AN ABSTRACT OF THE THESIS OF

Joshua K. Thomas for the degree of Master of Science in Mechanical Engineering presented on July 26, 2006.

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Abstract approved:

Robert K. Paasch

Aquatic insect population counts can be a good indicator of the health, or water quality, of rivers and streams. Stoneflies (*Plecoptera*) are particularly susceptible to pollution in streams. However, today's current method for obtaining these population counts requires biologists to examine individual specimens under a microscope for identification to the species level. This manual method has proven to be a cumbersome and inefficient way to monitor stream and river health. In response, an aquatic insect imaging device was designed, constructed and tested in an attempt to speed and automate the process of insect identification. The device was specifically designed to handle various species of stoneflies immersed in a fluid medium. Orientation methods are incorporated to acquire appropriate digital images useful for pattern recognition. This project suggests an alternative method for aquatic insect identification that could lead to more efficient biomonitoring.

Automating Aquatic Insect Identification through Pattern Recognition

by
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APPROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Joshua K. Thomas, Author

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Automating Aquatic Insect Identification through Pattern Recognition

1 Introduction

Stonefly larvae, aquatic macro invertebrates of the Order *Plecoptera*, are found in abundance in North America. Approximately 500 different species inhabit the fast-flowing waters in cool, clean streams across the continent [Webb 1999]. A few examples of different species of stonefly larvae can be seen in Figure 1.

Figure 1: *Calineuria, Doroneuria, Zapada, Moselia, Hesperoperla,* and *Yoraperla*

Stoneflies are characteristically susceptible to changes in dissolved oxygen concentrations in streams. Small changes in concentration from fertilizers, sewage and other sources can have radical effects on the local populations due to their extreme sensitivity. Thus, stonefly population counts provide valuable information about the health of streams.

The current method of identifying stonefly species is a daunting task. Visual inspection of body characteristics and patterning on the exoskeleton is used for identification. Because of their small size individual specimens must be placed under a microscope for viewing. In addition, some stonefly species have such subtle differences that identification requires a person with extensive knowledge and experience. Samples of several hundred specimens need to be identified for statistically legitimate inferences about the total population. For these reasons, currently it is not efficient to conduct this type of experiment to monitor the health of streams on a large scale.

Since 2002, an ongoing project study has been underway that may eventually lead to a solution to the stonefly identification enigma. A collaborative effort is being made between the disciplines of Entomology, Computer Science and Mechanical Engineering. Ultimately, the goal is to produce a fully automated device that will singulate, correctly identify and categorize the specimens to allow the ability to efficiently monitor stream health.

Contribution of all three disciplines to the project is essential. First, the project requires the collection and proper identification of several hundred specimens of various species of stoneflies. The specimens can then be individually placed into the mechanical system to obtain the proper digital images via a computer and digital microscope. A computer learning algorithm can be used to identify the species based on pattern recognition. Several pre-identified specimens can be used with the algorithm to first "teach" it the differences between the various species. The system's accuracy would then be tested with digital images of specimens not yet identified.

Previous work on the project resulted in a device, Prototype 4.0, capable of handling stoneflies and producing digital images useful for pattern recognition. It is discussed in more detail in the following section on literature review. The device had not been thoroughly tested on a large scale basis.

This report describes the design process, construction, testing and results of the mechanical system involved with digital image acquisition. Based on new and emerging customer requirements, various designs are evaluated by constructing and testing them. Evaluations are based primarily on image quality and efficiency but also include stonefly handling, device usability, and manufacturing and economics, among others.

2 Literature Review

Several attempts at automating insect identification exist. Computer aided methods have been devised to identify various types of insects based on specific identifying features. Live insects are also identified through acoustic and image analysis. The techniques are studied from various sources of journals, magazine articles, web pages and conference proceeding.

The “Optical Flying Insect Detection and Identification System” (OFIDIS) created by Moore Scientific and Qubit Systems Inc. was developed for insect identification. As the name suggests, OFIDIS is designed to automatically count and identify individual flying insects. By use of a photo sensor the reflected light off an insect’s wings and body is used to generate a species-specific harmonic waveform [Moore 2002]. However, this method would not be useful for our research since it requires live, flying insects.

The detection and identification of pests in soil is important in agriculture for control of infestations. Currently, a laborious technique of digging up the soil is the only successful method for identification of the infestations. By using sensors or microphones the sounds produced by insects during feeding, communication or general movement can be recorded and studied. Assuming that their sounds are distinguishable this may offer a method of underground insect identification [Mankin et al. 1998]. A similar study was conducted for acoustic identification and measurement of activity patterns of white grubs in soil [Zhang et al. 2003]. Again, for our purposes these offer little support as they require live insects.

Throughout time the accepted method to identify insects is based on visual inspection. Specimens can be identified by studying and matching pictures and certain identifying features of insects. There have been several attempts and studies in the past to identify insects through image analysis based on pattern recognition or shape analysis [Chesmore and Nellenbach 2001, Ellington et al. 2005, Howell 1982, O'Neill 2005, Tofilski 2004, Weeks et al. 1999, Yu et al. 2002].

For insects with wings there is typically a species-specific pattern of veins visible within the wings. Because the patterning of veins and shape of wings for different species is unique, they can be used to identify the insect. "DrawWing" is a current program capable of carrying out the image analysis to create coordinates of vein junctions and wing diagrams useful for classification [Tofilski 2004].

Wing analysis of insects is useful because their patterning is unique between species. Stoneflies do not have wings but they do possess unique patterning on the dorsal side of their exoskeleton. This suggests the possibility of using pattern recognition for automated identification of stoneflies. In support of this theory there currently exists a semi-automated species identification program called "DAISY" [O'Neill 2005]. This is a generic pattern matching system that can be taught to identify species of a range of organisms. It uses digital images and a point-and-click interface to operate. However, the accuracy of the device is not ideal, averaging less than 90%.

Previous work on automating insect identification at Oregon State University resulted in Prototype 4.0, shown in Figure 2. Pattern recognition techniques utilizing learning algorithms, coarse and fine classification, region detectors and descriptors were deployed in an attempt to automatically identify various stonefly species from digital images[Zhang W. 2006, Larios E. 2006]. Success rates range from 95% to 73%, depending on the species[Mortensen et al. 2006].

