



GlobalHAB symposium on automated in situ observations of plankton
Kristineberg Marine Research Station, Fiskebäckskil, Sweden
August 22-27, 2022
Session 2

Demonstrations of CytoSense's software

Zéline Hubert, Clémentine Gallot, Alexandre Epinoux & Luis Felipe Artigas

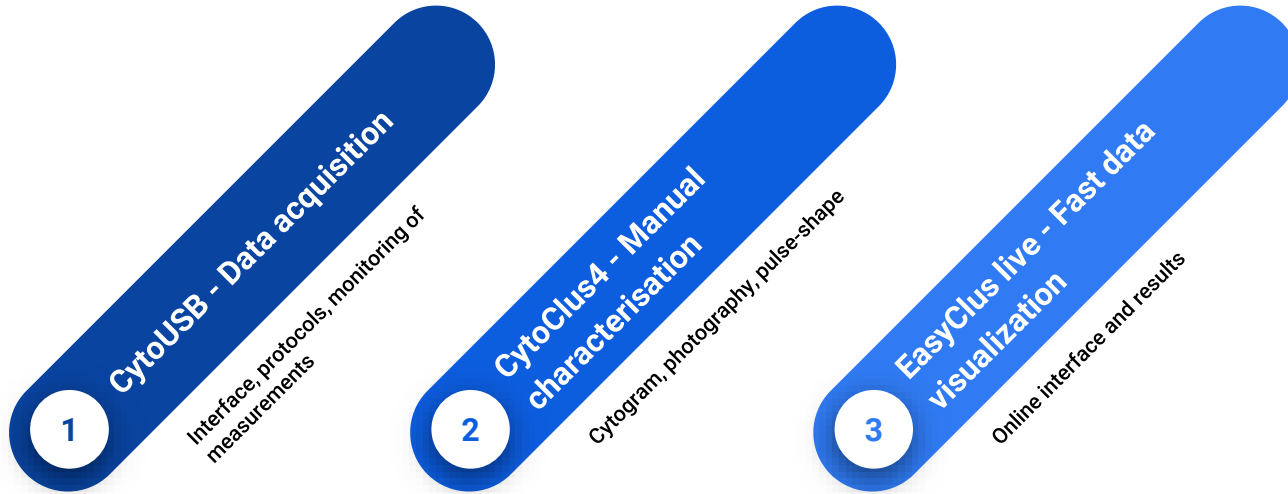


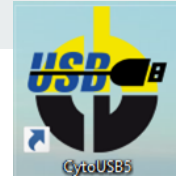
Laboratory of Oceanology and Geosciences
CNRS – UMR 8187 LOG - ULCO





From acquisition to visualization

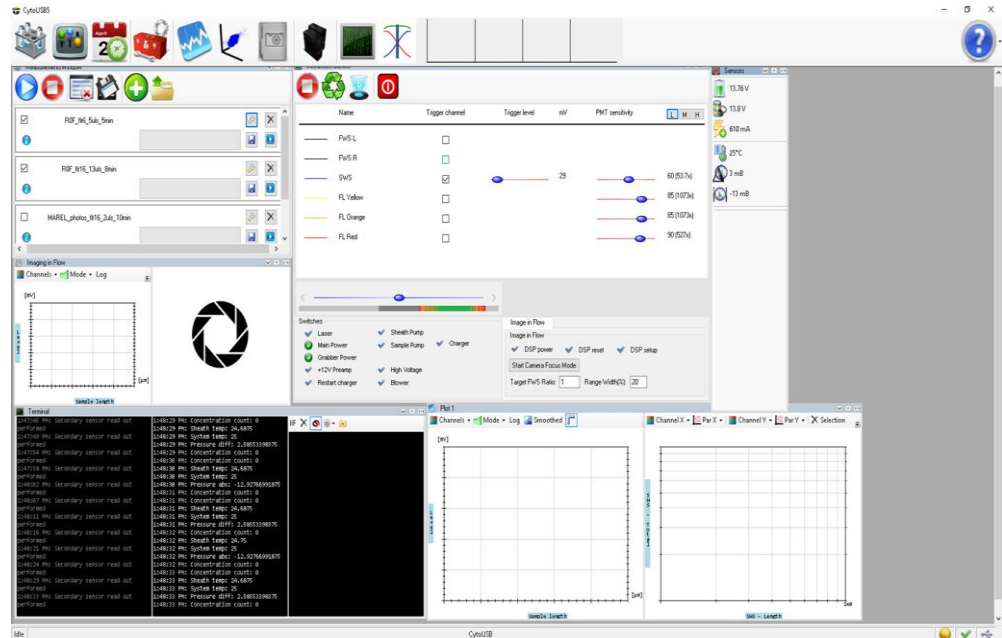




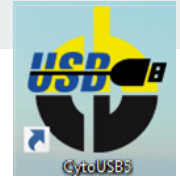
1 CytoUSB - Data acquisition

CytoUSB is CytoSense' data acquisition and instrument control software

- Observe data during measurements
- Schedule measurements at fixed times or at time intervals
- Check the instrument on the sensor readouts
- Remote control from your office computer or your cell phone



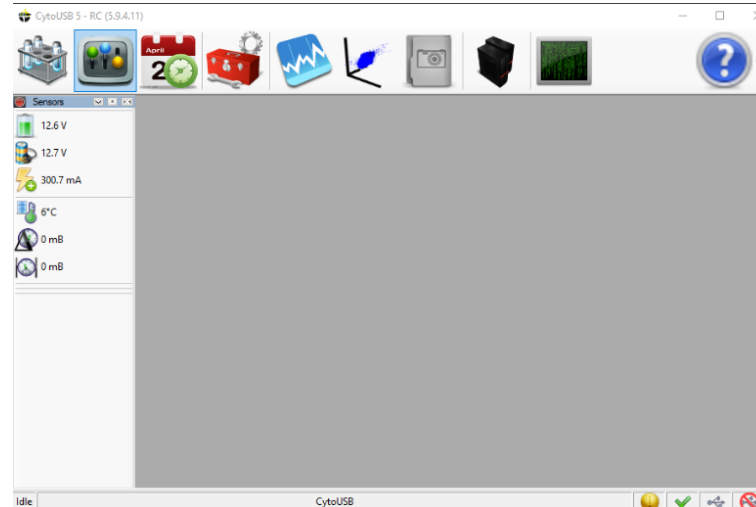
1 CytoUSB - Data acquisition



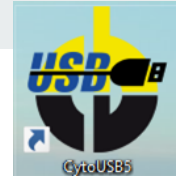
Upon opening the program, the following Menu will appear with the buttons (from left to right) use to open windows for:



