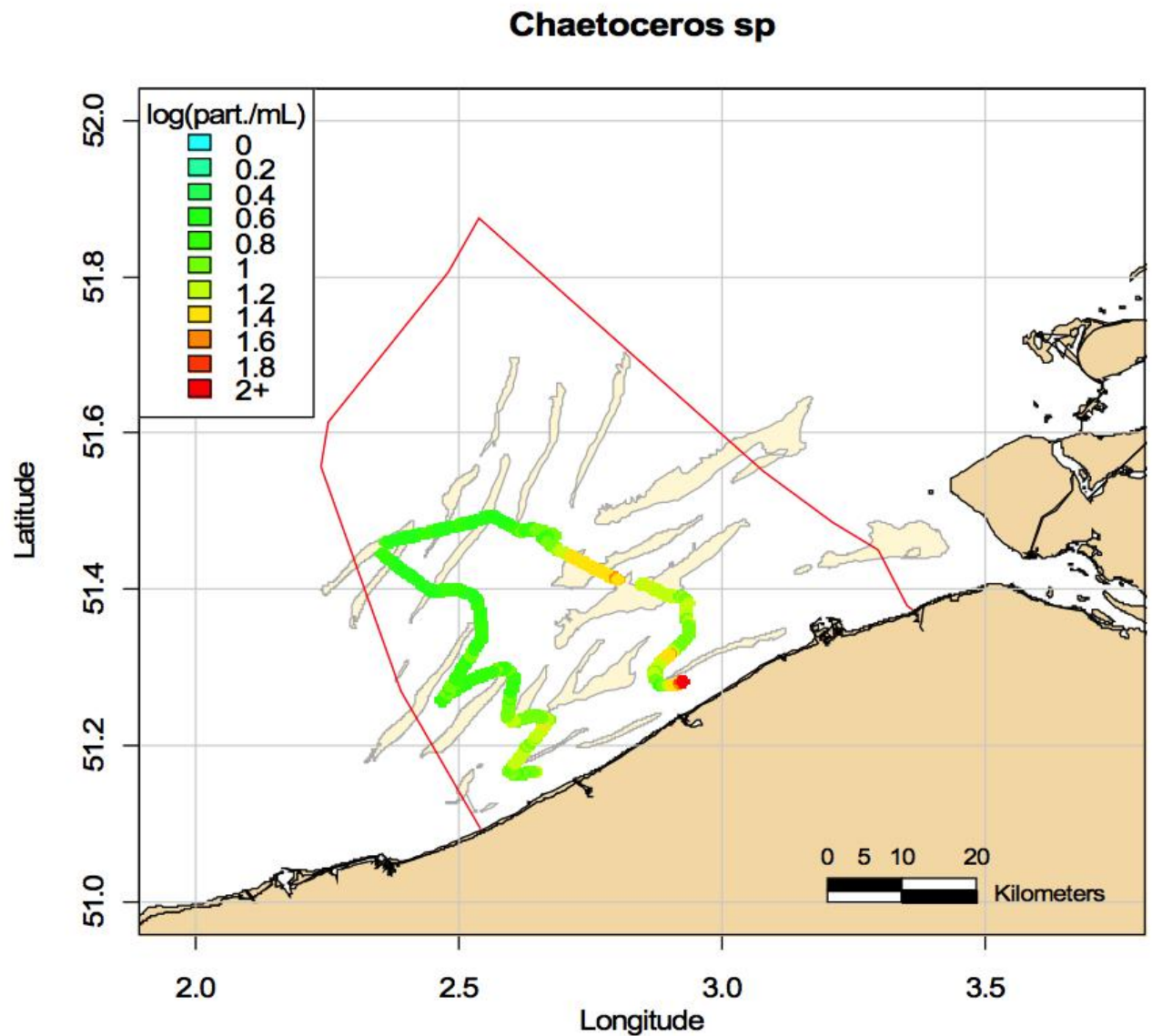
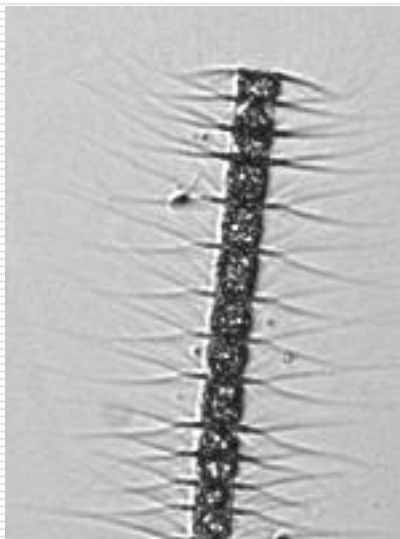
Small particles dominating near the coast and the Schelde estuary.