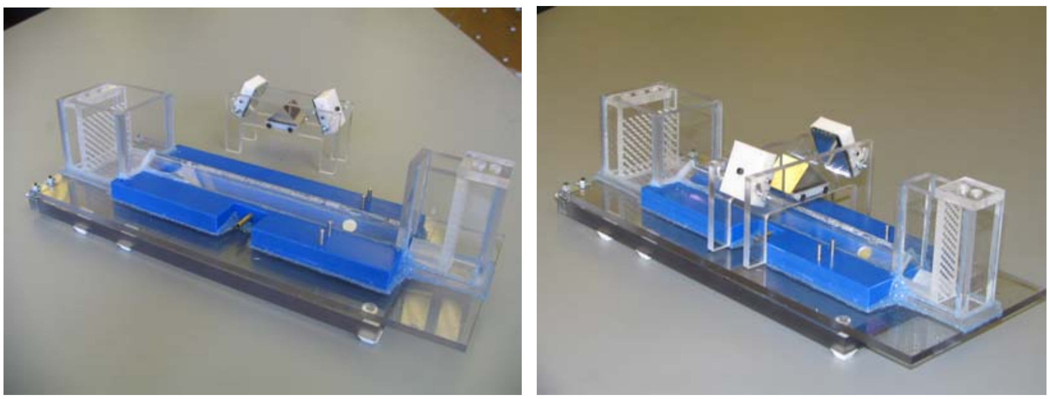

Figure 2: Prototype 4.0

The device consists of loading and exit basins connected by a transport tube to provide a means for transporting a single specimen from one location to another. The computer algorithms designed for insect identification require digital images of individual specimens. A clear fluid medium is used throughout the system to preserve the stoneflies. Pumps provide flow to transport insects from the loading basin to the imaging area and to the exit basin, and infrared sensors are used to automate the process to improve the efficiency of the device. Orientation of the insects at the imaging area is done via a side jet. Digital images from various views of a specimen may allow the creation of a three-dimensional model that can be used to identify it to the species level. The mirror configuration doubles productivity by capturing two views of the insect per image. A blue background in the images provides a good contrast with the insects and allows for easy segmentation [Sarpola 2004].

The requirements that emerged from the efforts in creating Prototype 4.0 are shown in Table I, which can be divided into categories relating to image quality, stonefly handling, device usability and other design requirements [Sarpola 2004]. No design values exist for the requirements “Number of calibration steps,” “Number of pages of operation instructions not including pictures” and “Number of user performed operations.” This is because Prototype 4.0 has not been tested on a large scale and no calibration procedure has been developed.

Table I: Prototype 4.0 requirements

Customer Requirements	Engineering Requirements	Targets	Prototype 4.0
Clear picture	Number of objects in image other than sonefly	<3	0
	Number of variable objects in image overlaying stonefly	0	0
	Number of gill and hair clusters visible	>2	7
	Percent of image with visible sealant	<5%	0
Good view of back of stonefly	Rotation of back with respect to image (degrees)	+/-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
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, med, large)	0	0,1,7
	Number of stoneflies stuck in jet hole per 40 (small, med, large)	<2	0,0,0
	Number of stoneflies stuck in loading basin per 40 (small, med, large)	<4	0,6,11
Processes range of stonefly sizes	Range of stonefly lengths that device can prcess effectively (mm)	5-20	8-12
	Range of stonefly diameters that device can process effectively (mm)	1-5	2-4
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 heigth in inches of main device	18x8x5	14x5.5x3
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 claibration	Minutes to setup	<20	13
	Number of calibration steps	<10	*
	Number ot setup steps	<10	10
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

3 Design Process

A more complete list of design requirements will allow for better performing prototypes to be built. To develop new design requirements extensive testing of existing prototypes is necessary. A detailed look at the performance of Prototype 4.0 is done and new designs are generated, manufactured, tested and evaluated.

3.1 Testing

Prototype 4.0 has met most of the target values shown in Table I. It produces clear images of the entire insect, showing several hair clusters and no visible objects other than the stonefly. The images appear well lit with a uniform background. This permits easy segmentation of the images as shown in Figure 3.

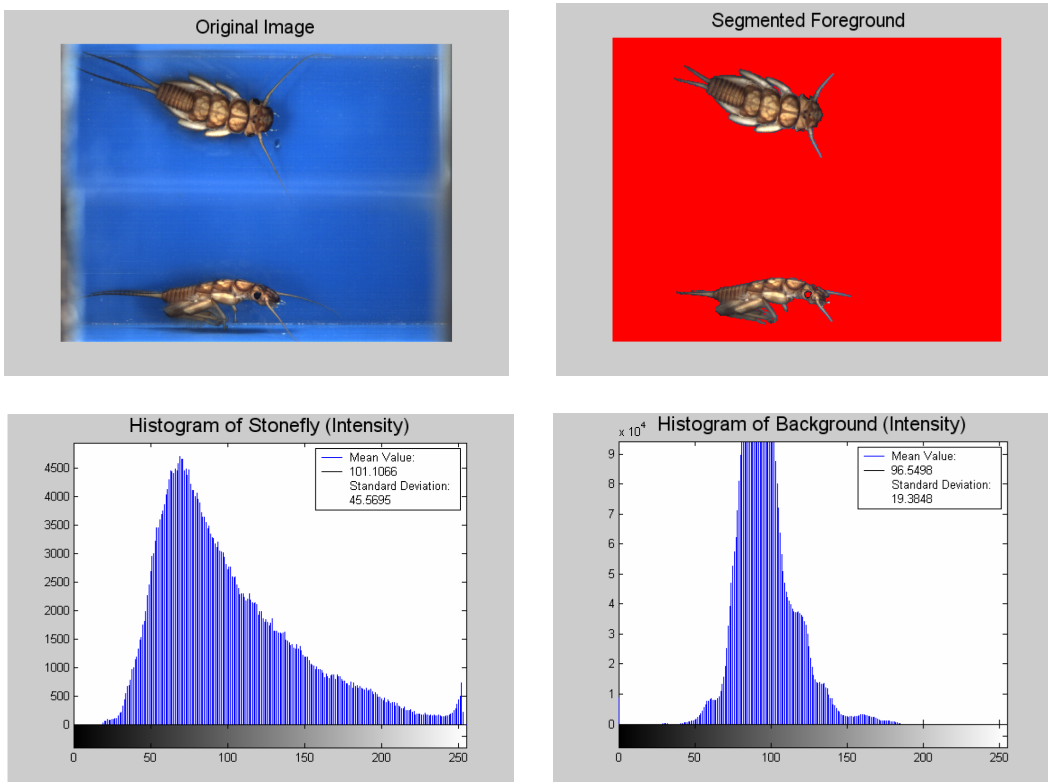

Figure 3: Prototype 4.0 histogram analysis

Figure 3 demonstrates the results of a computer algorithm that is used to segment the stonefly from the background by recognizing their contrasting colors. Intensities of the pixel values for the background and stonefly are used to create the histograms. A uniform background results in a narrow dispersion of pixel values. A wide dispersion of pixel values for the stonefly signifies that most of the colors of the stonefly are being recognized by the algorithm.

Testing of Prototype 4.0 revealed several areas of possible improvement. The failure rate of the IR sensors was inadequate, particularly for small stoneflies. The size of stoneflies that the device is able to process effectively does not meet the target value. Large stoneflies could get stuck at the entrance of the transport tube and at the IR mirror location. Small, medium and large stoneflies are considered to be specimens of length less than 8mm, 8mm-12mm and larger than 12mm, respectively. Figure 4 demonstrates the size range.

Figure 4: Stonefly size range

Usability of the system was easy, but efficiency of the device needs improvement. Because stoneflies tend to land on their backs the number of reorientations required for a dorsal view reached as many as 40 for smaller specimens. Constant lighting adjustment for each image also contributed to the inefficiency of Prototype 4.0. Lastly, image quality can be improved by eliminating bubble formation and shadowing.

3.2 3-D Representation

One method of insect identification investigated was to reconstruct three-dimensional models of each insect. With this kind of model a number of different parameters, or identifying features, could be used to identify the species. These identifying features may include the location and number of appendages and patterning found on the exoskeleton.

