

AN ABSTRACT OF THE THESIS OF

Matt J. Sarpola for the degree of Master of Science in Mechanical Engineering
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Title: The Design of an Aquatic Insect Imaging Device as Part of a System to
Automate Insect Classification for Biomonitoring

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Robert K. Paasch

Population counts of aquatic insects are a valuable tool for monitoring the water quality of rivers and streams. However, the handling of samples in the lab for species identification is time consuming. An aquatic insect imaging device was designed as part of a system to automate aquatic insect classification and was tested using several species and size classes of stonefly (Plecoptera). The device uses ethanol to transport specimens via a transparent rectangular tube to a digital camera. A small jet is used to position and reorient the specimens so that sufficient pictures can be taken to classify them with pattern recognition. This project demonstrates a possible design for an automated aquatic insect imaging device and is an example of the collaboration possibilities between the engineering and life science disciplines.

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The Design of an Aquatic Insect Imaging Device as Part of a System to Automate
Insect Classification for Biomonitoring

by
Matt J. Sarpola

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The Design of an Aquatic Insect Imaging Device as Part of a System to Automate Insect Classification for Biomonitoring

1 Introduction

Aquatic insect population counts are a valuable tool for monitoring environmental quality. The various species of aquatic insect larva provide an effective way to monitor the health of streams. For example, stonefly (Plecoptera) larvae are very sensitive to changes in water quality. On a scale from 0 to 10 with 10 being the most tolerant species, stonefly larva rank between 0 and 2 depending on the specific species [Hilsenhoff 1988]. They are usually the first organisms to experience population decline which make them a good early indicator that water quality is deteriorating. Since stonefly larva live in the stream continuously, they integrate stream health over time and are a better indicator than a single point in time measurement such as chemical analysis [Resh et al. 1996]. Several examples of stonefly larva are shown in figure 1.

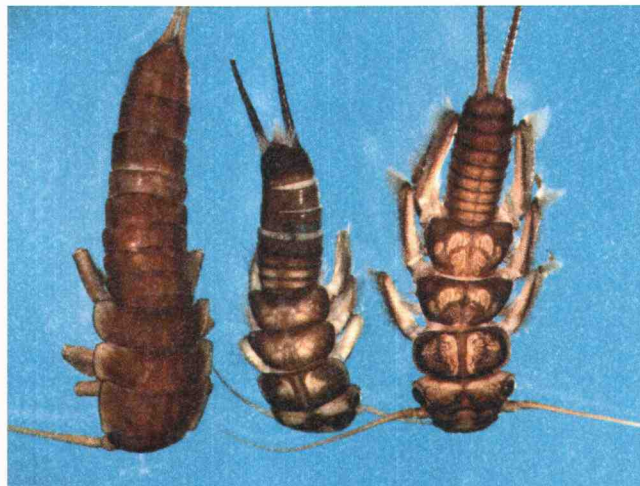


Figure 1: Stonefly Larva

Collecting and classifying stonefly population counts is time consuming. Several hundred individual insects must be collected and identified for a

statistically meaningful population count [Resh et al. 1996]. Accurate classification of species requires an expert who can differentiate the minute differences between some species. People with this knowledge are in short supply and don't have the availability to make stonefly population counts a viable method of monitoring stream health.

1.1 Solution Approach

An automated method of determining insect population counts is being developed by a multi-disciplinary team at Oregon State University to make stonefly population counts a viable method of monitoring stream health. The Computer Science Team is creating pattern recognition software to classify the species from a digital image while the Entomology Team is providing stonefly samples and classification expertise. This project involved designing a device that will automatically transport the insects to a digital camera and orient them for imaging.

The long term goal of the "Bug ID" project is to create a fully automated system for determining stonefly population counts. To determine the population counts for a stream or river using the system, a trained field worker will collect and preserve stoneflies using industry standard procedures. After collection, the stoneflies will be taken to a lab where the system will be set up. All the stoneflies will then be placed in the system together to be processed. The system will require no human interaction to carry out the classification after this point. First, the system will singulate the stoneflies and transport them one at a time to an area where they will be photographed multiple times. Between each photograph, the stonefly will be reoriented for different views. After sufficient pictures are taken and the stonefly is classified using pattern recognition software, the system will automatically sort the stoneflies into bins according to classification for further analysis if necessary. Once all the stoneflies are processed, the software will create a report detailing the results, including an estimate of water quality based on the species present.

This paper focuses on the mechanical design aspects of the project, specifically the automated imaging system. The project provides a case study that can provide insights into the design of a system for transporting and orienting irregular shaped objects, using a fluid as a transport medium, mechatronics as a means of automation, integrating mechanical devices with computer vision applications, and collaboration between the engineering and life science fields to solve challenging problems.

1.2 Mechanical Design Problem

The goal for the mechanical system is to provide a fast and easy way to produce consistent stonefly images that can then be used for classification. There are three main tasks for the mechanical device to accomplish in order to achieve this goal. These are singulating, transporting, and orienting the stoneflies. In addition, the system should be as automated as possible. It should also be noted that all stoneflies processed by the system and used during development are preserved in 70 percent ethanol.

Singulating stoneflies involves extracting them one at a time from a holding area and inserting them into the device. The stoneflies must be singulated because the system is designed to classify them one at a time. Inserting more than one stonefly could also cause an obstruction in the transport system. The challenges involved with singulating stoneflies include their tendency to become entangled with one another, handling the variety of sizes and shapes among different species and maturity levels, and removing them from a fluid filled holding area.

Transporting stoneflies involves moving a stonefly from the insert area to the field of view of the camera. After the stonefly has been imaged, it must then be removed from the field of view and sent to an exit area. The stoneflies must be transported via a fluid medium or they will quickly dry out and become brittle. In this state, they are easily damaged, and curl up, making imaging difficult. Fluidic transport is challenging because it is difficult to control the three dimensional

motion of the stonefly within the fluid. Consistently moving the stonefly along a predetermined route takes much experimentation and prototyping.

Orienting stoneflies involves positioning them once they are within the field of view so that sufficient images can be taken to classify them. Ideally the system would accurately and repeatably orient stoneflies into any desired orientation. This would allow the system to capture the necessary images as quickly as possible. Orienting stoneflies in a fluid medium is difficult when exact movements are required.

Automating the overall system is important in making it a viable way to collect stonefly population counts. If the system is not sufficiently automated, it will not be an improvement over existing methods. Automating the system requires sensors to monitor the status of the device, communication between the mechanical device and camera control software, and additional hardware and software to implement the automation. The challenge of automation is that it must be able to control all three device tasks and coordinate them with each other and the software.

For the system to be successful, all of the above tasks must be accomplished. This is a challenging mechanical design problem due to the fluidic constraints, the physical diversity among stoneflies, and the fragile nature of the insects. In addition, there is little prior work in the area of automating insect classification, particularly with regard to automated image collection, to provide possible direction for this project.

1.3 Designing with Ill-defined Customer Requirements

Ideally a well defined set of customer requirements is known at the start of any design project. These requirements guide the design process and the generation of possible solutions. In many design projects, including this one, these requirements are initially unknown or poorly understood. The customers may have a general idea about what they want but cannot provide specifics.

At the start of this project only a few customer requirements were known by the Computer Science Team who would use the images. This was because the image processing and recognition software development had just started and no known previous work existed in the area of automated insect imaging. The main requirement was that the images provided by the device needed to be as good as possible. A good image was loosely defined as one that provided an unobstructed view of the stonefly and was clear and well lit. A second requirement was that the stoneflies had to be in a fluid medium to keep them from drying out and curling up. It was also known that some form of transportation and manipulation had to be implemented to automate the system and provide views of the stoneflies in different orientations.

Several experimental setups were created from the initial ill-defined requirements to learn about the transportation of stoneflies in a fluid medium. Prototypes were developed from these tests, and images from the prototypes were given to the software development team. Feedback was given on how the images could be improved. Then the Mechanical Design Team made changes in the next prototype to attempt to resolve issues. This iterative process continued until the Computer Science Team was satisfied with the image quality. In addition, as the iterative process continued, the software development progressed which gave the Computer Science Team a better understanding of their needs.

The automated stonefly imager design process showed that a best guess prototype based on what little was known about customer requirements helped both teams better understand what was really needed. In some cases, several experimental setups may need to be created first to provide direction for the prototypes. The prototype then allows the customers to provide qualitative feedback which is used to create the next prototype. As this iterative process continues, and both designer and customer increase their domain knowledge, the customer requirements become more clearly understood and defined which allows the design of a quality product.

2 Literature Review

2.1 Automated Insect Classification Techniques

Automated classification of insects has been attempted with multiple techniques. One technique involves acoustic methods where insects such as grasshoppers and crickets are classified using signal analysis of the sounds they make [Chesmore 2001]. Another technique involves using an optical sensor to measure the wingbeat waveforms of mosquitoes [Moore 1991] and aphids [Moore 2002]. The most frequently attempted means of automated insect classification is pattern recognition. Most work on pattern recognition systems involves classifying insects such as bees and wasps by the unique venation in their wings [Dicky et al. 1992, Howell et al. 1982, Roth et al. 1999, Weeks et al. 1997, Weeks et al. 1999]. The distinctive patterns on stonefly larva make pattern recognition a natural choice for automated stonefly larva classification.

2.2 Automated Insect Classification with Pattern Recognition

Several automated insect classification systems have been developed that employ pattern recognition [Dicky et al. 1992, Howell et al. 1982, Roth et al. 1999, Weeks et al. 1999, Weeks et al. 1997]. However, none of these systems have an automated method for capturing images. Each sample must be prepared individually by hand. This usually involves removing an insect wing and then carefully preparing a slide. A large time investment is required to generate the number of images necessary to make meaningful population counts.

2.3 Patents with Related Content

Little previous work has been done on the transport and manipulation of insects. An automated device [US Pat. 6,688,255 B2. 2004] has been patented to move fruit flies between containers in order to feed them. It is to be implemented in laboratories doing genetic research on large colonies of fruit flies. The device automatically sedates the flies then blows them from an old vial to a new vial containing food. This is accomplished through a pair of robotic arms which grasp the two vials, a manifold, and a tube with compressed gas. Although the device deals with the added complexity of living specimens, it does not need to specifically orient them during or after transport.

Another patent involves a device [US Pat. 6,631,826. 2003] which transports, singulates, and counts identical objects through a fluid medium. The objects are held in a hopper and jets of air are used to send them into a channel for counting and singulation. Different gates can be put on the channel to suit various object shapes. The design concepts for this device could possibly be used to singulate stonefly larva if the air were replaced with a liquid. However, it is designed for identical objects and does not specifically orient them.

A third patent also presents a device [US Pat. 6,053,302. 2000] which provides a method for singulating and counting small objects. This device uses a rotating wheel with tubes attached along its circumference. Each tube has a vacuum applied to it which is used to grasp the objects. When the tubes reach a certain point in the rotation of the wheel, the vacuum is cut and the objects fall into a tube for counting. This concept could be used to singulate stoneflies and feed them to an orienting device. The main complication would be getting the machine to pick up only one insect at a time without entanglement.

2.4 Agricultural Technology Articles

A variety of areas were studied due to the lack of available literature specific to insect transport and orientation. One such area was agricultural automation techniques. Agriculture uses many machines in the harvesting, inspecting, and processing of food. Since crops are biological, and therefore have a variety of shapes and sizes, they provide some insight into how objects such as insects can be handled.

One method of determining the color of fruit in order for it to be automatically sorted employs a system of mirrors [Peleg 1985] to view the fruit from three different angles. The images from these three mirrors are composited to determine the average color of the fruit and it is then automatically sorted appropriately. A similar system of mirrors could be used to view insects from multiple angles giving a more complete picture.

A grain kernel singulation device [Melvin et al. 2003] was developed to singulate grains and then present them to an X-ray machine for inspection. This device used a rotating plate with a hole which moved under a hopper causing a grain to drop into the hole. The plate then moved the grain to the X-ray machine and finally the grain was deposited in another container through a second hole below the first. The main difficulty in applying this approach to insects is that soft insects will behave much differently than hard and fairly uniform grain seed.

There are numerous variations on the seed metering plate [Jayas et al. 1999, Zulin et al. 1991]. This approach to singulation uses a rotating wheel with numerous seed sized slots cut at a certain radius. A vacuum is applied to suck a seed into each hole. As the wheel rotates the seeds are brushed off at a certain point which allows the vacated slot to grab another seed. The removed seeds are then sent to a tube for such purposes as planting. A similar approach for transporting and orienting insects is a possibility.