Notice the influence of sandbanks (no interpolation!)



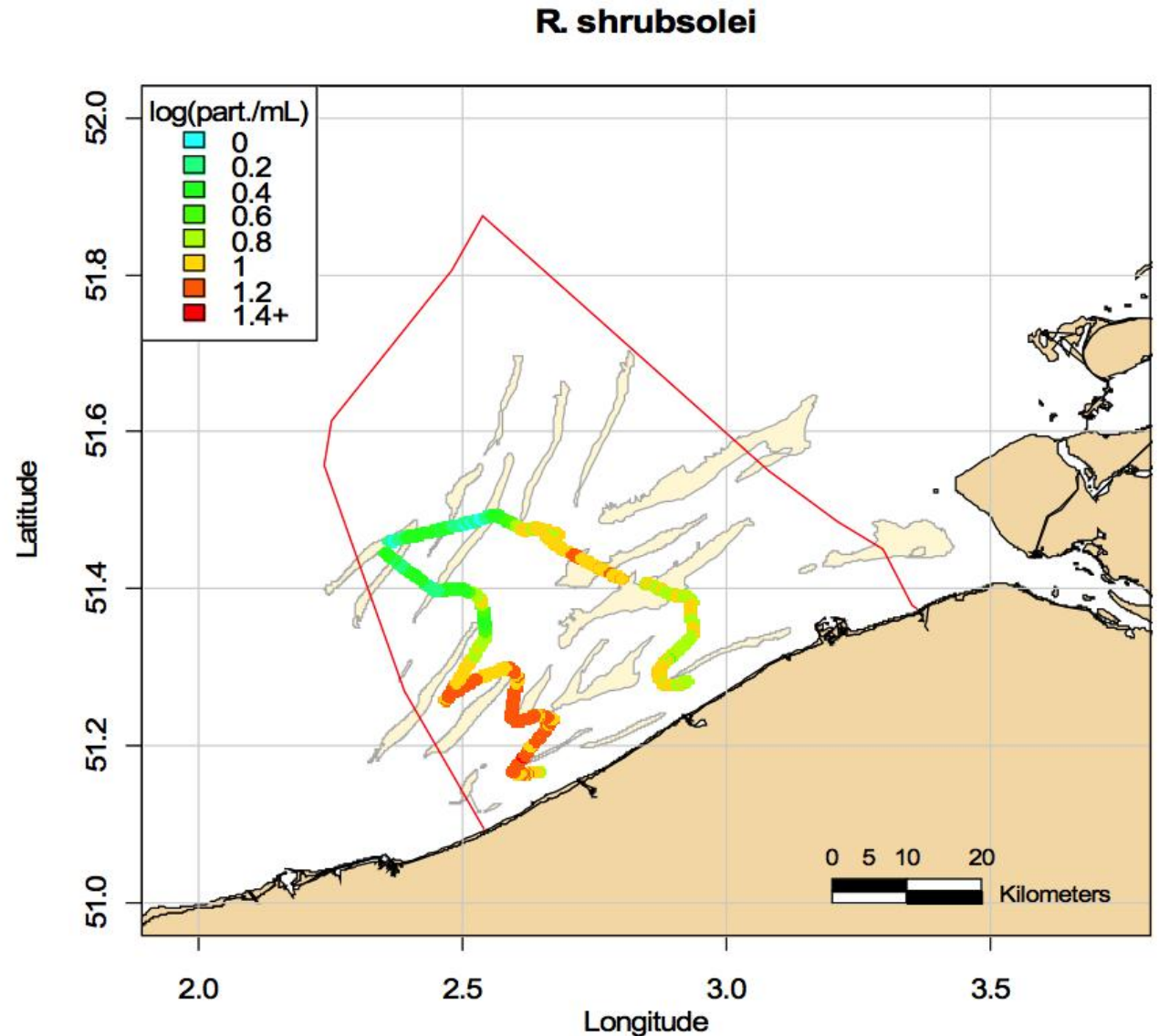
# Real-time monitoring - results

A neritic  
diatoms :  
*Chaetoceros sp.*



