

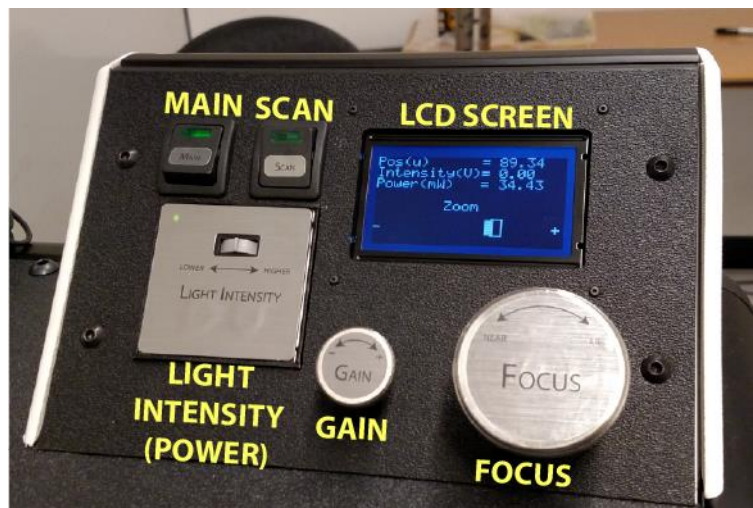
Zebrascope user manual

Date: 17/Mar/2016

Deliberate misuse of the Zebrascope to image tissues not approved on this protocol may introduce additional risks. These additional risks may include ventricular fibrillation, infection, and pneumothorax. Using the Zebrascope to access non-approved tissues is prohibited.

For the purposes of this document, please see the images below to identify key components and definitions for the machine orientation.

Zebrascope Tower and Dashboard view



Start procedures

- **Plug in the Power Cord**

- The main power cord resides at the rear of the machine near the bottom edge. This is black, standard three prong grounded plug for use with 60 Hz, 120V AC.

- **Turn on the MAIN switch**

- Left most switch on the dashboard



- The Machine will first check for a laser source. Until the laser has been enabled it will ask you to “Raise the laser power to 100”. If you try to raise the laser power using the “Power” dial on the dashboard and nothing happens it is most likely due to not having the laser on. Please wait until the laser is on before raising the laser power via the “Power” dial on the dash.



- Once the laser power is at an appropriate level, the machine will attempt to optimize. There are several scenarios that can happen and we would recommend getting the machine into or as near to its final location and booted up well before the intended use.
 - Please be patient as the machine is “Optimizing...”, it will eventually settle and report the following screen to the dash allowing for user control



1. If the “Optimizing...” screen does not change after >5 minutes, please turn off the “MAIN” switch, wait ~5 seconds and turn it back on. If the problem persists, please see troubleshooting section or contact Zebra Medical Technologies at fred@zebramedtech.com or (505)514-3353

- **Turn on the Computer and Monitor**

- Check to make sure the software can open and run if Computer has entered sleep mode after extended time of nonuse. If the computer freezes or will not recognize the software, simply restart the computer.
- Power up the monitor using the power button in the lower right hand corner of the monitor.

- **Turn on the Laser**

- Turn Key to “On” position and wait for blinking green LED to stay lit. This will coincide with a chime.



- The start/stop button on the laser will enable and disable the light. Leave the light on at this point for optimization.

This can be done at any time during the use of the machine. Note that the machine will not be able to image or optimize if the laser light is not enabled.



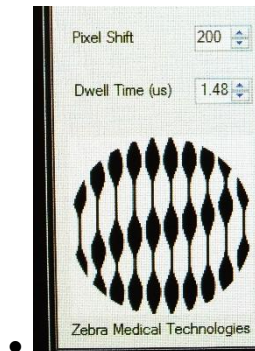
- If any of these steps fail, or the laser fails to produce light please contact Zebra at: fred@zebramedtech.com (505)-514-3353.

