I. Laser-Pointer Microheater assembly

Component List (see picture):
(a) Power Supply (HP 6218C Power Supply 0-50V/0-.2A, Hewlett-Packard)
(b) 75mW Red Beam Laser Pointer (Pulsar P75 Wicked laser)
(c) Hexagonal holder for laser pointer (Astro – 1, Beam of Lights Technologies)
(d) (1x) Post Mounted Translator (Thorlabs LM1XY), with focusing lens (Thorlabs LA1805)
(e) (1x) Post Mountable Fiber Clamp 250µm (T711-250 Thorlabs),
(f) 50 µm core Optical Fiber (AFS50/125Y 0.22 -NA 50µm Core Multimode Vis-IR Fiber, Thorlabs)
(10 m spool, use 13 cm lengths with scissors)
(g) (2X) Half inch posts and (2X) post holders (TR1, PH1, Thorlabs)
(h) Translation stage (Thorlabs MS1),
(i) 1/4"-20 kit (e.g. HW-KIT2, Thorlabs),
(j) (1x) Miniature Breadboard (MB8 - Aluminum Breadboard, 8" x 8" x 1/2", ¼-20 Threaded, Thorlabs)
(k) (3x) BA 1 regular bases (Thorlabs). The focal length (the distance between the lens and the tip of the optic fiber) should be 4cm (1.5").
(l) MS-series base plate (MS101)


Connect laser pointer to adjustable power supply and mount on a mounting plate

- Replace the batteries of the 75mW laser with a piece of wood with wires to make contact between the power supply and the electrodes of the laser.

- Connect the wires to the power supply and adjust the voltage to 3V (The power should be off at this time). Anything more than 3V could burn this 75mW laser diode. Keep the current lower than 150 mA, you shouldn’t need more than 70-80 mA to reach 40°C.

- Mount the 75mW laser on its holder base (C,K) and use a clamp to depress the laser pointer power switch.

- Mount lens (D) and fiber optic holder (E) on adjustable mounts as shown.

- Make sure the voltage is set at 3V, don’t go over this or you will blow out the laser point. Current will be adjusted to obtain the desired temperature, start with approx.50mA.

**Note:** You will keep the laser-pointer power button depressed and turn the laser ON/OFF from the power supply (ON/OFF button)
II. Digital thermometer Assembly: (see figure below)

- Use a **K-type thermocouple (t/c) (diameter .0005'') (Omega; CHAL-0005)** and a k-type male plug (**Omega; HFMPW-K-M**). The k-type thermocouple comes un-insulated on a white plastic holder and the ends are held together by red (positive end) and yellow (negative end) tape.

- Shorten the plastic holder so it can be mounted on the digital thermometer. To do this, lift the red and yellow tape with forceps to lift the T/C wires, leaving the other end as is (the heat detector). Cut the plastic 10 cm from the heat detector. Keep the cut end for the next step.

- Glue the plug to the cut end of the plastic with superglue. To do this, place the back side of the male plug on the glued portion of the plastic holder. Screw the negative end (yellow tape) of the thermocouple to the **positive** terminal on the plug, and the positive end of the thermocouple (red tape) to the **negative** end of the male plug (yes, this is backwards). Use tape to prevent the wires from contacting each other. Screw the plug together, and glue the cut end of the plastic on top to help protect the wires.

- Plug this unit into the digital thermometer (**OMEGAETTE HH303 Type K J Thermometer**). Turn the digital thermometer on for several minutes and verify that it correctly measures room temperature.

III. Optical Fiber Preparation:

- Remove the coating from both ends of the 13cm optical fiber by flaming using a cigarette lighter. Remove ~ 5 cm of coating from one end and ~ 2cm from the other.

- Use forceps to make a clean/perpendicular breaks at both ends. Check under a dissecting scope.
Coat the longer end of the fiber with permanent black ink. A easy way to do this is to extract the ink from a Sharpie using a ~27 gauge needle and 3mL syringe. Dry ~5 minutes.

Tape the coated end of the fiber optic to a ~0.5 cm wooden dowel (a dissecting probe handle works) to use to mount on a micromanipulator.

Place the non-coated end of the 13cm long optical fiber in an adjustable clamp approximately 4cm from the lens, leaving ~ 2mm overhanging.

Optional: an electrode puller can be used to produce smaller diameter tips

Remove the coating from a ~4 cm region near one end of the 13cm fiber optic by quick flaming.

Pull the optical fiber with a Sutter Micro-Pipette Puller (Model P-97) using the following parameters: Heat 640 mA/sec, Pull 120, Velocity 200, Time 200 seconds.

Pulled optical fibers are usually 10 micrometers in diameter. We used forceps and break the tip to the desired size.

IV. Calibrate/Adjust the temperature at the microheater tip

Place one or two drops of water top of the k-type thermocouple (the little ball). Using the micromanipulator, gently touch the coated tip of the fiber to the t/c.

Optimize the temperature obtained at the tip by focusing the laser light on the uncoated end of the fiber (move the lens in the x/y direction), and by adjusting the distance between the lens and the uncoated fiber. Once the maximum temperature is achieved, adjust the current to produce a temperature of ~40°C. For our setup, ~70mA produces the desired temperature.

Notes: The coated optical fiber tip must touch the heat detector in the middle. Avoid vibration, you don’t want to scratch the ink off of the tip.
V. Embryo Mounting and Local Heat Shock

- Mount anesthetized embryos or larvae in a drop of ~1% Low Melting Temperature (LMT) Agarose (Sigma) on a slide or Petri dish. Position as desired before agarose hardens.
- Touch the calibrated fiber tip to the embryo. Contact needs to be made, but keep it gentle.
- Turn the power supply ON, and laser heat shock for 25 minutes at 40°C.
- Embryos can be left in agarose to monitor transgene expression (e.g. GFP fluorescence). Cover with ERM so they don’t dry out. Alternatively, embryos/larvae can be freed immediately.

VI. Have fun!