# Real-time monitoring - results

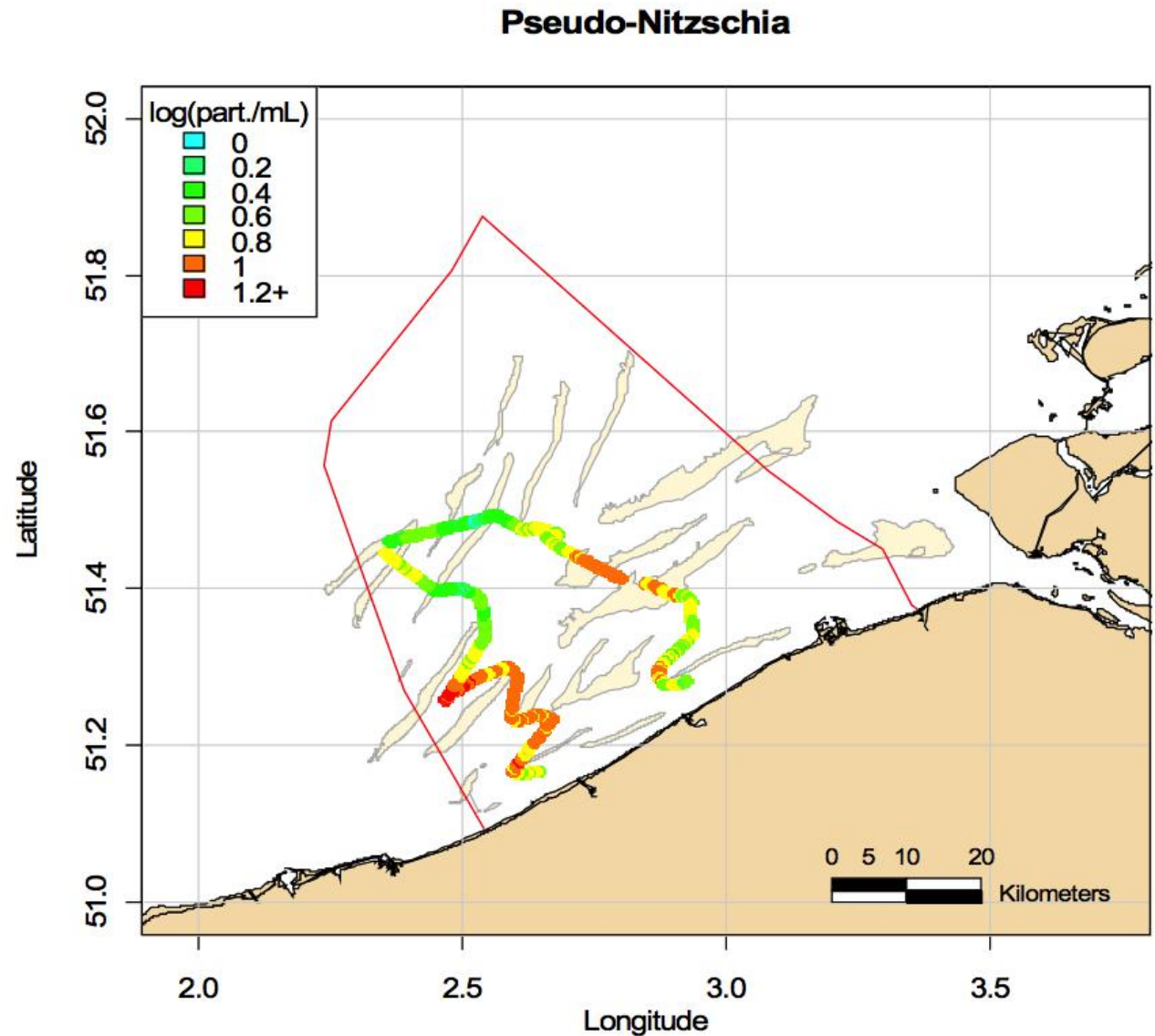
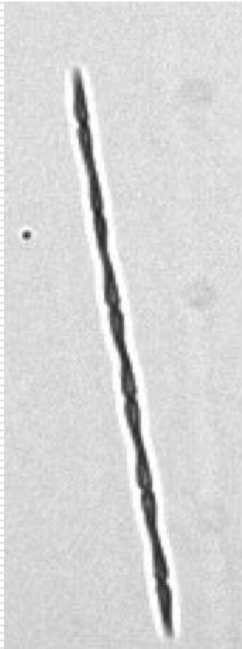
*Rhizosolenia  
shrubsolei*  
with a very  
different  
distribution  
pattern.