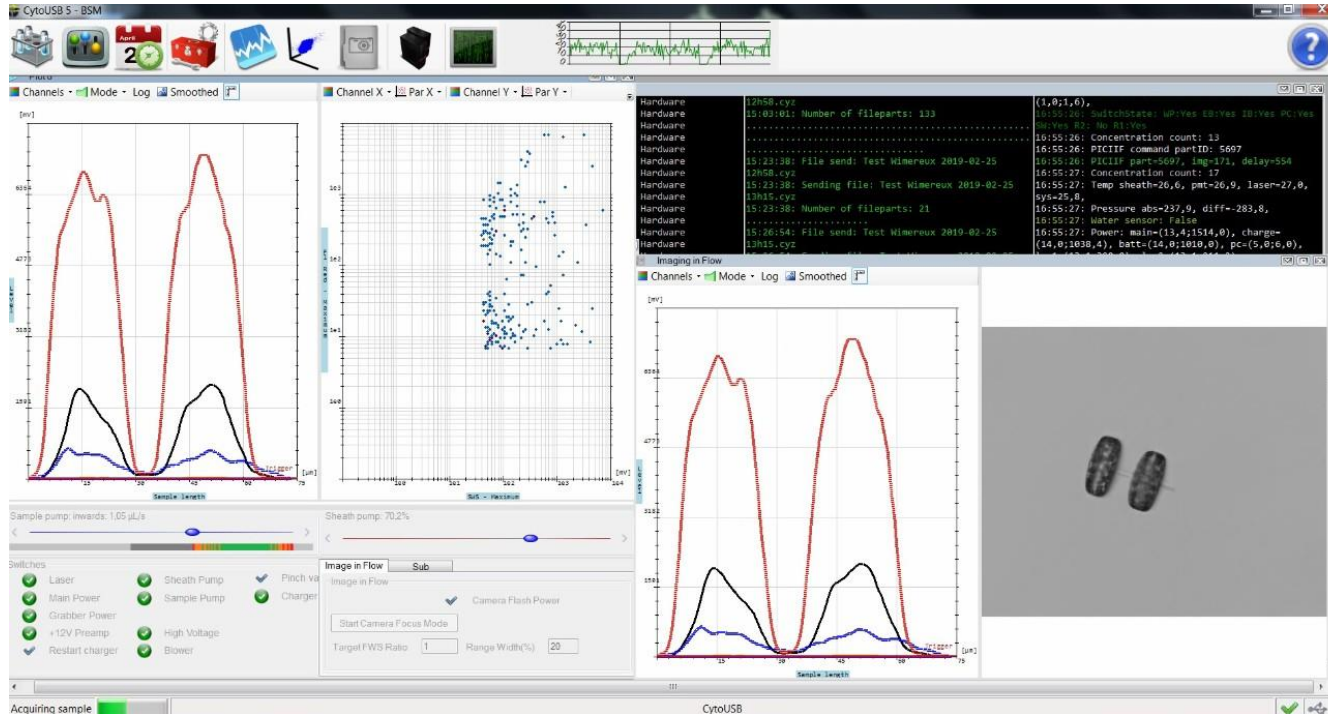
- Measurement Overview
- Instrument Control
- Schedule
- Instrument Settings
- Real-Time Plot
- 3D Plot
- Imaging In Flow
- File Server
- Terminal



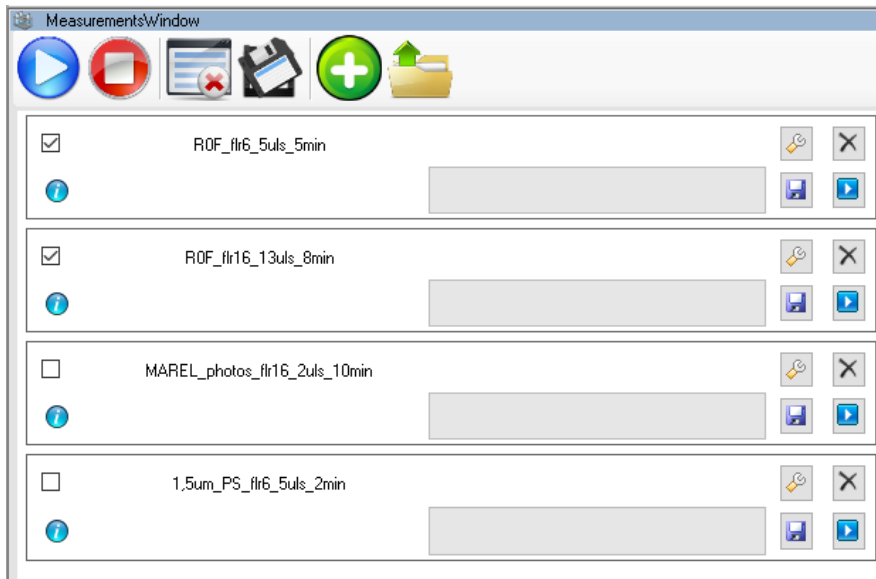
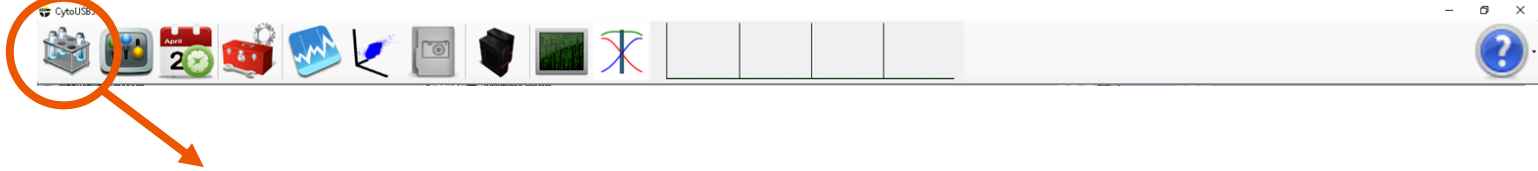
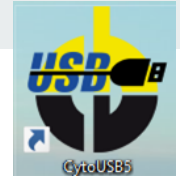
1 CytoUSB - Data acquisition



CytoUSB Layout options

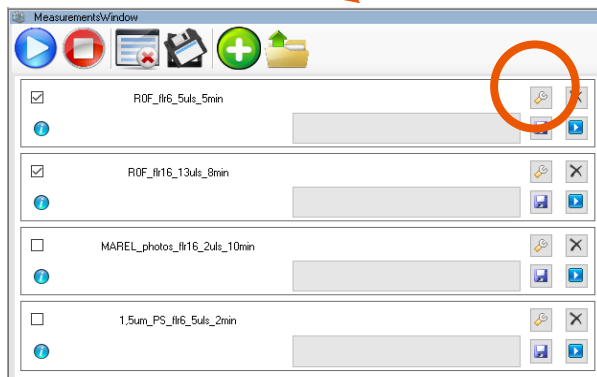
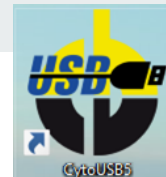


1 CytoUSB - Data acquisition



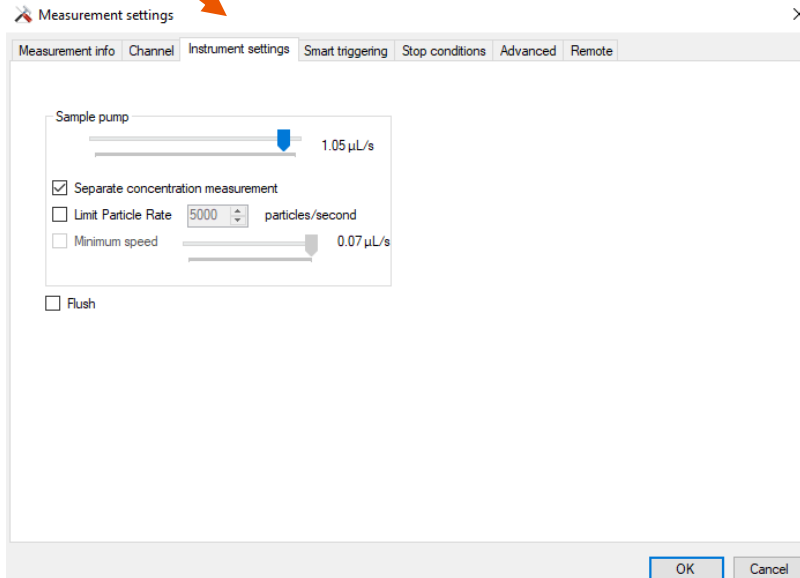
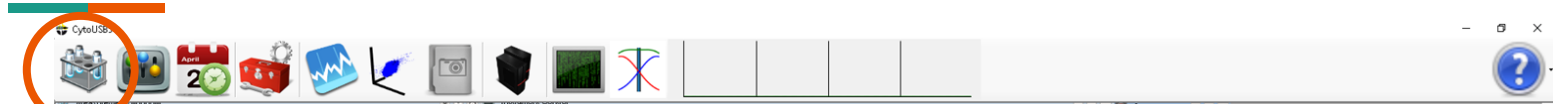
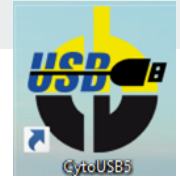
- Run all measurements listed below
- Stop any task
- Delete all measurements
- Save measurements set
- Create new measurement and show Measurement window
- Open and add measurement settings to list
- Ability to run different protocols

