Microscope Enclosure for Temperature Regulation and Light Isolation

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Featured Application: Here we present the design of a temperature regulation and light isolation microscope enclosure. This design can be readily adapted to the specific configurations of a custom imaging system.

Abstract: Light isolation and temperature regulation are often required for microscopic imaging. Commercial enclosures are available to satisfy these requirements, but they are often not flexible to the variety of custom systems found in research laboratories. We present the design for an affordable enclosure which utilizes aluminum t-slot profiles to support opaque expanded PVC panels. Temperature is regulated by exchanging the enclosure air with an external heater. In addition, we demonstrate baffles integrated into the enclosure improve temperature uniformity. Example designs for both upright and inverted microscopes are given, providing a starting point for creating a system-specific custom enclosure.

Keywords: microscopy; fluorescence microscopy; enclosure; incubator; environmental chamber; temperature regulation

1. Introduction

Fluorescence imaging of biological systems often requires an environment in which the temperature of the sample is regulated. For example, live cell microscopy typically involves imaging at the natural temperature of the biological system [1,2]. Alternatively, sometimes it is advantageous to image biochemical processes at non-natural temperatures, or to vary the temperature to affect reaction kinetics [3–7]. Three methods are typically utilized for controlling sample temperature during imaging: (1) whole room regulation, (2) whole image system regulation, and (3) local sample regulation.

For imaging at room temperature, the microscope’s laboratory room conditions are sometimes sufficient for regulating the sample temperature. To image above or below room temperature, the temperature of the entire room can be increased or decreased using the building’s HVAC system or the entire microscope can be placed in a lab-specific hot or cold room. A potential advantage with whole-room regulation is that the entire microscope, table, and image sample are all at approximately the same temperature. In theory, this situation minimizes temperature variations in the microscope which could affect sample temperature or optomechanics stability [8–10]. However, in practice, depending on the quality of the building’s HVAC equipment, laboratory air temperature may vary with spatial position and time. HVAC equipment may also produce air drafts. Together, these conditions can result in unpredictable sample temperature and image instability, including lateral and axial (focal) drift [8,9,11]. Without proper compensation, this drift compromises the ability to acquire time-lapse images.
To isolate the system from the room conditions, it is advantageous to enclose the microscope in a box. The temperature within the box can then be regulated independently of the room by heating or cooling the air within the enclosure [12]. This mimics whole-room control where the goal is to have the entire system and sample at the same temperature. However, it is typically easier to create a more consistent temperature for the imaging system in a smaller space that is isolated from the building HVAC. In addition, it often is not practical to cool or heat an entire room. Finally, even when not heated or a cooled, a whole-system enclosure provides protection from drafts in a laboratory.

Another method to regulate sample temperature is locally, directly at the position of the sample. This can be achieved with devices such as resistive heaters, Peltier coolers, or water heater/cooler jackets attached to the sample holder or the objective [10]. Local temperature control enables quick equilibration and temperature changes, which can be useful for imaging at a series of different temperatures in a relatively short period of time. A disadvantage is the potential for poorly controlled temperature cycling resulting in image instability and inconsistent sample temperature [10]. In practice, and depending on the imaging requirements, a combination of whole room, whole system, and local sample regulation may be used to achieve the best imaging conditions. However, even the best combination of regulation systems may still require some type of active or post-imaging drift compensation [9,11,13–19].

During biological imaging, it is also often necessary to isolate light entering and exiting the imaging system. Eliminating external light is essential for most measurements of luminescence. Even for fluorescence microscopy, an acceptable signal-to-noise requires lowering the background light by isolating the imaging system from external light sources, including room lights and ancillary lab equipment [10,20]. This situation also minimizes undesirable fluorescence excitation and premature bleaching. Although it may be possible to reduce external sources, such as turning off room lights, this is not always practical, possible, or sufficient. An enclosure for the whole system has an advantage in that it can isolate laboratory lights from the sample and microscope optics. In addition, a whole-system enclosure can also provide a level of safety to laboratory occupants by isolating bright illumination sources, including UV-rich arc lamps utilized for conventional fluorescence microscopy and lasers used in specialized techniques such as confocal and multi-photon laser scanning, STED, TIRF, STORM/PALM, and SIM [13,14,21–25].