- **Prepare the Zebrascope for Imaging**

- Turn the “Gain” knob on the dash all of the way down (counterclockwise)



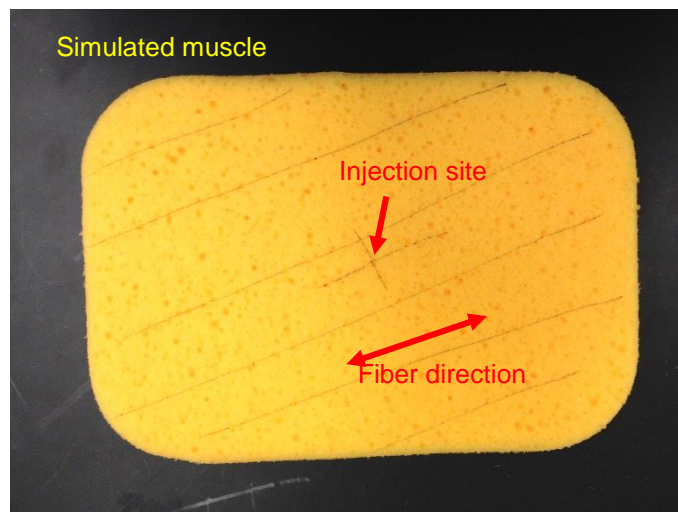
- Turn on “SCAN” switch. It is the rightmost of the two black switches on the dashboard.
- Start the imaging software on the computer desktop.
- Open the software settings file.
- After opening the software, you will need to change two key settings that do not initialize upon startup. These are the “Dwell Time” and the “Pixel Shift”.
 - Set the “Pixel Shift” to 200.
 - Set the “Dwell Time” to 1.48 μ s.



- **After probe and microscope are in place for subject imaging (see below):**
 - Click “Start Scan” within the software to begin scanning
We recommend a “Gain” value of \sim .7 and a “Power” value of \sim 75mW to begin with. Note that these values should be adjusted in real time in order to optimize your on-screen images.
 - Use the appropriate static image or video settings to acquire images or videos, respectively.

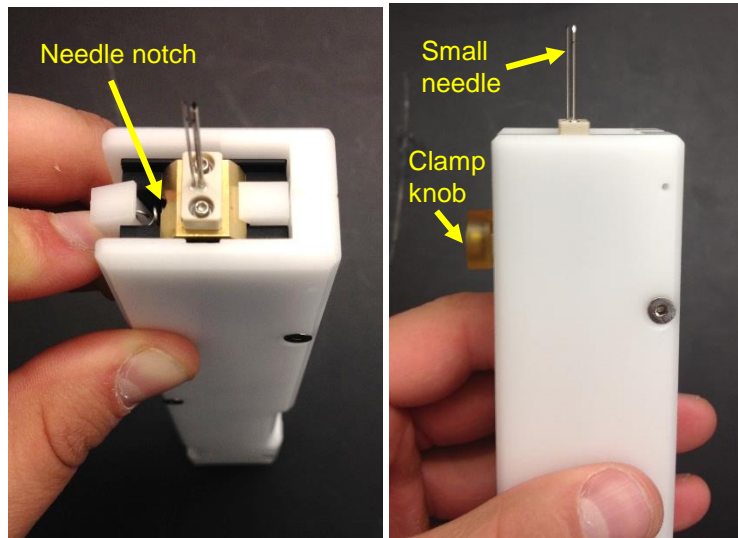
Imaging procedures: *In vivo* imaging

- **Prepare subject and probe**
 - **Instruct subject to wear laser safety goggles that you provide.**
 - Carefully choose the positioning and posture of the subject to access the muscle of interest. We recommend a position that allows the microscope to sit nearly statically without any support. You want to minimize torque on the needle since excessive needle torque may hurt the subject and likely alters the local sarcomere length.
 - Mark the injection site on the subject. I draw a large cross with one arm parallel to the muscle fibers. The long axis of the microscope should align parallel to the muscle fiber direction. For a complex muscle, use ultrasound imaging to identify a plane in which the fibers align. Remember that even if you find such a plane, it is usually best to keep the microscope normal to the subjects limb surface, so it is likely you can get the fibers in one plane, but they may be at an angle in the final image.