A method for constructing a three-dimensional model would be to capture a digital image of a stonefly suspended within the bounds of a conical mirror as shown in Figure 5. The resulting image would be distorted due to the curvature of the mirror, but with knowledge of the specific geometry of the mirror the image could be undistorted with computer algorithms. This method was not implemented due to complexity.

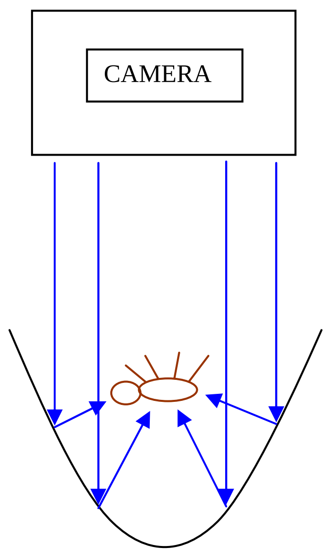

Figure 5: Conical mirror sketch

Another method to create a three-dimensional model is to locate common points between standard digital images of the stonefly at various orientations. A series of images with common points that circumvent the perimeter of the stonefly would be needed for an entire three-dimensional construction. This method was attempted by using images that had been taken with Prototype 4.0. The mirrors on Prototype 4.0 provide images at a 90 degree angle of separation. It was found that the angle of separation between the images was too great to locate enough common points for reconstruction.

A simple device, called "The Rotisserie," was constructed to further investigate the required angle of separation for three-dimensional reconstruction. The device needed to generate clear, well lit images with a uniform and consistent blue background. While immersed in a fluid, the stoneflies needed to be accurately rotated at measurable increments of 5 degrees between images.

The stonefly is pierced longitudinally with a needle which is attached to a controlling arm via a rubber grommet. A rubber grommet is also used as a seal for the side of the imaging bin. Bearings and an aluminum frame support the controlling arm and imaging bin. The angle of rotation is measured with a protractor and the apex of the triangular support. Rotation of the insect is achieved by manually rotating the end of the aluminum controlling arm. Blue HDPE was used for the background. The completed device is shown in Figure 6.

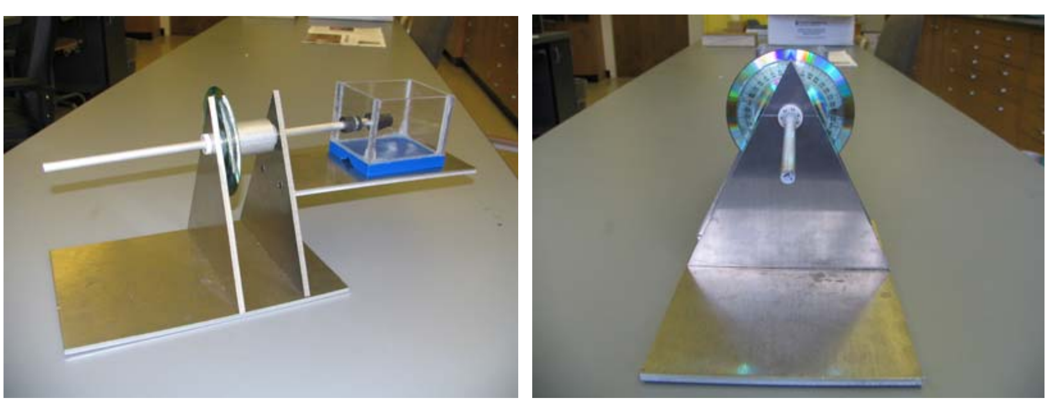

Figure 6: "The Rotisserie"

"The Rotisserie" was a successful device, generating images that were sufficiently clear and well lit. The blue background is fairly consistent and the formation of bubbles is absent. Rotation of the stoneflies was precisely controlled with the protractor. Images taken with "The Rotisserie" are shown in Figure 7. A complication apparent in the images is the presence of the needle. Its reflection makes segmentation difficult. Painting the needle blue would allow it to be segmented with the background.

Construction of a three-dimensional model within the required time and computational constraints proved problematic due to difficulties in locating common points among images. This method for identification was not further pursued. Pattern recognition based on strictly dorsal images was thought to be more feasible.

Figure 7: “The Rotisserie” images at 0, 60, 135 and 270 degrees

3.3 Stonefly Free-Fall

To obtain good dorsal images, a method for consistently orienting the insects was investigated. During testing of Prototype 4.0 it was found that the stoneflies tended to land on their dorsal side. Testing was conducted to further the probability of stoneflies landing on their dorsal side by free-fall through a fluid. Stoneflies were individually dropped into two beakers filled with water to a height of 8 inches. Wide and narrow beakers were tested to verify if contact with the sides of the beaker when falling would have an effect on the results.

The testing verified the method, demonstrating a 97.5% and 99.2% probability of landing dorsal side down in the wide and narrow beakers respectively. The specimens were chosen at random to ensure that the statistical results could be extended to a larger population. Small, medium and large specimens of *Calineuria*, *Hesperoperla*, *Doroneuria* and *Yoraperla* were included in the experiment. Results are shown in Table II.

Table II: Stonefly free-fall

Genus	Wide beaker	Narrow beaker	
Doroneuria	30	30	Dorsal down
	0	0	Dorsal up
Yoraperla	28	29	Dorsal down
	2	1	Dorsal up
Hesperoperla	29	30	Dorsal down
	1	30	Dorsal up
Calineuria	30	30	Dorsal down
	0	0	Dorsal up
Total	97.5%	99.2%	

4 Prototype 5.0

Pattern recognition based strictly on dorsal images requires a design that can attain dorsal images more efficiently than Prototype 4.0. The design requirements for Prototype 4.0 were still applicable. The new functional characteristic of Prototype 5.0 is the ability to capture images from beneath the stoneflies to attain dorsal views. A configuration of mirrors and legs to support the main platform provide the ability of the camera to view the bottom side of the transport tube. The blue background, constructed from ultra high molecular weight (UHMW) polyethylene, was placed on the top side of the rectangular shaped transport tube. The mirror configuration is shown in Figure 8.

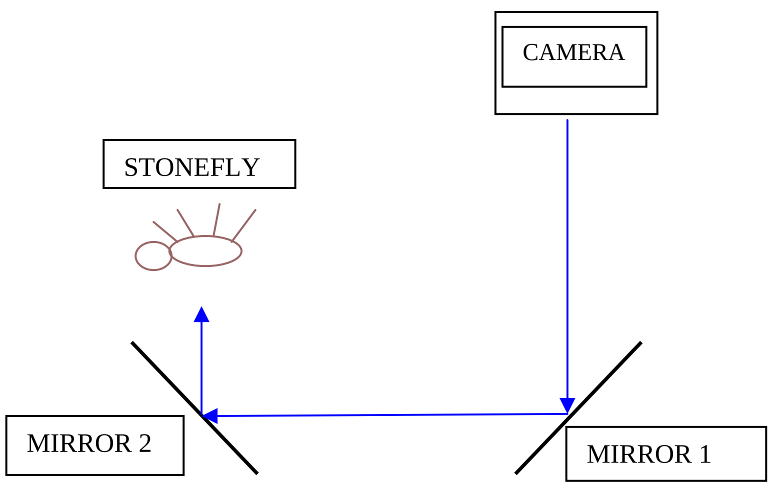

Figure 8: Mirror configuration

The device was designed to sit on the table on which the microscope sat. Prototype 5.0 is shown in Figure 9. The photo was taken after a cut had been made in the side of the device.