Microscope enclosures are available from commercial suppliers. However, a commercially designed and manufactured enclosure for a custom imaging system is often expensive. Some commercial systems also do not provide light isolation capability. To satisfy custom imaging systems, many research labs construct homemade enclosures out of various materials such as wood board, foam, plexiglass, polycarbonate, and PVC. The quality of these enclosures varies, with some suffering from light and air leaks, temperature gradients, and poor accessibility. From experience constructing multiple generations of enclosures, we have found that user-friendly and stable enclosures can be easily designed and constructed from lightweight expanded PVC board held by lightweight aluminum t-slot profiles mounted securely to an optics breadboard. These materials create a stable enclosure, which also does not significantly raise the system’s center of mass, which can cause vibrational instabilities with a floating optics table.

Considering the necessity of customization, we developed an enclosure platform that can be adapted to a variety of imaging systems. The design consists of vertical aluminum t-slot profiles which securely support black expanded PVC panels. These vertical posts create a stable structure for holding the panels, while also securely sealing the panels to create a dark interior. The vertical posts also enable easy access to the inside of the enclosure because the panels can readily slide along the posts. In addition, air baffles are incorporated to distribute air throughout the enclosure, producing a more uniform temperature when compared to an enclosure without baffles.
2. Design and Methods

2.1. Design Overview

The enclosure design consists of aluminum t-slot profiles holding 6 mm thick black expanded PVC boards (80/20 Inc., Columbia City, IN, USA). These boards form the sides and roof panels of the enclosure (see Figure 1, Supplementary Figures S1–S3 assembly diagrams and photos). T-slot profiles are available from multiple manufacturers and can be custom cut to the desired length. The expanded PVC panels can also be purchased from multiple manufacturers and can be hand cut with an electric saw, though ours were CNC-machined from technical drawings provided to the panel supplier (80/20 Inc. through distributor Knotts Company, Berkeley Heights, NJ, USA). The total cost of t-slot profiles and custom cut panels was approximately USD 1000. The basic structure consists of four vertical corner posts threaded into an optics breadboard holding the microscope (1020 and 1010 with threaded 1/4”-20 end holes, 80/20 Inc.). Alternatively, the vertical posts can be mounted to a t-slot profile base frame if the microscope is not sitting on an optics breadboard. The vertical posts hold four side panels in their t-slot channels, and these channels also allow the panels to slide up and down to provide large clearance access to the interior of the enclosure. The roof panel is supported and attached to the tops of each of the vertical post via 1/4”-20 screws. Incorporating air distribution baffles, ocular viewing access, focus knob access, and camera ports makes the design more nuanced than this basic description. Below, these various accommodations are discussed.

![Figure 1](URL)

**Figure 1.** Inverted and upright enclosures. Inverted microscope enclosure designed around an (a) IX-81 frame (Olympus Scientific Solutions America, Waltham, MA 02453 USA) and an (b) Olympus IX-83 frame. (c) Upright microscope enclosure designed around a Thorlabs Cerna system (Thorlabs Inc., Newton, NJ, USA).

2.2. Baffles

Input and output air distribution baffles were incorporated on the left and right sides of the enclosure (Figure 1, Supplementary Figures S1–S3). These baffles distribute air uniformly throughout the enclosure (see Section 3). Each baffle has an inside and outside panel, with the outside panel having a large hole for connecting an air hose to an external heater/cooler (Supplementary Figures S1 panels 2 and 9, S2 panels 6 and 9, S3 panels 3 and 4) and an inside panel with an array of small holes to distribute/collect air within the enclosure (Supplementary Figures S1 panels 1 and 8, S2 panels 7 and 8, S3 panels 1 and 2). The total area of the small holes was set similar to the area of the external heater/cooler air duct. The two panels are held together by a single vertical post with two t-slot channels separated by 1” (1020, 80/20 Inc.). This creates a baffle space for supplying and returning conditioned air. Due to the multiple side panels and connected air ducts, it is impractical for these panels to slide and therefore they are capped by the roof.
2.3. Oculars

The oculars need to be accessible from outside the enclosure to enable direct viewing of the sample. On an inverted microscope, the oculars typically exit the microscope from the front of the frame, which we accommodated for by creating an upside-down U-shaped cutout in the front panel (Supplementary Figures S1 panel 15, S2 panel 4). This cutout slides over the front of the microscope, placing the ocular assembly outside of the enclosure. The design of the cutout varies with microscope type (Figure 1a, Olympus IX81; Figure 1b, Olympus IX83).