# Real-time monitoring - results

Yet another  
distribution  
pattern :  
*Pseudo-*  
*nitzschia*.



---

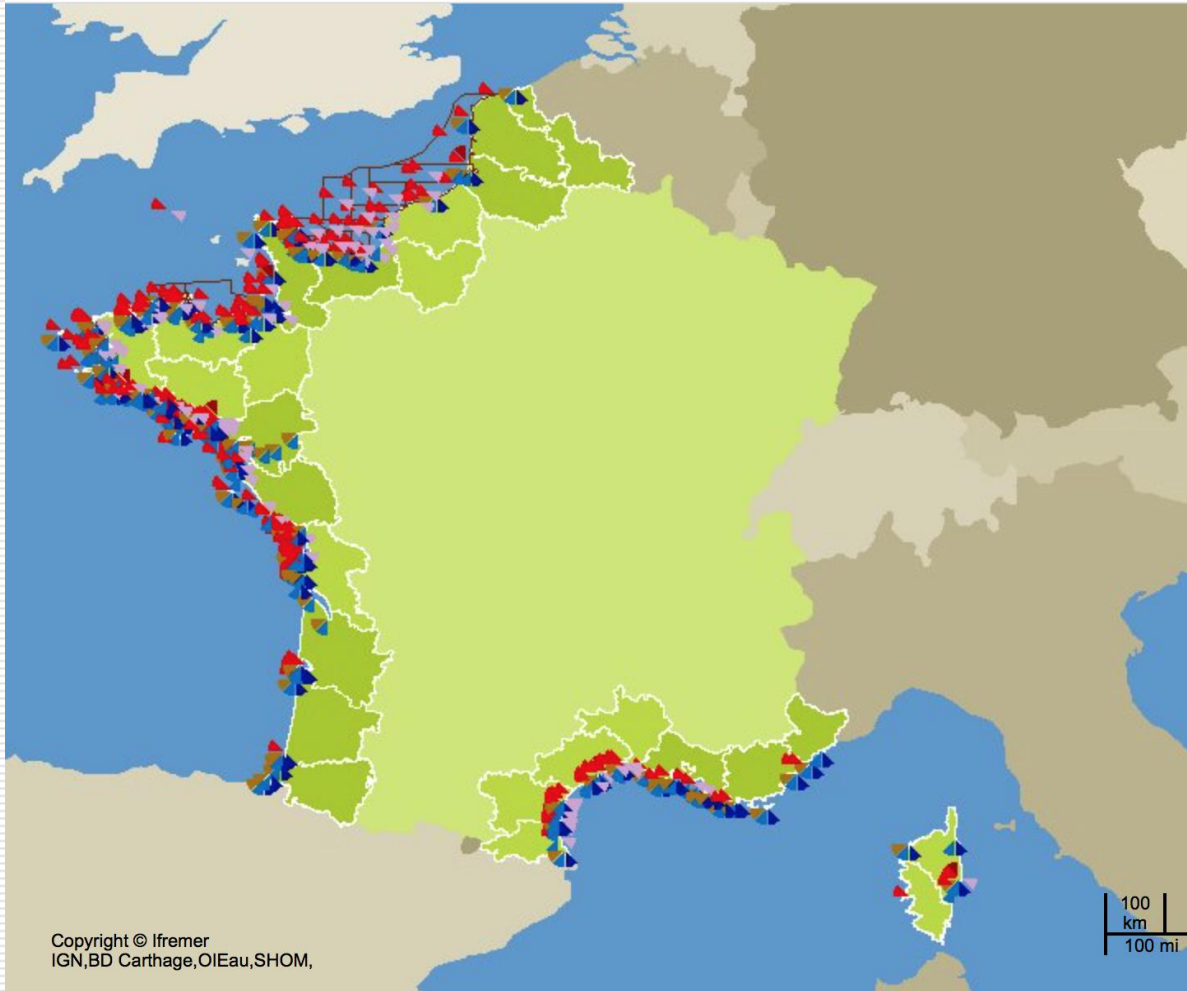
What about a higher taxonomic discrimination ?