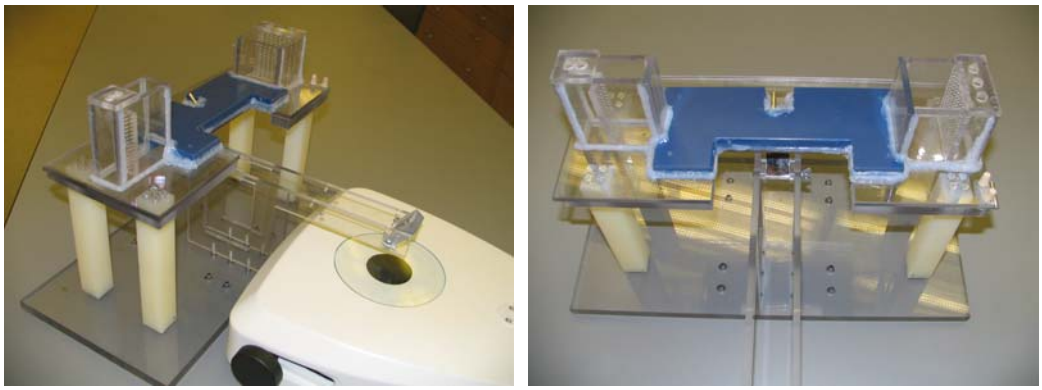

Figure 9: Prototype 5.0

During initial testing, several problems were discovered. The quality of the images was poor due to the inability to attain a focused image. The initial attempt to solve this problem was to change the magnification lens used on the microscope however, this had no influence on the outcome. The thickness of the polycarbonate was tested to determine if the images were being distorted by refraction of light rays passing through the material. The transport tube of Prototype 4.0 had been constructed with 0.0625 inch thick polycarbonate. The polycarbonate used in Prototype 5.0 was 0.25 inches thick. To test the theory a stonefly was manually placed on the microscope platform and various polycarbonate sheets of thickness 0.5, 0.25 and 0.0625 inches were placed above the insect. The images resulting from the different polycarbonate thicknesses are shown in Figure 10.

Figure 10: Images through polycarbonate thicknesses of 0, 0.0625, 0.25 and 0.5 inches, respectively

The polycarbonate does have an effect on the focus of the images. The difference between the control and the 0.25 inch polycarbonate images are detectable but minor. The poor quality of images from Prototype 5.0 is not likely due to the thickness of the polycarbonate.

Another theory to explain the poor image quality is imperfections in the mirrors. The industrial grade quality of the mirrors may not be good enough for the design of Prototype 5.0. To test the theory a single mirror was used to capture images of a stonefly at various distances from the mirror. A sketch is shown in Figure 11 to better illustrate the tests.

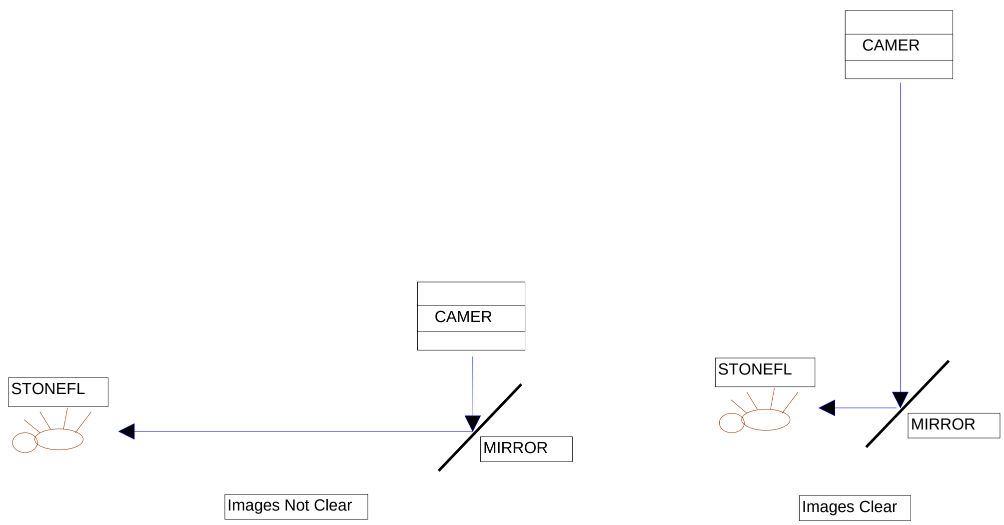

Figure 11 - Mirror testing sketch

The results were conclusive and it was discovered that the position of the insect in relation to the mirror was important. The images became more distorted the further away the stonefly was positioned from the mirror. To resolve the problem either the mirrors needed to be moved as close together as possible or new high quality mirrors needed to be purchased.

Shadows were also evident in the images. The shape of the transport tube made it difficult to eliminate shadows in the corners. Shadows from the insect itself are also still evident.

It was also discovered that the width of the transport tube may be a little large. Although acceptable, for future prototypes the width would likely be decreased. In addition, it would be ideal to make the transport tube a little taller as well. However, due to the design of Prototype 5.0 and the lack of availability of thick UHMW polyethylene the height could not be increased.

Functioning of the side jet was not ideal. It was manufactured to enter the transport tube horizontally and did not create the needed vortex to keep the stoneflies in the imaging area. Prototype 4.0 suggests that the jet should enter the transport tube at a 45 degree angle.

Finally, a permanent lighting system was needed for more consistency between imaging sessions. Pattern recognition algorithms could then be more accurate in detecting differences in the insects, not the lighting patterns. The time to setup the device would be reduced by not needing to manually adjust the lights each time.

5 Open-Channel Flow & “Fuzzy” Background

Bubble formation and the background are important for image consistency, segmentation and ultimately, insect identification. A new design idea that operates by using open-channel flow was constructed and tested to evaluate its effectiveness in combating bubble formation. An out-of-focus background was also tested and evaluated in an attempt to improve image consistency.

5.1 Open-Channel Flow

By leaving the top of the fluid in the transport tube open to the atmosphere, the bubbles formed would float to the surface and burst. This is called open-channel flow. A simple device was constructed to test the theory. No images were taken with the device but stonefly handling was evaluated.

Made of polycarbonate, the device sits on the base of Prototype 5.0. For open-channel flow the fluid height throughout the system is the same when at rest. Flow is produced by creating head in the system with the use of pumps. A side jet was used to orient the insects. The device is shown in Figure 12.

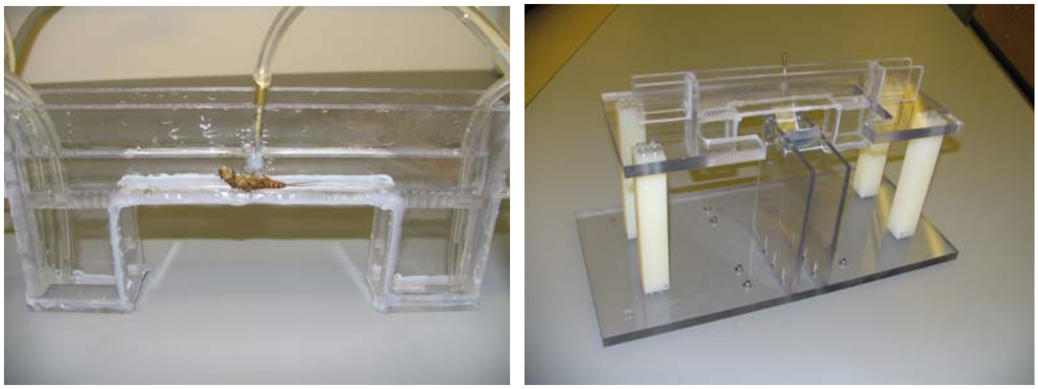

Figure 12: Open-channel device

Initial testing revealed that the device did eliminate bubble formation as hypothesized, however excess time was needed to attain equilibrium in the system after running the pumps. The insect swayed back and forth several times before coming to rest, negatively affecting the ability to get the specimen to the imaging area. This method was not further pursued.