There are also simpler devices which do not use a vacuum [Churchill et al. 1991] but instead have a wheel on which large number of samples can be hand

positioned. In one application the samples are rotated into the field of view of the camera for inspection. This requires that the operator only be present to load the wheel which has a fifty seed capacity. A more automated approach is desired for this project.

Another method of singulation is vibratory bowl feeders [Casady 1989]. These use vibration to cause seeds or other objects to travel single-file up a ramp which wraps around the inside of a bowl. A solenoid can be placed at the top of the ramp to more effectively control the rate at which seeds exit the bowl. As with most agricultural applications, the techniques mentioned are not usually used with objects suspended in a fluid medium which creates added difficulty.

In addition to agricultural technology, other design concepts for transporting, sorting, singulating and orienting parts were examined [Sclater and Chironis 2001]. Although the concepts presented are proven in industry, they do not work for manipulating stoneflies. The concepts are for parts which are much more uniform, rigid, and geometrically simple.

3 The Design Process and Development History

At the beginning of the project, the pattern recognition and imaging software was not yet developed. As a result, there was little constraint as to what the mechanical apparatus had to accomplish beyond transporting the insects into the field of view and providing the clearest image possible. This gave the mechanical design a lot of flexibility but made determining design requirements very difficult.

Since transportation into the field of view was a necessary function, and little was known about stonefly transportation, it was a natural place to start experimentation. It was also important to determine transportation techniques as early as possible because they would have a large impact on how the other functions of the device could be accomplished.

Additionally, it was important to get prototype images from the device for the Computer Science Team to use. This would allow them to develop the pattern recognition software with images of similar quality to images from the final system. It was decided to focus on the transportation, orientation, and imaging aspects of the device first and develop a singulation method at a later time that could be integrated with the existing device. The remainder of this section details the development of the automated imaging system.

3.1 Prototype 1.0

3.1.1 Prototype Description

The first prototype was a half inch square acrylic tube about one foot long that was filled with water and capped at both ends. A stonefly was inserted before capping the tube. Acrylic was chosen as it was the only clear plastic tubing that was readily available from distributors with a square profile. The square profile

was important because a round profile would provide distorted images when viewed from the outside. By tipping and rotating the tube it could be seen how the stonefly would move through the water inside. This was a quick and easy first step and the small size of the tube made it easy to transport to group meetings so that all involved could see first hand how the stonefly moved.

3.1.2 Testing Results

These experiments showed that the stonefly would sink in the tube when the tube was held upright, that the stonefly could be rotated by rotating the tube, that the various appendages of stoneflies can be easily trapped in bubbles, and that a square acrylic tube has sufficient clarity to provide images that are undistorted.

It should be noted that initially the stonefly floated in the tube due to being preserved in ethanol. After its body had soaked up enough water it began to sink. This inconsistent behavior occurred throughout the experimentation with future prototypes and made working with the stoneflies more difficult. To make the problem more challenging, the time it took a stonefly to soak up sufficient water varied greatly between specimens from several minutes to several hours. These observations gave qualitative insight into how stoneflies would behave in various fluids and as they were moved between fluids.

3.2 Prototype 2.0

3.2.1 Prototype Description

The second prototype was made by using a three foot section of the same acrylic tubing used for prototype 1.0. This time the tube was positioned between two plastic basins (Rubbermaid 3919 0.85 ml containers). The tube and basin were connected through a square hole. Silicon sealant was used to prevent leaks. A Sonic-Tronics Mark X pump was used to pump water from one basin to the other to

provide longitudinal flow through the tube. Stoneflies were inserted into the tube with forceps one at a time and then observed. Figure 2 shows prototype 2.1 which is identical to prototype 2.0 except for the side jet and flexible tubing at the middle of the acrylic tube. Prototype 2.0 is not shown because it was destroyed before a picture was taken.



Figure 2: Prototype 2.1

3.2.2 Testing Results

The pump provided a mass flow rate of approximately 7.2 g/s when connected to a 12V 200 mA power supply. This was sufficient to move the stoneflies through the tube at about 5-8 cm per second depending on the size of the stonefly. When a stonefly was inserted into the tube it would usually stay in the orientation it entered the tube. If it did reorient, it was generally due to hitting the wall of the tube and spinning a small amount. This was much more prevalent with the smaller stoneflies as the larger stoneflies could not easily rotate in the tube. The smaller stoneflies could also be sucked up by the pump and destroyed. This necessitated putting a fitting on the uptake of the pump that was small enough to keep the stoneflies from entering the pump. For this project small stoneflies were defined as those less than 8mm in length, medium stoneflies were 8mm-12mm in length, while large stoneflies were greater than 12mm in length. Length was measured from the front of the head to the end of the abdomen. Figure 3 shows an individual from each group.

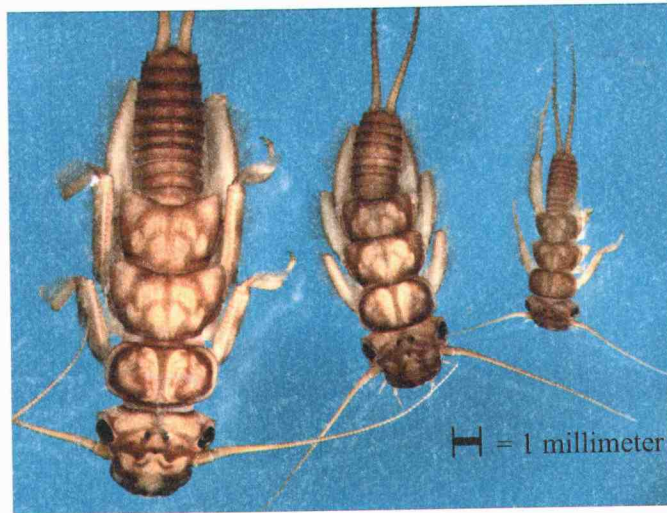


Figure 3: A Large, Medium, and Small Stonefly

During testing many bubbles formed in the tube, a problem for two reasons. First, the stonefly appendages can easily get trapped in the bubbles. When this happens they get stuck in the tube and the tube must be tilted manually to release them. Secondly, the bubbles form in the imaging area of the tube which results in poor image quality. These bubbles make pattern recognition extremely difficult as they confuse the program as to what objects are important in the image and provide unwanted variability.

In an attempt to eliminate the bubbles, the water in the tube was replaced with ethanol. It was intended that the lower surface tension of the ethanol would reduce, if not prevent, the formation of bubbles. With ethanol in the tube, although bubbles did form, they were fewer in number and were easier to remove by tipping the device manually. This produced enough longitudinal flow for the bubbles to be removed from the tube. However, it was discovered that ethanol is not chemically compatible with acrylic. Ethanol caused the tube to craze after several hours, making it opaque. As a result, prototype 2.0 was inadvertently destroyed and was replaced with prototype 2.1 which was initially identical. After prototype 2.0 all acrylic prototypes were only used with water.

3.3 Prototype 2.1

3.3.1 Prototype Description

Prototype 2.1 was built to replace the destroyed prototype 2.0 and was identical except that three side jets were inserted into the side of the acrylic tube. The side jets were designed to spin and orient stoneflies. For this and future prototypes, the side jets and longitudinal flow were powered by Sonic-Tronics Super X DC pumps. One side jet was inserted at the top of the tube, another at the bottom, and another entering the tube in the middle at a 45 degree angle. The side jets were made by joining a 10 μ l pipette to a plastic fitting designed for flexible tubing. The connection was sealed with a small O-ring and some silicon sealant.

3.3.2 Testing Results

Three different side jet configurations were tested to see which, if any, spun the stoneflies best. A profile schematic for each of the configurations can be seen in Figure 4. After testing, it was found that the side jet at a 45 degree angle worked best. The side jets at the top and bottom tended to push the stonefly into the side of the tube while the angled side jet caused the stoneflies to rapidly spin in place by creating a vortex over approximately a 3 inch region centered on the side jet.

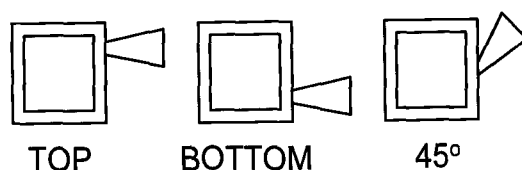


Figure 4: Side Jet Configurations

Prototype 2.1 was the first prototype used to capture images of insects. All images for this project were taken with a Qimaging MicroPublisher 5.0 RTV 5

megapixel color digital camera. The digital camera was attached to a Leica MZ9.5 high-performance stereomicroscope at 0.63x magnification. A 0.32 objective was placed on the microscope to increase field of view, depth of field, and working distance. All images were lighted using gooseneck light guides powered by Volpi V-Lux 1000 cold light sources.

The initial idea to capture images was to move the stoneflies through the tube at a sufficiently slow rate and then slowly spin them with a jet or other device. While spinning, the camera would rapidly take pictures to get images of the stonefly from all different sides. Testing of the camera revealed that quality images of moving stoneflies would not be possible due to interlacing problems with the camera.

The color CCD camera used to capture images was interlaced. This meant that it first captured an image with the odd numbered red and green pixel rows and then another image with the even numbered blue and green pixel rows. The two images were then put together to form the complete color image. Between these two images, the stonefly moved and caused a blurring as shown in Figure 5.



Figure 5: Picture of Moving Stonefly

Some possible software fixes for this problem were proposed but ultimately it was determined that it would be easier to stop the stonefly in the field of view of the camera.

The 45 degree side jet could be used stop a stonefly when there was longitudinal flow through the tube. A stonefly inserted in the tube would move through the tube until it reached the vortex which would catch and spin it. To stop a stonefly both the longitudinal flow and the side jet would be turned on. When the stonefly reached the side jet it would begin to spin in place and then the longitudinal flow would be turned off. After the longitudinal flow had stopped, the side jet would be turned off and the stonefly would settle to the bottom of the tube where its image could be captured. The stonefly could also be reoriented by pulsing the side jet for a couple of seconds and allowing the stonefly to settle into another random orientation. Another concept that was initially explored with this prototype was the use of mirrors to take pictures of stoneflies from multiple angles. This would allow for single images to contain more information and thus decrease the number of pictures necessary to classify each stonefly. Figure 6 show both a concept and an early test of this technique.

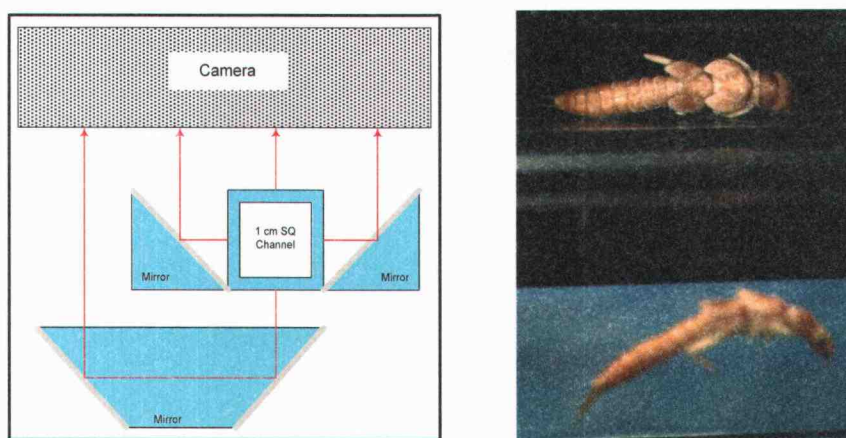


Figure 6: First Mirror Concept and Image

With this concept, the focal length for the images is different and it is therefore possible to focus on only one of the images at a time. The bottom image in Figure 6 is the real image looking through the tube while the top image is the reflection of a mirror next to the tube. It is still possible to get two images that are in focus using this method if the images focused on are from the two 90 degree mirrors in the schematic on the left. A similar method was used for prototype 2.2.

3.4 Prototype 2.2 and 2.3

3.4.1 Prototype Description

Prototype 2.2 was almost exactly like prototype 2.1 except the acrylic tube was only about 18 inches long and rotated 45 degrees from the horizontal. Prototype 2.3 was the same as 2.2 except it had sturdier construction and the basins were shorter to make loading the stoneflies into the basin from above easier. A mirror frame was designed to fit around the tube and create two views that were 90 degrees apart as shown in Figure 7.

