Microscope Manipulator

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Abstract: There has been an insurgence of research involving zebrafish embryos within the last few years, primarily due to the ease with which an observer can view changes in their internal physiology caused by external and internal factors. It has been proposed that a digitally controlled micromanipulator be constructed to create a fast scanning system to image and irradiate a large sample of zebrafish embryos efficiently. It is required that the stage not exceed 6 cm in height and have a minimum stepping resolution of 200 μm.

§1. **Problem Definition**

Accurate scanning and re-positioning of samples under a dissecting microscope is inefficient with the equipment currently available to the client. The current stage is too large and the imaging and positioning hardware and software is outdated. The primary goal of this project is to develop a fused digitally interfaced stage and custom imaging technique that can systematically do the following: scan a sample of zebra fish, analyze the fused images, store the positions of each zebra fish and reposition the sample to the localized positions. An example of a micromanipulator system is shown in Fig. 1.

Fig. 1 – Micromanipulator set-up with intergrated analog monitoring equipment.

§2. **Motivation**

Zebrafish as Early Vertebrate Model:

Zebrafish embryos are becoming more popular in the scientific community as vertebrate models. Zebrafish embryos are transparent during their embryonic stage and develop *ex utero* (Xu). This transparency allows for observation of organ and skeletal development on the cellular level *in vivo*. This is preferable to the researcher as they are able to pinpoint and monitor the specific area or multiple areas of interest. The zebrafish genome project is nearing completion, and will afford the scientific community many opportunities for studying this vertebrate development.

The client specifically studies the inflammatory response of zebrafish cells due to radiation exposure, and its relationship to cell apoptosis (programmed cell death). The zebrafish is irradiated with approximately 50 keV of non-ionizing high energy photons. The mechanisms of inflammatory response of the zebrafish may be elucidated as a result of this cutting edge technique, which would ultimately provide insight into the mechanisms of radiation poisoning in other vertebrates.

Cell Apoptosis

Apoptosis is the programmed destruction of cells by their own lysosomal enzymes (Campbell and Reece, 2002). The exact pathway for human apoptosis is not currently known and is an active area of research. The cell receives a signal to die from various signaling regions of the body (e.g. central nervous system, paracrine system and endocrine system) which initiate leakage of suicide proteins from the outer membrane of the mitochondria. Post mortem, the remaining pieces of the dead cell are then engulfed and digested by neighboring cells, allowing the embryo to stay free of the harmful proteins. An example of the failure of apoptosis in the morphogenesis of the human is webbed fingers or toes (Campbell and Reece, 2002). Apoptosis has become very prevalent in current cancer research, due to tumor cells' resistance to this mechanism.

§3. Product Requirements

The table must move freely in the XY plane and have a step precision of at least 200 μm. In order to clear the microscope lens, it cannot exceed 6 cm in height. It must be large enough to hold a 6 cm diameter Petri dish and have a range of motion that is wide enough

to scan the entire dish. Because each zebrafish will be irradiated, the table must withstand 50 keV of ionizing radiation without demonstrating adverse effects or retaining any radioactivity. There should be no limit on the number of uses the micromanipulator can endure.

The camera used will be purchased and integrated with the microscope. It must have sufficient resolution to see the effects of the radiation on individual fish. It must also mount easily and securely on the eyepiece and should be directly connected to the operating computer for online analysis.

The imaging and positioning software must be integrated, automated, and PC compatible. Image processing software will be required to localize all embryos in a sample so that they can be irradiated one at a time. It will also have to create a composite image of the Petri dish using the many small images gathered by the imaging camera. All components should be interfaced with the PC using USB or Firewire technology, but a serial port connection would suffice.