1 CytoUSB - Data acquisition



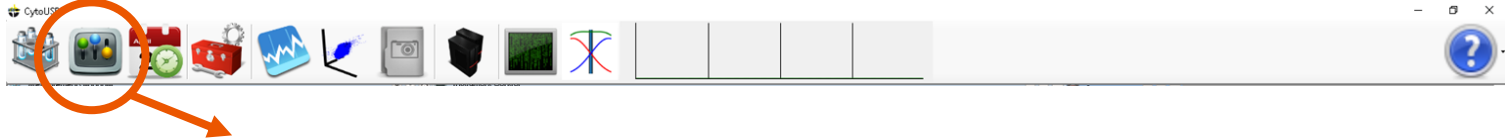
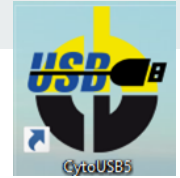
- set the Trigger Channel and its levels, as well as PMT sensitivities (low, medium, high)

1 CytoUSB - Data acquisition



- In the “Instrument settings” you can set:
- the flushing speed of the sample pump (by moving the slider),
 - limit particle rate (for high particle loading),
 - include flushing in-between samples (by ticking on the box “Flush”). Depends on low/high particle loading
 - Flushing advised to avoid cross contamination between different samples

1 CytoUSB - Data acquisition



Name	Trigger channel	Trigger level	AV	PMT sensitivity
PuS L	<input type="checkbox"/>			
PuS R	<input type="checkbox"/>			
SWS	<input checked="" type="checkbox"/>	29		60 (53.7%)
FL Yellow	<input type="checkbox"/>			85 (107.3%)
FL Orange	<input type="checkbox"/>			85 (107.3%)
FL Red	<input type="checkbox"/>			90 (82.7%)

Sample pump: off

Switches

- Laser
- Sheath Pump
- Main Power
- Sample Pump
- Charger
- Grabber Power
- High Voltage
- +12V Preamp
- Blower
- Redstart charger

Image in Flow

Image in Flow

- DSP power
- DSP reset
- DSP setup

Start Camera Focus Mode

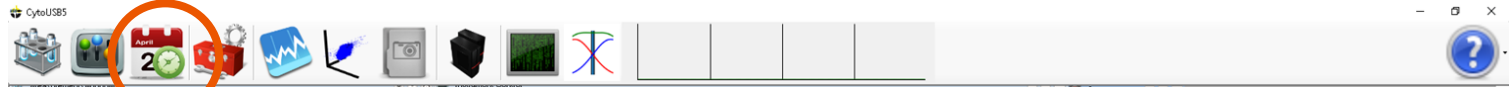
Target PuS Ratio: 1 Range Width: 20

In Instrument Control Window you can easily monitor your measurement and perform some simple actions:

- Stop the measurement
- Backflush (cleaning the sample inlet)
- Flush the sample inlet
- Shutdown (turns off the instrument)

In the Instrument Control window you can also switch manually: laser, sheath pump, sample pump, etc.

1 CytoUSB - Data acquisition

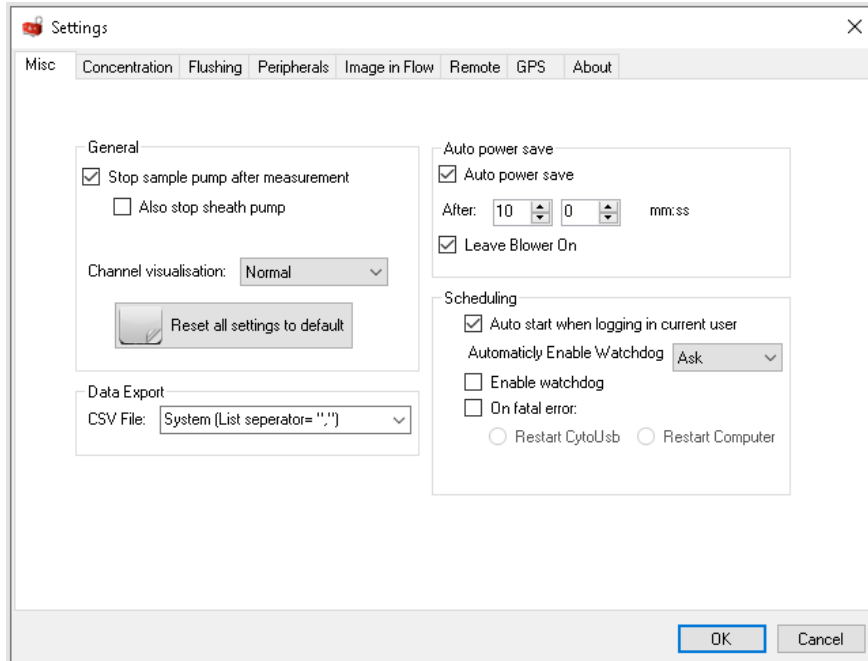
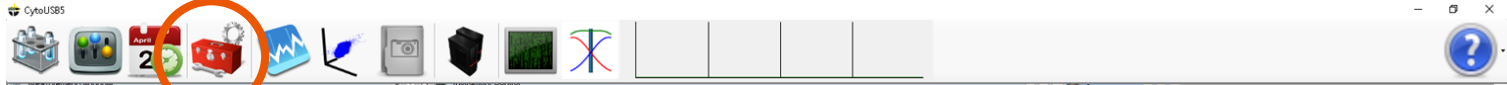


Schedule

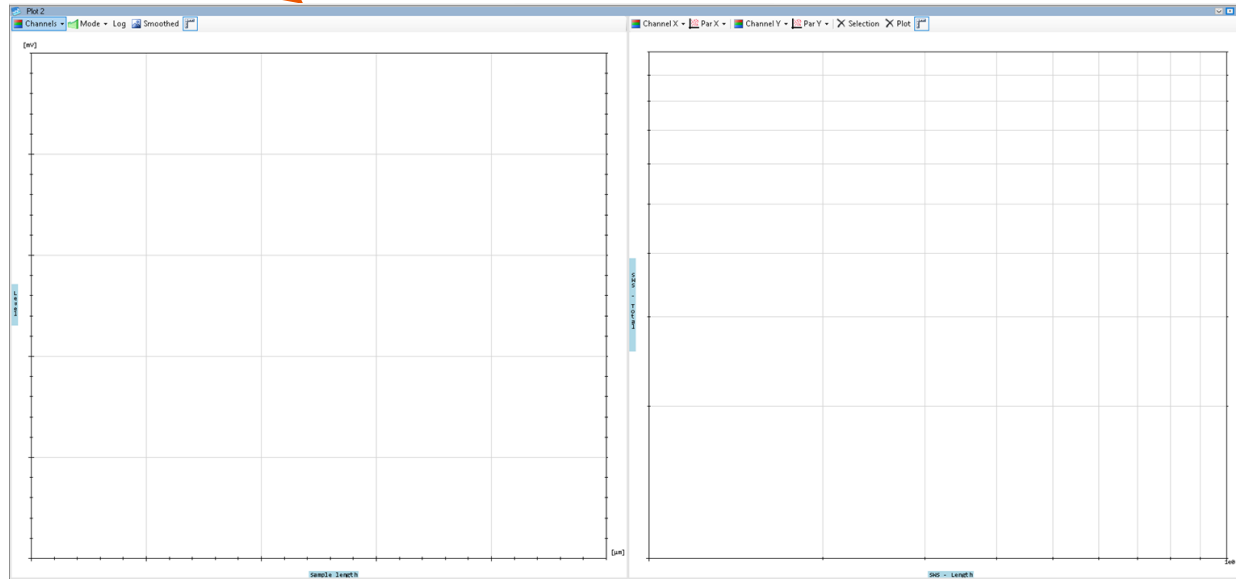
Schedule task wizard | Shutdown

	Enabled	Start	Finish	GPS	External_Trigger	Name	Number_Of_Measurements	Times_scheduled_per_day	Scheduled_days	ShutDown_Afterwa
▶	<input checked="" type="checkbox"/>	12/22/2022 2:02 PM		<input type="checkbox"/>	<input type="checkbox"/>	New task	4	-		<input type="checkbox"/>