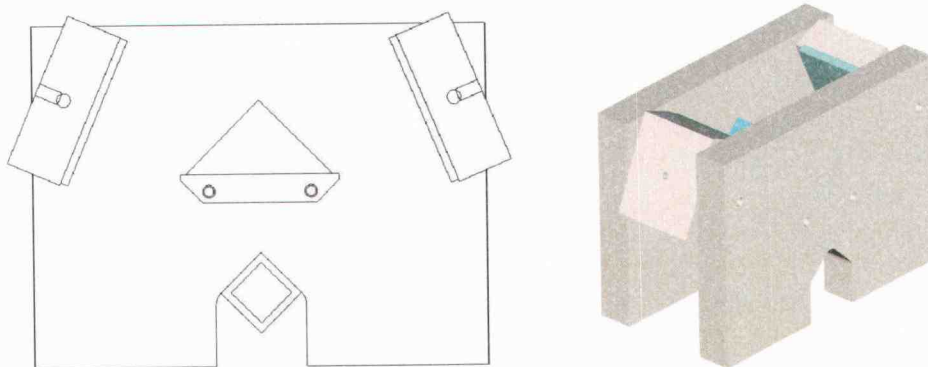


Figure 7: Mirror Frame Configuration

At this point, the Computer Science Team was considering two possible approaches to the design of the classification software. One involved acquiring a single approximately straight on view of the highly patterned back of the stonefly.

This is defined as a dorsal view and is shown in the right image of Figure 8. From this image, pattern recognition could be done for classification. The mirror setup doubled the images of the stonefly and, therefore, doubled the chance of acquiring a dorsal view. If the stonefly was oriented such that a dorsal view was impossible, it could be reoriented as necessary with the side jet. Another approach involved creating a three dimensional model of the stonefly which would be used for classification. This required images of the stonefly from many different angles. Again, the mirror setup significantly reduces the number of images required for this approach. It should be noted that the two images in figure 8 are slightly offset vertically due to a slight misalignment in the initial mirror frame manufactured.

3.4.2 Mechatronic Automation

Prototype 2.2 was the first prototype to incorporate mechatronic control. The kit used to automate the system was an ME101 Tekbots board developed by the Electrical and Computer Engineering Department at Oregon State University. This kit was chosen for its ability to run motors similar to the Super X pumps, low cost, ease of use, and readily available technical support. The system was designed with one input that signaled when a stonefly was present and two outputs that ran the side jet and longitudinal flow pumps. A 12V 300 mA AC adaptor was used to power the system.

Initially, the longitudinal flow would be on and a stonefly would be inserted. Once the stonefly got near the side jet, a switch would be pushed to turn on the side jet and turn off the flow. The side jet would continue to spin the stonefly for five seconds until the longitudinal flow stopped completely. Next the side jet would turn off for five seconds so that an image could be captured and then the side jet would pulse for one second. This was repeated five times to provide a total of ten images of the stonefly using the mirror setup. These images were captured manually. In the final design the camera control software will capture the images.

After imaging, the longitudinal flow would turn back on and remove the stonefly from the field of view. Then the system would reset.

The mechatronic board had only enough power to run one pump at a time. This could be a problem in that sometimes the side jet would not turn on in time to catch the stonefly. It was desirable to run both pumps simultaneously to guarantee that the stonefly would be caught. In order to provide the board with sufficient power, the AC adapter used to power it was upgraded from 300 mA at 12 V to 1500 mA at 12V. This was enough power for all components of the system.

After the initial mechatronic design was tested, the switch used to signal the presence of a stonefly was replaced by an infrared (IR) sensor made specifically for the Tekbots ME101 board. This made the system more automated since all the user had to do was drop a stonefly into the system and then take pictures. The IR sensor works by sending out a beam of IR light. When the sensor gets back a sufficient amount of that light as determined by its sensitivity adjustment, it triggers.

Experimentation revealed that it was very difficult to trigger the sensor with the stoneflies moving through the tube. Stoneflies do not easily reflect IR light back to the sensor because of their generally dark color and irregular shape. To get around this difficulty, the control logic of the IR sensor was reversed. A mirror was placed opposite the sensor on the other side of the tube to continually reflect IR light. Then the mechatronic program was changed so that the sensor would trigger when its light was blocked instead of reflected. This proved to work reliably on large and medium sized stoneflies but was only able to detect small stoneflies about half the time.

3.4.3 Testing Results

The mechanical performance and stonefly handling of prototypes 2.2 and 2.3 were good. However, a number of imaging problems were discovered. First, as can be seen from figure 8, many bubbles formed in the tube. This is due to the surface tension of water. Bubbles make it more difficult to separate the stonefly

from the background by creating holes in the extracted image where there are bubbles. Also, the bubbles can confuse the pattern recognition software.



Figure 8: Prototype 2.3 Image

The bright white sections of the background are from clear silicon sealant used to make the side jet water tight. This non-uniformity in the background also makes it more difficult for the imaging software to extract the stonefly from the background. Ideally the background would be a uniform blue that provides good contrast with the stonefly. This makes it easy for the software to differentiate between the background and the stonefly.

It is easy to provide a blue background while looking straight on with only a single view as in early pictures. This is accomplished by placing a blue piece of paper below the tube on the reflector that lights the image from below. However, with the tube at a 45 degree angle, the background is no longer directly below the tube but off to the side. A large piece of blue paper can be placed below the tube so that it can be seen from both views but this blocks the light from below resulting in a poorly lit image. To compensate for this, the exposure time must be increased by a factor of three or four resulting in a slower processing time and slightly blurry pictures.

3.5 Prototype 3.0

3.5.1 Prototype Description

This prototype was the first to be custom manufactured. Earlier prototypes relied largely on modified parts. The system works the same way as previous prototypes with a tube, basins, and a side jet. It was made primarily of polycarbonate and high density polyethylene (HDPE). The side jet itself is exactly the same as in earlier prototypes except that now it is held in place by a mounting block. The system is shown in figure 9. One picture shows the system with the mirror setup and the other without it. In both pictures the longitudinal flow would be right to left. The mechatronic board can also be seen in the picture on the right.



Figure 9: Prototype 3.0

Since the system is made out of polycarbonate and HDPE, it is compatible with ethanol. If ethanol is used as the fluid in the system, bubbles are much less of a problem. Fewer bubbles form and the ones that do are easier to eliminate due to the lower surface tension of ethanol as compared to water. The vortex created in the imaging area with the side jet also serves to wipe away the bubbles. Another advantage of ethanol is stoneflies always sink in ethanol making it is easier to predict how they will move within the system. A 70% by volume ethanol solution

is used in this system. This solution was chosen because it is the entomology standard for preserving stoneflies.

Blue HDPE was used to construct the bottom of the tube to create the necessary blue background. The two blue HDPE pieces are triangular in shape and are attached to the base plate through screws. A single extruded polycarbonate angle piece was going to be used for the tube top, but extrusion lines resulted in an unacceptable loss of clarity. Instead, two pieces of polycarbonate were glued together at a 90 degree angle to form the transparent top half of the tube.

When properly lighted, the blue HDPE provides a blue colored background which makes extracting the stonefly from the image easier. A small amount of silicon sealant was placed in the notch between the polycarbonate and HDPE to seal the tube. Figure 10 shows the tube cross section.



Figure 10: Prototype 3.1 Tube Cross Section

3.5.2 Mechatronic Automation

Some additions were made for the mechatronics of prototype 3.0. The board was equipped for serial communication with a computer by soldering on some additional electronic components. Serial communication was then implemented and a graphical user interface for the system was created using MATLAB. Serial communication is important because, once the pattern recognition software is developed, the software and device must be able to coordinate their activities to achieve maximum automation. The functions of the MATLAB graphical user interface were to mock-up the future software and make the device more user friendly for testing. It allowed the user to turn the system off

and on as well as confirm when sufficient images had been captured and reorient the stonefly. The graphical user interface is shown in figure 11.

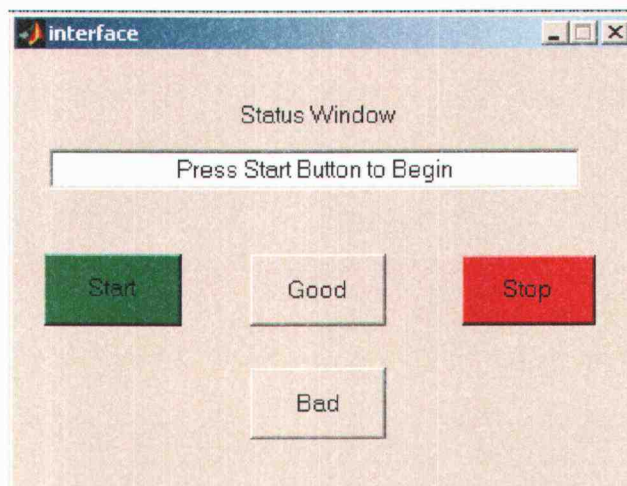


Figure 11: Graphical User Interface

In addition, another IR sensor was added to the system to increase the chances of detecting the smaller stoneflies. One of the main reasons small stoneflies are difficult to detect is that they generally have a tumbling motion through the tube. As they pass by an IR sensor their current orientation may not allow them to block enough light to trigger the sensor. By placing a second sensor slightly downstream of the first sensor and on the opposite side of the tube, it is unlikely that stoneflies will pass by both sensors with an unfavorable orientation. Testing showed this setup detected small stoneflies reliably, with an increase in detection rate from approximately 50% to approximately 95%. The mirrors to reflect the IR light were mounted in slots milled into the HDPE using Adhesives Research ARclad 9030 double sided splicing tape that tolerated ethanol well.

3.5.3 Testing Results

Both basins for the prototype were made out of polycarbonate pieces that were glued together. Initially the two basins were too low, at only 1.3 inches tall,

and the ethanol would frequently overflow the walls. To solve this problem, additional pieces of polycarbonate were glued on top of the existing basins to increase their height to approximately 3 inches. The narrow loading basin was designed such that once a stonefly was dropped in; it would automatically be loaded into the tube. This was accomplished by having the return of the longitudinal flow pump discharge directly into the back of the basin as shown in the left picture of figure 12.

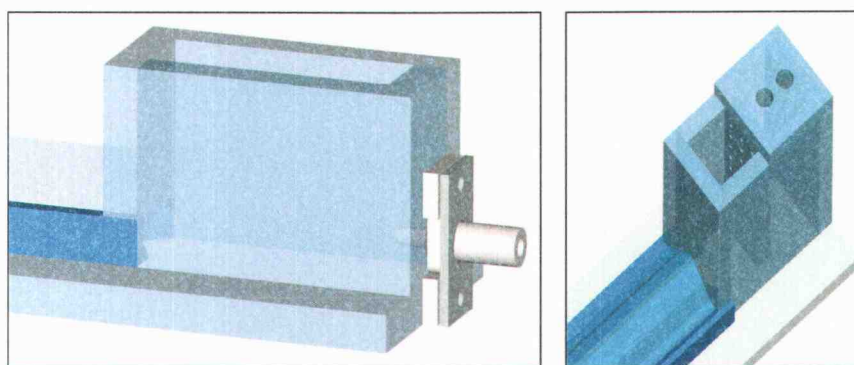


Figure 12: Original and Modified Loading Basin Setup

The return flow connection was sealed by using an O-ring and a pair of screws to tighten the interface. Unfortunately, this method did not work well. Instead of being shot into the tube by the return jet, the stoneflies just moved around within the basin due to the turbulence created by the fast moving fluid.

A number of different ideas were tried to fix this problem. The idea that worked best returned the fluid into the back of the basin from above. A piece of polycarbonate with many small holes was then placed in the middle of the basin. This piece covered the entire width of the basin and prevented the turbulence of the return flow from affecting the stoneflies as they were dropped in the front of the basin. The many small holes allowed fluid to flow through the plate and created a smoother flow that directed the stonefly into the tube. This design is shown in the right picture of figure 12.

The system worked well from a mechanical standpoint after making the above changes, but a number of issues were discovered in terms of the ability to create satisfactory images. First, due to the fact that the HDPE was opaque, it was not always possible to see the entire stonefly. Once they sank to the bottom of the tube, small parts of them became obscured by the HDPE and the silicon sealant used to seal the tube. Although most of the stonefly could be seen, it was important for the pattern recognition software to see all of it. An example image from this prototype is shown in figure 13.