5.2 "Fuzzy" Background

A method to improve the consistency of the background was to place the blue background far away from the focal point of the camera. Details of an object can not be seen when not in focus. Scratches, bubbles and other imperfections and inconsistencies on the background would not be visible. Inconsistent lighting on the background would also be less apparent.

Due to time constraints only a small test was performed to see how the background looked when out-of-focus. The digital microscope was focused on a stonefly and a piece of blue UHMW polyethylene was positioned about 4 inches behind the focal point. The background was very consistent, verifying the hypothesis. An image of the test is shown in Figure 13. The effect of shadowing was not evident.

Figure 13: "Fuzzy" background

The methods described for open-channel flow and "fuzzy" background provide valuable information. The techniques were not incorporated into any current designs, but they could be useful for future devices.

6 Prototype 5.1

Performance of Prototype 5.0 could be improved by moving the mirrors close together, reorienting the side jet and adding permanent lighting. Reshaping the transport tube would also help eliminate shadowing, but would require re-manufacturing most of the device. Due to time constraints this was not pursued. The requirements for Prototype 5.1 are focused images, consistent lighting and the ability to orient the insects within the imaging area.

Prototype 5.1 was produced by modifying Prototype 5.0. First, the side jet was reoriented to enter the transport tube at a 45 degree angle downward. The mirrors were relocated in close proximity to each other and the transport tube. New mirror supports were constructed and a notch was cut into the side of the device. The device is shown in Figure 14.

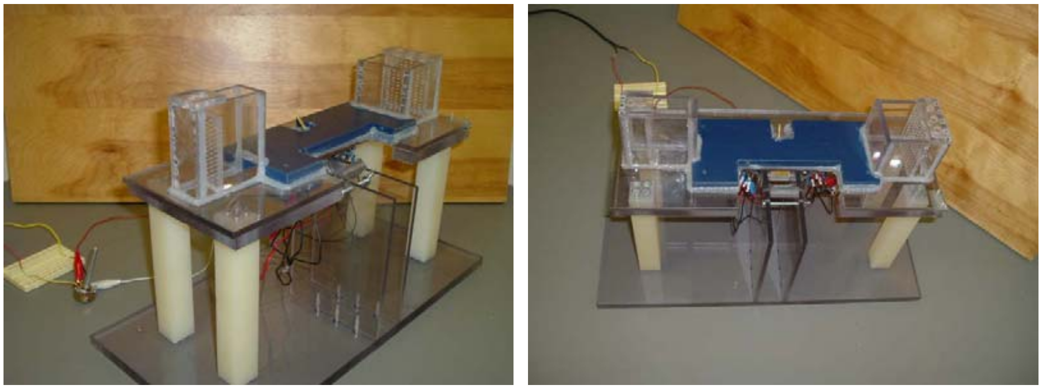

Figure 14: Prototype 5.0 with modifications

6.1 Lighting

A lighting setup was constructed by using light emitting diodes (LED). Eight LED lights, emitting an ultra bright white light at a maximum 3000 millicandela each, were mounted to aluminum pieces with standard plastic holders. Brightness was controlled with a potentiometer. The setup is shown in Figure 15.

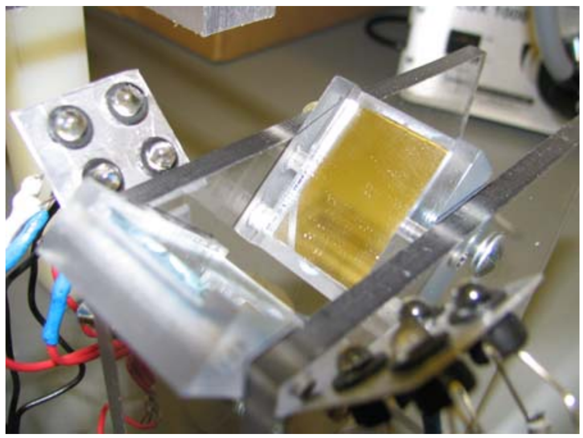

Figure 15: 3,000 millicandela light emitting diodes

The LED lights were tested but found to be insufficient. Images appeared in a bluish tint and the luminosity was not acceptable. An image taken with the LED lights operating at their maximum rated power is shown in Figure 16.

Figure 16: Image with small LEDs

New LED lights providing 10,000 millicandela each were purchased in an effort to increase the luminosity of the insects. They are shown in Figure 17.

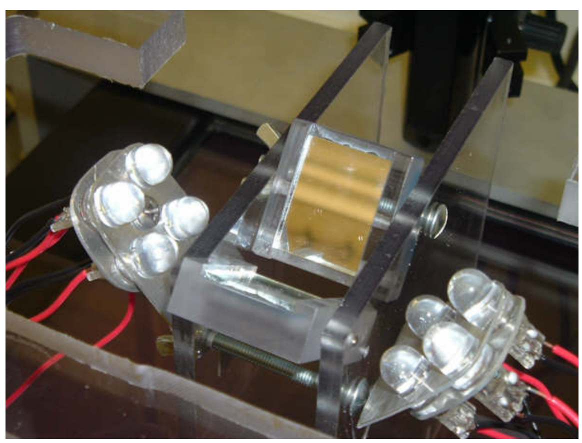

Figure 17: 10,000 millicandela light emitting diodes

Different mounts for the lights were constructed and a larger 15V power source was used. The effect of the new LED lights was immediately clear. The additional brightness made a difference, but was still unacceptable. An image taken with the new LED lights is shown in Figure 18. Using LED lights for illumination was abandoned.

Figure 18: Image with large LEDs

The best lighting that had been tested was from the fiber optic light sources. By constructing light fixtures the gooseneck fiber optic lights could possibly be fixed in place. However, the excessive stiffness of the gooseneck light guides would make it difficult to accurately position them the same way each time the device is set up, resulting in inconsistent lighting levels from imaging session to session. More flexible light guides would be easier to mount consistently. Two twin-arm flexible light guides were purchased.

The light fixtures were designed with the ability to adjust the position of the fiber optic light guides and fix them in place. An aluminum clamp, rod, bar stock and set screws were used in the final design shown in Figure 19. Once in the appropriate position the set screws are used to fix the lights. Machined pieces of plastic were designed to fit over the ends of the fiber optic lights. Thin, translucent pieces of HDPE were attached to the plastic fittings to act as diffusers.