Challenges with the IFREMER Rephy network

# IFREMER Rephy

## Lieux de surveillance actifs REPHY

Inventaire cartographique



- French coasts
- All the dots on the map are stations included in the survey
- Starting to use the FlowCAM + Zoo/PhytoImage v.5 since mid 2014

(map from IFREMER)

# Typical results with > 40 classes

Global error : 26 %

## Error per class:

	Error (%)		Error (%)
Drop	7	Lauderia_Schroederella	24
Pollen	7	Thalassiosira_spp	25
C.fusus	8	Fecal_pellets	25
L.danicus	9	Long_thin	26
L.undulatum	12	Dark	32
Thalassiosira_spp_cells	13	D.fragilissimus	33
G.flaccida	14	D.brightwelli	35
Black_opaque	15	Aggregates	36
D.tripos	17	P.alata_indica	40
Centrales_spp	17	G.delicatula	46
Mues	18	Membranous	48
G.striata	18	R.imbricata_styli	50
Pseudo-nitzschia	19	Fibers	50
Euglenophyceae	20	Dictyochophyceae	53
T.subtilis	20	Protoperidinum_spp	68
Short_thin	22	Chaetoceros_spp	69
Clear	23	Larvae_crustaceans	71
N.longissima_Cylindrotheca	23	Ceratium_spp	79
A.glacialis	23	P.alata	93
Bubbles	23	C.danicus	95
Granular	24	C.decipiens	97

- On average,  $\frac{1}{4}$  wrongly classified
- Large discrepancy of results: some classes are OK, other are completely wrong

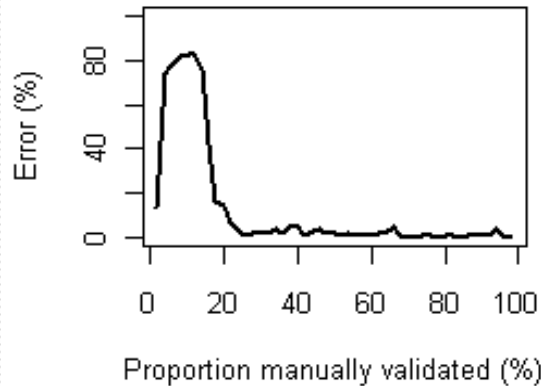
**Manual validation is required to lower the error down to acceptable levels for all classes**

(from Tunin-Ley et al, 2011)

