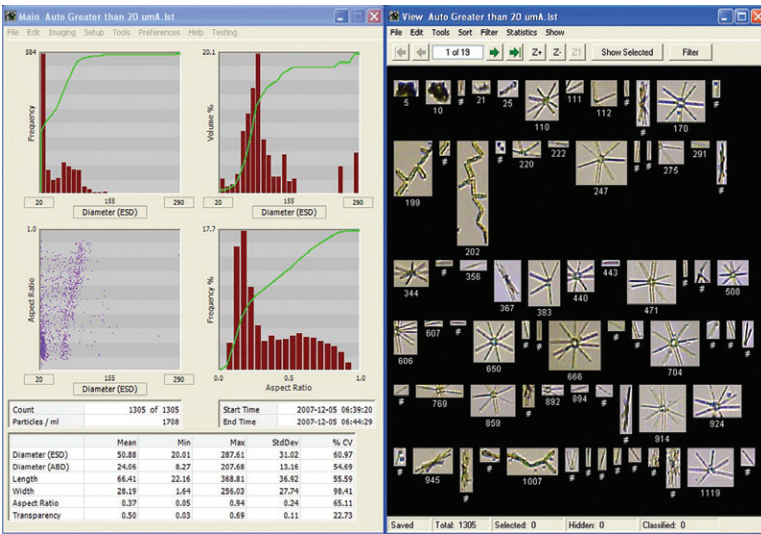




## VisualSpreadsheet<sup>®</sup>: Intelligent Pattern Recognition for Particle Analysis

FlowCAM<sup>®</sup> is an imaging-based particle analysis system, and produces large quantities of data for any given sample run. An image of each particle in the sample is captured, along with up to 26 different measurements for each image. This is quite different than most other particle analysis instruments, which store only a measurement of Equivalent Spherical Diameter (ESD) for each particle. Having the particle images available, along with the wealth of measurements made for each particle, enables highly sophisticated pattern recognition operations to be made automatically. This process duplicates standard microscopy techniques without the need for a human operator, allowing for much larger quantities of sample to be processed in significantly less time. The greater number of data points analyzed yields a much higher degree of *statistical significance* for the results.

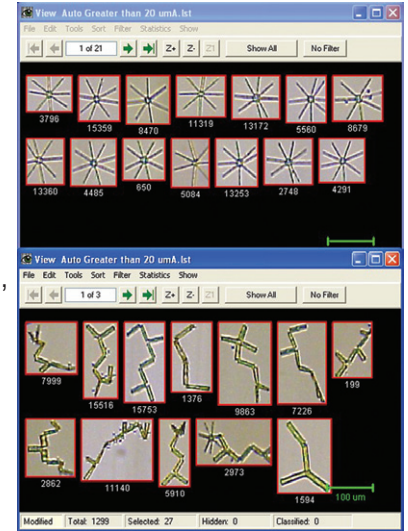


### How it works:

The image above shows the results of a FlowCAM run for a drinking water reservoir sample. In this particular sample, the object is to quantify the amount of two particular taste and odor causing algae, *Asterionella* sp. and *Taballaria* sp. which may be found in the water supply. This is normally done manually through a microscope, which is time consuming, thereby not allowing for a large amount of sample to be analyzed.

FlowCAM's integrated software, VisualSpreadsheet, can automate this process automatically using pattern recognition.

This process does not occur without first having an operator "train" the system so that it knows what to look for. However, the process of training the system is a one-time occurrence; once the system has been trained, it can be used to look for the same set(s) of particles in *any* sample. To train the system, the user merely browses through the particle images and highlights target particles of the type one

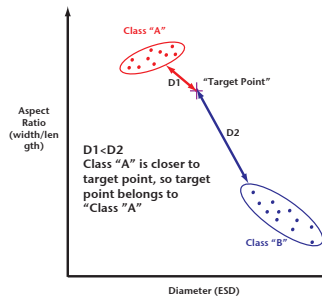


desires to find. These are then added to a "library" stored in the software. Multiple libraries can be built and applied against any given sample. The image above shows the two libraries defined for this example: *Asterionella* sp. on top and *Taballaria* sp. below.

Once the libraries are built, the pattern recognition (classification) is invoked simply from the VisualSpreadsheet menu structure. Although the two classes shown above are relatively simple to distinguish by eye, this is computationally a fairly advanced discrimination to make mathematically. In *statistical pattern recognition*, the libraries are used to statistically describe a point in a multi-dimensional space representing each class. For each particle measurement being used, the statistics of the library images (mean, standard deviation, CV) are used to calculate a "weighting factor" for each variable so that the measurements which most clearly define the particles are weighed heavier than those that do not.

Once each class is defined by its point in the multidimensional space and the classification is invoked, then each particle in the sample run is then plotted in the same multidimensional space and compared to the points for the classes. Each particle is then assigned to one of the classes based upon the class point which is the *minimum distance* away from the sample point.