Figure 13: Prototype 3.0 Image

Lighting the images also proved to be very challenging. Due to the new tube construction the image could no longer be lighted from below. The mirror setup also made it difficult to light the image since the mirror frame covered most of imaging area. To light the image, two flexible fiber optic lights were positioned just above the imaging area and directed toward the mirror setup. In this way, a small amount of light was diffused through the HDPE frame for the mirror setup. However, since there was only a small amount of light the exposure time had to be increased. This resulted in a slightly blurry image and also caused the clear silicon sealant to become very bright. These bright spots made it difficult for the computer

software to extract the stonefly from the image because the software confused the high contrast bright spots with the stonefly.

To fix this problem, both the material and geometry for the mirror frame piece were changed. First the material was changed to polycarbonate which allowed light to pass through it. Second, the profile of the frame was changed so that it no longer fit right over the tube. A cutout above the tube was created, as shown in figure 14, so that the light could pass by the frame and directly light the image. This decreased the necessary exposure time to achieve a sufficiently lighted image from approximately 500 ms to 100 ms. The silicon sealant was also painted with a flat black paint to reduce the bright glare that it produced.

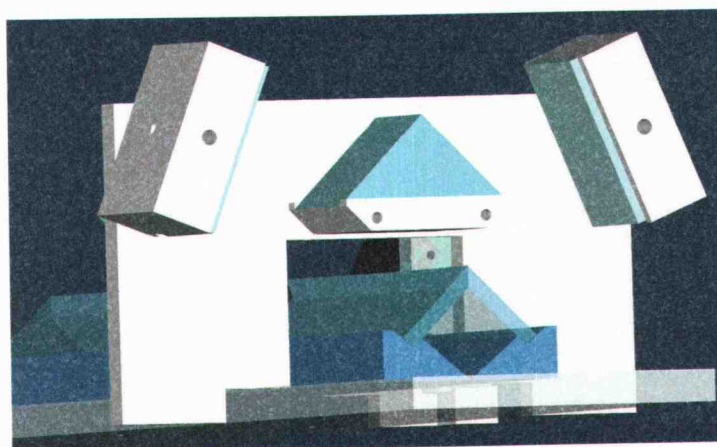


Figure 14: Modified Mirror Frame Geometry

Another imaging difficulty that arose was that the blue HDPE showed a lot of circular tool marks that reduced the uniformity of the background. Although they looked relatively small upon visual inspection, when viewed under the microscope and camera system, they were very evident. These marks can confuse the pattern recognition software like bubbles do. Tests showed that sanding the HDPE parts with 600 grit sandpaper eliminated most of the marks, while sanding with 2000 grit sandpaper provided an exceptionally smooth and uniform

background. The marks are seen in figure 15. It is difficult to see the marks in figure 13 due to insufficient lighting.

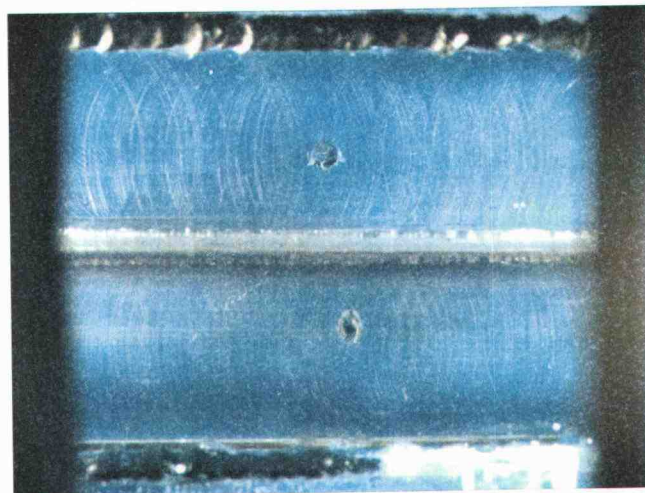


Figure 15: Tool Marks on HDPE

3.6 Prototype 3.1

3.6.1 Prototype Description

The second custom manufactured device was similar to the first and had identical mechatronics. Many of the modifications to the first prototype were designed into the second. These included the return jet discharging into the top of the loading basin, the divider in the middle of the loading basin, increasing basin height, removing tool marks from the blue HDPE with high grit sandpaper, and changing mirror frame geometry to allow for better lighting. Other minor design changes included using higher grade abrasion resistant polycarbonate to reduce the possibility of scratches in the imaging area and increasing the tube cross sectional area by increasing the length of the polycarbonate angle legs from 0.4 to 0.5 inches so that large stoneflies could be transported more easily. Photographs of prototype 3.1 are shown in figure 16.



Figure 16: Prototype 3.1

Prototype 3.1 worked well in terms of stonefly handling but the imaging capabilities were not sufficient. One of the biggest problems from the previous prototype was that the entire stonefly could not be seen. To solve this problem the angle between the two bottom walls of the tube was increased from 90 degrees to 100 degrees as shown in figure 17. The angle between the two polycarbonate pieces was kept at 90 degrees.

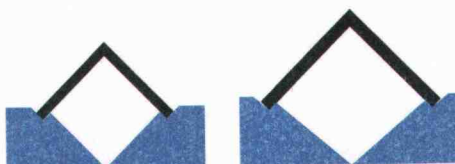


Figure 17: Prototype 3.0 and Prototype 3.1 Tube Cross Sections

Increasing the angle between the two bottom walls of the tube had the effect of pulling the walls away from the stonefly image. When a stonefly settled to the bottom of the tube, all of it could now be seen. Another factor contributing to the lack of stonefly visibility was the silicon sealant which was difficult to apply to such a small area. As can be seen in figure 13 from prototype 3.0 the legs of the stonefly are obscured by excess sealant. To solve this problem the silicon sealant was carefully applied to prototype 3.1 using a syringe with a small nozzle. This allowed precise application.

The lighting methods for prototype 3.1 also improved. Several lights were added to the setup which allowed the exposure time to be decreased. With just two fiber optic lights an exposure time of 100 ms was required, with four lights the time could be reduced to 60ms, and for six lights the time could be reduced further to 30ms. Lower exposure times result in sharper images and allow pictures to be taken more quickly. The extra light also allows for more flexibility in terms of other camera settings and for the application of diffuse lighting techniques. These techniques reduce the amount of light but diffuse it for a more evenly lighted image. Tests were done with diffuse lighting on prototype 4.0 to achieve more even lighting as well as reduce shadows, glare, and specular highlights.

3.6.2 Testing Results

Further tests were done with this prototype to reduce the formation of bubbles. Switching fluids from water to ethanol did not completely prevent bubbles from forming. Testing showed that bubbles were still forming due to cavitation in the pump. The fluid was moving quickly and thus reducing its pressure below the vapor point causing bubbles to form.

Two modifications were made to reduce cavitation. First the nozzles on the uptake end of the pumps were removed. These 1/16 inch nozzles were originally placed on the pump uptakes to prevent stoneflies from being sucked into the pumps. The ethanol experienced less of a pressure drop with the nozzles removed because it did not have to accelerate as much to enter the small orifice. A setup similar to the loading basin was created in the exit basin to keep stoneflies from being sucked into the pumps with the nozzles removed. A filter consisting of a piece of polycarbonate with many drilled holes was placed between the pump uptakes and the area of the exit basin where the imaged stoneflies were deposited.

The second step to reduce cavitation was reducing the speed of the longitudinal flow pump. This was done by adding an additional Super X pump. Since the mechatronic board could supply 300 mA total, when both pumps were

run simultaneously their speeds were reduced while keeping the fluid mass flow rate sufficiently high. These modifications significantly reduced the number of bubbles that formed. The example image in figure 18 shows the lack of bubbles and more uniform background.

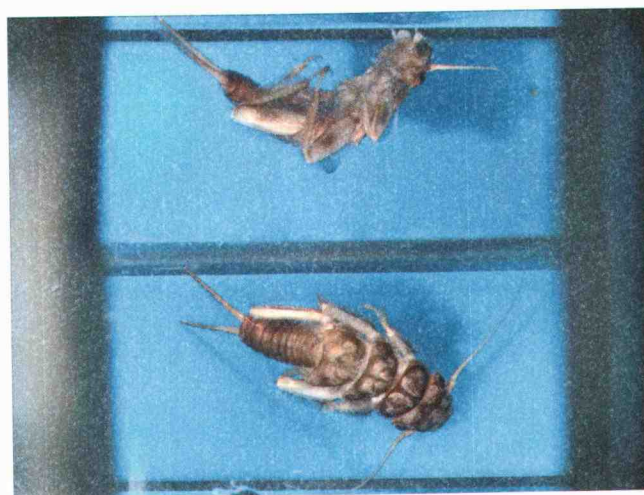


Figure 18: Prototype 3.1 Image

A new side jet design was tested with this prototype. It was created downstream of the current side jet design. The new side jet was made by drilling a stepped hole. Then a piece of 5/32 inch diameter brass tubing was press fit into the larger section of the hole for the flexible tubing from the pump to fit over. A chamfer bit was used to create a converging section that directed the fluid into a 0.020 inch diameter hole which served as the side jet. This design eliminated the need for a side jet mounting block, simplified the side jet and tube interface, and reduced the size of the hole so stoneflies are less likely to get their appendages stuck in it. The new side jet performance was comparable to that of the existing side jet design and replaced it for prototype 4.0.

One of the most important lessons learned from prototype 3.1 was the need for alignment and assembly features. When the prototype was assembled some parts were misaligned and did not fit well together while others were assembled incorrectly. The biggest misalignment problem was that the two blue HDPE parts

did not meet at the center of the bottom of the tube. As a result, a gap existed which showed up as a black line near the top and bottom of the images. This made it difficult to extract the stonefly from these sections. Incorrect assembly resulted in the mirrors for the IR sensors being downstream of the imaging area and the IR mounts in the wrong place. Fortunately these problems only affected the mechatronics and the prototype could still be used for mechanical and imaging testing. Steps were taken with prototype 4.0 to address these issues.

3.6.3 Determining Appropriate Exposure Time

A method for determining an appropriate exposure time was also developed during this stage in the design process. If the exposure time is too short, the stonefly will be too dark to give sufficient contrast to its various features and patterns. If the exposure time is too long, the image will saturate and information will be lost as lighter parts of the stonefly are washed out. Figure 19 shows three images which have exposure times of 20ms, 80ms, and 160ms left to right. These images were lit with four fiber optic lights. The 20ms image lacks contrast and the detail of the stonefly cannot be seen. In the 160ms image, parts of stonefly such as the legs are washed out. The 80ms image provides a balance between the two extremes.

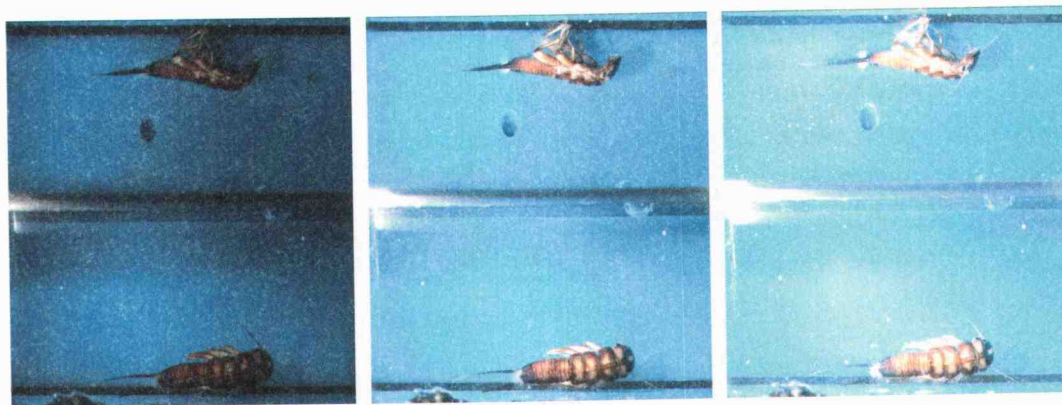


Figure 19: Images with Different Exposure Times

The method developed involved determining the standard deviation of the stonefly pixels from a segmented image for a range of exposure values. Curves like the one below could be obtained from this information. Some of the data points on the “HES” curve in the figure 20 were derived from the images shown above.