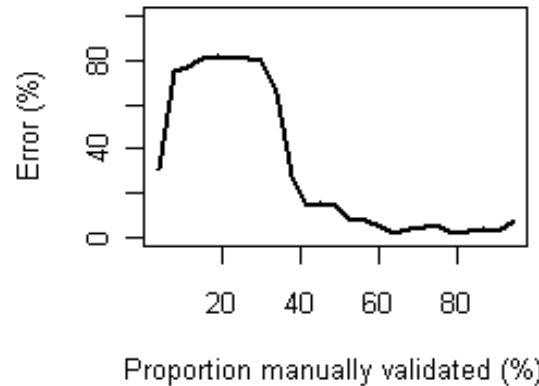


# Detection of suspect particles

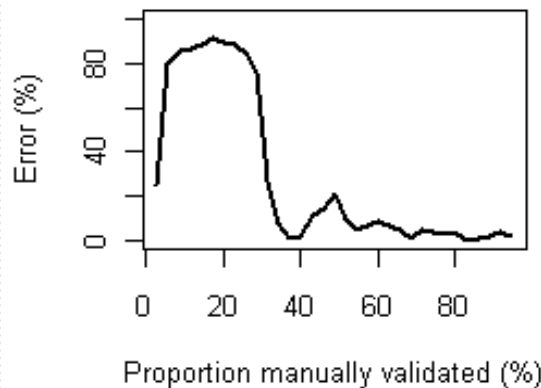
W06 : Error in manual validation



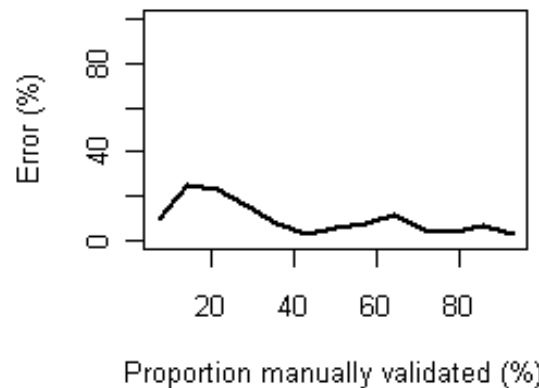
W07 : Error in manual validation



W08 : Error in manual validation



T34 : Error in manual validation



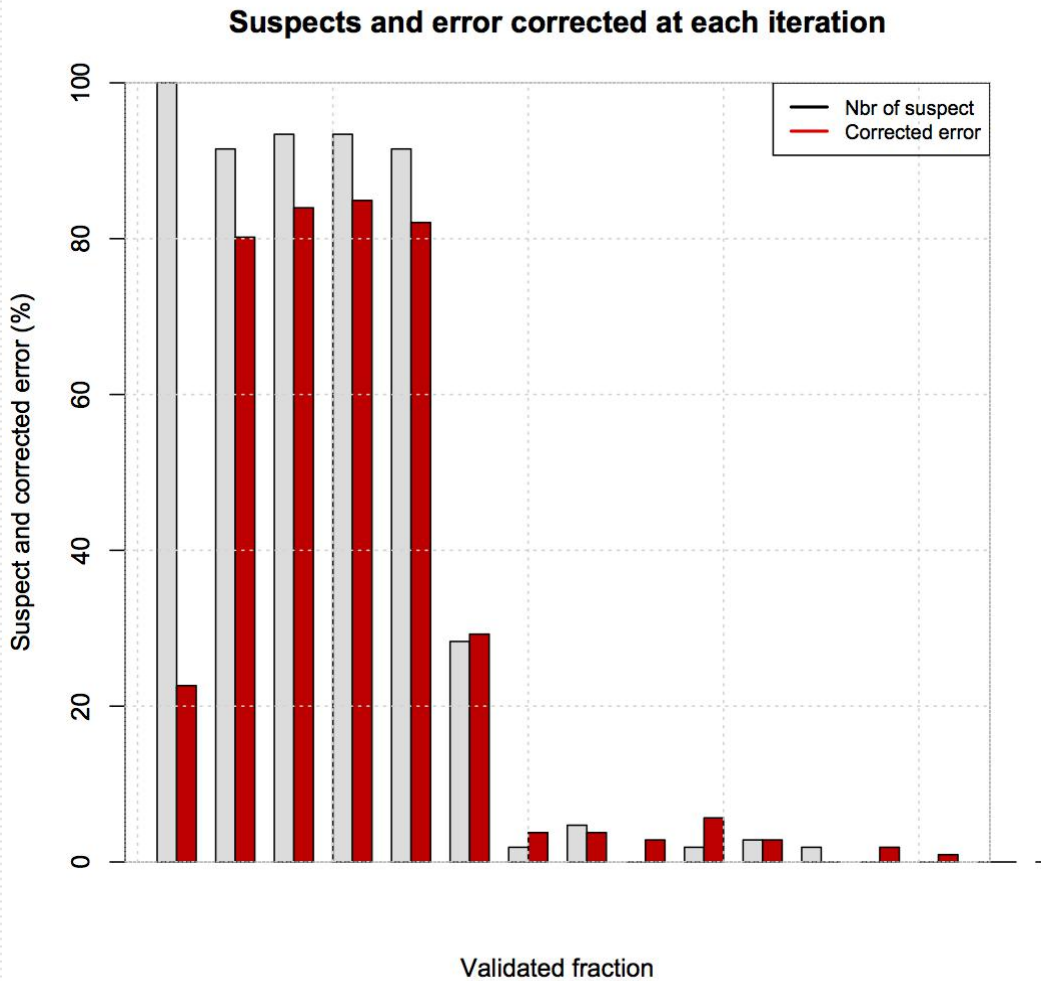
In addition to the classification, is it possible to estimate a probability of misclassification ?

