

Using Example FlowCam Aquatic Image Libraries

SUMMARY

Example libraries can be a great way for new FlowCam users to gain experience with plankton identification and provide a starting point to creating their own personalized libraries. Yokogawa Fluid Imaging Technologies has collected images from aquatic samples gathered across North America, and is providing the following libraries to all FlowCam users:

Freshwater Libraries:

- *Aphanazomenon*
- *Asterionella*
- *Cylindrospermopsis*
- *Dolichospermum*
- *Fragilaria*
- *Lyngbya*
- *Microcystis*
- *Pediastrum*
- *Planktothrix*
- *Rotifers*
- *Scenedesmus*

Marine Libraries:

- *Asterionellopsis*
- *Chaetoceros*
- *Corethron*
- *Cylindrotheca*
- *Detonula*
- *Dictyocha*
- *Dinophysis*
- *Diplopsalis*
- *Ditylum*
- *Eucampia*
- *Favella*
- *Gonyaulax*
- *Gyrodinium*
- *Leptocylindrus*
- *Licmophora*
- *Mesodinium rubrum*
- *Navicula*
- *Odontella*
- *Pleurosigma*
- *Nauplii*
- *Prorocentrum micans*
- *Protoperidinium*
- *Protoperidinium steinii*
- *Pseudo-nitzschia*
- *Rhizosolenia*
- *Scrippsiella*
- *Skeletonema*
- *Synchaeta*
- *Thalassionema*
- *Thalassiosira*
- *Tintinnopsis*
- *Tripos furca, lineatus, and muelleri*

HOW TO USE LIBRARIES WITH DATA ACQUIRED ON A DIFFERENT FLOWCAM INSTRUMENT

The libraries provided here are intended as examples: while they may perform well on your data, that performance depends on a variety of factors, including instrument-specific variables (e.g. model, objective, background intensity mean, Capture settings, mode of

data acquisition,) and sample-specific characteristics (e.g. organism size, health, and preservation method). Therefore, while users are welcome to test these libraries on their own samples, we advise you to create your own to optimize performance. These libraries were built using FlowCam 8000 and a 10X objective. They should not be applied to datasets at other magnifications.

OPTIMIZE LIBRARY PERFORMANCE TO SEMI-AUTOMATE IDENTIFICATION OF ORGANISMS

When building a FlowCam image library, the mantra you must memorize is *unique* and *uniform*. Automated identification with images is based on morphology (size being the primary point of differentiation) to identify related particles. Are the images unique compared to other images in the sample? Are the images within the library uniform?

The unique and uniform mantra is illustrated with *Ceratium* and *Chaetoceros*, a common marine diatom (Figures 1 and 2, respectively).



Figure 1. *Ceratium* images captured with FlowCam 8400 in Trigger mode using a 10X objective, as displayed in VisualSpreadsheet



Figure 2. *Chaetoceros* images captured with FlowCam 8100 using a 4X objective, as displayed in VisualSpreadsheet

Ceratium is very unique compared to *Chaetoceros*. It is also very uniform. A horizontal orientation does not affect identification. It is relatively easy to automate identification of *Ceratium* with FlowCam data. *Chaetoceros* is unique compared to *Ceratium*, but *Chaetoceros* is not uniform. It can be long, short, curved, or straight. The *Chaetoceros* shown on the previous page should be split into multiple libraries. Here is one way (of many ways) this could be accomplished: *Chaetoceros* - Short Straight, *Chaetoceros* - Short Curved, *Chaetoceros* - Long Straight (Figure 3), *Chaetoceros* - Long Curved (Figure 4).

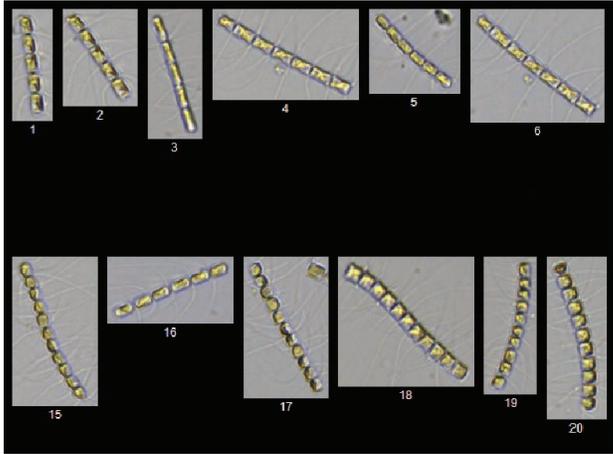


Figure 3. An example of a *Chaetoceros* - Long Straight library. Images captured with FlowCam 8000 using a 10X objective, as displayed in VisualSpreadsheet.

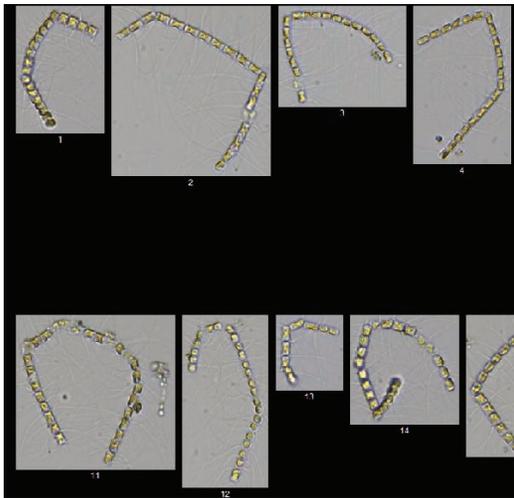


Figure 4. An example of a *Chaetoceros* - Long Curved library. Images captured with FlowCam 8000 using a 10X objective, as displayed in VisualSpreadsheet.

These libraries are intended as a starting point. Some users may find that the libraries function well with their data sets without manipulation. Others may find they need to supplement the libraries with some of their own data to better represent the exact conditions found in their sample.

If you are following the “rules” for library building and the library still isn’t performing well, open the library and turn on Particle Edge Trace (Show > Particle Edge Trace). This will show you what the FlowCam software “sees” (compared to what the human eye sees) so that you can better relate to how the data is being characterized.

MASTER LIBRARIES

Please note, you cannot move particles from one library to another in VisualSpreadsheet 4 (this is possible in VisualSpreadsheet 5 and 6). For VisualSpreadsheet 4 users, here is a workaround using *Chaetoceros* as an example, as this genus has many species and manifests in many forms.

1. Create a “master” library: Ex. “Chaetoceros Master” and start adding all types of *Chaetoceros* to it.
2. Once the library has a sufficient number of images (typically 40-60 images with edge gradients over 60, depending on your sample), go to File > Save As and save the library under a new name. Ex. “Chaetoceros - Long Straight” and “Chaetoceros - Long Curved”.
3. Delete the particles that are no longer relevant in the new library. For example, delete the short and curved *Chaetoceros* particles from the “Chaetoceros - Long Straight” library. Do not alter the “Master” library in case you need to reference it again.
4. For additional library training and resources, contact Yokogawa Fluid Imaging.