Startup Procedure

- **Starting the Inverted Microscope:**
  1. Turn on both switches on the Remote Control
     left = computer + right = microscope
  2. Turn on the Computer (button on the tower)
  3. Make sure the network says 1Gbit, on Taskbar near clock
  4. Start Zen 2009 software, Start System

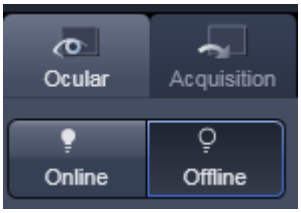
☆ Upright scope information is on the last page ☆

- **Starting the 2-photon laser:**
  1. Skip to the next section if you do not need the 2-photon laser
  2. Start Mai Tai control
  3. Choose **COMM port 5**, OK
  4. Click the ON button and hold until you see emission
     - Allow laser to warm up:
       - Laser output will be between 2.5- 3 amps
       - Pulsing button should be green
  5. Open the shutter by pressing and holding until you see yellow
  6. Minimize the Mai Tai control (do not close the software)

- **Humidity and CO_2 Incubation:** (only if needed)
  - Turn on the CO_2 regulator
  - On the Microscope touch screen, go to Control – Incubator
  - Turn on each of the three heating components and the CO_2
  - If the CO_2 is not correctly going to 5%, turn everything off and restart the Control module

Setting up your Sample on Inverted

- **Zen Software:**
  - Make sure the Beam Path is set to Ocular, by pressing the 'Online' button



- **Microscope Screen:**
  - Home - Load position (obj. down)
    ▲ for Working position (obj. up)
    - TL Illumination (transmitted light shutter) on/off
    - RL Illumination (reflected light shutter) on/off
    See page 3 for X-cite Illuminator
  - Microscope (Control)
    - Objective Change
      - 40x objective has adjustable working distance
        low 0.15, normal 0.17 (170µm), high 0.19
        Black = R/T Red = 37°C
    - Reflector change fluorescence filters (see pg 3)
      change to DIC (see pg 4)

- **Microscope Buttons:**
  - Right side - **front/top**: load and work positions
    - **back/bottom**: objective
    - **middle**: transmitted light shutter
  - Left side - X-cite shutter

☆ Remember to add water or oil to the objectives ☆
(check the text on the objective)

X-Cite Fluorescence:

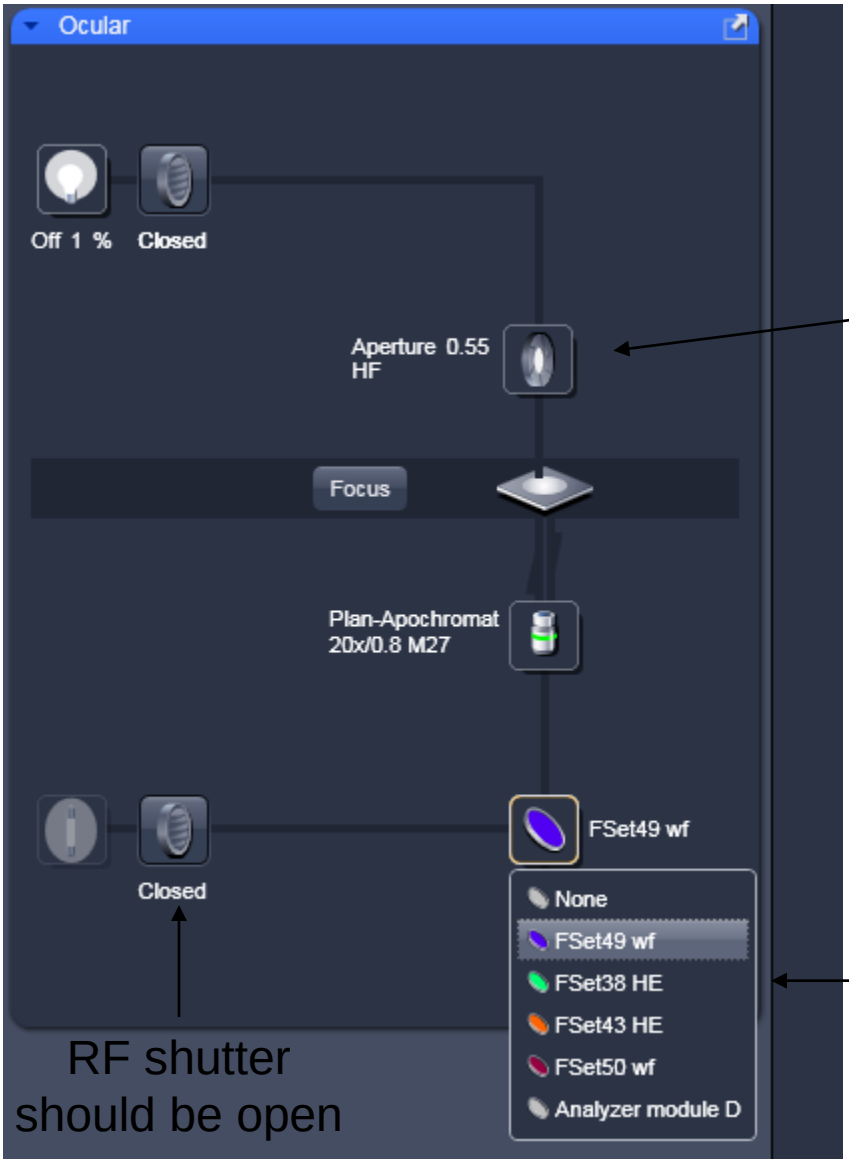
The X-cite Illuminator is helpful for focusing in on fluorescence in your sample