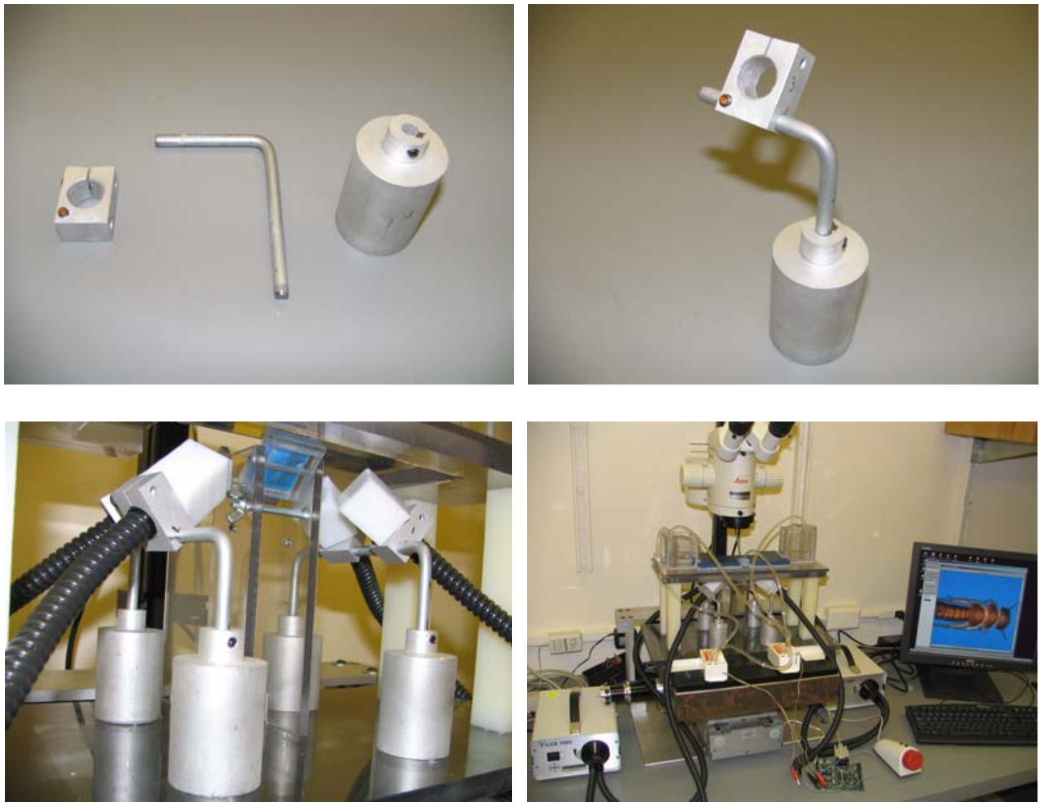

Figure 19: Prototype 5.1 fiber optic light fixtures and complete setup

6.2 Testing

Approximately 3,500 images of 8 different specimens were captured with Prototype 5.1 over a two week period. The effectiveness of the device was analyzed and it was immediately discovered that there was a new problem with focusing. The 0.32x objective could not be adjusted enough to get a focused image. The 1.0x objective focused much better, but required contact with the top of the device. A 0.63x objective was purchased to solve the issue. The new objective worked well, providing a balance between the other objectives, allowing focused images at an acceptable distance from the top of the device.

The repositioning of the side jet was an improvement. It created a vortex that spun the insects in place. It was not ideal however, because the insect did not always stay within the imaging area. A tight vortex may not have been created because the shape of the transport tube was rectangular, not square.

The new light fixtures functioned well with the ability to hold the fiber optic lights in position for several weeks. Light from the sources was effectively diffused with the diffusers. However, the diffusers were excessively large, restricting movement of the light fixtures.

Small index marks were made on the light fixture pieces so that if ever disassembled, they could be mounted back in the same position. Similarly, marks were made on the intensity adjustments on the Volpi cold light sources to ensure consistency.

The images produced by Prototype 5.1 are acceptable. More precise positioning of the lights could reduce the shadows found in the corners of the transport tube and provide a more consistently lit imaging area. Reconstruction of the transport tube could also reduce shadows. Increasing the intensity of the lights could be experimented with more extensively to better light the insects. An image taken with the final device and its histogram analysis can be seen in Figure 20.

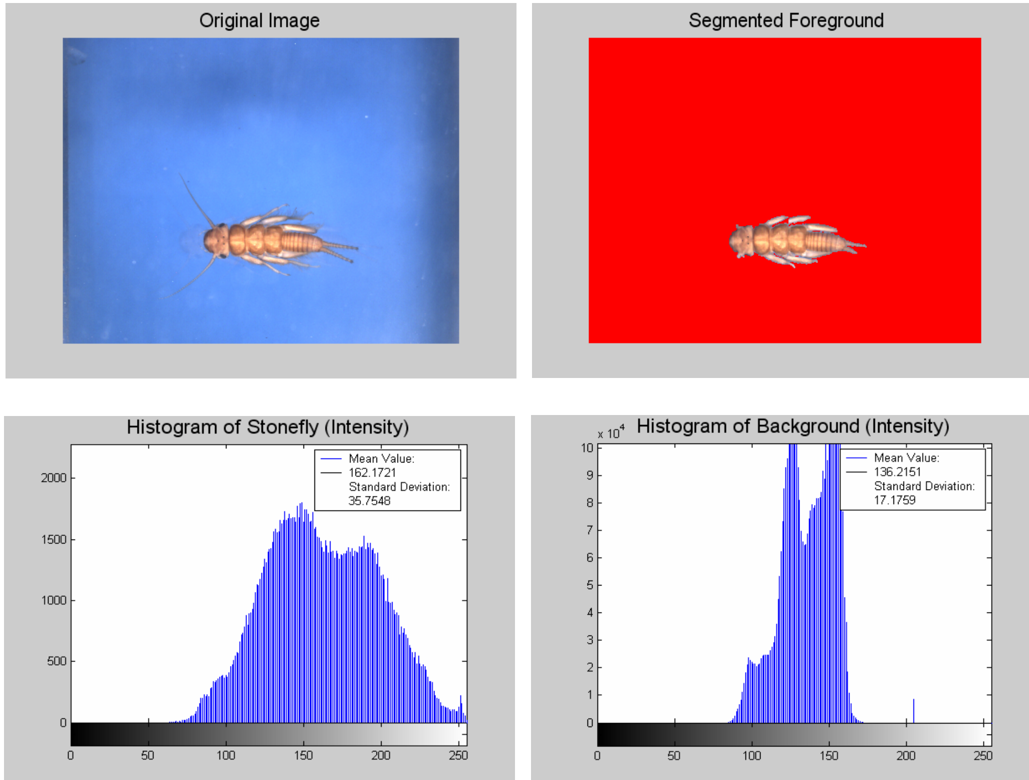

Figure 20: Prototype 5.1 histogram analysis

The image quality for the prototype is very good based on the histogram analysis. The wide dispersion of the stonefly histogram means that a large amount of hue and intensity differentiation is being achieved. The narrow dispersion of the background histogram signifies the uniformity of the background. Standard deviations are used for the measures of dispersion. For a meaningful analysis the segmentation algorithm was applied to thirty different specimens for each of eight different species, including *Calineuria*, *Doroneuria*, *Hesperoperla*, *Isoperla*, *Moselia*, *Sweltsa*, *Yoraperla* and *Zapada*. The averaged results are shown in Table III and are further discussed in the following section.