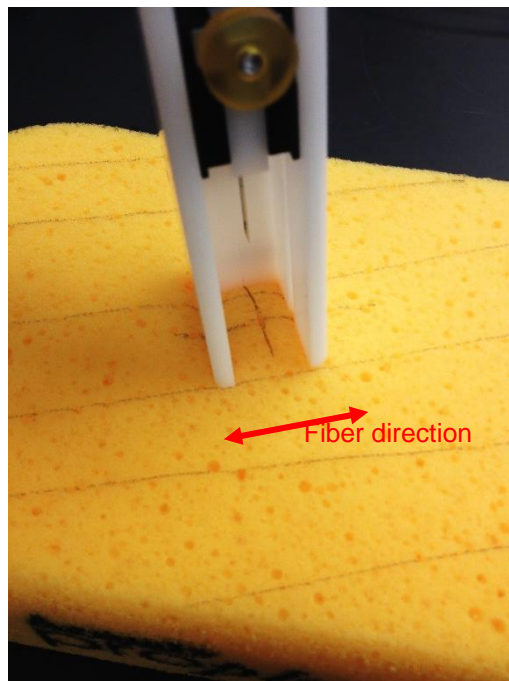


- Sterilize the probe with either an autoclave or plasma (sterad) processes. We recommend plasma since this will prolong the life of your probe. The probes withstand approximately 5-10 autoclave cycles before the epoxy joints denature.
- Use gloves and an alcohol swab to disinfect the injection site.
- Clamp the probe in the injector as shown in the following image. The probe notch and probe reflector (the smaller of the two needles) should point towards the open window on the injector. The injector clamp requires a combination “twist-push” motion to open. Twist the knob clockwise and push towards the back end of the injector (away from the open window) to raise the moving clamp jaw.

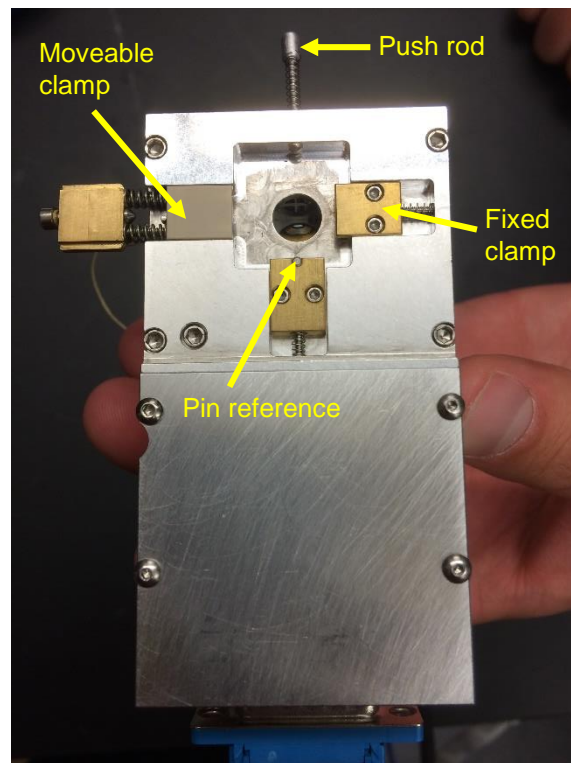
- Release the clamp knob and it will secure the probe.



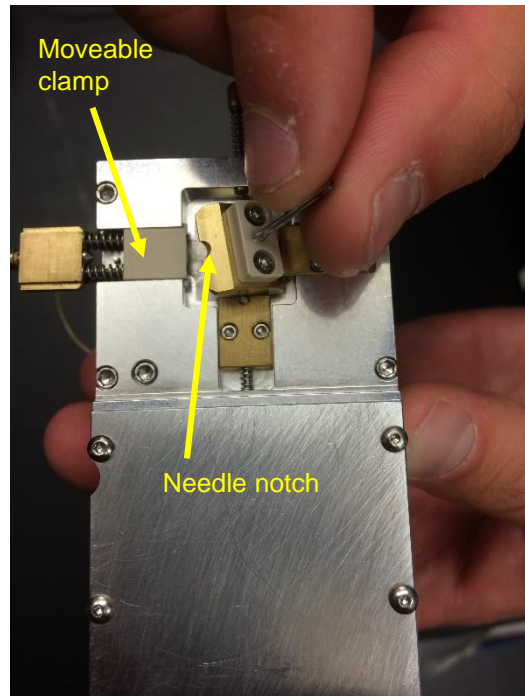
- Retract the probe plunger until it clicks and locks in place.
- Line up the injector as shown in the image. The fiber direction (as indicated by the cross hair) should be parallel to a line between the notches on opposing walls on the open end of the injector. The notch on the back wall of the injector should line up with the other arm of your cross.



