Detection of Invasive Species Using Birefringence Imaging Particle Analysis

Objective

Many fresh water lakes and rivers are being assaulted by invasive species of plankton and other organisms. These "invaders" can be brought into water bodies by ballast water from ships, on the hulls of boats (both commercial and recreational), or by any means where water from one source is exchanged with another. The introduction of these invasive species which are not native to the ecosystem can cause serious problems.

Of particular concern has been the detection of invasive mussels in the Great Lakes in America, most notably the zebra and quagga mussels, and their rapid spread to other bodies of water.

Adult Zebra Mussel

These mussels are "filter feeders", which means that they remove particles from the water. On the positive side, this can make the water clearer and reduce pollutants. On the negative side, these mussels consume large amounts of phytoplankton, which are an important source of food for young fish. They also retain much of the pollutants they filter, which are then ingested by birds and fish that feed on the mussels. Finally, these mussels are so prolific (a single adult can produce up to 1 million eggs/year) that they quickly begin to cover any hard surface available, ranging from water intakes to buoys. They can actually clog up pipes or intakes used for cooling or drinking water.

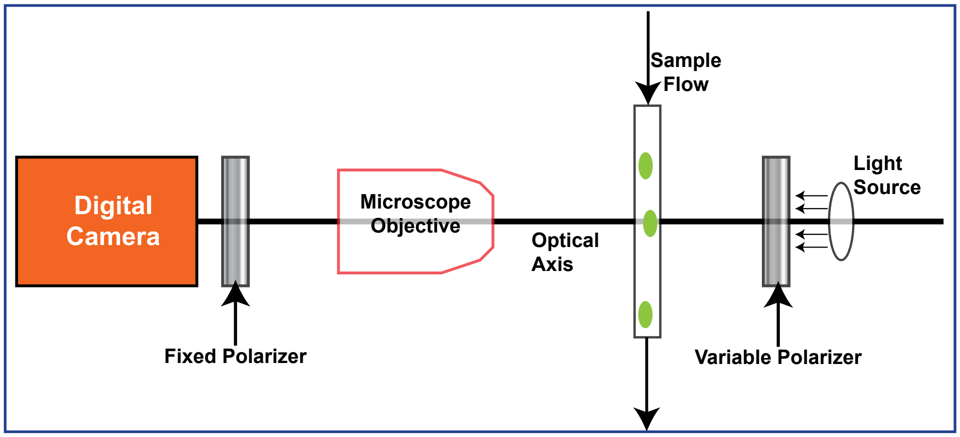


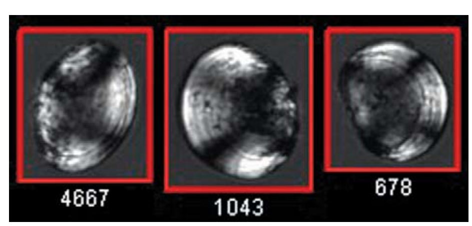
Figure 1: Optical configuration for birefringence particle imaging (patent pending).

While adult mussels can be as large as 1 ½ inches in size and easily seen by the eye, sampling for larval stage (veligers) mussels is the preferred method for detection, if possible. Since these mussels are so prolific, early detection is critical in order to have a chance of proactively curbing their spread. Detection of the veligers shows the initial presence of the species, when it is possible to take certain steps to slow down their spread. Unfortunately, the veligers are so small (50-250µm) that they can only be seen through a microscope. Adding to this problem is the fact that in early stages, the veligers will be present in very sparse amounts, meaning that a large volume of water needs to be analyzed through a microscope in order to spot them early.

Method

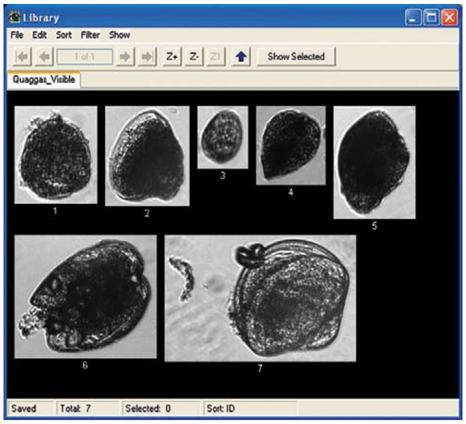
The FlowCAM© is ideally suited for the early detection of mussel veligers, as it can process a significantly larger amount of sample "in-flow", much faster than could conceivably be done with manual microscopy. Using the VisualSpreadsheet© automated pattern recognition algorithms, one can quickly distinguish the particles in the sample which appear to be mussel veligers. Unfortunately, since the veligers look very similar in shape to some plankton and detritus, some error can occur in the process.

Fortunately, the skeletons of these organisms are calcareous, and therefore will exhibit birefringence under cross-polarized light. The FlowCAM can be outfitted for birefringence imaging merely by placing a fixed polarizer in front of the camera, and a variable polarizer between the light source and the flow cell (see Figure 1 above). The result of this is that the camera sees a dark field with white images only when a birefringent particle passes through the flow cell (see below).