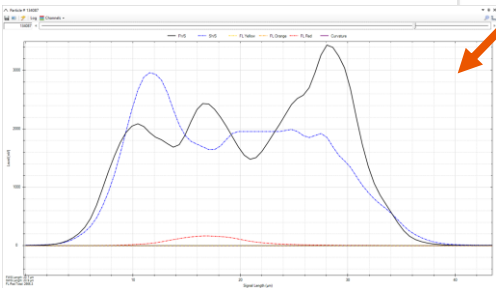
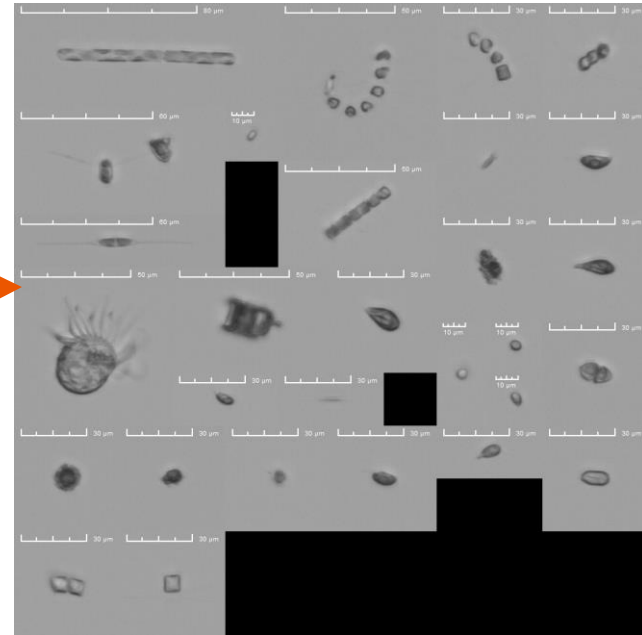
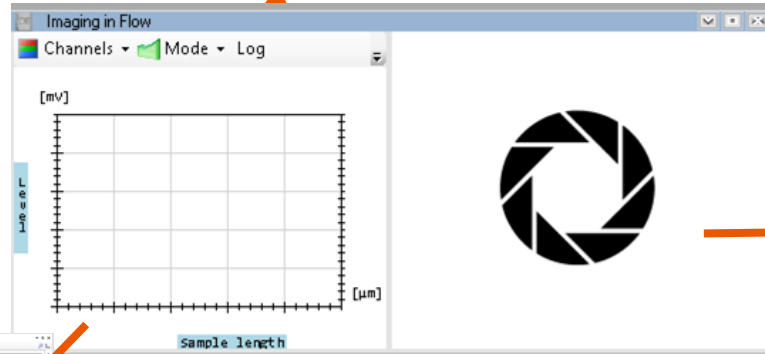
1 CytoUSB - Data acquisition



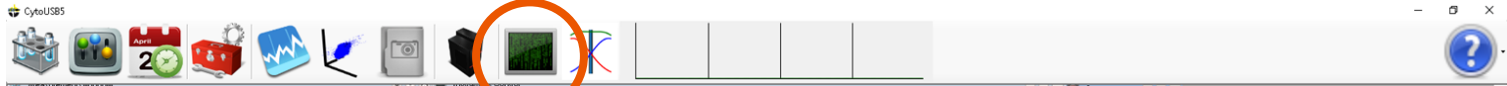
1 CytoUSB - Data acquisition



1 CytoUSB - Data acquisition



1 CytoUSB - Data acquisition



```
Terminal
2:00:11 PM: Secondary sensor read out performed
2:00:16 PM: Secondary sensor read out performed
2:00:21 PM: Secondary sensor read out performed
2:00:25 PM: Secondary sensor read out performed
2:00:29 PM: Secondary sensor read out performed
2:00:33 PM: Secondary sensor read out performed
2:00:38 PM: Secondary sensor read out performed
2:00:42 PM: Secondary sensor read out performed
2:00:47 PM: Secondary sensor read out performed
2:00:51 PM: Secondary sensor read out performed
2:00:55 PM: Secondary sensor read out performed
2:01:00 PM: Secondary sensor read out performed
2:00:56 PM: Sheath temp: 24.8125
2:00:56 PM: System temp: 25.5
2:00:56 PM: Pressure abs: -15.5132039025
2:00:56 PM: Concentration count: 0
2:00:57 PM: Sheath temp: 24.8125
2:00:57 PM: System temp: 25.5
2:00:57 PM: Pressure diff: 5.1710679675
2:00:57 PM: Concentration count: 0
2:00:57 PM: Concentration count: 0
2:00:58 PM: Sheath temp: 24.875
2:00:58 PM: System temp: 25.5
2:00:58 PM: Pressure abs: -15.5132039025
2:00:58 PM: Concentration count: 0
2:00:58 PM: Concentration count: 0
2:00:59 PM: Sheath temp: 24.8125
2:00:59 PM: System temp: 25.5
2:00:59 PM: Pressure diff: 7.75660195125
2:00:59 PM: Concentration count: 0
2:01:00 PM: Concentration count: 0
2:01:00 PM: Sheath temp: 24.8125
2:01:00 PM: System temp: 25.5
2:01:00 PM: Pressure abs: -15.5132039025
2:01:00 PM: Concentration count: 0
```

2

CytoClus4 - Manual characterisation



- Software package to process and analyse data from Cytobuoy instruments
- Works with .cyz files
- Current version is CytoClus4
- You can download it by Cytobuoy website.

2

CytoClus4 - Manual characterisation



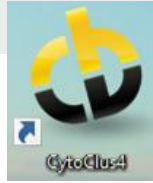
Main layout



When you hover your mouse pointer over the different buttons, you can read a short description of each tool.

2

CytoClus4 - Manual characterisation



A- Load file

The screenshot illustrates the manual characterisation process in CytoClus4. It shows a file explorer window on the left with a list of files under the folder 'Fichiers_bruts'. An orange box highlights the 'Database' dropdown menu, which is also shown in a larger view on the right. This view includes a yellow folder icon and a dropdown menu with the example text 'Example: "beads"'. An orange arrow points from this view to the 'Preferences' dialog box on the right. In the 'Preferences' dialog, the 'Folders' tab is active, and a table lists the folders to be added. The '+' icon in the top right of the table is highlighted with an orange box.

Show	Name	Include sub folders	#sub folders	# files
<input checked="" type="checkbox"/>	Fichiers_bruts	<input checked="" type="checkbox"/>	30	444
<input checked="" type="checkbox"/>	Fichiers_photos	<input checked="" type="checkbox"/>	11	140