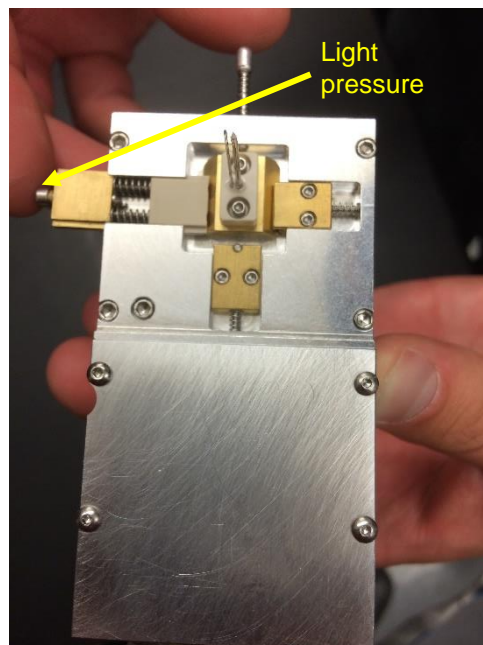
- Insert the needle by pushing the button on the top of the injector. Make sure the injector is normal to the skin surface.
 - Without moving the injector, “twist-push” the locking nob to release the button.
 - Carefully lift the injector off the skin, leaving the needle in the muscle. It is best to remove the injector by pushing it away from you slightly before lifting to avoid snagging the needle and pulling it out as you remove the injector.
- **Connect the microscope to the probe**
 - The clamping mechanism works as indicated in the following images. The needle notch should face the moving plastic clamp, not the fixed brass clamp.



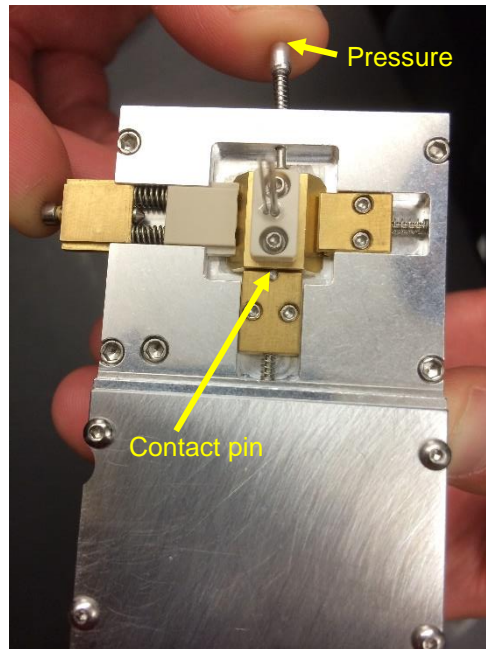
- Carefully place the microscope over the trapezoidal exposed block on the needle. Position the needle as close to the final clamping surfaces as possible to minimize needle motion (needle motion may hurt the subject).



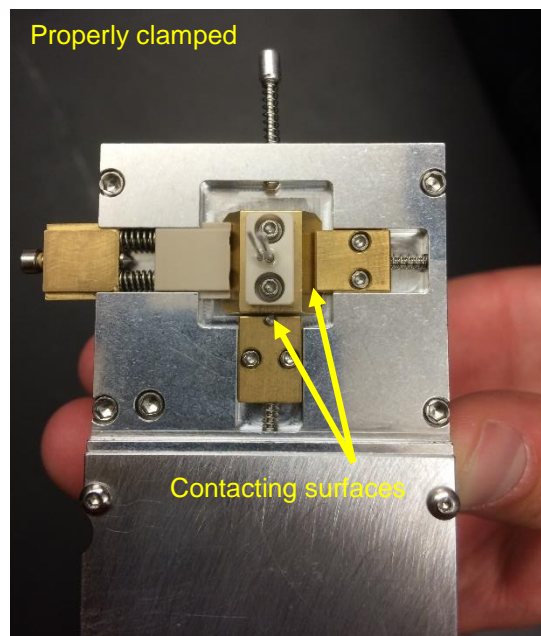
- Begin squeezing the moveable clamp until it engages the needle. You should feel the resistance of the clamping springs.