§4. **Background:**

Stepper Motors:

Stepper motors are small electronic devices that accommodate linear and angular translations through the utilization of electromagnetic principles. There are three primary types of stepper motors including variable reluctance (VR), permanent magnet (PM) and hybrid stepper motors (Ericson). Due to the inner workings of each stepper motor, hybrid

stepper motors can facilitate the smallest step sizes, whereas VR and PM are generally used for large step sizes. Hybrid stepper motors combine the best characteristics of the VR and PM by providing high resolution similar to VR and torque comparable to that of PM steppers (Ericson). All steppers have a common magnetic basis for their operation. Control of the rotation is achieved by running specific currents through various combinations of windings in the stator; the rotor is in effect manipulated by the resultant magnetic fields (See figure 2 for stepper motor diagram). Pre-determined combinations of current input cause specific step sizes.

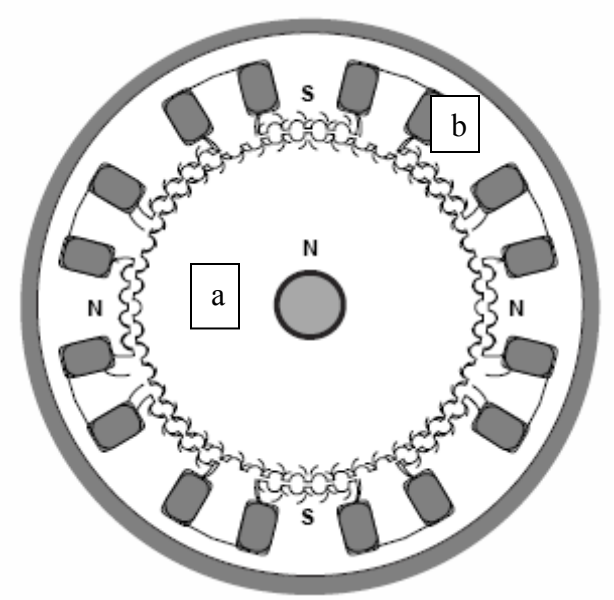

Fig. 2 – Hybrid stepper motor schematic. Portion a is the rotor whereas b is the stator. (Courtesy of http://www.library.solarbotics.net /pdflib/pdf/motorbas.pdf)

Stepper motors can be operated using several different modalities, including full-step, half step and micro-step. Full-step operation causes the stepper motor to rotate an entire stepper increment (step). For hybrid steppers, this is usually 1.8 degrees. A gear ratio would be utilized to make this angular displacement realized in a linear translation of the stage. The linear translation is quantified by the equation $s=\theta r$, where θ is the angular displacement, r is the radius of the gear and s is the linear displacement of the stage. Another modality that can be used is the half-step mode. This mode allows for a stepper motor rated for 1.8 degrees per step to move .9 degrees, by compromising the torque of the motor. Half-step modes offer greater consistency in step angles and less overall jerk

in the system. A more recent development in stepper motor control is the micro-step mode. This approach allows for rotations as small as $1/256^{th}$ of a full step, and can function at various speeds. This is the most effective, but most complex method for stepper manipulation.

Another factor that must be considered when deciding upon a stepper motor is the holding torque of the motor. This measure is the amount of torque that a given motor can exert at a stand still, which is roughly equivalent to the maximum torque. This will be looked at qualitatively, since a quantitative model of the required torque to cause stage translation is unavailable and the torque of a given motor when correlated to applied load exhibits a non-linear relationship. A motor with a maximum torque will be used if affordable, given that other facets of the high torque stepper motor are similar to lower torque models.

§5. **Designs/Parts:**

Overview:

In order to meet the client's requirements, several disparate components will be fused to accomplish the expected task. The following components are necessary if a custom stage prototype is to be manufactured: stage, 2 stepper motors, PCI (Personal Computer Interface) hardware, camera with mount, framer grabber, image analysis software and a custom program to mesh all portions. The logistics of understanding and applying all of these disparate facets will be a formidable task. The widespread use of such devices should simplify the task considerably because of the quantity of resources available.

Although this is an untraditional method for reporting design alternatives, it is the most effective way because of the complexity of the required design.