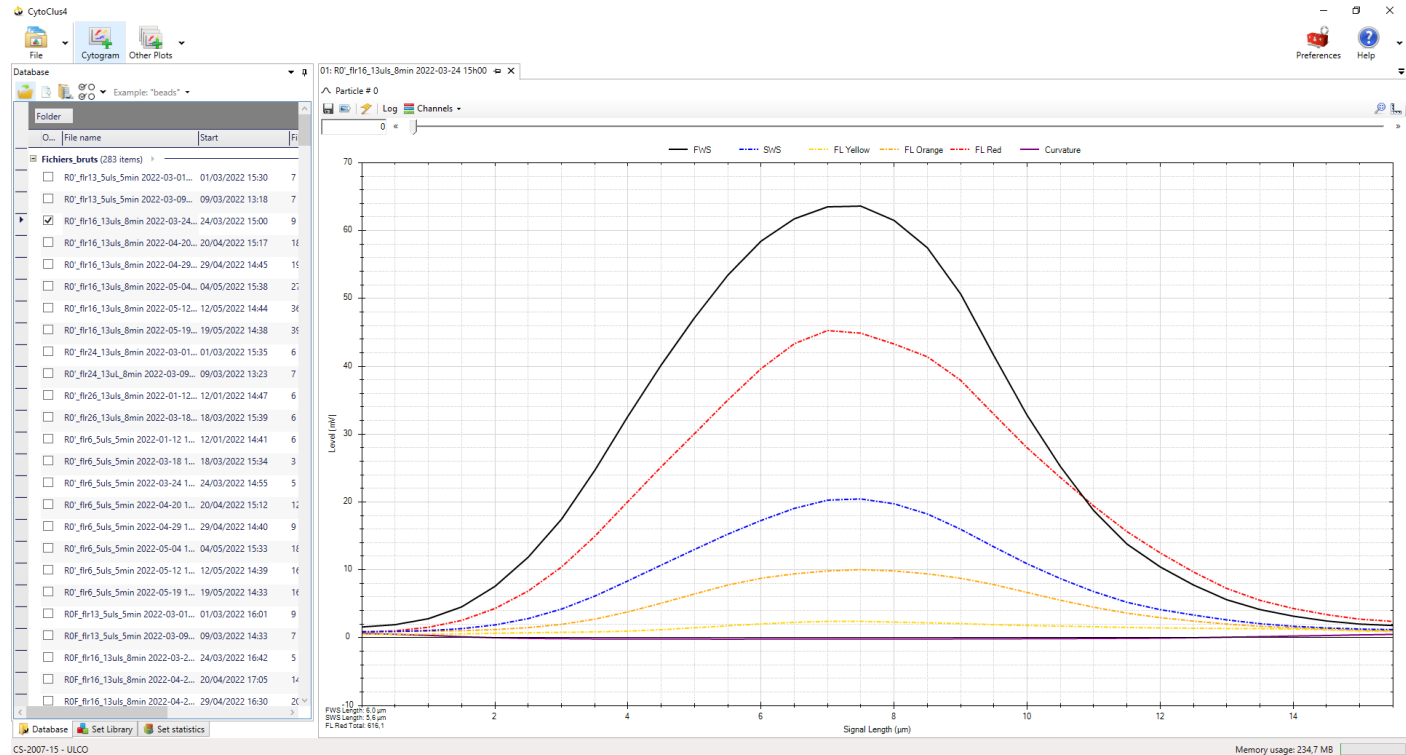
to choose folders you wish to add

2

CytoClus4 - Manual characterisation



A- Load file



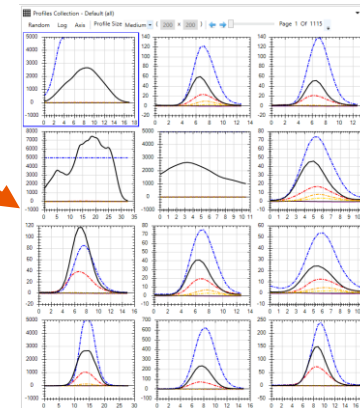
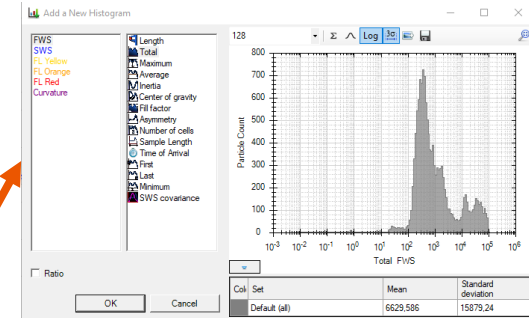
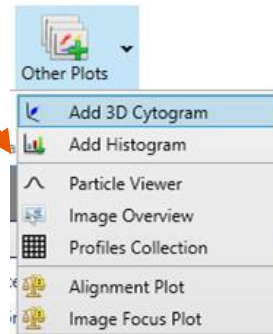
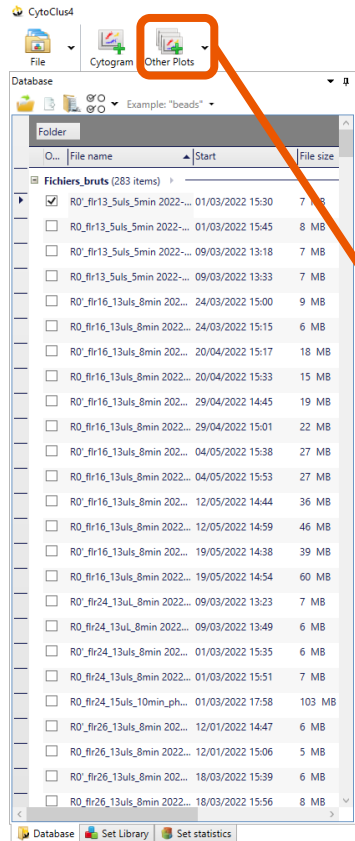
The default page layout is showing a “particle view”.

2

CytoClus4 - Manual characterisation



B- Plots



Shows collection of pulse shape profiles 19

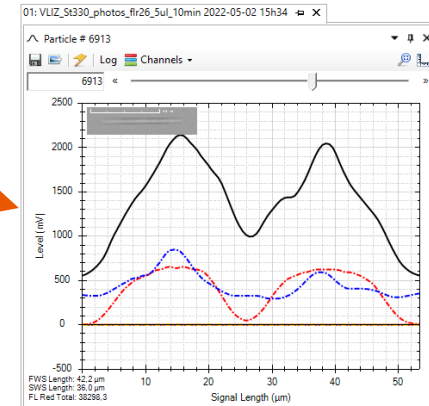
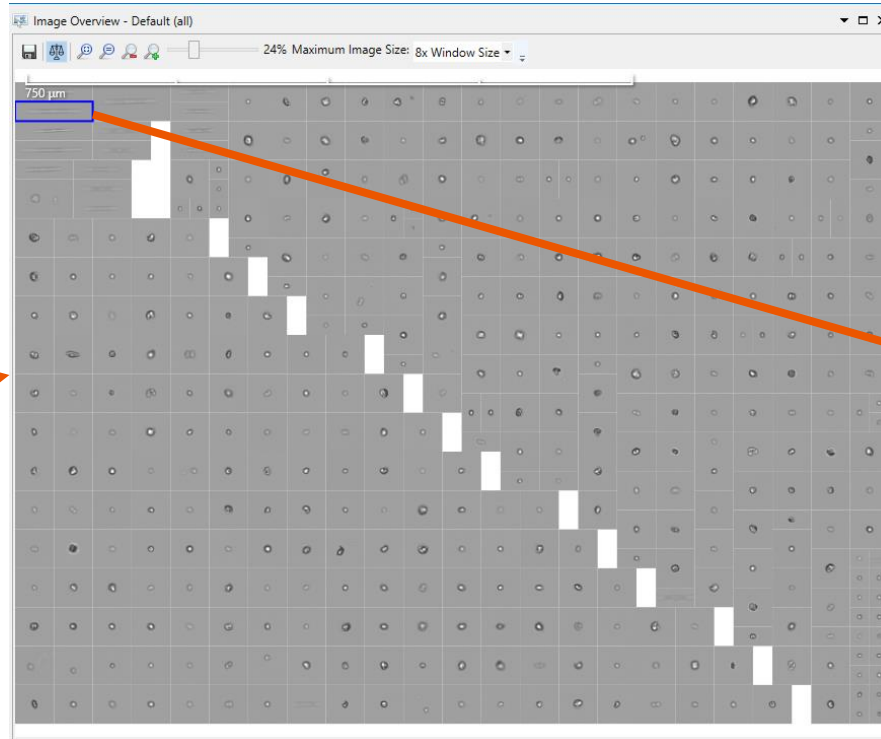
2

CytoClus4 - Manual characterisation



B- Plots

- Other Plots
- Add 3D Cytogram
- Add Histogram
- Particle Viewer
- Image Overview**
- Profiles Collection
- Alignment Plot
- Image Focus Plot