**Yes : suspect detection**

Application on four different samples

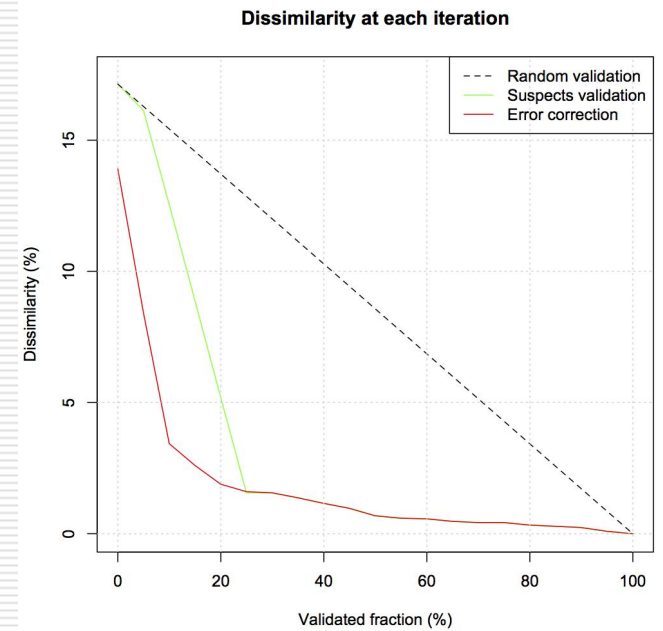
**Suspect particles contain a large fraction of the error in most cases**