Steppers/Stages:

One option for satisfying the client's requirements is to buy a commercial stage, which would include all of the hardware necessary for digitally interfacing with a computer to control stage movement. This system would have sufficient resolution, but would be expensive. One commercial option is the H105 Proscan II, manufactured by Prior Scientific, (Rockland, MA). This stage, when interfaced with a PCI (Personal Computer Interface) High Speed Stepper Motor Controller, also manufactured by Prior Scientific, has a minimum step size of .04 μm and an operation area of 15.4 cm x 15.4 cm, surpassing the requests of the client. This stage is limited to XY translation. A similar option, which would be slightly less expensive, is the Prior Scientific ES111. It would have a minimum step size of 1 μm and the ability to translate in the XY plane in an area of 17.4 cm by 7.6 cm, if interfaced with the same PCI hardware. In both circumstances, a microscope camera would still be necessary to analyze zebrafish samples.

Another option for goal realization is the fusion of several disparate devices, some commercial and others custom. It is estimated that a custom stage could be micromachined through the University of Wisconsin Machine Shop for approximately $750 (this estimate is based upon 25 hours of work at $30 per hour, and excludes costs of materials). The primary, and most time consuming facet of this option would be the fabrication of a stage, although it would be possible, but difficult to find a compatible

non-motorized commercial stage. A large amount of time would have to be dedicated to designing the stage, and creating a model using modeling software. Regardless of stage choice, stepper motors (one is shown in Fig. 3) will be controlled by a personal computer, which will instigate stage translation. Stepper motors are a component that is commonly found in medical devices (Ericson).

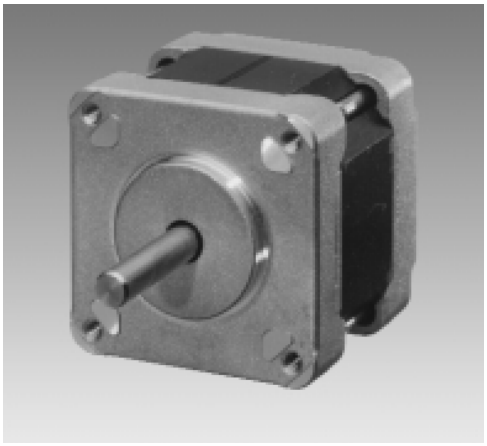

Fig. 3 – Shinano stepper motor.

One company that is prevalent in the production and distribution of stepper motors is Shinano Kenshi Corp. (Culver City, CA), focusing on hybrid stepper motors. One Shinano hybrid stepper model that could be used is the SST-40C2011. This model requires a 6 volt DC source and has a full step size of .9 degrees. If a gear with a radius of .5 cm is used at full step mode, this would cause a linear translation of 78.5 μm. This is sufficient for the intended use. There may be a problem with torque, since this model has the lowest holding torque of all the models considered (for explanation of holding torque, see Background section).

A second Shinano model that has been considered is the SST-41D1100, which has the same specs as the 2011, except a step size of 1.8 degrees and slightly increased holding torque. The 1.8 degrees at full step mode would translate to be 157 μm per step with a gear of radius .5 cm. The cost of both of these models, based upon comparable models, should be approximately $50 – 100 per unit.

Another company that manufactures stepper motors is NMB Technologies Corporation (Chatsworth, CA). A satisfactory model is the 17PM-K401V, which requires an input voltage of 3.4 volts, has a step size of 1.8 degrees, and has two times the holding torque of either of the Shinano models. These stepper motors cost $25 each, considerably cheaper than the expected cost of the Shinano models.

The final company that was investigated was Danaher Motion (Wood Dale, IL), which specializes in high precision PM stepper motors. The specific model that has been considered is the K42N, which has a step size of 1.8 degrees, but a torque 8 times that of the NMB stepper model. This would certainly be sufficient to translate the stage. At this time, the price of this model is unknown, but a quote for 2 units has been requested.