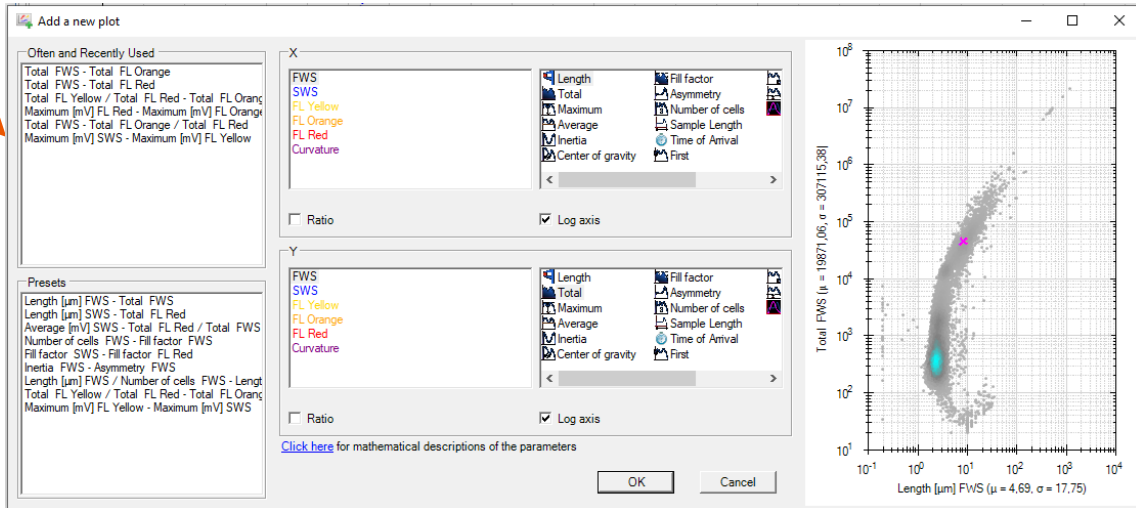
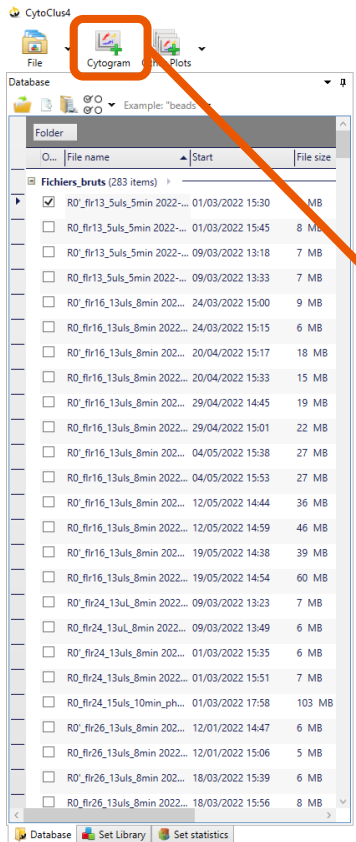
To see the all images taken during the measurement

2

CytoClus4 - Manual characterisation



C- Cytograms



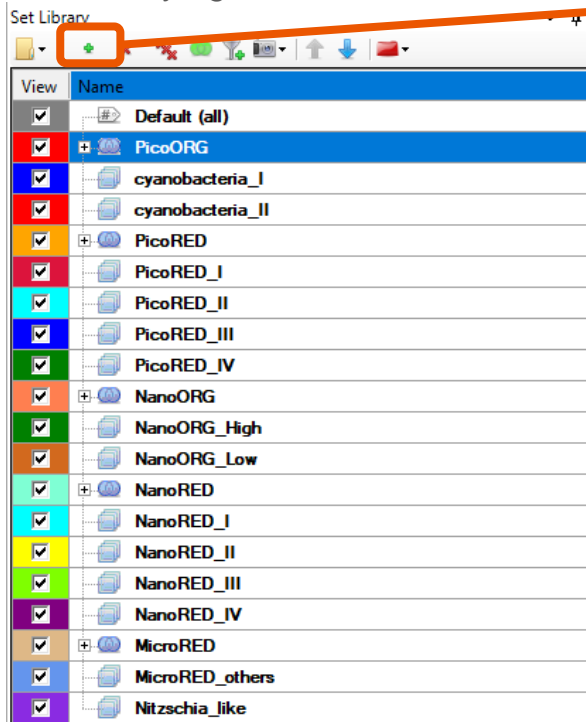
14 properties per particle ; 6 channels (FWS, SWS, FLR, FLO, FLY, Curvature)
To visualise each particle in a scatterplot

2

CytoClus4 - Manual characterisation



C- Cytograms



Create a new Set

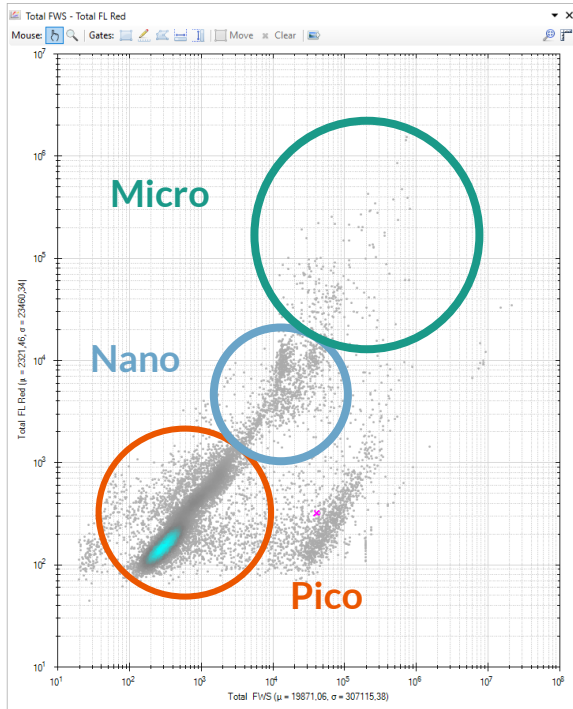


Draw clusters with different tools

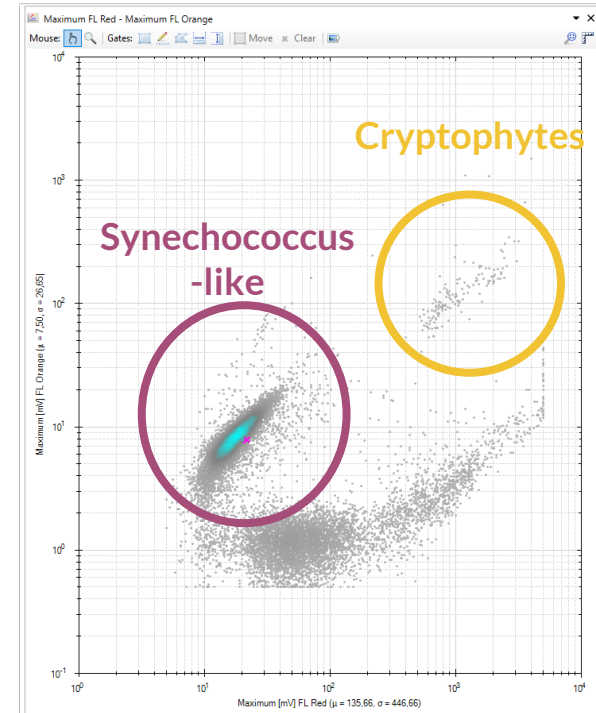
CytoClus4 - Manual characterisation



C- Cytograms



- The main ones are:
- 1/ Tot FWS - Tot FLR
 - 2/ MaxFLR - Max FLO
 - 3/ Max SWS - Max FLY
 - 4/ Length FWS - Tot FLR
 - 5/ Tot FLY/FLR - Tot FLO/FLR