On an upright microscope, the oculars are typically on the top of the system. On our upright Thorlabs Cerna microscope, the oculars and camera are housed in a trinocular assembly (LV-TI3, Nikon Instruments Inc., Melville, NY, USA) on top of the system (see Figure 1c). To enable access to the eyepieces, we created a hole in the roof panel which fits the bottom shape of the trinocular assembly, sealing the enclosure around its base (Supplementary Figure S3 panel 9). This hole is positioned near the front of the microscope so that the oculars reach over the front of the enclosure to provide unobstructed viewing access. However, having the oculars positioned over the enclosure’s top front edge prevents vertical sliding of the front panel. Instead, internal access is provided through a side sliding panel which fits into the channels of an upper and lower horizontal t-slot rail (Supplementary Figure S3 assembly diagram and panel 11). These rails are attached to the vertical posts with screws and t-nut fasters (3059 and 3382, 80/20 Inc.), with perpendicular access holes drilled in the horizontal rails to enable screw tightening. The left side of our upright enclosure contains the illumination optics, which are accessible with a second vertical sliding front panel (Supplementary Figure S3 panel 8). The horizontal rails continue in front of this panel to provide a track for the horizontal sliding panel (Figure 1c).

2.4. Focus Knobs

Focus knobs on an Olympus IX-81, and most microscopes, are located at front bottom of the frame. Access to these knobs is through pockets created below the front panel (Figure 1a, Supplementary Figure S1 assembly diagram). These pockets are on each side of the microscope and consist of two panels glued together at a right angle with cyanoacrylate (All Purpose Krazy Glue, Newell Brands, Atlanta, GA, USA). Each pocket is supported on its top front by a horizontal t-slot profile (1010, 80/20 Inc.) attached to the front vertical posts with a corner mounting plate (4150, 80/20 Inc.). These horizontal t-slot profiles also support the bottom of the main front sliding panel. The bottom back of each pocket is fixed in position to the optics table with table clamps (CL5, Thorlabs). On the inverted Olympus IX-83 frame (Figure 1b) and the upright Thorlabs Cerna (Figure 1c), the focus knobs are controlled by an external focus assembly which sits outside of the respective enclosure, and thus, the pockets were not included. Focus knob pockets for an upright microscope with knobs on the frame can be added by relocating the lower horizontal sliding rail to a position above the optics breadboard. This horizontal rail could be supported by vertical t-slot profiles. Then, pockets could be created under the horizontal rail, providing access to the knobs which are typically located near the bottom front of the frame.

2.5. Cameras

The camera(s) on an inverted microscope typically exit on the lower left and/or right side of the microscope frame. The camera can be placed inside or outside the enclosure, though the camera should be placed outside if the enclosure temperature exceeds the camera’s temperature range. Figure 1a and Supplementary Figure S1 assembly diagrams illustrate a pocket within the right-side baffles for a right-side mounted camera which is external to the enclosure. Horizontal t-slot profiles (1010 and 1020, 80/20 Inc.) are attached to vertical posts via corner mounting plates (4150, 80/20 Inc.). This design can be replicated or swapped for a left-side port. A design with more space, like for an image splitter between a camera and microscope, consists of a full opening under the front panel (left side of Figure 1b, Supplementary Figure S2 assembly diagram). In this case, horizontal
t-slot profiles are held with mounting plates and inside mounting brackets (4150 and 4115, 80/20 Inc.), which then hold expanded PVC cutout panels. On the upright system, the camera sits on the top of the trinocular assembly (Figure 1c), which sits above the enclosure (see Section 2.3).