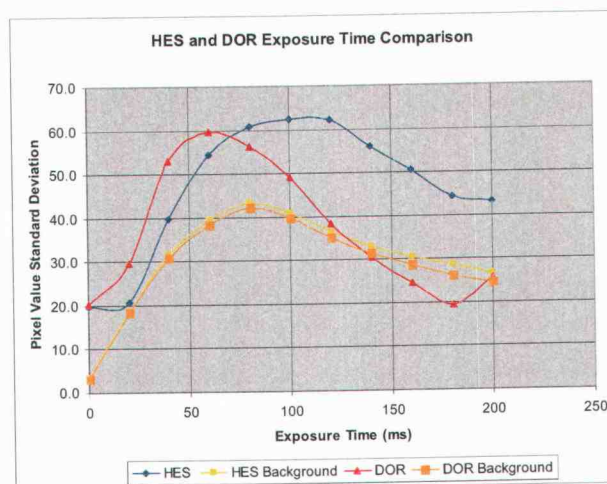


Figure 20: Graph for Determining Optimal Exposure Time

Figure 20 compares the results of two different stonefly species, *Hesperoperla pacifica* (HES) and *Doroneuria baumanni* (DOR). The background curves are the same for both species as would be expected. The two stonefly curves show the same shape where the curve initially ramps up to a peak then slowly decreases. This shows that as exposure time is increased from zero, more information is visible in the stonefly. There are more colors and variations present which result in an increase in standard deviation. However, as exposure time continues to increase, the curve levels off. This shows that the image is beginning to saturate. Darker parts of the stonefly are becoming more visible but lighter parts are being washed out so the net gain in standard deviation is close to zero. When the curve begins decreasing this shows that the washout effects are overcoming what little increase in contrast there is for the darker parts on the stonefly. It is

desired to minimize saturation because it results in a loss of information. These curves show that the appropriate exposure times are located in the area where the curve is ramping up. The peak of the curve should be avoided as this is where saturation effects begin to take over.

It should be noted that *Doroneuria* begins to saturate before *Hesperoperla*. This is due to the fact that the *Doroneuria* species is a lighter color than *Hesperoperla*. A picture of *Hesperoperla* can be found in Figure 13 and a picture of *Doroneuria* can be found in Figure 18. The color differences between various species and insect maturity levels have to be taken into account when determining the best overall exposure time. For these two species an optimal time is around 40ms. This will give sufficient lighting to both species but should avoid any major saturation issues. A histogram equalization can be carried out later to give more contrast to images of darker stoneflies. Since it will not be known what species will be encountered during the collection of population counts, the exposure time must be set to a value that will work best with the most types of species. This will have to be determined in the future as more species are collected and analyzed. It might also be possible to have a number of exposure times based on what species are expected. If a sample comes from a region expected to be highly populated with *Hesperoperla* the camera could be optimally set up to photograph them.

4 Prototype 4.0

4.1 Prototype Description

Prototype 4.0 was similar in concept to previous designs with two basins connect by a tube and a jet to stop and orient the stoneflies. Polycarbonate was the primary material used in the design. Design changes mostly involved making manufacturing and assembly easier. The bottom of the tube was made out of a single piece of blue HDPE. Making the part out of a single piece of material avoided the black lines at the top and bottom of prototype 3.1 images. The lines were due to the fact that the two pieces that made up the bottom of the tube did not touch and created a gap. Alignment features such as dowel pins were used to position the part on the base plate and a non-symmetric screw pattern was used to fasten the HDPE part to the base plate so that it would be oriented correctly. The larger HDPE part also made securing it in a mill for fabrication easier. Figure 21 shows the mechanical device and figure 22 shows the entire setup on the microscope.

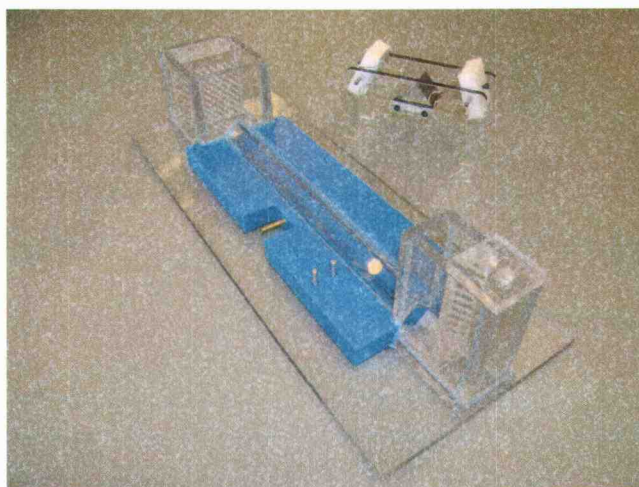


Figure 21: Prototype 4.0

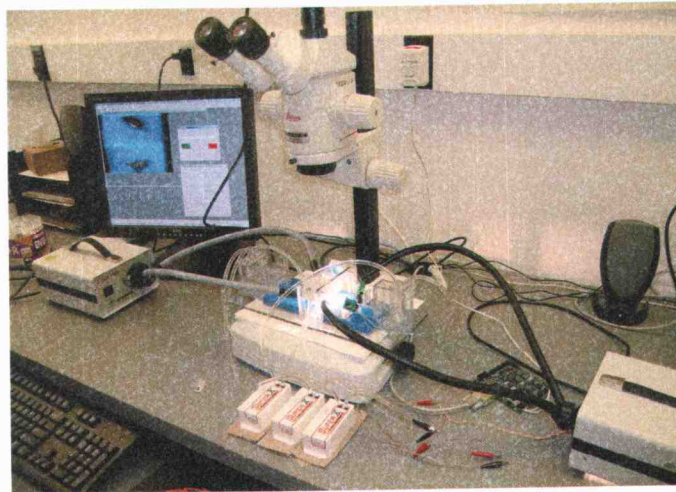


Figure 22: Complete System Setup

The loading and exit basins were made out of polycarbonate as with previous prototypes and were assembled with polycarbonate glue. Base plates were built for each basin to assist with assembly and alignment. These plates had cutouts that the walls of the basins could be fit into. The main base plate also had cutouts to position the basin base plates. With previous prototypes the basin walls were glued directly to the base plate which made attaching and aligning them difficult. The basin base plates also elevated the bottom of the basins to the same level as the bottom of the tube. The loading basin had a “laminar flow plate” as with the previous prototype and the exit basin had a similar feature to prevent stoneflies from being sucked into the pumps. There were also two small triangular shaped blocks placed in the bottom of the loading basin so that the bottom of the basin matched the contour of the bottom of the tube. This made it easier for stoneflies dropped into the basin to enter the tube.

Other design changes included the mounting of the IR sensors. For prototype 4.0 the IR sensors are secured to a small polycarbonate mounting block with screws. These blocks are then attached to the device through the use of dowel pins which can be seen in figure 21. The mounting blocks were permanently glued to the base plate in earlier devices. The new design makes it much easier to attach the IR sensors to the mounting blocks as well as decouple the mechatronics from

the mechanical device. Additional changes to the IR setup included circular mirrors instead of square mirrors to reflect the IR light. This made machining the mirror slots easier. The IR sensors can be seen attached to the device in figure 23.

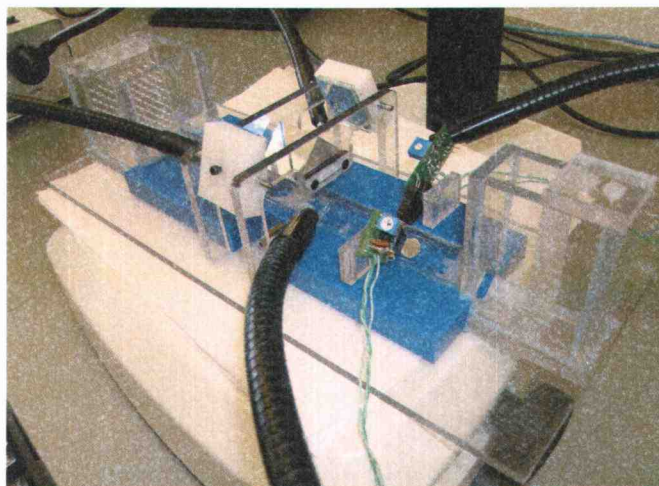


Figure 23: Device on Microscope Stage

The new polycarbonate mirror frame design can also be seen in figure 23. In order to increase image area lighting, two large pockets were milled into the each side of the mirror frame. These can be seen between the square mirrors and the triangular shaped mirror. The pockets provided a place where gooseneck fiber optic lights could be placed as shown in figure 23. There are no obstructions between the goose neck lights and the imaging area. This allowed for superior lighting relative to previous prototypes. Direct lighting from the four point sources does result in some shadowing from the stonefly itself. These shadows were reduced by placing several pieces of thin translucent HDPE, which are not shown in figure 23, between the light sources and the imaging area.

4.2 Mechatronic Automation

The overall operation of the mechatronic automation remained the same except for a few modifications to increase the reliability of the control and reduce stress on the DC pumps. It was observed that the IR sensors would periodically trigger even though no stonefly was present. Although testing was inconclusive as to the cause of the malfunctions, it was speculated that there was a grounding problem with the electronics. The malfunctions could also have been a result of the fact that the IR sensors were being used in an inverted method with the mirrors.

To solve the problem, the way in which the mechatronic board registered triggers was changed. Instead of triggering on one signal, the IR sensors had to receive two signals that were 10ms apart. A 10ms delay was chosen because the smallest stonefly was observed to produce a signal with a minimum duration of 12ms. Larger stoneflies produced signals in the 100 to 200ms range.

Implementing the delay between signals eliminated any signals that were the result of electrical noise. After the change no more false triggers occurred. Although this method reduced the sensitivity of the IR sensors, testing showed that they were still reliable. Large and medium sized stoneflies, 8mm in length and larger, were detected 100 percent of the time. Small stoneflies, less than 8mm in length, were detected greater than 95 percent of the time.

The jet pulses used to orient the stonefly were also changed. In previous designs the side jet would pulse a random amount of time between 0.5 seconds and 1.5 seconds. This usually resulted in the stonefly spinning rapidly and then settling on its back. To improve the chances of the stonefly landing with its back to the camera, the side jet operation was changed so that it gave two quarter seconds pulses about a quarter of a second apart. The intent was to rotate the stonefly just enough so that it would turn over instead of doing many rotations and then settling into an undesirable position.

The new side jet pulse method worked better for medium and large stoneflies and about the same for smaller stoneflies. With the new method it took

approximately 5 to 10 pulses to rotate medium and large stonefly for a back view. The quick duration of the pulses meant that the actual orientation time was less than 10 seconds for the medium and large stoneflies. Previous methods of side jet pulses would take 20 to 30 seconds to perform. For smaller stoneflies it could take upwards of 40 pulses. The smaller stoneflies would start spinning rapidly even with the short duration of the pulses. Solutions to this problem will be discussed in the future work section.

The final change to the mechatronics involved the timing of the DC pumps. Originally both the longitudinal flow and the side jet were on while waiting for an insect. This stressed the mechatronic board as it was powering all the pumps simultaneously while waiting for a stonefly to be inserted. Additionally the side jet pump was being excessively stressed. It only had to be turned on when a stonefly was inserted into the device.

To solve this problem the pump operation was staggered so that only the side jet or longitudinal flow was on. During operation of the final design, the longitudinal flow is turned on while the device is waiting for a stonefly to be inserted. Once the device detects a stonefly, the longitudinal flow is turned off and the side jet is turned on after a 30ms delay. The side jet then orients the stonefly as necessary. Once a satisfactory picture is taken, the longitudinal flow is turned on and the system resets waiting for a new stonefly to be inserted. In this way the pumps run the minimum amount of time necessary for operation and the board never has to power the jet pump and the longitudinal flow pumps simultaneously.

4.3 Calibration

It was important to develop a calibration method for the device. This would allow for important tasks such as feature measurement from the images. There are no standard calibration objects that would work for the design. The calibration object needed to fit inside the tube, be transported by the system, and tolerate ethanol. It also had to have a precise pattern of known dimensions that was easily

seen by the camera. To accomplish these tasks a custom object was manufactured. Figure 24 shows a model of the object and a photograph of it inside the tube.

