Cryogenic Optical Microscope (Phase III)

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Abstract
In phase III of this project, the goal is to develop a completed design that will further increase the working time as well as integrate the optics of the custom microscope developed at Harvard Medical School. The design must keep the sample under -140°C for at least one hour. Thermal analysis showed that simply changing materials of the system would not extend the viewing time to the desired duration. However, analysis showed that improving the nitrogen flush system substantially increases the imaging time. There were two main changes that were made to the system: the upper chamber was modified to make it a less massive insulating component and the nitrogen flush will now be used as an active re-cooling apparatus rather than the original passive method. As before, the nitrogen flush is used to keep the sample and objective dry from moisture.
The Need for Project

A cost efficient method to keep biological samples under -140°C to further study viruses such as Polio using TEM and OM microscopes.

Currently the scientists at Harvard Medical School are trying to investigate the Polio virus. A custom optics system was developed to achieve this goal. However, it was unable to keep the Polio sample at a temperature less than -140°C for long enough to get satisfactory images. The scientists require approximately 45 minutes to obtain sufficient images of the sample. Creating a system that would hold the sample under -140°C for over an hour would give the scientists a cost efficient method to investigate the Polio virus. This system will be beneficial to not only the study of the Polio virus, but increasing the easy at which other viruses are studied.

The Design Project Objectives and Requirements

The objective of this project is to extend the working time of an optical microscope operating at cryogenic temperatures while minimizing vibrations. Design Objectives

The main objective of this project is to design and construct an optical microscope that operates at cryogenic temperatures in order to maintain the integrity of the sample being viewed, which is encased in vitreous ice. As with any microscope, vibrations in the system have an adverse affect on the image seen. The design must maintain the biological sample at cryogenic temperatures of below -140°C while keeping the area moisture free. Any moisture entering the system will cause the formation of cubic ice which can destroy the sample and distort the microscope’s images.

Design Requirements

Requirements for the design include: eliminating vibrations, creating a moisture free environment and maintaining the biological sample at cryogenic temperatures for 45 minutes to achieve satisfactory images without degrading the integrity of the sample.
Design Concepts considered

The Hilsch tube, Nitrogen Jet, and LN₂ delivery system were considered and parts of each were implemented in our final design concept.

Three initial design concepts were researched and considered based on the recommendations of Phase II and our interpretation of the project. All designs are based on the concept of re-cooling the system using nitrogen gas to maintain cryogenic temperature and a moisture free environment.

Hilsch Tube

The Hilsch tube takes a stream of gas and separates the high energy molecules from the low energy molecules. The performance of the Hilsch tube depends on the input pressure, and the cold fraction which is a parameter that is specific to the device. The cold fraction is defined as the mass flow rate of the cold output divided by the mass flow rate of the compressed air input. These devices are most commonly used for spot cooling in a machine shop environment with compressed air as the working fluid. The following concerns had not been previously addressed: Nitrogen gas will be used as the working fluid because it is readily available, and compressed air is not cold enough to reach the desired temperature of -140 °C. All of the nitrogen must be adequately vented to prevent any health problems for the operators. Another concern is the output flow rate; having too large of a flow rate will cause vibrations to the sample.

Nitrogen Jet

Nitrogen gas will go into an insulated coil and will be dispersed onto and above the sample area. The stream moving above the sample area will reduce the thermal gradient of the objective moving to within working distance of the sample. The coil will wrap around the cryogenic chamber through its existing insulation. At the top of the cryogenic chamber there will be holes in the coil that disperse the nitrogen gas to the sample and to the area above the sample. The coil going through the insulation will keep the nitrogen below -140 °C and absorb vibration from the massive flow rate through the coil. This will allow the sample to be spot cooled between imaging.
**LN\textsubscript{2} Delivery System**

This design concept focuses on maintaining the liquid nitrogen level surrounding the copper slug and using evaporative cooling to keep the sample area dry and re-cool the system. Currently, liquid nitrogen surrounds a copper slug under the biological sample in the bottom chamber to help keep it cold. Once imaging has begun it is not possible to refill the liquid nitrogen once it has evaporated away. This design will allow for a constant level of liquid nitrogen in the bottom chamber which will keep the copper slug at a lower temperature for a longer period of time.

**Recommended Design Concept**

The recommended design is an object that holds and maintains medical samples under -140°C using the evaporation of LN\textsubscript{2}. The ability to add LN\textsubscript{2} to the system allows for the user to extend their imaging time of the sample.

COSMOSWorks was used to determine which design concept would extend the present viewing time best. It was decided to use the LN\textsubscript{2} delivery system. This design would allow for an extended operation of the system and allow for the ability to re-cool the system after imaging has begun.

Upon deciding on the LN\textsubscript{2} delivery system, several adjustments were made to the design. Having an external reservoir attached to the bottom chamber became impractical due to the movement of the bottom chamber to scan the sample grid. To eliminate this problem, the reservoir was made smaller and placed on the base plate directly in front of the bottom chamber. This reduced the initial volume of liquid nitrogen in the reservoir but since this design allows for the reservoir to be re-filled easily, this is not a concern.

There were also changes made to the existing bottom chamber. The volume of the copper slug was decreased and the nanogel insulation was removed from the PTFE (Teflon\textsuperscript{©}) dewar to increase the amount of liquid nitrogen available in the bottom chamber. The removal of the insulation will assist in the evaporation of the nitrogen. In initial testing, it was advised to place a heater in the bottom chamber to assist in the boil off of the liquid nitrogen. A resistive heater was placed into the bottom chamber and was used to increase the flow of nitrogen gas to the sample area.
The top chamber was changed to a Teflon shroud with a stainless steel insert. The stainless steel insert will contain the objective and act as a thermal mass to the system. This will work in conjunction with the copper slug creating radiative surfaces to keep the sample cold.

Finally, the COSMOSWorks transient thermal analysis revealed that the recommended design will have a theoretical working time of approximately 2.5 hours while staying below -140 °C. Testing will take place in the next few weeks to find the experimental working time of the redesigned dewar. The main advantage to this concept is its ability to re-cool the system. Thus, creating a system where the operator can run the microscope as long as needed without removing the biological sample.

**Financial Issues**

Machining costs of components and the cost of materials that would withstand cryogenic temperatures were the main concerns in the design.

The cost of a motorized translation stage is too large, so the operator must use a manual translation stage.

There are some materials such as Kevlar that have a better thermal conductivity, but the material and machining costs were far too high.

The cryo-probe, which the design is built around, costs around $30,000 and has not yet been made available to us.

**Recommended Improvements**

Creating a system that can be completely controlled from one console would allow for ease of use of the microscope.

It would greatly help the user to have everything integrated on one single base of control. A motorized x-y translation stage for the assembly, and a control based liquid nitrogen delivery system would allow the user to remain in one place. Currently, the user would have to move around the system or have a second person present for assistance.