2.6. Illumination

Illumination sources can be inside or outside the enclosure, though a hot arc lamp inside the enclosure may have insufficient ventilation and could disrupt the enclosure temperature stability. One approach is to put a hole in the back panel for an external laser beam to enter the enclosure (Supplementary Figure S1 assembly diagram and panel 12). Alternatively, a slot can be made for a liquid light guide from an external arc lamp (Supplementary Figure S2 assembly diagram and panel 2). The additional panel on the back is fixed and sits under the liquid light guide, whereas the upper panel slides up and down over the light guide. This allows the light guide to remain fixed to the microscope while the back of the microscope can still be accessed via the top sliding panel. If an arc lamp is utilized, a similar fixed lower panel could be constructed which fits around the mounting tube of the illuminator, while a sliding upper panel still provides rear access to the microscope. In an upright microscope, rear horizontal sliding panels could be used to accommodate an arc lamp, which is typically located near the top back of the system.

2.7. Access Doors

In addition to sliding panel access to the enclosures, smaller doors are incorporated in the front panels of all the designs for accessing just the sample (Figure 1, Supplementary Figures S1–S3 assembly diagrams). These doors allow access to the sample while minimizing significant exchange of conditioned air. The doors are attached to the front panel via metal piano hinges (15665A901, McMaster-Carr, Elmhurst, IL, USA), which are attached to the panels with screws and nuts. To hold the doors securely closed, thin neodymium disk magnets (5862K79, McMaster-Carr) are in slots carved into the opposing edges of each door panel. Small handles consisting of screws threaded directly into the door panels make it possible to easily open the doors.

2.8. CAD Design

The upright and inverted microscope enclosures were all designed in FreeCAD version 0.19, a free and open-source computer-aided design program. In FreeCAD, panels were 2D drawn using Sketcher workbench, 3D extruded into 6 mm thick solids in Part workbench, and then assembled using the A2plus plugin. Panel technical drawings are given in Supplementary Figures S1–S3, and the FreeCAD design files are available on a file repository (https://github.com/JohnsonLab-Hofstra/MicroscopeEnclosure, accessed on 22 July 2021). These can be a starting point for creating a custom enclosure. (Note: our optics breadboards have imperial unit hole spacing (1") and therefore we used imperial unit t-slot profiles (1") and designed the panel sizes around these units. We recommend using metric profiles and metric panel cuts if a metric table is used.)

2.9. Temperature Regulation

The temperatures in the enclosures were regulated with an external heater (AirTherm or AirTherm SMT, World Precisions Instruments, Sarasota, FL, USA). Our heaters were approximately USD 3000. However, other options such as a cooler or humidity regulation machine could also be incorporated. In our systems, each heater has supply and return air hoses which mount to the external baffle holes. In addition, each heater also has a temperature feedback sensor which we placed in the enclosure near the sample.

2.10. Temperature Measurements

Temperature measurements were acquired with type T thermocouples (5SRTC-TT-T-24-72, Omega Engineering Inc., Norwalk, CT, USA) attached to a data acquisition module
(TC-08, Omega Engineering Inc.) which was data-logged to a computer. The thermocouples were calibrated at 37 °C. During data collection, temperature samples were collected every second.

3. Results

To assess temperature uniformity, we measured the temperature with and without the baffle panels at eight locations throughout the upright enclosure (Figure 2a). These measurements were conducted without the microscope in the enclosure. Temperature was monitored with the enclosure starting at room temperature (~20 °C) and the heater setpoint at 37 °C (AirTherm SMT, World Precision Instruments). The measured temperature then increased over approximately 300 s, before stabilizing into oscillations (Figure 2b). These oscillations were attributed to heater feedback and could be altered by adjusting feedback parameters (the factory default parameters were used for these measurements). Without the baffle panels, we observed that the average temperature range across the eight probes was 5 °C. This average range was calculated by finding the difference between maximum and minimum probe temperature readings at each time point and then averaging over a time period when the enclosure temperature was near the 37 °C setpoint (500 s to 1000 s). We also observed that the highest temperature probe readings were near the air supply at the bottom of the enclosure (Figure 2b).

