

## ZEN 2008

1. Double click on the **ZEN** icon to open the application.
2. Press **Start System**.
3. Press a) **SMART SETUP** and set up a new configuration, or b) select and **LOAD** a saved configuration, or c) open a saved image and press **REUSE**. The laser(s) you need will be turned on automatically.

There are currently 1700 entries in the Smart Setup dye database. Choose the one(s) you want to use. Then choose among **FASTEST**, **BEST SIGNAL**, and **BEST COMPROMISE**. The histogram will show the amount(s) of channel cross-talk, if any, so you can optimize your data collection. Best signal reduces the amount of cross-talk, but results in slower scan speeds. For live samples with more than one signal, switching lasers by **LINE** rather than **FRAME** may result in better data collection. Choose between frame and line in Setup manager/Image setup.

Objective	Zoom	Prism
10X	4X	
20X	4X	DIC II
40X	3X	DIC III
63X	2.8X	DIC III
100X	2.2X	

For finding your sample, fast scan speeds (7-9) and 8 bit depth are sufficient. Begin with a pinhole of **1 AU**, and adjust it accordingly if needed. When collecting multiple channels simultaneous, set the middle channel (ChS) to 1 AU; the other channels will be adjusted automatically.

Use **range indicator** to avoid red pixels (saturation) and reduce blue pixels (underexposed) for signal optimization. Adjust saturation by changing the **master gain** and underexposed areas with **digital offset**. The new **DIGITAL GAIN** will increase the signal without increasing the noise.

For publication quality images, use slower scan speeds, averaging, and 12 or 16 bit depth. You might also try the **OPTIMIZE** button in Online acquisition/Acquisition mode to see if that algorithm gives you a satisfactory image.

You should regularly save your images while you are working and close them. When you have ~1 GB in images open, ZEN will crash and you will lose unsaved data. Remember to save reference images in a folder called "Reference Images" in your documents so you can reuse them later. Only save images to the directory that is set up for you during training. Do **NOT** mount any servers. This puts your data integrity at risk as well as the ZEN system.

When you are finished collecting images, exit ZEN and logoff. Follow the shutdown procedure if you are the last user of the day.

## Microscope stand IMAGER.Z1

You should begin with the **10X objective**.

Press **Microscope** on the touch screen. You should see the **Objectives** and **Reflector** tabs.

1. To load a slide, press **LOAD POSITION** on the touch screen. The stage will lower, and then place the slide on the slide holder and move it under the clips. You can move the stage towards you with the joystick to make it easier to take slides on and off the stage.

2. Press the **UP ARROW** with the **LINE** above it to return the stage to the previous work position. Use the fine adjustment to focus your image. If you press **set work position**, the stage stays in the load position and you can use the coarse adjustment to manually raise the stage.

3. Press **OCULAR** in ZEN (upper right corner, next to camera and LSM) or **VIS** on the right of the microscope stand by the oculars to view your sample through the oculars. You can control the shutter through the ZEN software or the RL-Shutter on the touch screen.

4. In ZEN **Light Path**, press the **Ocular** tab. Turn on the transmitted light by clicking on the bottom left right icon (light bulb), then press **ON**. Open the shutter by pressing the shutter icon on the top left of the box or the RL-Shutter/ Open on the touch screen. You can control the light intensity with the dial on the microscope stand when the shutter is open or by using the slider in the dialogue box.

5. To use the X-cite box, make sure you have turned the switch on. Choose the filter you want from the Reflector tab. Press RL-Shutter/Open on the touch screen. If you have transmitted light on, you can control the light intensity with the dial on the microscope stand when the shutter is open or by using the slider in the dialogue box. You may want to turn off the transmitted light when using X-cite excitation.

6. If you are using DIC, click on the condenser icon in the Light Path dialogue box, and choose the prism that you want. Remember to click the front lens switch so that it is in line with the light to get a DIC image. Also, remember to check to see that the Köhler illumination is correct.

7. You cannot go back to the 20X objective if you have immersion media on the slide.

8. When you are finished, please gently clean the objectives with **lens paper** if you used immersion media objectives. Please return the microscope to the **10X objective**.