Table III: Prototype 5.1 histogram results

	Background		Stonefly	
Species	**Mean**	**Std. Deviation**	**Mean**	**Std. Deviation**
Calineuria	125.2	17.7	121.5	38.4
Doroneuria	130.8	17.8	129.3	34.8
Hesperoperla	123.6	20.2	98.5	35.6
Isoperla	137.4	17.8	135.6	36.8
Moselia	124.9	19.7	122.3	28.5
Sweltsa	130.8	17.5	126.0	31.6
Yoraperla	127.1	23.0	122.4	37.9
Zapada	127.1	21.5	111.8	35.9
Total Average:	**128.4**	**18.9**	**120.2**	**34.9**

The Prototype 5.1 histogram analysis meets or exceeds the performance of previous designs in most aspects. The images appear clear and well lit, allowing a good view of the important patterning features on the dorsal side. The insects are fairly easily segmented from the uniform background with a minimal amount of the stonefly lost, as shown in Figure 20. Adjustments in the lighting may allow for even better segmentation.

7 Comparison to Design Requirements

A new list of design requirements was generated to evaluate Prototype 5.1. Most of the requirements remain the same as for Prototype 4.0 (Table I), but several new requirements were added based on experience with using the device. The requirements for the device are divided into four sub-categories: Image quality, Stonefly handling, Device usability and Other design requirements.

7.1 Image Quality

The design requirements, goals and performance for those requirements related to the quality of the images are shown in Table IV.

The overall quality of images produced by the prototype is very good compared to the targets listed in the table. The images are clear and consistent, which are the overarching goals of the system. All of the goals in the table are met by the design. Some of the design values for the prototype are estimates based on extended use of the system. For example, the value for “Percent of stonefly lost when segmented” is an estimate based on visual inspection of the segmentation analysis. The specification for “Degree rotation of stonefly with respect to direct dorsal view” is also an estimate. Most images appear to be direct dorsal views, but some specimens tend to land somewhat on their side. The number of shadows has increased due to those visible in the corners of the transport tube. Due to the lighter color of the stonefly in Figure 20, the mean stonefly pixel value is out of the target range, but the results from Table III show that the average for several different specimens is acceptable. The requirements in the Table IV related to the histogram analysis come from the averages shown in Table III, which all but one met the target values. The standard deviation of the stonefly pixel values is less than acceptable. This may be due to the darker colors of the stonefly not being recognized by the segmentation algorithm, or in general the stoneflies used to collect the data are lighter in color.

Table IV: Image quality

Customer Requirements	Engineering Requirements	Targets	Prototype 4.0	Prototype 5.1
Works in different lighting	Has lighting adjustment	Yes	Yes	Yes
Clear images	Maximum pixel value for stonefly	<255	254	254
	Number of variable objects in image overlaying stonefly	0	0	0
	Percent of image with visible sealant	<5	0	0
	Image exposure time in milliseconds	<250	245	250
Good lighting	Candlelight power provided by lights (Candela)	>10	>>10	>>10
	Maximum number of shadows	2	1	2
Dorsal images	Degree rotation of stonefly with respect to direct dorsal view	+/-15	0	+/-5
Consistent background	Maximum number of shadows	2	1	2
	Number of materials visible in background	1	1	1
	Standard deviation of background pixels values	<30	19.4	18.9
Digital images	Generates digital images (pc compatible)	Yes	Yes	Yes
No debris	Average number of debris visible per image	<1	<1	<1
No air bubbles	Average number of bubbles visible per image	<3	<1	<1
No saturation	Number of saturations visible per image	<2	<2	<2
Easy image segmentation	Percent of stonefly lost when segmented	<10	5	5
Can see entire stonefly	Standard deviation of stonefly pixels values	>40	45.6	34.9
	Mean stonefly pixel value	100-150	101	120.2
	Percent of stonefly feet visible that are not obscured by body of stonefly	100	100	100
Blue background	Blue background	Yes	Yes	Yes
	Number of colors visible in background	1	1	1

Testing of Prototype 5.1 led to additional requirements. New requirements related to lighting include "Has lighting adjustment" and "Candlelight power provided by lights" which were added to ensure that the lights had sufficient intensity and could be adjusted for different external lighting conditions. For uniformity of the background only one material should be visible in the background. The images produced need to be digital images so that computer algorithms can be easily applied. The final addition to the requirements relating to image quality include "Blue background" and "Number of colors visible in background." These requirements help guarantee a consistent background.

7.2 Stonefly Handling

The goals and performance of the system with respect to stonefly handling are shown in Table V.

The device does well with respect to stonefly handling. It is greatly improved as compared to Prototype 4.0. By using the mirror setup to capture images from beneath the number of reorientations required for a good dorsal view is greatly reduced. Because the stoneflies tend to land on their backs, previous prototypes required up to 40 reorientations before attaining a good dorsal view. With Prototype 5.1 more than 1 reorientation is seldom needed, vastly improving the efficiency of the device.

Table V: Stonefly handling

Customer Requirements	Engineering Requirements	Targets	Prototype 4.0	Prototype 5.1
Works quickly	Time to image one stonefly (seconds)	<45	40	30
	Seconds to reorient stonefly	<3	1	2
	Number or reorientations required for good back view	<5	30	1
Robust	Range of stonefly length that the device can process (mm)	5-20	8-12	5-18
Stoneflies do not jam	Number of stoneflies stuck in loading basin per 40	<5	6	3
Preserves specimens	Uses fluid transport	Yes	Yes	Yes
	Transport fluid >70% ethanol/water mixutre	Yes	Yes	Yes
	Number of appendages lost per stonefly during processing	0	0	0
	Body segments damaged per stonefly during processing	0	0	0

Processing the largest stoneflies is still difficult. A small number tend to get stuck in the loading basin and are hard to transport through the system. Although the range of stoneflies that the device can process is acceptable, there exist species significantly larger than 20mm in length. Future prototypes may adjust the target values to accommodate for those larger specimens.

Again, some of the values listed are estimates. In some cases the number of seconds to reorient a stonefly is much more than the listed value. This occurs when the side jet forces the specimen out of the imaging area. To improve consistency of the images the fluid used in the system was modified from water to an ethanol/water mixture. The requirement “Transport fluid >70% ethanol/water mixture” was included to decrease bubble formation and allow specimens to quickly sink.

7.3 Device Usability

Device usability refers to the ease and performance of functions of the device that require human intervention. The evaluation of the final prototype is shown in Table VI.

A close look at Table VI shows that with regards to device usability by the operator the device performs very well. The apparatus meets all the requirements. It is fairly easy and straightforward to use. For initial setup more tools are needed as compared to Prototype 4.0, but because the light fixtures can be permanent the steps and minutes to setup Prototype 5.1 are reduce. Without having to adjust lighting for each picture fewer steps are required to image with Prototype 5.1.

The performance of the prototype has not been evaluated for three of the requirements. “Number of pages of operation instructions not including pictures” and “Number of user performed operations” could not be evaluated simply because the device is not yet ready for the market. Also “Number of calibration steps” could not be evaluated because a procedure for calibrating the camera has not yet been developed.