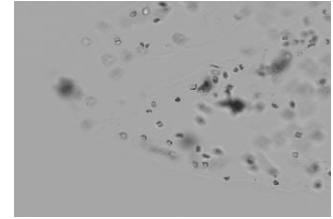
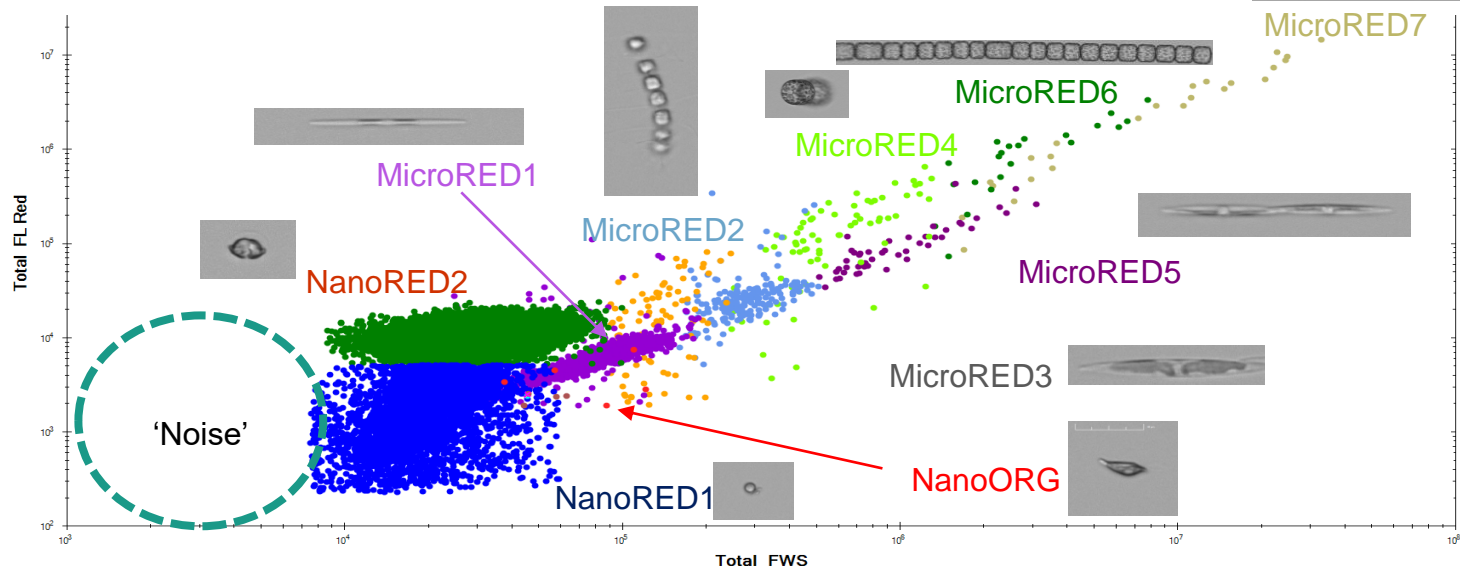
2

CytoClus4 - Manual characterisation (extended)



→ Microplankton protocol (small cells treated as « noise »)

→ Focus on nano- and microphytoplankton cluster (7 sub-clusters distinguished based on images and pulse-shapes, a hard work)



2

CytoClus4 - Manual characterisation



D- Export data

CytoClus4

File Cytoqram Other Plots

Database

Example: "beads"

Folder

O...	File name	Start	File size
[-]	Fichiers_bruts (283 items)		
<input checked="" type="checkbox"/>	R0'_flr13_5uls_5min 2022-...	01/03/2022 15:30	7 MB
<input type="checkbox"/>	R0'_flr13_5uls_5min 2022-...	01/03/2022 15:45	8 MB
<input type="checkbox"/>	R0'_flr13_5uls_5min 2022-...	09/03/2022 13:18	7 MB
<input type="checkbox"/>	R0'_flr13_5uls_5min 2022-...	09/03/2022 13:33	7 MB
<input type="checkbox"/>	R0'_flr16_13uls_8min 202...	24/03/2022 15:00	9 MB
<input type="checkbox"/>	R0'_flr16_13uls_8min 2022...	24/03/2022 15:15	6 MB
<input type="checkbox"/>	R0'_flr16_13uls_8min 202...	20/04/2022 15:17	18 MB
<input type="checkbox"/>	R0'_flr16_13uls_8min 2022...	20/04/2022 15:33	15 MB
<input type="checkbox"/>	R0'_flr16_13uls_8min 202...	29/04/2022 14:45	19 MB

Exports

General export settings

Settings file

Output directory D:\Zeline\Donnees\DYPHYRAD\Cytometrie\2014\Donnees_exportees_fgps

Load Save Select

Exports

Summary Text file

Listmode parameters

Statistics per set

IIF images

Generate overview image

CYZ File (for set)

GPS Data

Auxiliary sensor logs

Pulse shapes

Sets

Default (all)

PicoORG

cyanobacteria_I

cyanobacteria_II

PicoRED

PicoRED_J

Select All

Listmode parameter settings

Channels

FWS

SWS

FL Yellow

FL Orange

FL Red

Curvature

Select All

Parameters

Length

Total

Maximum

Average

Inertia

Center of gravity

Fill factor

Asymmetry

Number of cells

Sample Length

Select All

Format

CSV

FCS

MAT

Select All

Selected files: 1

Run Interactive OK Cancel

There is also a possibility of running analysis in an interactive mode



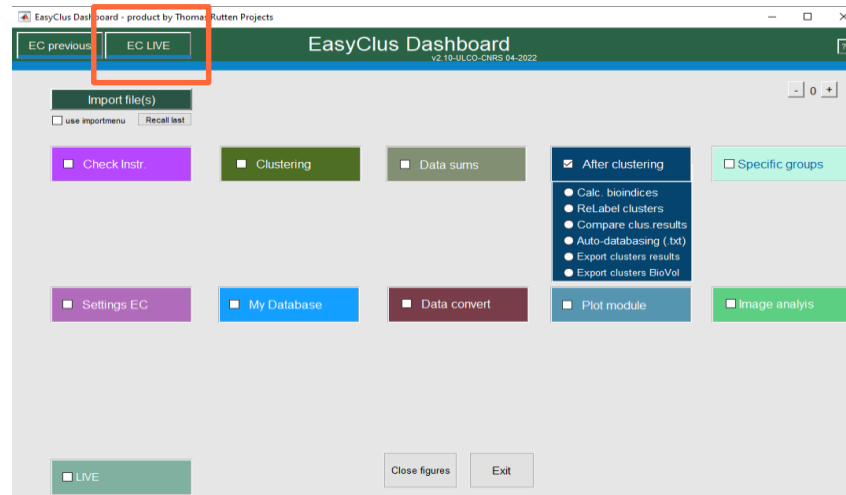
3

EasyClus live - Fast data visualization

- EasyClus software supported on MatLab developed by Thomas Rutten
- Feature we use: EasyClus Live

During long in situ deployments:

- Useful to quickly visualize what is happening in the water and the state of the machine



EasyClus live - Fast data visualization



menu Easyclus live

EasyClus Live 2.0

v2.10-ULCO-CNRS 04-2022

Live-location name:

Protocol NOT available

Live process settings

Totals

Size fractions

Clustering DESIGN 2

Bioindices

Beads Charts

Preprocess raw fcmdata autodatabasing on

Export all as txt autoIMAGdbse on

images mosaic on

Figures visibility on

Leave figures open until next file is imported

or

Release NEW location by copy below

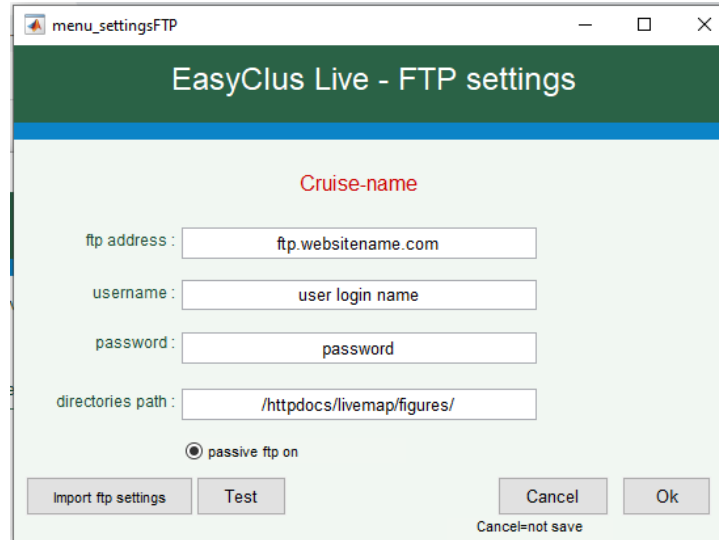
Use .cyz

Process cyz- (or .mat) files from directory:
C:\EasyClusv210results\datafiles\

open menu filter filenames

results to internet

Copyright Thomas Ruitten Projects

A screenshot of a software dialog box titled "menu_settingsFTP" with a dark green header bar containing the text "EasyClus Live - FTP settings". The dialog has a light green background and contains several input fields and a radio button. The fields are labeled "ftp address", "username", "password", and "directories path". The "ftp address" field contains "ftp.websitename.com", "username" contains "user login name", "password" contains "password", and "directories path" contains "/httpdocs/livemap/figures/". Below the fields is a radio button labeled "passive ftp on" which is selected. At the bottom, there are four buttons: "Import ftp settings", "Test", "Cancel", and "Ok". Below the "Cancel" button, the text "Cancel=not save" is displayed.