- Before fully clamping on, use the push rod to ensure that the back face of the needle is in contact with the pin reference on the back fixed brass clamp.



- With the moving clamp engaged and the needle resting against the back clamp, continue to squeeze the moving clamp until it engages the spring-loaded latch. You will hear a distinct clicking sound.
- Ensure the electrical stimulation wire to the needle is firmly connected to external stimulator.



- Either hand hold or secure the device with a fixture (e.g., strap) of your choice.

- Follow the same imaging procedures described in the previous section to collect images.
- The image scale is **0.16 $\mu\text{m}/\text{pixel}$** , for a field of view of $82 \mu\text{m} \times 82 \mu\text{m}$ (512×512 pixels)
- **Electrical stimulation of motor units**
 - Parameter limits: Pulse width less than 0.5 ms, frequency of 1 Hz or slower, maximum voltage 4 V.
 - Begin stimulation at 0 V and ramp by 0.1 V or less.

Rapid end procedure

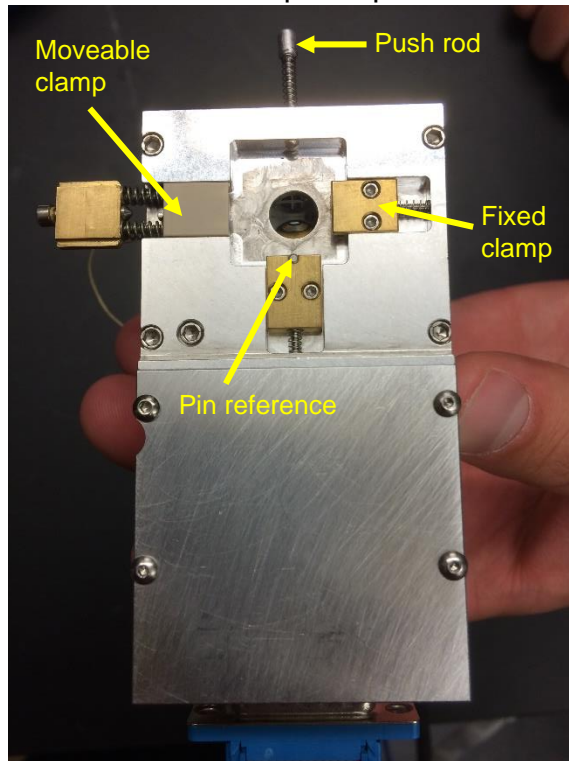
Alternative to “Standard end procedure” shown below. Use this procedure in any case where the probe needs to be removed quickly (e.g., subject requests to end the procedure as soon as possible).

- **FIRST, press the “Start/Stop” button to disable the laser** (important for ensuring no radiation exposure).
- **While holding the microscope in place, remove any fixture around the limb.**
- **Pull probe straight out of muscle, and apply bandage to insertion site.**
- **Remove any restraints from the subject’s leg.**
- **Then –**
 - Properly store or dispose of blood-contaminated probe in accordance with sharps safety standards.
 - Cover the microscope output with the “Moveable clamp”.
 - Turn off the “Scan” switch on the far right of the dashboard.
 - Turn the “Gain” knob all the way down (Counterclockwise).
 - Turn the laser key to the “Off” position.
 - **Subject may now remove laser safety goggles.**
 - Turn off the “Main” switch on the left of the Dashboard.
 - Shut down the computer.

Standard end procedures

- **Turn off the “Scan” switch on the far right of the dashboard.**
- **Turn the “Gain” knob all the way down (Counterclockwise).**
- **Turn off the laser** (important for ensuring no radiation exposure).
 - Press the “Start/Stop” button to disable the laser.
 - Turn the key to “Off” position”.

- Turn off the “Main” switch on the left of the Dashboard.
- Remove probe from subject.
 - While holding the microscope in place, remove any fixture around the limb.
 - Pull probe straight out of muscle, and apply bandage to insertion site.
 - Remove any restraints from the subject’s leg.
 - Subject may now remove laser safety goggles.
 - Properly store or dispose of blood-contaminated probe in accordance with sharps safety standards.
 - Cover the microscope output with the “Moveable clamp”.



- Shut down the computer.