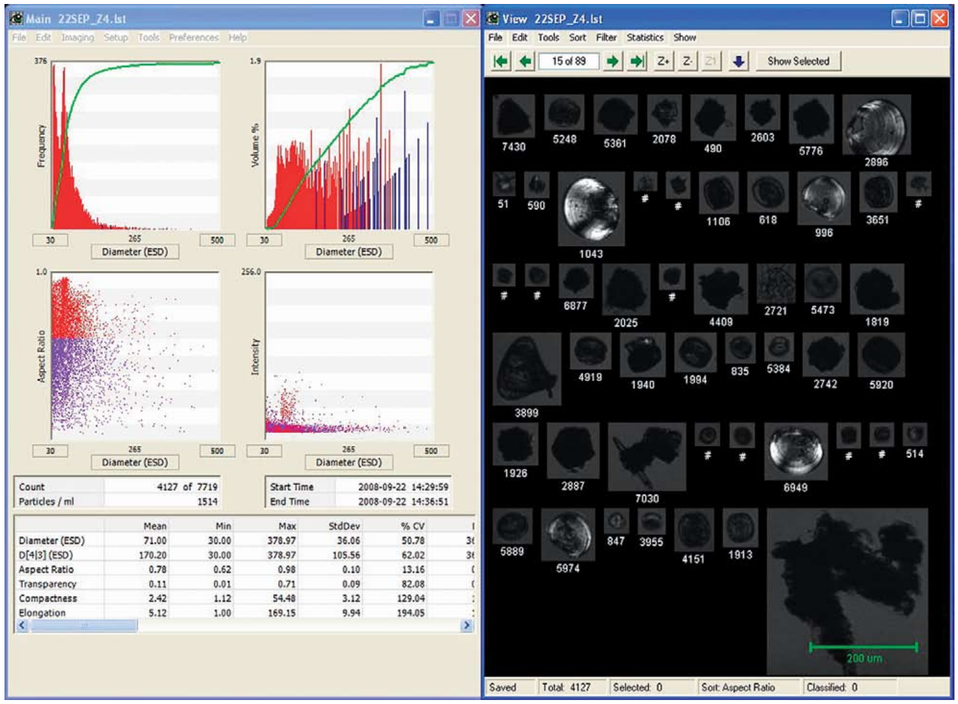
Zebra Mussel veligers: birefringence images captured under cross-polarization. Note distinctive "Maltese Cross" pattern.

The image below shows how quagga mussel veligers appear when imaged by the FlowCAM using standard



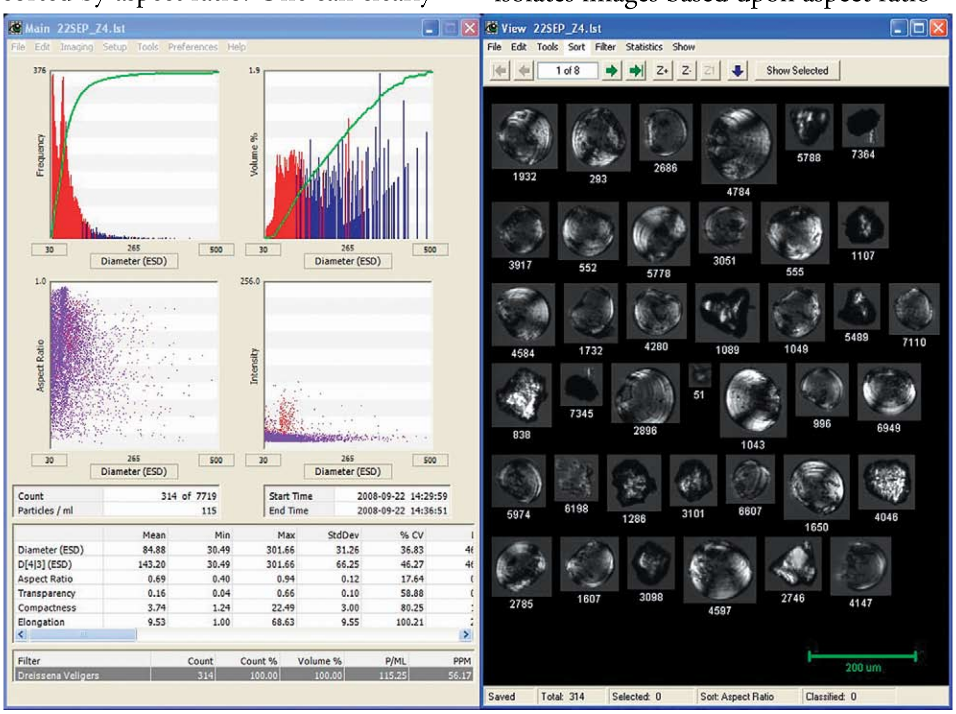
lighting: While these images are relatively distinct to the eye, the only significant mathematical differentiation they share in common is aspect ratio (width/length). However, when imaged under cross polarized illumination, the mussel veligers become much easier to distinguish.

The image to the right shows all particles found in a lake water sample when imaged using cross polarization, sorted by aspect ratio. One can clearly pick out the veligers based upon gray scale intensity from similar shaped particles (detritus, *Coscinodiscus*, etc.).



Birefringence images of lake water sample.

So, by merely constructing a “filter” in the VisualSpreadsheet software that isolates images based upon aspect ratio and gray scale intensity, one can quickly isolate just the veligers as shown below.



Birefringence images of zebra mussel veligers in lake water sample.

Using the FlowCAM with cross-polarization to detect invasive species of mussels is extremely simple, saves enormous amounts of time and eliminates the human error that may be introduced through manual microscopy. Because this technique detects the larval stage (veligers) of the species, it provides for significantly earlier detection of these invasive species. Additionally, since the FlowCAM images a moving stream of sample, it has the capability to process much larger quantities of sample in a very short time, enabling detection of very sparse populations.

The design of the FlowCAM is flexible enough that the instrument can also be used to detect taste and odor causing algae as well as cyanobacteria in the same water samples. Contact Fluid Imaging Technologies today to learn more and discuss having a sample of your water tested!

What’s in Your Water?©

FLUID IMAGING TECHNOLOGIES FlowCAM

Fluid Imaging Technologies, Inc | 65 Forest Falls Drive, Yarmouth, ME | 04096 | (207) 846-6100 | www.fluidimaging.com