menu_settingsFTP

EasyClus Live - FTP settings

Cruise-name

ftp address :

username :

password :

directories path :

passive ftp on

Cancel=not save

EasyClus live - Fast data visualization



menu Easyclus live

EasyClus Live 2.0
v2.10-ULCO-CNRS 04-2022

Live-location name:

Protocol available

Live process settings

Totals

Size fractions

Clustering DESIGN 2

Bioindices

Beads Charts

Preprocess raw fcmdata autodatabasing on

Export all as bxt autoIMAGEdbse on

images mosaic on

Figures visibility on

Leave figures open until next file is imported

Use .cyz

Process cyz- (or .mat) files from directory:
C:\EasyClusv210\results\datafiles\

open menu filter filenames

Filter files option

Pico	<input checked="" type="radio"/> use filetext tag1 filter
Micro	<input checked="" type="radio"/> use filetext tag2 filter
	<input type="radio"/> use filetext tag3 filter
	<input type="radio"/> use filetext tag4 filter <input type="checkbox"/> =susp.mat
	<input type="radio"/> use filetext tag5 filter

results to internet

Copyright Thomas Rutten Projects

3

EasyClus live - Fast data visualization

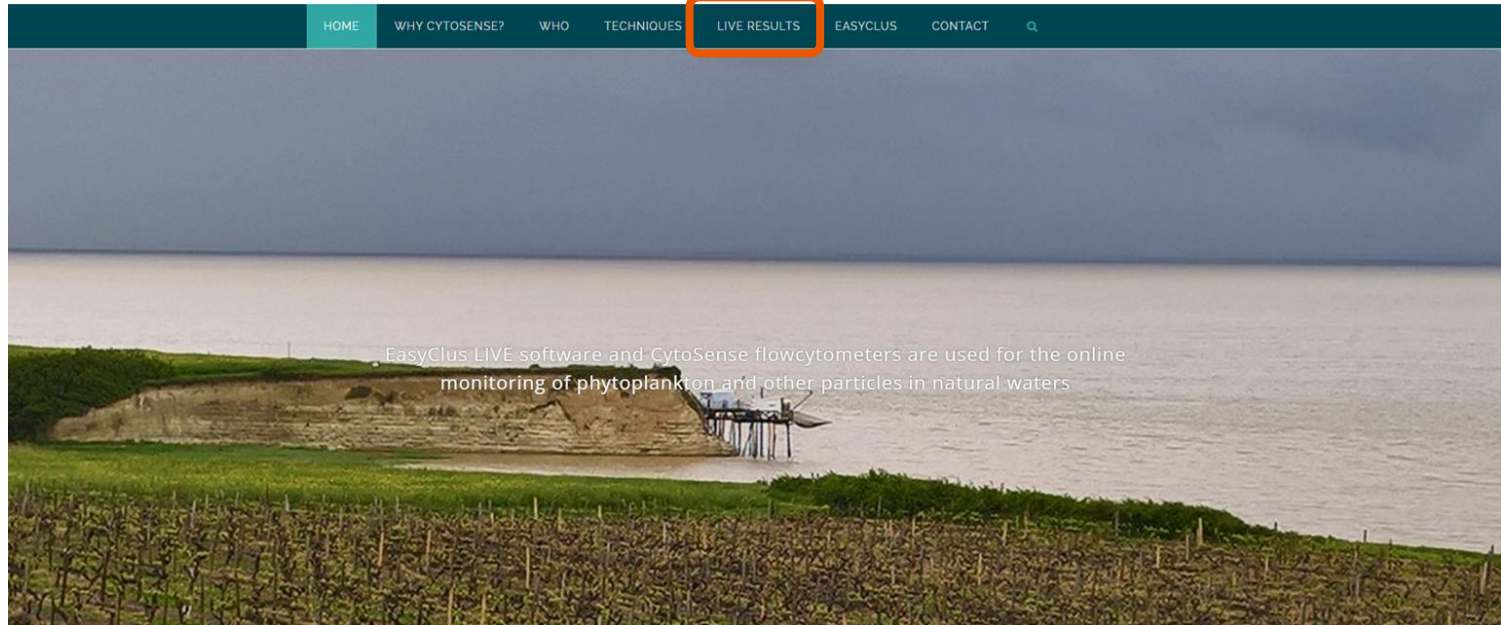


https://fytoplankton.nl/ULCO-CNRS/Marel/phytoplankton_livloc.shtml

PhytoplanktonLIVE

Thomas Rutten Projects

Online automatic phytoplankton monitoring at several locations performed by flowcytometry (CytoSense-CytoSub)

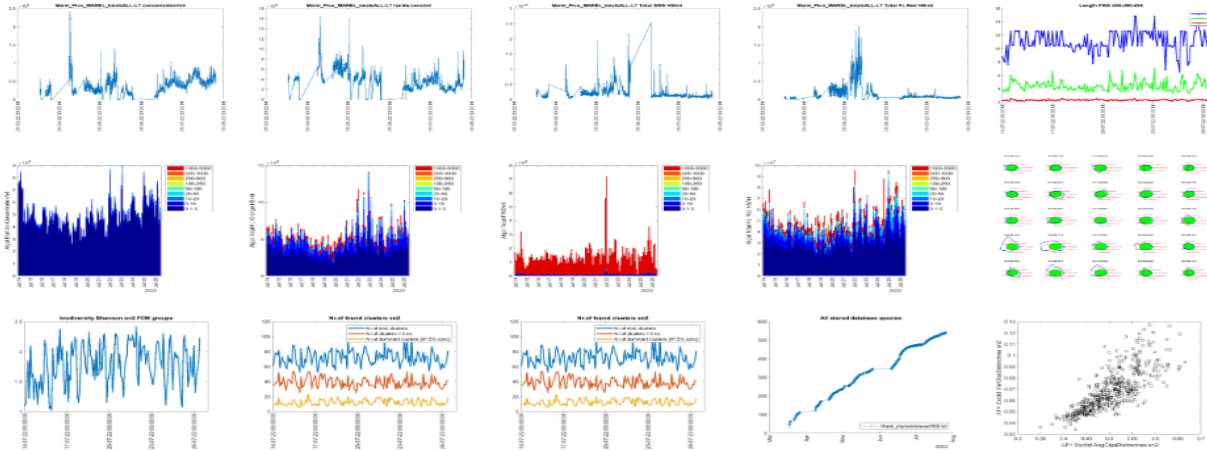




HOME

Live Results Marel

[to image_gallery](#) (if available)



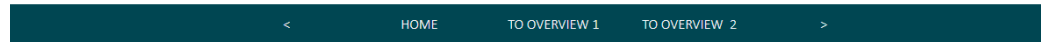
[data.html](#)
[data.html](#)
[data.html](#)
[data.html](#)
[data.html](#)
[data.html](#)
[data.html](#)
[data.html](#)

Disclaimer



ktonLIVE.com

aplankton monitoring at several locations performed by flowcytometry (CytoSense-CytoSub)



[To overview](#)

Live Results Marell

Check latest Sample

Analysis volume

383 μ l

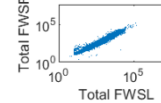
Total events

21329

Part/sec.

6318 ev/s

26-07-2022-10:01



Traffic lights Dashboard

Checks of the instrument that indicates whether the instrument is working properly or not.
Green light = ok
Red light = not ok

Int.Pressure

200 mBar

Int.diff.Press.

-274 mBar

% ev. Mean \pm 10%

54.2 %

Ratio L/R

1.09

Temp.Laser

31.5 C

Temp.Sh.Flow

27.6 C

Temp. PMT

30.0 C

Temp. System

30.5 C

%FL Red HSflat sign.


0.03 %

Laser1 Curr.

0.0 mA

Laser2 Curr.

0.0 mA



**Thank you for your
attention!**