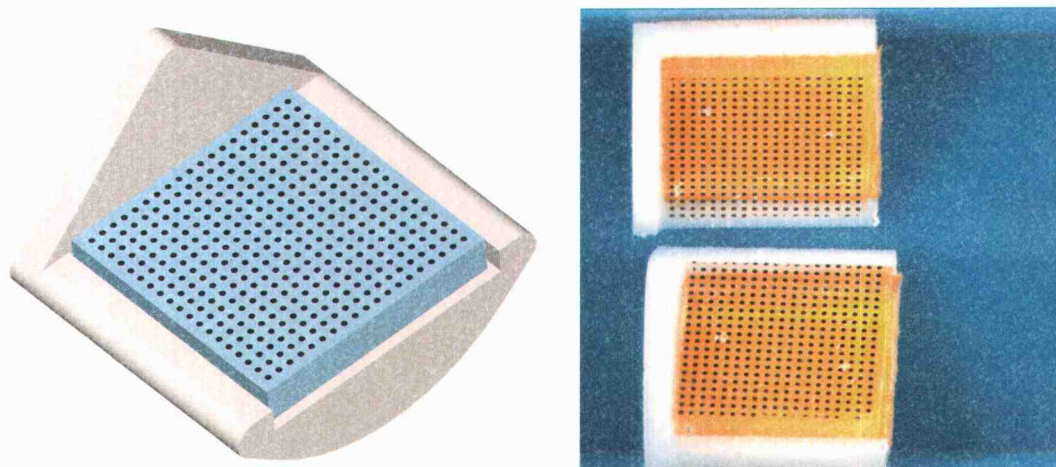


Figure 24: Calibration Object

The object was created with a rapid prototype machine that creates parts out of ABS plastic. An Edmunds Industrial Optics Grid Distortion Target was used for the dot pattern shown. This required cutting out a 10mm square section of the glass optics target and then attaching it to the ABS plastic carrier with the same ethanol resistant double sided tape used to attach the mirrors inside the tube. Each dot has a diameter of 0.25mm and the center to center spacing between the dots is 0.5mm. The orange color is from the tape. Due to its small size and low mass, the object could easily fit inside the tube and be transported by the longitudinal flow. The object could also be shifted within the tube by the side jet for multiple viewing angles. A calibration procedure and the accompanying software are under development by the Computer Science Team.

4.4 Testing Results

Prototype 4.0 performed well in the areas of stonefly handling and imaging. Small and medium sized stoneflies move easily through the tube while larger

stoneflies can get stuck in the entry basin on occasion. They usually get stuck when an appendage or antenna comes into contact with the front edge of the basin just above the tube entrance. In these cases they must be manually adjusted with tweezers to enter the tube. It would be possible to add a jet into the entry basin that could be pulsed to dislodge stuck stoneflies. Also, if the stoneflies are carefully inserted into the basin head first they very rarely get stuck. A singulator that could accurately position stoneflies head first into the entry basin could avoid jamming problems. Once inside the tube, all sizes of stoneflies are transported easily with some small exceptions when they get caught on the IR sensor mirrors.

One small mistake in the design is the size of the IR sensor mirror slots. It was desired for the mirror slots to be as tight a fit as possible so that stoneflies could not get appendages caught in the small space between the mirror and the slot. Past prototypes showed that crevasses and holes provide features for stoneflies to get caught on. This is due to the fact that stonefly feet are specifically adapted for grasping the wet and slippery surfaces within a stream. As a result of making the fit between the mirror and slot very tight, the mirror did not fit entirely into the slot. Each mirror slightly protruded and provided an edge that the stonefly would occasionally get caught on. Future devices should increase the diameter of the slots approximately 0.015 inches and adjust the tolerances so that the slot will not be undersized.

Image quality for the prototype is very high. The image is well lit with a uniform background. This allows for easy image segmentation and gives excellent contrast to the patterns on the stonefly. An example image is shown in figure 25. Note the small 0.020 inch diameter side jet hole which is the result of the new side jet design initially tested with prototype 3.1. The design provides a more uniform background and prevents the stonefly from getting stuck in the hole. Blurs at the edge of the image are the sides of the mirror frame.

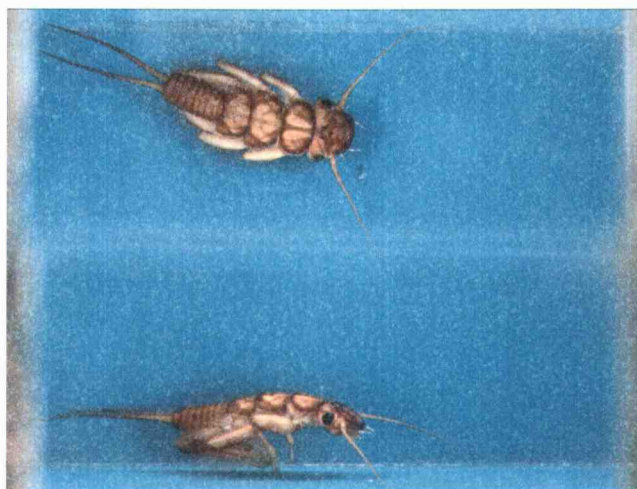


Figure 25: Prototype 4.0 Image

Another imaging improvement over the previous prototype is the significant reduction in shadows. As mentioned previously, this was accomplished by diffusing the light through thin translucent HDPE. Exposure time had to be increased from approximately 25ms to 245ms to sufficiently light the image with the diffuser. Using thin opaque HDPE to diffuse the light produced the best results and completely eliminated shadows. However, this method required an increase in exposure time to 700ms which is above the 250ms exposure time target. A quantifiable analysis of image quality and comparisons of prototype image quality are further discussed in the image validation section.

4.5 Comparison to Design Requirements

A list of design requirements was created to define goals with respect to image quality, stonefly handling, device usability, and other areas. These goals are used to establish the success of the design. Goals and measures of success were determined by discussions with the Computer Science Team and evaluating quality images. The following tables display the customer requirements, engineering specifications, design goals, and actual design performance.

4.5.1 Image Quality

Image quality measures the output of the device. This is the most important goal for the device. The images have to be of sufficient quality that they can be easily processed and the stonefly can be classified.

Table I: Image Quality

| Customer Requirements | Engineering Specifications | Targets | Design |
|-------------------------------|---|---------|--------|
| Clear Picture | Number of objects in image other than stonefly (bubbles, scratches, etc) | <3 | 0 |
| | Number of variable objects in image overlaying stonefly | 0 | 0 |
| | Number of gill and hair clusters visible (can see individual gills and hairs) | >2 | 7 |
| | Percent of image with visible sealant | <5% | 0 |
| Good view of back of stonefly | Rotation of back with respect to image | +/- 15° | 0 |
| Well lighted image | Image exposure time in milliseconds | <250 | 245 |
| | Mean stonefly pixel value | 100-150 | 101 |
| | Maximum number of shadows | 2 | 1 |
| Can see entire stonefly | Percent of stonefly feet visible that are not obscured by body of stonefly | 100% | 100% |
| Easy image segmentation | Standard deviation of background pixels values | <30 | 19.4 |
| | Minimize jet hole diameter in millimeters | <1 | 0.5 |
| | Percent of stonefly lost when segmented | <10% | 5% |
| Stonefly image not saturated | Standard deviation of stonefly pixels values | >40 | 45.6 |
| | Maximum pixel value for stonefly | <255 | 254 |

The device met all the goals for image quality with the most important being the visibility of the stonefly in its entirety, a uniform background, and a well lit image. Standard deviation of the background pixel values and stonefly pixel values change from stonefly to stonefly and are also dependent on the lighting setup. However, acceptable values can be achieved for most insects. The values shown here are for the image shown in Figure 25.

The engineering specification “Percent of stonefly lost when extracted” is estimated by looking at segmentation program results. Antenna, cerci, hairs, and eyes are most often lost to segmentation. In some cases these parts are critical to identification by entomologists. It is not known how critical these parts will be to computer classification. A general segmentation method was used to segment the images. A custom segmentation method that is specific to the images will improve the segmentation process. The image quality is discussed in more detail in the image validation section.

4.5.2 Stonefly Handling

Stonefly handling is the ability of the device to efficiently and reliably handle a wide size range of stoneflies without damaging them. This is a challenging area due to the diversity of stoneflies and their fragile nature.

Table II: Stonefly Handling

| Customer Requirements | Engineering Specifications | Targets | Design |
|--------------------------------------|---|----------|----------|
| No stonefly damage | Number of appendages lost per stonefly during processing | 0 | 0 |
| | Number of body segments damaged per stonefly during processing | 0 | 0 |
| Positions stoneflies in minimal time | Seconds to reorient stonefly | <3 | 1 |
| | Seconds between stonefly insertion and initial positioning | <5 | 5 |
| | Seconds between final positioning and resetting for new stonefly | <5 | 3 |
| | Number of reorientations required for good back view (small, others) | <10 | 40, 5-10 |
| Stoneflies do not jam | Number of stoneflies stuck at mirror slots per 40 (small, medium, large) | 0 | 0,1,7 |
| | Number of stoneflies stuck in jet hole per 40 (small, medium, large) | <2 | 0,0,0 |
| | Number of stoneflies stuck in loading basin per 40 (small, medium, large) | <4 | 0,6,11 |
| Works with range of stonefly sizes | Range of stonefly lengths that device can process effectively | 5mm-20mm | 8mm-12mm |
| | Range of stonefly diameters that device can process effectively | 1mm-5mm | 2mm-4mm |

The device was successful in stonefly handling but it cannot handle the largest and smallest stoneflies reliably. Most notable is the number of jams for larger stoneflies. Medium stoneflies also jammed but to a lesser extent. Some solutions to this problem were mentioned in the testing results section of prototype 4.0. Although small stoneflies had no jamming problems they could take upwards of 40 reorientations to acquire a good dorsal view. For these reasons, the range of stoneflies that the device can effectively process is more narrow than desired. The size range given is an estimate.

Processing a stonefly with the device will rarely damage it. The only case where damage occurs is if the stonefly had been previously damaged during collection or if the stonefly is run through the device many times. During standard operation a stonefly will only be run through the device several times at most. There is no concern that the device is too rough when handling stoneflies.

4.5.3 Device Usability

Device usability measures the function of the device as it interfaces with people. This involves the ease of use, setup, and shutdown for the device.

Table III: Device Usability

| Customer Requirements | Engineering Specifications | Targets | Design |
|-----------------------------|---|------------|--------------|
| Maximum automation | Number of user performed operations | <3 | * |
| | Coordinate operation with computer and microscope via serial or USB | Yes | Yes |
| Easily transported | Number of people required to transport device | 1 | 1 |
| | Maximum weight in pounds for all components | 5 | 3.5 |
| Fits on microscope stage | Maximum length, width, and height in inches of main device | 18 x 8 x 5 | 14 x 5.5 x 3 |
| Doesn't make a mess | Minutes to clean up after processing complete | <5 | 3 |
| | Number of leaks | 0 | 0 |
| Easy to use | Minutes to learn to use and setup the device | <15 | 10 |
| | Number of pages of setup instructions not including pictures | <3 | 1 |
| | Number of pages of operation instructions not including pictures | <2 | * |
| | Number of hands required to operate | 1 | 1 |
| Quick setup and calibration | Minutes to setup | <20 | 13 |
| | Number of calibration steps | <10 | * |
| | Number of setup steps | <10 | 10 |

The device met all the requirements that could be measured. "Number of user performed operations" and "Number of pages of operation instructions not including pictures" could not be evaluated because the overall system has not been completed. Likewise, "Number of calibration steps" could not be evaluated because a calibration procedure has not been developed. Both these engineering specifications will be evaluated in the future. Other device usability engineering specifications were evaluated using the current device. The design values may change as the project progresses and additional setup and operational procedures are added.

4.5.4 Other Design Requirements

These design requirements didn't fit into the three other major categories. They are important considerations in the design.

Table IV: Other Design Requirements

| Customer Requirements | Engineering Specifications | Targets | Design |
|-----------------------|---|---------|--------|
| Minimize cost | Cost of components and materials | <\$1000 | \$750 |
| | Hours to manufacture in OSU Mechanical Engineering shop | <40 | 35 |
| Looks good | Percent of people involved with project who approve of appearance | 100 | 100 |
| | Number of easily visible scratches | <5 | 0 |
| | Total volume of sealant on device | <4ml | 6ml |
| Rugged | Transported without breakage or developing leaks | Yes | Yes |
| | Months until maintenance required | >3 | 2 |

The cost design value of \$750 includes the full cost of several bulk material buys such as large sheets of polycarbonate and HDPE. These materials were used in the construction of prototypes 3.0, 3.1, and 4.0. Only a portion of these materials were used in the construction of prototype 4.0. Other than the materials, significant costs included the mechatronic components and mirrors.