Table VI: Device usability

Customer Requirements	Engineering Requirements	Targets	Prototype 4.0	Prototype 5.1
Easy to use	Steps required to image	<4	4	3
	Minutes to learn to use and setup the device	<15	10	10
	Number of pages of operation instructions not including pictures	<2	N/A	N/A
	Number of hands required to operate	1	1	1
Easy to maintain	Average time between repairs (months)	>2	2	3
Has numerous features	Focus capability	Yes	Yes	Yes
	Bug positioning capability	Yes	Yes	Yes
Maxiumum automation	Number of user performed operations	<3	N/A	N/A
	Coordinate operation with computer and microscope via serial or USB	Yes	Yes	Yes
Easy to install/set up/calibrate	Number of pages of setup instructions not including pictures	<3	1	1
	Steps to setup device	<10	10	5
	Minutes to setup device	<15	13	10
	Number of tools required for setup	<2	0	2
	Number of calibration steps	<10	N/A	N/A
Safe	Average annual user injuries	0	0	0
Easy to repair	Average minutes for repairs	<20	5	5
	Uses USCS tools for repair	Yes	Yes	Yes
	Average number of tools for repair	<3	<1	<1
Faults easily identified	Average minutes needed to identify problems with device	<5	3	3
Easy to clean	Minutes to clean device	<5	3	3
	Number of tools required for cleaning	<2	0	0

Some fundamental requirements were added that can be seen in the previous table. “Focus capability” and “Bug positioning capability” were included to ensure that the proper images can be focused. Another requirement was added so ease the setup process by minimizing the number of tools required for setup. To ensure that the device is safe to use “Average annual user injuries” was included. To ease the repair and cleaning process the following requirements were added: “Average minutes for repairs,” “Uses USCS tools for repair,” “Average number of tools for repair," "Average minutes needed to identify problems with device" and "Number of tools required for cleaning."

7.4 Manufacturing and Economics

Requirements related to the manufacturing ease and economic awareness are shown in Table VII.

Table VII: Manufacturing and economics

Customer Requirements	Engineering Requirements	Targets	Prototype 4.0	Prototype 5.1
Easy to assemble	Number of tools for assembly	<4	1	4
	Time for assembly by skilled machinist (min)	<30	10	20
	Number of steps for assembly	<10	2	5
Parts easy to manufacture	Time to manufacture parts by skilled machinist (hrs)	<30	35	20
	Number of machining tools needed for manufacture	<5	4	4
Minimize scraps and rejected parts	Amount of scrap material acquired during manufacturing (lbs)	<3	1	1
Low cost of materials	Material cost ($)	<200	150	175
Materials readily available for production	Days needed to attain materials	<7	10	5
Minimize number of parts	Number of parts	<25	25	23
Uses standard parts and fasteners	Uses USCS parts/fasteners	Yes	Yes	Yes
Low purchase price	Purchase price ($)	<2000	N/A	N/A
Inexpensive to maintain	Average annual repair cost($)	<25	5	N/A
Easy to package and transport	Minutes to package device for transportation	<15	8	10
	Total weight (lbs)	<15	5	12
	Number of people required to transport device	1	1	1
Minimizes packaging space	Packaging volume (ft^3)	<2	<1	1
	Required floor space for device (ft^2)	<2	1	1.5

Again, the product meets all of the target values. “Purchase price” and “Average annual repair cost” have not been evaluated. The listed material cost includes the bulk cost. More material was purchased than was used in the device, so the actual cost of material used would be slightly less than that listed. Also, the cost listed includes only the main device itself consisting of primarily the polycarbonate, nylon and polyethylene. Prototype 5.1 is more complicated than Prototype 4.0, requiring more tools and time for assembly, but the parts are easily manufactured.

The new requirements added include “Number of tools for assembly,” “Time for assembly by skilled machinist,” “Number of steps for assembly,” “Number of parts” and “Uses USCS parts/fasteners” which were used to evaluate the ease of assembling the device. To evaluate the ease of manufacturing the parts the requirement “Number of machining tools needed for manufacture” was included. For economic concerns the requirements “Amount of scrap material acquired during manufacturing” and “Average annual repair cost” were added. Other new requirements include “Days needed to attain materials,” “Minutes to package device for transportation,” “Packaging volume” and “Required floor space for device.”

7.5 Other Design Requirements

Design requirements not falling into any of the previous categories are shown in Table VIII.

The remaining targets listed in the table have been accomplished by Prototype 5.1. Prototype 5.1 is more efficient largely due to the design of the mirror setup, reducing the number of insect reorientations needed. The device works faster than Prototype 4.0 but the improvement listed in the table is an estimate. The percent of dorsal images attained by Prototype 4.0 was hard to determine since many were captured at odd angles.

Table VIII: Other design requirements

Customer Requirements	Engineering Requirements	Targets	Prototype 4.0	Prototype 5.1
Aesthetically appealing	Fasteners/adhesives not visible	Yes	Yes	Yes
	Percent of people involved with project who approve of appearance	100	100	100
	Number of easily visible scratches	<5	0	4
Works as it should (identifies species)	Percent of images which are dorsal views	100	40	100
	Number of leaks	0	0	0
Enduring/durable	Transported without breakage or developing leaks	Yes	Yes	Yes
More successful than previous models	Percent faster the device images as compared to previous model	>20	N/A	25
Fits under digitial microscope	Total height in inches	<24	4	11
	Mirror height in inches	4-12	3	6

For aesthetics the new requirement “Fasteners/adhesives not visible” was added. “Percent of images which are dorsal views” was included after discovering that three-dimensional representation would not work. To estimate the improved efficiency of Prototype 5.1 the requirement “Percent faster the device images as compared to previous model” was added. Lastly, “Total height in inches” and “Mirror height in inches” was included to ensure the proper size constraints of the digital microscope.

8 Conclusion and Future Work

Prototype 5.1 has performed well and has met most of the requirements that have been developed for it. Still much about what exactly is needed for the device is not yet known. Targets will be adjusted and requirements will change for better performing prototypes.

There still remains a significant amount of work that needs to be done before reaching the ultimate goal. A working singulation device needs to be designed and constructed that will have the ability to select single individuals out a large sample and transport it to the imaging device. In addition, a sorting mechanism is included in our overall vision. Currently, work is underway to address this issue. All three components of the system can then be fused together and automated with computer control.

With the current imaging device there are still improvements that need to be made before the possibility of marketing can be addressed. By modifying the shape of the transport tube shadowing can be reduced and reorientation may be more efficient. More effort in the placement of the fiber optic lights and intensity adjustments could lead to a more evenly lit imaging stage, allowing for better segmentation.

Further automation needs to be incorporated into the imaging device. Currently the transporting, orientation and imaging are done by hand through use of the computer. The IR detectors used in earlier prototypes and automation of the pumps for proper positioning of the insects in the imaging platform could be better redesigned for the current and future prototypes.

Continuing work with the open-channel flow could eliminate bubbles in the imaging area. Similarly, further exploration of the “fuzzy” background idea could result in more consistent images with the possibility of being able to adjust brightness of the insects without affecting that of the background.

Overall, the progress that has been attained is this project gives an optimistic outlook to the potential of the insect identification system through pattern recognition. The achievements thus far provide a possibility for an automated stonefly imaging device. Further work and improvements could eventually lead to mass production of the device with the ability to identify several types of insects, not only stoneflies. The final design would allow insect population counts to be a more realistic and efficient means for monitoring stream health.

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