In all cases, to increase the resolution (e.g. decrease minimum step size), a half-step or micro-step method could be used. These step modalities are performed by the PCI hardware; therefore, the primary factors affecting our stepper choice will be cost and holding torque instead of step size.

Computer Interface:

In order to control these stepper motors, driver hardware must be purchased. Using this hardware, steps can be realized using a digital signal. Instructions for phase activation for each step mode are universal, so the main concerns for driver hardware are reliability,

cost and connection compatibility. These can be determined by reviewing specs of specific models and, if needed, contacting the manufacturer.

Labjack Corporation (Lakewood, CO) is a major supplier of driver equipment that is used widely in the engineering world. Their model U12 could be implemented in the micromanipulator design. If the motors are operated in full step mode, 8 bits of output would be sufficient. The Labjack U12 supplies 12 bits of data transfer, and is affordable, with an overall cost of $118. It uses a USB interface and can be controlled using Labview, C, Matlab and other programming languages. If microstep driving is desired, this model will not be adequate.

A cheaper model that is currently available is the Ocean Controls Stepper Motor Interface Card, which can be assembled by the purchaser or bought in its constructed form. There is a price difference of approximately $10 ($50-60), so due to the added convenience of the assembled version, it would be the preferred choice. The KT5158 Bipolar Stepper Motor Driver is another option from Ocean Controls. This model has a simple programming language that can easily be interfaced with other programs (e.g. Matlab, C, Labview, etc.). Again, microstep driving would not be possible with this hardware.

For a microdriving solution, external drivers can be used, but Advance Microsystems Inc. provides digitally interfaced microdriving hardware. They offer resolutions of $1/8^{th}$ of a step and can be controlled using standard programming languages. Their model DR-308

allows for 1/8th step sizes, which would be ideal for the necessary application. This model would cost approximately $250.

If a commercial stage is purchased from Prior Scientific, the hardware necessary must be purchased from them and is called the High Speed Stepper Motor Controller. This commercial set up allows for a simple interface between stage and drivers and allows for iterations of .01 µm, well beyond the constraints necessary.

Fig. 4 - Pixelink PL-A662

Camera:

The client requires a digital camera to replace the inefficient analog camera which is currently implemented and has been used in past studies. The camera will be used for the systemic scanning of the entire Petri dish to make a composite image. Once the composite image is formed a virtual grid will be placed on the entire image, and in conjunction with image fusion software, locate the exact position and orientation of each individual zebrafish. This information will be used to supplement the irradiation process and allow for post-irradiation still frames to be taken. The client expressed concerns with regards to their current analog camera. It is possible to convert analog video signals to digital, but inefficient, due to cost, reinvestment in old supplies, poor resolution and overall necessity for maintenance. A more efficient process is to acquire a capable digital camera. The goal is to find the cheapest and highest quality camera available, which is

Fig. 5 - PAXCam EDU

able to perform the necessary image capture with minimal error. To connect a digital camera to a microscope three possible solutions exist: high quality microscope cameras, low quality microscope cameras, and use of a consumer camera in conjunction with custom lenses and adapters.

High quality microscope cameras are the best option, but are inherently the most expensive. The two companies which are under investigation are Pixelink (Ottawa, ON) and PAXcam (Chicago, Illinois). The Pixelink PL-A662 (Fig. 4) camera has a maximum resolution of 1.3 mega pixels and comes in either color or mono-chromatic. The camera cost is $1,500 and $1,400 for the color and mono-chromatic, respectively. The frame rate is 60 frames per second (fps) at 640x480 pixels and 12.7 fps at 1280x1024. The PAXcam EDU camera (Fig. 5) can capture 1.3 mega pixel images and is only available in color. The frame rate of the PAXcam camera is 60 fps at 640x480 pixels and 15 fps at 1280x1024. The PAXcam EDU camera also includes the Image Analysis Software PAXit. Pertaining to the image fusion, the most important features of the software are data processing and image overlays. In essence the PAXcam camera and Pixelink camera are essentially identical except for the added software which is included with the PAXcam camera. The major problem involved with the use of these cameras is their price. Since these are the highest quality option, both cameras will allow for optimal data collection and would contribute to the overall quality of the proposed study.