The increase in "Total volume of sealant on device" is due to patching leaks. These leaks occurred when the device was initially constructed. It would be possible to reach the target value for sealant if no leaks or only minor leaks occurred during assembly. The device was transported by hand between labs and buildings for two months without serious incident. It appears sufficiently rugged for such transportation. After two months, maintenance was performed by applying a small amount of additional sealant to fix a small leak. This leak could be due to excessive or rough handling.

5 Image Validation: Quantifying Image Quality

As with any design project, the performance of the design has to be quantifiably measured in order to determine if improvements are being made during the development process. These metrics are ultimately used to determine if the device meets the design requirements. To quantify the image quality of this design, a MATLAB program was written by one of the computer science graduate students.

This program first separated the stonefly from the background, which is called segmentation, and then created histograms of the background and stonefly pixel values which ranged from 0 to 255 for these 8 bit images. First this allowed for a qualitative evaluation of how well the program did at segmenting the stonefly. Secondly it allowed a quantitative evaluation of the uniformity of the background and dynamic range of the stonefly. Standard deviation of the pixel value histograms was used as a measure of dispersion for the histograms.

It was desirable for the background to be as uniform as possible and thus have a narrow dispersion. This allows the segmentation program to more easily differentiate between the stonefly and background. For the stonefly, a wide dispersion was desired so that the maximum amount of hue and intensity differentiation could be obtained. It is also important to avoid saturation for the background and stonefly at both the light end and dark end of the spectrum. When parts of the image are saturated, all information contained in those areas is lost. To summarize, a quality image has a uniform background, a stonefly with a broad dynamic range, and no saturation.

Baselines for both the background and stonefly dispersions were obtained by running the evaluation program with a high quality image made by hand. The results are shown in Figures 26 through 31 with the original image, the segmented stonefly, the histogram of the stonefly, and the histogram of the background respectively.

5.1 Baseline Image Results

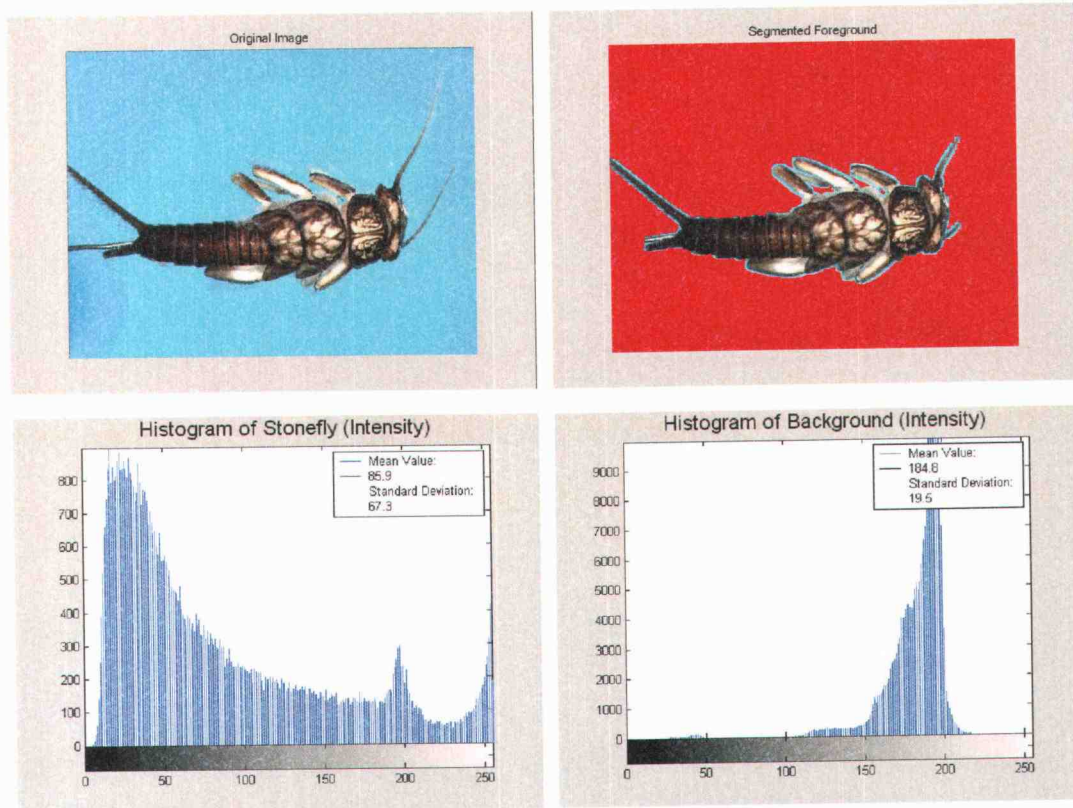


Figure 26: Baseline Image Analysis

The baseline image in figure 26 shows excellent values for the dispersion of both the stonefly and background. Dispersion of the stonefly covers almost the entire range of pixel values and the background dispersion is very narrow. However, there is too much saturation resulting in a loss of information for some of the legs. This image was created by placing a stonefly on a needle and then positioning it appropriately for the camera. It was submerged in a petri dish containing ethanol. The blue background was created by placing a glossy piece of blue paper on the bottom light reflector of the microscope.

5.2 Prototype 2.1 Image Results

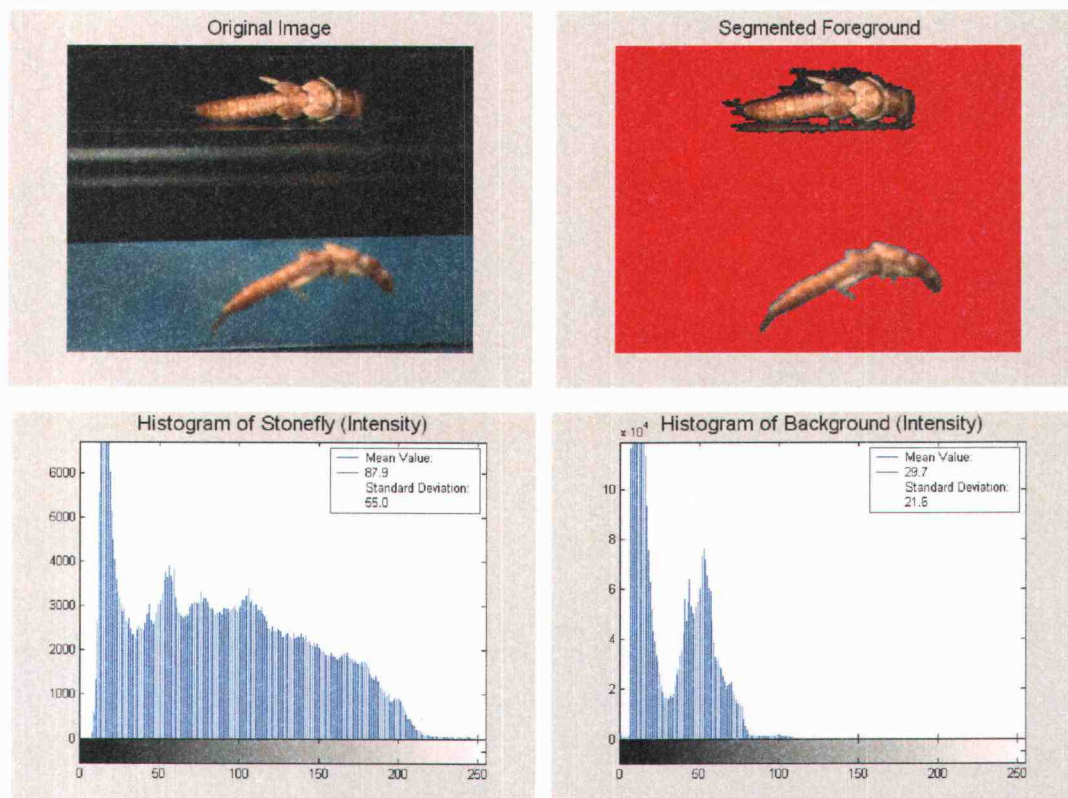


Figure 27: Prototype 2.1 Image Analysis

Dispersion as measured by standard deviation for the prototype 2.1 image in figure 27 is fairly good. The stonefly dispersion is slightly offset by the higher than acceptable number of black pixels that were extracted with the image. This is due to the inability of the program to differentiate between the insect and the dark background. The background dispersion is narrow but shows two peaks, one for the black background and one for the blue background. Although this prototype produced good images, one of them is out of focus and the prototype lacked the functionality of a side jet to manipulate the stoneflies. However, the prototype was a good first step for testing the mirror concept and imaging capabilities of the camera and microscope.

5.3 Prototype 2.2 Image Results

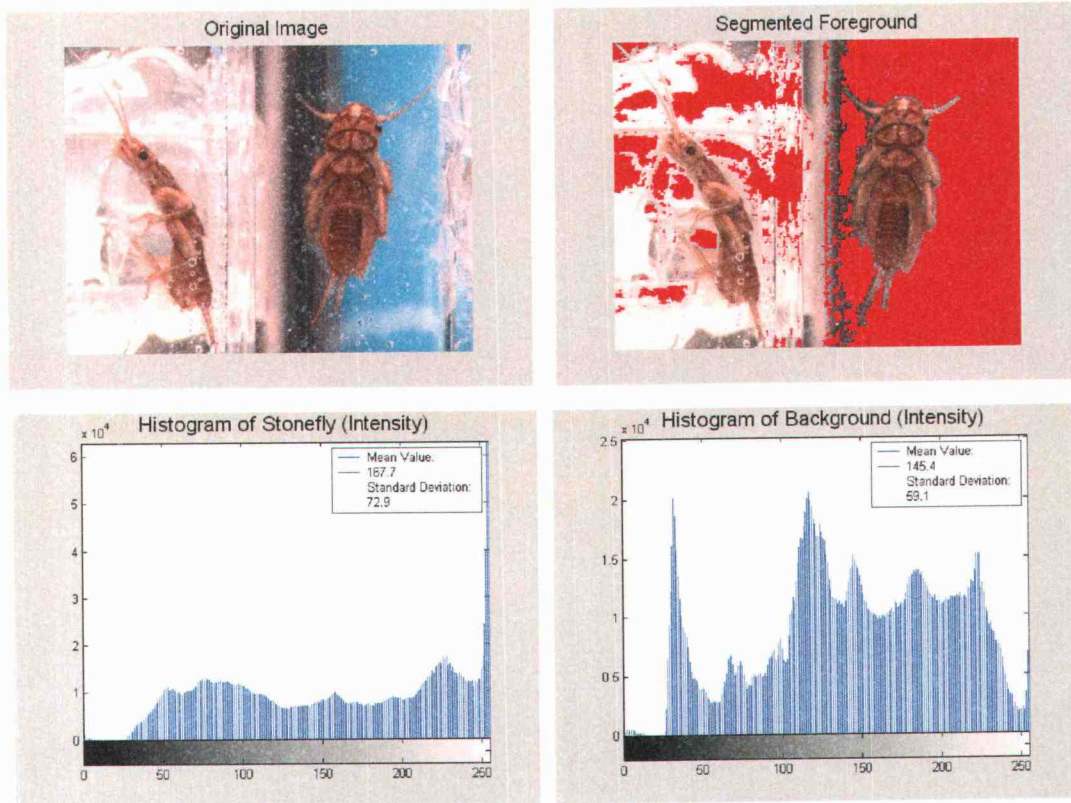


Figure 28: Prototype 2.2 Image Analysis

Prototype 2.2 was the first prototype to include a manipulating side jet. As can be seen in figure 28, the silicon sealant on the side jet is highly saturated and confuses the segmentation program. It is difficult for the program to differentiate the stonefly from the background. The dispersion for the stonefly looks quite good and covers a wide range of values. As would be expected there is an extremely large spike on the right side of the histogram for all the saturated pixels. Background dispersion is unacceptably high at about double the baseline image value because the side jet makes a uniform background impossible. The prototype clearly is incapable of producing an image that can be accurately segmented due to large background variability and saturation.