When the input and output baffle panels were added, the average temperature range across the probes was also 5 °C (Figure 2c). Additionally, the baffles altered the temperature landscape, with higher temperatures occurring closer to the top of the enclosure. We attribute this difference to (1) the baffles distributing the air more uniformly across the entire height of the enclosure, and to (2) warm air rising within the box. The confluence of these together resulted in the top of the box being warmer than the bottom. In addition, the baffles also damped out the responsiveness of the temperature feedback, increasing the length of time for the temperature to initially equilibrate. The magnitude of the feedback oscillations also decreased. Specifically, for the enclosure without the baffles (Figure 2b), the average standard deviation of the eight probes was 0.5 °C, while the average standard deviation with the baffle panels (Figure 2c) was 0.3 °C.

To account for the rising warm air in the enclosure, we closed the top half of the supply air baffle panel holes with tape. This resulted in a significant decrease in the average temperature range, with a new range of 2 °C between the probes (Figure 2d). Biasing the incoming air towards the bottom appeared to compensate for rising hot air. The average temperature standard deviation with respect to time also decreased to 0.2 °C. A measurement of temperature variation over a long duration, 48 h, was also acquired (Figure 2e). Temperature measurements were acquired every minute and the average temperature standard deviation of the eight probes was 0.1 °C. This measurement indicates the enclosure is stable for long time-lapse measurements.

Next, we placed eight temperature probes at different locations in an enclosure with a microscope (Figure 3a). Like the previous experiment, we monitored the probes as the temperature increased from room temperature to 37 °C. In addition, the heater’s temperature sensor was attached directly to the objective, as opposed to being in the air. In this experiment, we observed some of the probes went significantly beyond 37 °C, particularly those located on the air supply side (right side) of the enclosure or top of the enclosure. The probe mounted directly to the objective did not significantly overshoot the setpoint temperature. (Note: In this experiment, we used a less powerful, older generation heater (AirTherm, World Precision Instruments) and the enclosure volume was about twice the volume of the upright enclosure. Therefore, the equilibration period was significantly longer.)
Figure 2. Temperature in the upright enclosure with and without baffle panels. (a) Thermocouples were placed at 8 locations in the enclosure. Probes 2, 4, 6, and 8 were 15 cm above the table, while probes 1, 3, 5, and 7 were 30 cm above the table. Probes 1, 2, 5, 6, 7, and 8 were 17 cm from front of the box, while probes 3 and 4 were 38 cm from the front. The heater feedback temperature sensor was positioned at the same location as probe 2. No microscope was in the enclosure during the measurements. (b) Temperature versus time for the enclosure without the air distribution baffle panels. The enclosure was initially at room temperature, and monitoring began after the heater setpoint was set to 37 °C. (c) Temperature versus time for the enclosure with the air distribution baffle panels. (d) Temperature versus time for the enclosure with upper half of air supply baffle holes closed (top 6 rows). (e) Temperature versus time for enclosure over 48 h with the same baffle configuration as Figure 2d.
Enclosure temperature with the heater temperature sensor attached directly to the objective of a microscope. (a) Thermocouples were placed at 8 locations in an inverted microscope enclosure (same as Figure 1a). Probe 1 was attached, along with the heater feedback probe, directly to metal housing of the objective. All the other probes were in the air. Probes 2 and 6 were on the left side of the microscope (5 cm from the outgoing return air baffle panel), probes 3, 4, and 5 were on the right side of the microscope (10 cm from the incoming supply air baffle panel), and probes 1, 7, and 8 were in the center. Probes 1, 3, 5, 6, and 7 were all at a similar height from the table as the objective (30 cm), while probes 2 and 4 were closer to the table (15 cm), and probe 8 was the farther from the table (60 cm). Probes 1, 2, and 5 were 20 cm from the front of the enclosure, while probes 3, 5, and 6 were about 40 cm, and probes 7 and 8 were 65 cm (near the back). (Note: In this figure, the microscope stage, brightfield illumination optics, and top and front panels are not illustrated.) (b) Temperature readings were collected after the setpoint of the heater was changed to 37 °C. The probes closer to the incoming supply air baffle panel experienced higher temperatures than those shielded by the microscope. In addition, while equilibrating, the air temperature went significantly above 37 °C.