Lower quality camera microscopes are another option which should be considered. Lower quality microscope cameras are cameras which have been modified for use in a microscope. The cameras are not specifically created for microscope use, but have converted to be used as such. One option for this camera type is manufactured by Microscope Depot (Tracy, CA). The reviewed camera is the model S-05165 (Fig. 6), which features a resolution of 1.3 mega pixels and a frame rate of 30 fps at 640x480 pixels and 10-20 fps at 1280x1024. The cost of this model is $400. The benefits of this camera include the price, which is considerably lower than that of the higher-end models. The drawbacks of this camera are the lower performance and lower quality which results from deficient testing conditions and features for the camera because the original purpose was not for use in a scientific setting.

Fig. 6 - Microscope Depot S-05165

The final option to be considered for imaging is a consumer camera. Nearly all consumer cameras can be connected to a suitable adapter for use in any microscope. Most adapters are approximately $350. This option includes a variety of major concerns. The first of which is that the client does not already own a digital camera, so included in the total cost of this option would be the cost of a suitable digital camera. Also, one of the major concerns is testing, which occurs for these cameras in well lit conditions, but under the intense light of a microscope the lighting conditions may radically downgrade the functionality of the camera. Another problem is the inability of one to replace the

primary lens of the digital camera, thus the quality of the microscope may be degraded because of the inadequacy of the standard lens. These problems can be alleviated by purchasing additional lenses that can be integrated with commercial cameras, a common solution for these issues.

Image Analysis:

The ultimate function of the scanning system will be the scanning of the Petri dish by the computer and locating the zebrafish embryos. A typical image of the organisms in the dish is shown in Fig. 7a . This is representative of a larger population, which may include approximately 200 fish in the 3x3 cm dish. The image is input to Matlab with the *imread* command. By using the *fft2* command it is possible to take the 2-dimensional Fast Fourier Transform (FFT). The *fftshift* command brings the DC component into the center of the image and a more representative picture of the transform is created (Fig. 7b). The spatial edges in this figure correspond to perpendicular edges in the Fourier Domain.

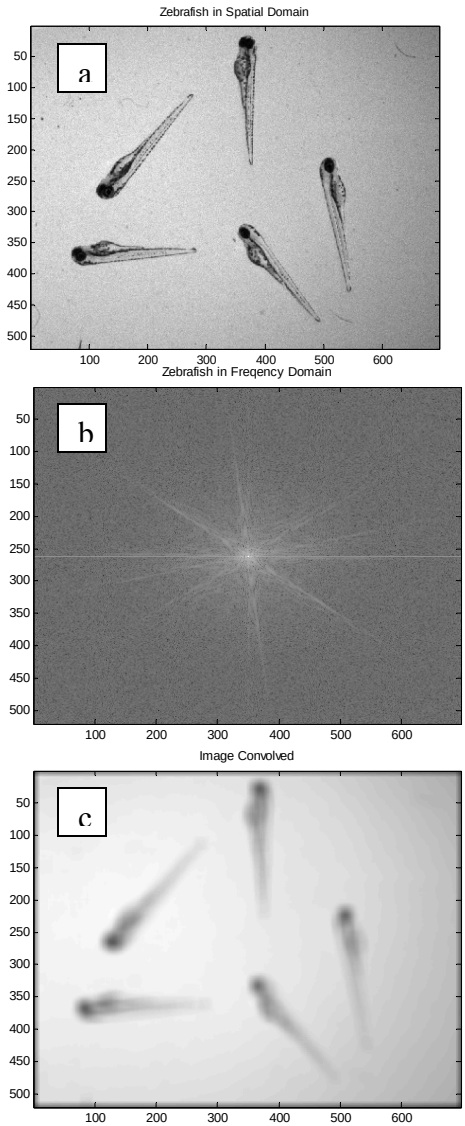

Fig. 7 – a. Sample of zebrafish in Petri dish environment. b. Fourier Domain representation of zebrafish sample. c. Zebrafish convolved with filter.