5.4 Prototype 3.0 Image Results

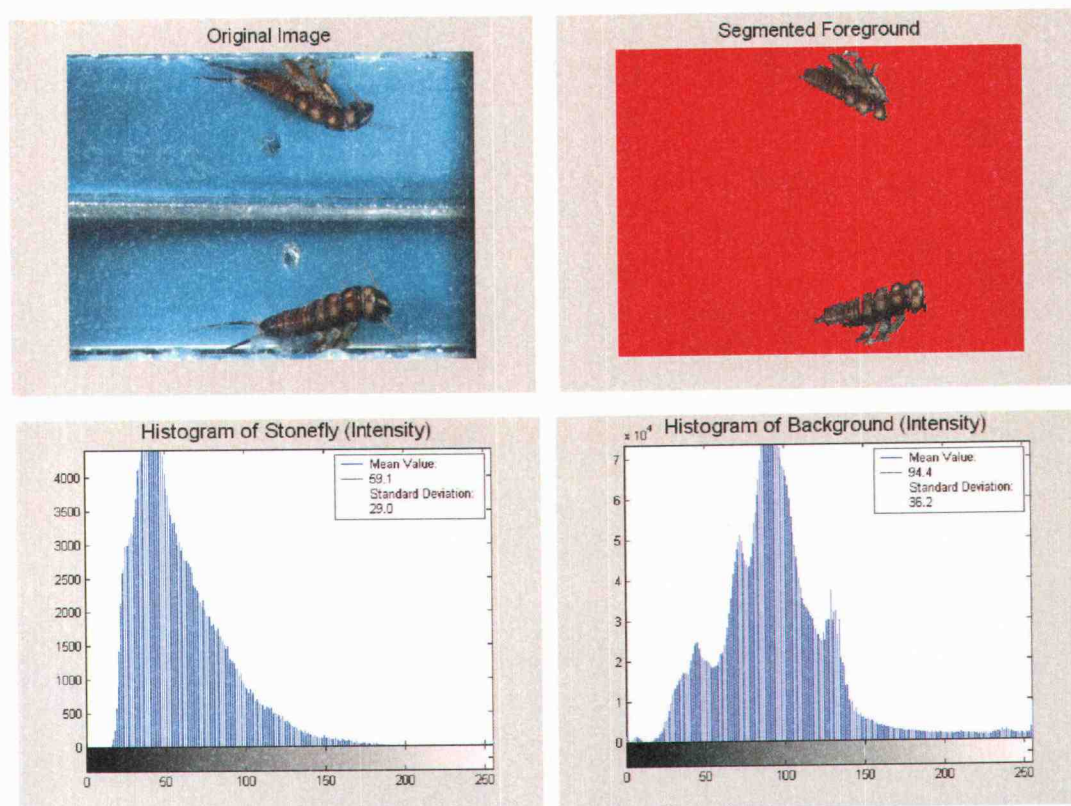


Figure 29: Prototype 3.0 Image Analysis

Although the prototype 3.0 image in figure 29 is an improvement over prototype 2.2, it is still unacceptable. Problems with both histograms are due to poor lighting. The image is too dark which makes darker parts of the stonefly blend in with the background and make it difficult to see the color variations of the stonefly. Note how the stonefly histogram is shifted to the left when compared to the other images. Improving the lighting would simultaneously widen the stonefly dispersion and narrow the background dispersion. It is also apparent that background uniformity should be increased by improving the finish on the blue HDPE parts and reducing the silicon sealant at the top and bottom of the image.

5.5 Prototype 3.1 Image Results

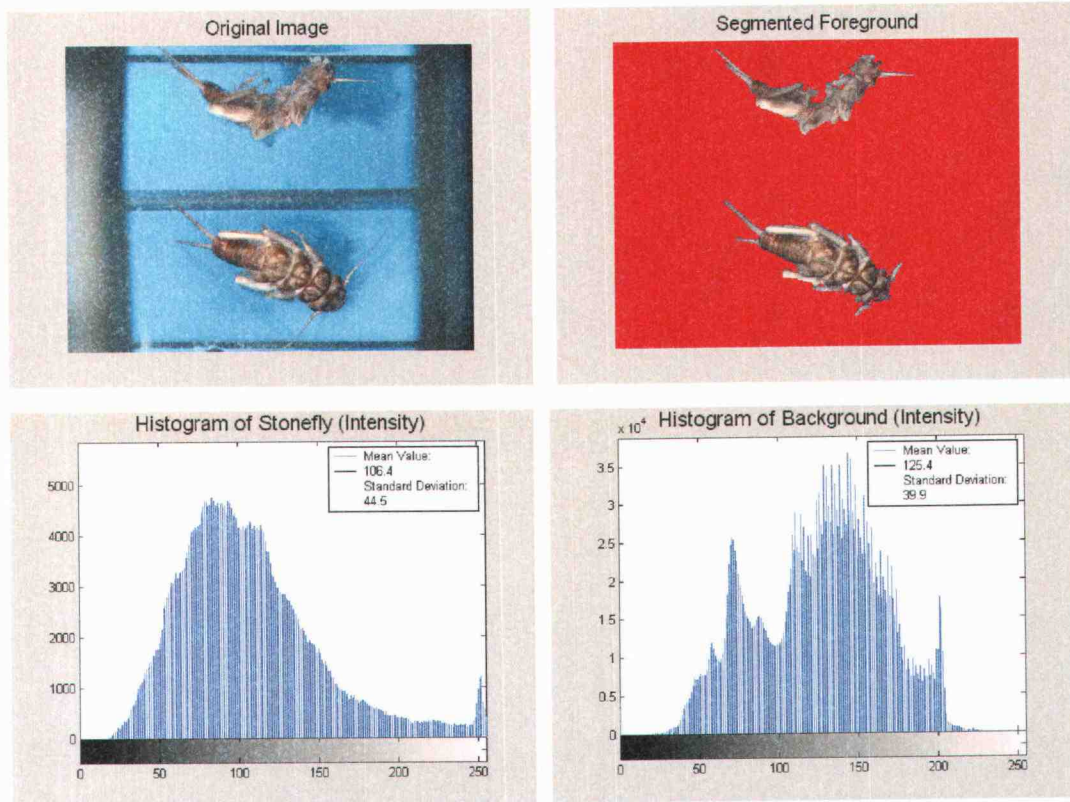


Figure 30: Prototype 3.1 Image Analysis

Images produced by prototype 3.1 were very good as shown in figure 30. The program was able to easily segment most of the stonefly, particularly from the back view. Dispersion for the stonefly pixels was approximately normal with a small tail on the right from slight saturation. These images were much better lit than earlier prototypes. Stonefly standard deviation improved by about 50% when compared with the previous prototype. The background standard deviation did not improve numerically. However, the program was able to better extract the stonefly due to the lighting which made the stonefly distinct from the background. The histogram for the background is from a cropped image that eliminated the black bars on the left and right. This gives a better measure of actual background uniformity as the black bars do not interfere with segmentation and can easily be cropped from the final image.

5.6 Prototype 4.0 Image Results

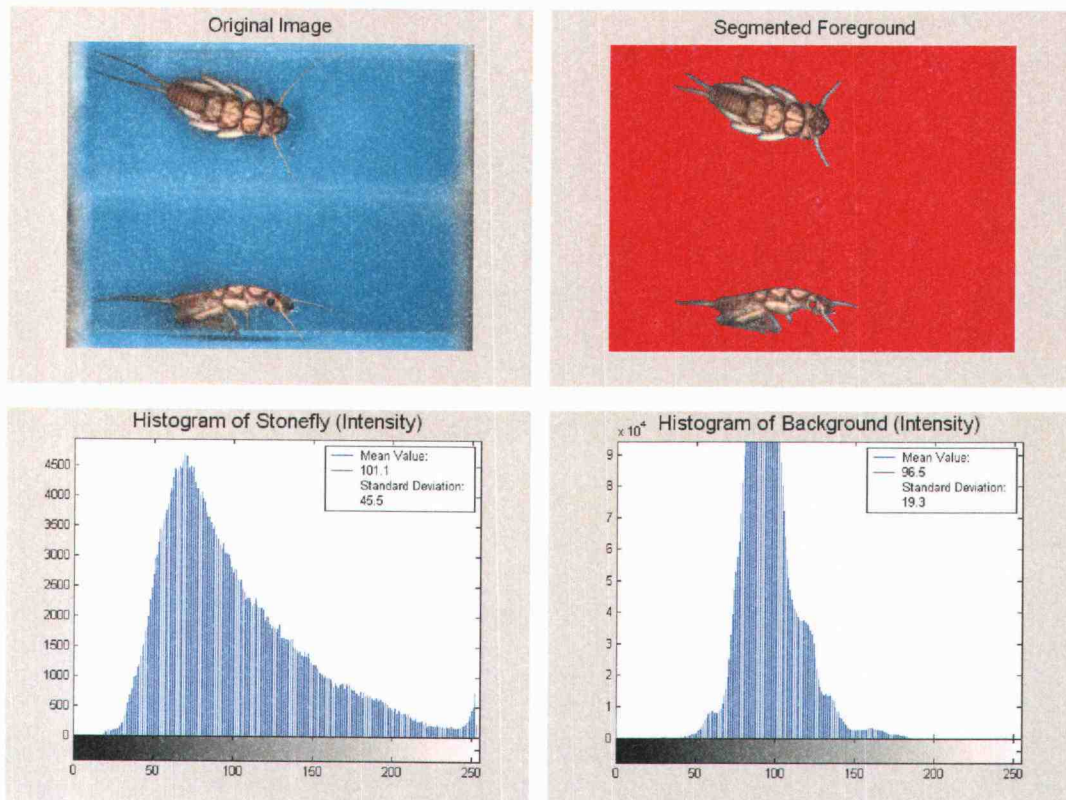


Figure 31: Prototype 4.0 Image Analysis

Images from prototype 4.0 are excellent as shown in figure 40. The stonefly image has a fairly wide dispersion that meets the design target of a standard deviation greater than 40 with no saturation at either end of the spectrum. Image processing such as histogram equalization could be used to further increase the dispersion of the stonefly to levels that are comparable to the baseline image. The background dispersion is the narrowest for all the images with a standard deviation of 19.3. This is well below the target value of 30 and a 50% improvement from the previous prototype. The image also has significantly less shadows than the prototype 3.1 image. Reduction of shadows allows for better image segmentation. The improvement in segmentation is particularly obvious in the side views for prototypes 3.1 and 4.0. In addition, due to the construction of the prototype, the black lines at the top and bottom of the prototype 3.1 image are gone.

6 Recommendations for Future Work and Conclusions

Several improvements must be made before the device can meet the goals of being completely automated and classifying stoneflies as quickly as possible. Most importantly a singulation and insertion device must be developed. Currently stoneflies must be inserted into the device by hand one at a time. Ideally, the user will be able to insert an entire sample of between 200 and 400 individuals to be processed at once. Some references mentioned in the literature review could provide direction for the singulation and insertion device design. This device will have to be integrated with the existing system.

The second major improvement is to increase the reliability and speed of the device when orienting stoneflies into correct dorsal view position. Currently it takes approximately 5 to 10 side jet pulse to correctly orient the stonefly in most cases, and upwards of 40 times for small stoneflies. This is due to the fact that most stoneflies tend to land on their backs with their legs pointing towards the camera. This fact could be exploited by mounting the entire system upside down with respect to its current orientation. With the system mounted upside down, stoneflies would tend to naturally orient themselves into the correct position. It is estimated that this would decrease the number of side jet pulses needed to reorient the stonefly to less than three for all sizes of stoneflies. The device would have to be redesigned to work upside down.

Other areas for improvement include the design of a sorting system for processed stoneflies, an automated way to eliminate bubbles that form in the imaging area, creation of an image calibration procedure, and further improvements in fabrication and assembly methods.

This project demonstrates a possible design for an automated stonefly imaging device. The device, once integrated with pattern recognition software, provides a method for the automated classification of stoneflies. A production

model will allow stonefly populations counts to be a more efficient method for monitoring stream health.

The methods used to transport and orient stoneflies could be extended to other applications. Different types of insects could be classified using devices specifically designed for the insects of interest. Automated inspection methods for irregularly shaped and fragile objects could also use the methods presented.

Additionally this project is an example of the collaboration possibilities between the engineering and life science disciplines. There are many challenging interdisciplinary problems to be solved and it is hoped that projects such as this will encourage more collaboration in the future.

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