# Step by step validation of the suspect items



Gray bars = suspects

Red bars = error corrected after manual validation

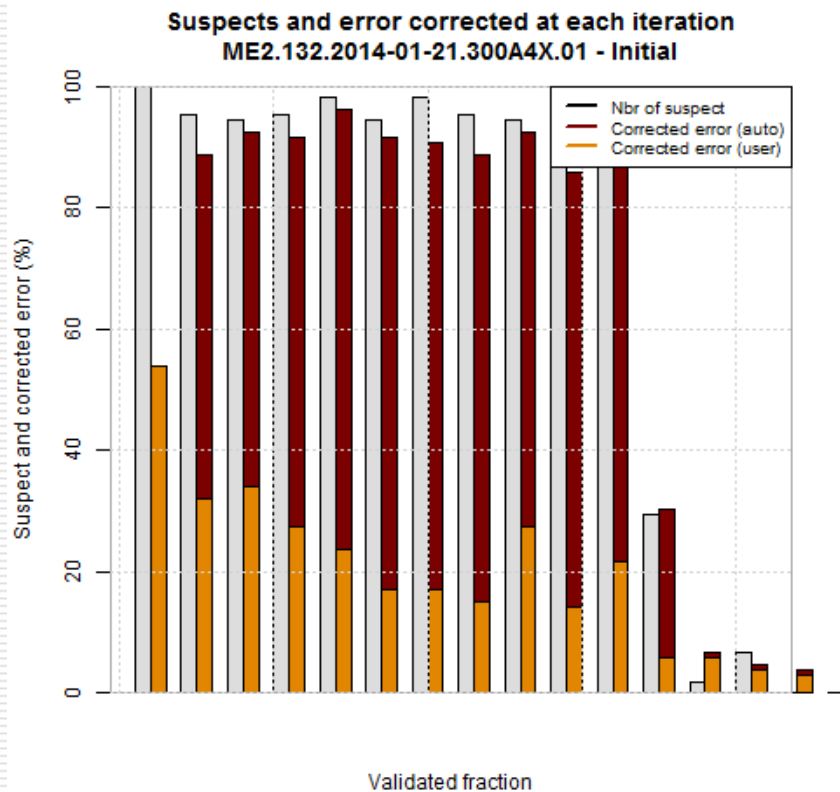


# Active learning and statistical error correction

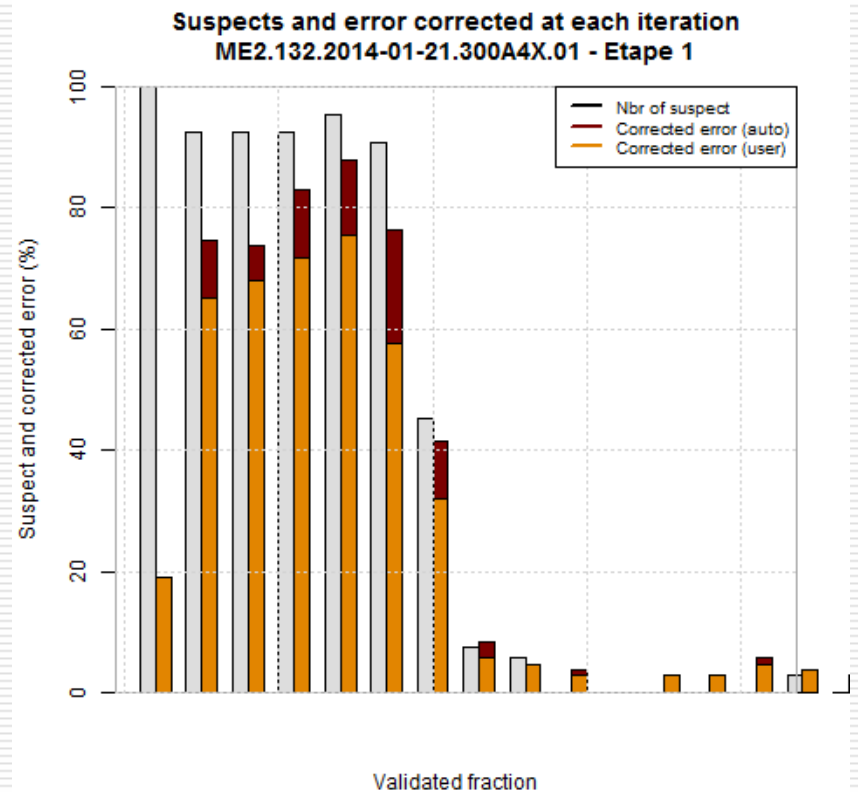
Brown bars = error statistically corrected

Orange bars = error that the user must correct

## Without adaptative training set



## With adaptative training set

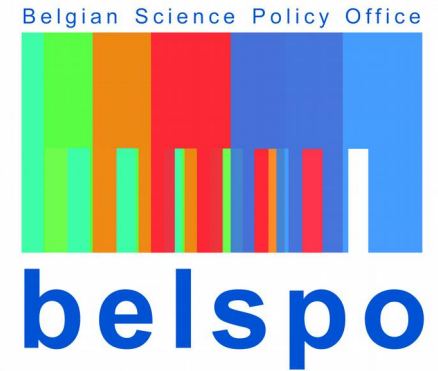


# Conclusions & perspectives

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- Image analysis (FlowCAM) combined with supervised analysis usable in real-time for coarse classification of phytoplankton
- Deployment in routine survey network with higher discrimination needs manual validation to lower the error (e.g., IFREMER - Rephy)
- Validation of suspects combined with statistical error correction greatly reduces the number of items to validate for a given error rate





Study in collaboration with *IFREMER*, also funded by the *Belgian Science Policy*