This will be the first step in the image analysis portion of the project. This, however, does not identify the locations of the zebrafish embryos. A general sense of

individual orientation has been gathered, but this can not be communicated to the fused micromanipulator system. The next step in the process is to convolve the image with a basic filter. This convolved image is shown in Fig. 7c. This removes a considerable amount of noise in the background of the image, while maintaining the position and orientation of the fish. The eye of the fish is a defining characteristic that may be used in the future to more precisely localize each fish. The potential possibilities of furthering the filter design are discussed in the Future Work section.

Evaluations of Designs:

The first part of the system which requires analysis is the microscope stage. The two options to be considered are a commercial stage and a customized micro-machined stage. The commercial stage would guarantee a sufficient end product, but costs would be substantially higher than that of a custom design. The large cost of buying a whole system is the impetus for the current project. It is proposed that a cost effective alternative be designed and constructed for use in the client's research. By using a custom micromanipulator, the benefits of the commercial micromanipulator systems are sacrificed, but customization should allow for the most cost effective and directly applicable solution to the problem. To create a functional prototype, a detailed design of the stage would have to be created, which could be given to a machinist for fabrication. Micromachining is expensive, and would most likely be the most costly and time-consuming phase of the prototype production.

The next part which needs to be analyzed is the stepper motors. Of the steppers which were analyzed the different types need to be further discussed to determine the best possible fit for the movement of the stage. The two basic specifications which need to be taken into account are degrees per step and the holding torque. The degree per step statistic is crucial because this determines the accuracy which the steppers will achieve. The smaller the degree of the steppers the higher the precision will be. This process can be manipulated by using different driving hardware and stepping modalities. The holding torque is critical because enough torque needs to be generated to cause linear stage translation. In general, there is not a significant difference in the cost of the steppers for this to be of concern in determining which stepper should be selected.

The computer interface comes with decisions which need to be made, but the answers result directly from the decisions made for the previous portions. There are two choices for the design which will ultimately determine which driving hardware will be required: commercial versus custom. If the commercial stage is selected, options are limited, so a final decision will be relatively easy. The second option would be a computer interface to control our chosen stepper motors. Two areas which would need to be considered would be the number of bits of information necessary to drive the two motors (dependent on desired stepping modality) and the output specs. The stepping modality chosen (i.e. full step, half step or micro-step) will ultimately decide what type of driver will be purchased. If a half step mode is used, 8 bits would be required for the computer to control the stepper motor. The Labjack model allows for 12 bits of data transfer which would be sufficient. This connects to a computer through the USB connection. The

Oceans Controls interface uses a serial port and should also be sufficient for half step modality. The final option is the AMS model, which could ideally allow for precision of up to $1/256^{th}$ of a step, surpassing the necessary requirements set by the client.

The final component which needs to be reviewed is the camera choice. There are three basic options for a microscope camera: professional quality microscope cameras, lower quality microscope cameras, and consumer quality cameras paired with microscope adapters and lenses. The professional quality cameras provide the best possible results and also provide software which will help in image composition. These cameras include the PAXcam and Pixelink. The cost of these cameras is substantially higher than the competition, ranging from $1200-$1500. The lower quality microscope cameras function essentially the same as the above camera, but were not originally intended to be used for microscope application, so the cameras have not demonstrated their qualities through rigorous product testing, which the higher quality cameras have been shown to fulfill. These cameras also do not include additional software which will aid in image capture and analysis. These options are much less expensive and cost between $400 and $900. The final option is the use of a consumer quality digital camera, which would be attached to the microscope using a custom camera adapter. This option has considerable deficits. The first is that the client does not have an adequate digital camera so purchasing one would be necessary, although it has become more common for labs to own digital cameras. In addition to the necessity of purchasing a camera, an adapter must be purchased which would cost $350 minimum. Consumer digital cameras are also documented as having defects which are not noticeable under normal light conditions, but

become apparent under microscope lighting. Also, the primary lens on the camera cannot be removed, so additional costs would be incurred to purchase the required supplementary lenses.

