

LSM Pascal Instructions

Before you do anything, sign-in to the logbook.

Startup (see Pascal and ArKr figures):

1. If you plan on positioning your sample using epifluorescence, turn on the **Mercury Arc Lamp** located on the shelf, and wait until the beam is steadily lit. If used previously in the day and still warm, the lamp should be allowed to fully cool before powering it on. It should left on for at least 20 minutes if used.
2. Make sure the small **Silver Toggle Switch** on the **ArKr Laser** is in the 'Standby' position, then flip the **Power Enable** toggle switch to 'On'. Turn the **Laser On** key to the 'On' position, wait 30 seconds, then flip the **Silver Toggle Switch** to 'Run'. Be careful not to adjust the light setting knob.
3. Turn on the **Main Powerstrip**, located on the shelf behind the scope.
4. Turn on the **Brightfield Lamp** next to the scope.
5. Start the computer with the case power button, and log in.

Start the Pascal software on the Desktop

- Select **Online Mode**, then click on the **Start** button.
- Select **Acquire** on the main toolbar.

To view your sample with reflected or transmitted light:

- Generally, you will position your sample and locate your imaging region under the scope using white light or epifluorescence:
 - **On the microscope:** Put the dual silver sliders in the **VIS** position (top in, bottom out).
 - **In the software:** In the **Micro** control panel, select an appropriate **Reflector Turret** for epifluorescence, or the **Transmitted Light** icon for brightfield. Select a low (5x-20x) powered **Objective**.
 - Position your slide on the stand and focus on your specimen.

To begin scanning:

- **On the microscope:** Put the dual silver sliders in the **LSM** position (top out, bottom in).
- **Config** control panel: Construct an appropriate filter and channel configuration for your dyes (see the **Optimizing Your Images** guide). Click on the **Config** icon to see if a preset configuration already exists for your dye(s).
- **Scan** control panel: Select the **Channels** subpanel. Click **Find** to automatically detect approximate brightness and contrast settings.
- Click on **Palate** in an image window, and select **Range Indicator** to label where saturation is occurring in your image.
- Continue scanning using the **Fast** (low-quality) or **Cont.** (slower, final quality) buttons. Optimize the image by refining settings and refocusing as needed, and click the **Single** button to capture a final image.
- To store your files, select **File** ® **Save**, then open an existing database or create a new one (**Open** or **New MDB**). Store all saved files in a folder you create on the

computer. When you are finished imaging, burn your databases and images to a CD or transfer them to the network (don't leave them on the computer).

Shutdown procedure:

- If someone will be using the scope immediately after you, exit the LSM software, log out, and flip the **Silver Toggle Switch** on the ArKr power supply to 'Standby'.
Otherwise:
- Exit the LSM software and shut down the computer.
- Turn off the **Brightfield Lamp**.
- Turn off the **Main Powerstrip**.
- Flip the **Silver Toggle Switch** on the ArKr power supply to 'Standby', and turn the **Laser On** key to 'off'. Wait 10 minutes for the fan to continue cooling the laser before flipping the **Power Enable** toggle switch to 'off'.
- Turn off the **Mercury Arc Lamp**.
- Make sure objectives are clean and dry. Be gentle, and use lens paper with a little methanol if necessary. Don't apply direct pressure to the objective tip; hold the lens paper by the edges and lightly drag it over the surface.
- Cover the microscope and clean the work area.
- Finally, sign-out of the logbook.