The illustration to the right shows a very simple example of how the classification process works. In this example we have plotted two classes in a two-dimensional space defined by aspect ratio and ESD diameter. When a target particle point is plotted in the same space, its distance to both classes is calculated, and the target point is assigned as *probably* belonging to the class that is nearest.



The distance to the assigned class is expressed as a *filter score*, which represents the *normalized distance* to the class. Based upon the class statistics and normalized distance, each target particle is then determined to be a member of the class or not.

After the classification is finished, each particle that has been assigned to a class appears in a separate image window for each class. All remaining particles which are “unclassified” remain in the original image window. In applications where *absolute enumeration* is required (such as this example where the particles are very sparsely distributed), the user can interactively add/subtract particles from each class or from the remaining unclassified images. Detailed statistics are generated for each class which are easily exported to be incorporated into reports.

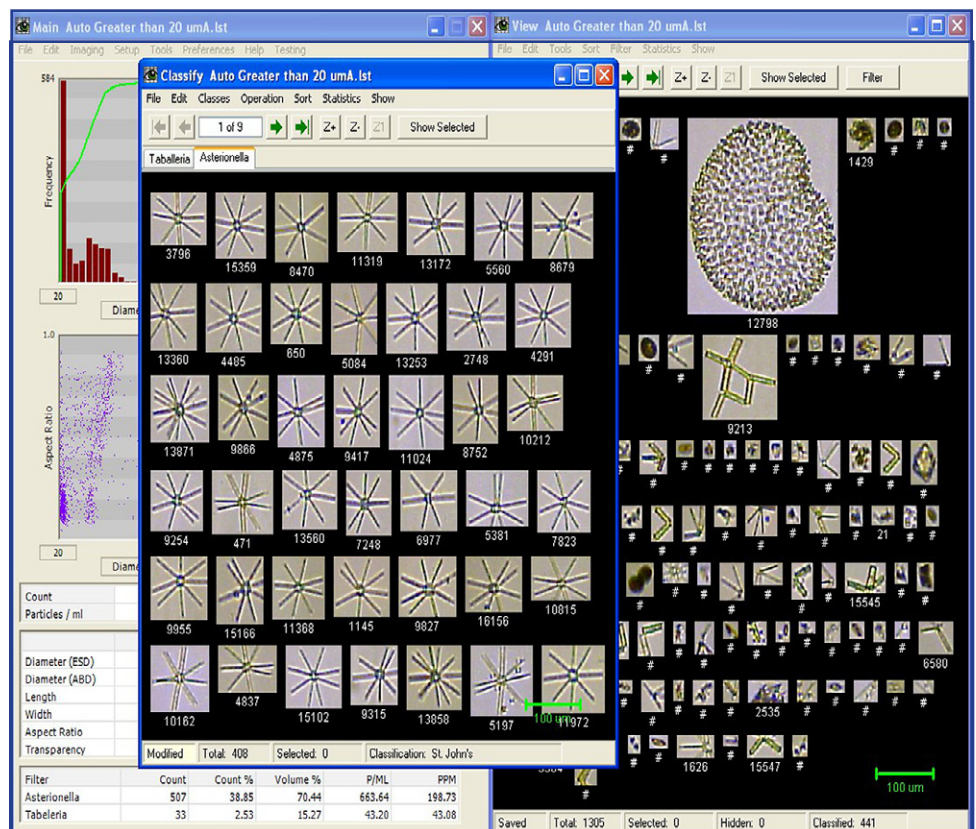
The same libraries can be used again on any new sample that needs to be analyzed. This means that the same statistical comparison is being made for each analysis, so that the results are statistically normalized for comparison purposes.

Although this example is a relatively simple one applied to algae in drinking water, the same technique is being successfully used on many other types of samples, including foods, beverages, chemicals, pharmaceuticals and many others. In all cases, VisualSpreadsheet is able to replace manual microscopy methods, analyzing far more statistically significant amounts of data in a fraction of the time it would take to accomplish manually.

## VisualSpreadsheet Pattern Recognition Benefits

- High-speed automated classification of particles
- Intuitive *image-based* user interface
- Re-usable particle libraries
- Microscopy results in a fraction of the time
- Detailed, exportable statistical results
- Uses up to 26 different particle measurements for a high degree of discrimination

For a more detailed explanation of how pattern recognition works in particle analysis, you may download our white paper, “*Particle Image Understanding - A Primer*”, available on the Fluid Imaging web site at [www.fluidimaging.com](http://www.fluidimaging.com). Or call us to discuss your potential application in detail at (207) 846-6100.



Results of the automated statistical classification. The “Classify” window shows the particles identified as members of each class. Note there are two “tabs” in this window, one for each class. In this case the window shows the particles classified as *Asterionella*, but clicking on the “tab” labelled *Tabellaria* would show the particles classified as that type. Particles in the right hand window are the particles left over as “unclassified”. Note also in the lower part of the left hand window that exact statistics (including count and concentration) for each class are summarized.