4. Discussion

Incorporating air distribution baffles improved the temperature uniformity throughout the enclosure. However, there are some design considerations if air baffles are included. First, with side mounted air baffles, warmer air will rise within the enclosure (Figure 2c), which may create temperature gradients in the system. This could result in unexpected instabilities in the optics. To improve the temperature gradient, we biased the incoming supply air towards the bottom of the supply baffle panel (Figure 2d). This scheme is similar to the typical air duct configuration in a cold-climate home, with the supply vents near the floor. After adjusting the baffles, the temperature varied approximately 2 °C between probes and the time variability of individual probes was approximately 0.2 °C. This time-dependent variability is dependent on the heater/cooler feedback system. The addition of a local sample temperature regulator may help provide an even more precise sample temperature [10].

We also observed with side airflow baffles that the air on the return side was cooler than on the supply side (Figure 3b). This problem may potentially be reduced by flowing the air from bottom to top vertically through the enclosure, resulting in both sides experiencing similar heated air. However, the frame of the microscope may still shield the sample itself from a vertical air flow, so probe measurements should still be conducted if temperature stability is critical.

Vertical air flow could be achieved by incorporating air baffle panels in the floor and ceiling of the enclosure. Specifically, the supply panel could sit just above the table surface, fitting around the microscope base and any optics mounted to the table, and the return air baffle could be incorporated into the roof of the enclosure. Alternatively, it may be possible to use the optics breadboard itself as the bottom baffles. This would require an internally open optics breadboard, and the ability to close all the external mounting holes. However,
if this option is possible, this design would significantly simplify the supply of air from below the microscope, and still allow mounting holes to be used on the breadboard. Our optics breadboards do not have this option, and we need frequent access to the mounting holes; therefore, we decided to utilize the simpler side baffle design.

Another consideration is placement of the heater’s temperature sensor. When we attached the feedback probe directly to the objective (Figure 3), we discovered the air temperature in the enclosure went significantly above 37 °C, particularly near the supply air baffle (Figure 3b). This is expected because the heater temperature sensor sits on a thermal mass, which requires significant heat to increase its temperature. The objective itself (probe 1 in Figure 3) did not go significantly above 37 °C, but the air temperature went above 37 °C when the heater sensor was below the setpoint. Heating the air significantly above 37 °C may result in unexpected overheating of other optical components, which could have adverse short- and long-term effects on the instrument. Thus, placing the sensor in the air, although slower to heat the microscope, may cause fewer problems.

The microscope and optics table behaving like heat sinks should also be considered. Specifically, depending on how the microscope sits on the table, it may not be possible to raise the microscope temperature to the setpoint because of heat loss through the optics table. Heat transfer can be reduced by minimizing metal contact between the frame and table. For example, the frame could be isolated from the table with small rubber feet. Thermal insulation could also be placed between the microscope and the table, though the microscope may not be as vibrationally stable. The necessity of isolation can be studied for a specific system by placing temperature probes on the microscope and the sample.

Here, we have presented designs for both inverted and upright microscopes. Although there are a wide variety of different shapes and sizes for both commercial and custom imaging systems, our design scheme, with vertical t-slot profiles securing side and roof panels, can be readily adapted to most systems. The main considerations for building a system specific enclosure are the size and location of the (1) focus knobs, (2) oculars, and (3) cameras. In addition, the location of baffle panels, if desired, should also be considered. To account for the location of these components, we recommend first creating a rough 3D CAD model of the microscope, following by designing 2D panels that fit around the microscope. Finally, assemble the 2D panels around the model to ensure everything fits properly before purchasing t-slot profiles and cutting the panels.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11156812/s1, Figure S1: Inverted Enclosure 1 Technical Drawings. Figure S2: Inverted Enclosure 2 Technical Drawings. Figure S3: Upright Enclosure Technical Drawings.

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