Potential Problems and Resolutions:

The first obstacle that must be dealt with is stage choice. A concrete estimate must be received from the University of Wisconsin machine shop prior to any decisions being made about any other parts. It may be worth the additional cost to buy a commercial operation if there is minimal cost efficiency increase and considerable quality decrease by using a custom micro-machined stage integrated with the aforementioned components.

If a custom design is decided upon, the drivers and steppers must be coalesced to create a functioning digitally interfaced micromanipulator stage. Most stepper motor drivers can be connected to a USB 2.0 port, which makes them nearly universally compatible with most PCs. The steppers must be set up in such a way that there is sufficient communication between the steppers and the computer, in order to record relevant coordinate data. This iterates that a coordinate system must be established, another task that must be accomplished for prototype fruition.

The camera chosen for the project must be able to clamp onto the microscope to function correctly. The cameras researched all come with kits for microscope mounting, but if these do not function adequately, custom solutions will be necessary for successful integration into the system.

The fish are anesthetized while being scanned, but the possibility of the anesthesia wearing off during the scanning process must be considered. If this were to occur, it would be expected that minimal movements would occur because they would be coming out of anesthetized state. Our filter will not be able to handle moving objects, but the blur from the image should still be able to be seen and its location determined. It is left to the client to decide whether such trials should be discarded, or if another means for coping with such circumstances should be considered (e.g. manual inspection of blurred fish or entire sample). Time and cost considerations will be important when making this decision.

Compatibility between all of the parts of the microscope system is going to be the most difficult portion in prototype construction. All of the disparate components will need to be interfaced using standard and custom connections when necessary. There needs to be cross-talk between all facets of the design to ensure satisfactory functionality. In addition, the software that will be used for frame grabbing will need to be able to stitch the larger image together, but this needs to be imported and read in Matlab; therefore, it must be in a format that is compatible with Matlab. Matlab is compatible with many different image files (.gif, .jpg, .bmp, .hdf, and more), and is compatible with the .tif file that was provided by the client. If the final image produced is in a format that is not able to be read in the program, it will have to be converted to a compatible format.

Future Work:

Parts Research:

The project at hand is very complex, and therefore requires an expanse of background knowledge before any portion can be completed. Further research on each part of the design will be necessary to insure compatibility and reliability. Basic tutorials of stepper motors, stage operation, camera specs and image analysis are available to the public and will be used extensively during various phases of the design process.

Image Analysis:

In order to determine and record the exact locations of the zebrafish embryos in the Petri dish, a grid system needs to be applied to the image. This grid system must correspond to the correct number of pixels on the screen that show the locations of the fish. Once a grid system is in place, the program must be able to communicate the position of the fish to the table which will then be accurately moved underneath the scope. A possible safeguard process could be implemented to determine that the post-radiation image acquired is consistent with the original scan.

Another challenge in the image analysis portion will be devising a more advanced filter for the image. A proposed filter would be approximately 25 x 25 pixels, and focus on the eye as the defining characteristic of each of the fish. The filter will need to be convolved across the image in all rotational and translational possibilities. The drawback to this process is that it will be computationally intensive and, in effect, be time consuming.

Integration:

Due to the multifarious nature of this project, it will be necessary to check the compatibility of each part with all others. Each part has specific inherent requirements, which will cause various compatibility issues to arise. To avoid extra cost because of unnecessary parts, all connections will be thoroughly researched and confirmed prior to any purchases. The fusion of all required components will be an ongoing process and problems will arise. All difficulties of integration cannot be predicted at this time, but will be dealt with on a rolling basis. The design will become exponentially more complex as more portions are fused to create the final prototype, which translates to becoming more difficult to resolve.

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