Turn on the X-cite Illuminator (back-right, behind the scope)

On the Microscope touch screen:

- Go to Home
- Turn on the RF Illumination
- Go to Control – Reflector
- Choose DAPI, GFP, Cy3 or Cy5 (see below for wavelengths)

Alternatively, in the software:



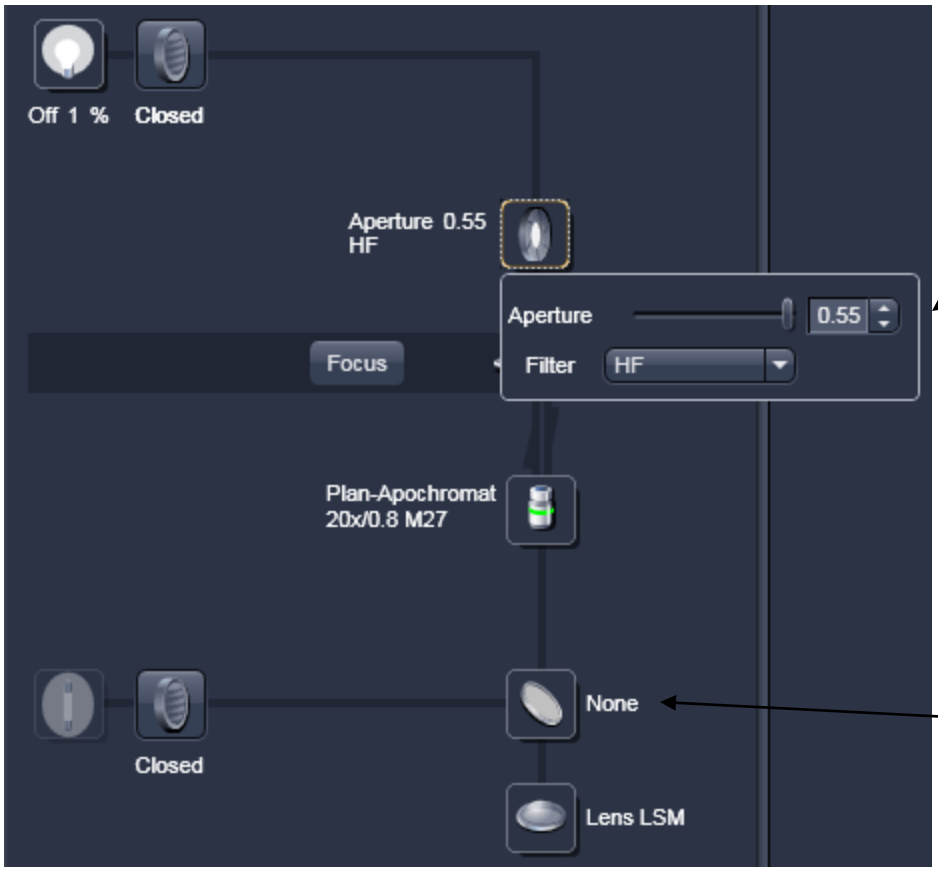
Keep aperture open to the max & use HF Filter

Filter set:	Ex/Em:
Blue	365/445nm
Green	470/525nm
Red	550.605nm
Far Red	640/690nm

** Cy5 may not be visible to the eyes **

Regular Brightfield & DIC

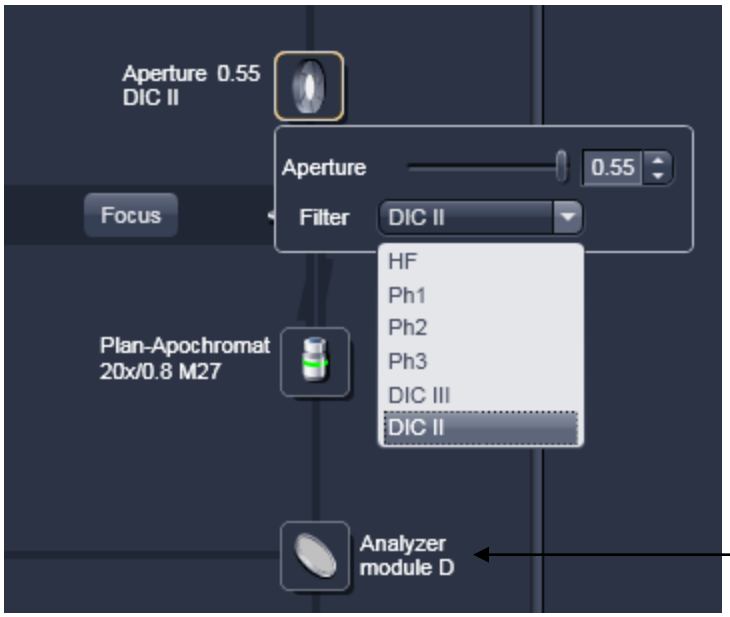
Brightfield Imaging (Under the Ocular tab)



Keep aperture open to the max & use HF Filter

No filters in place here

DIC



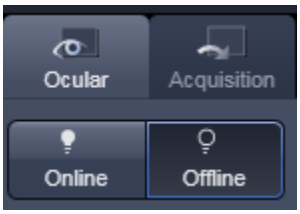
For:	Use:
20x	DIC II
40x	DIC III
60x	DIC III

Analyzer module D

** This can also be done on the Microscope touch screen. Go to Control - Reflector and choose DIC.

Acquisition: Laser Scanning Mode

- **Switch from Ocular to Acquisition:**
  - Make sure the Beam Path is set to Acquisition, by pressing the 'Offline' button under the Ocular tab
  - Go to the Acquisition Tab



- **Starting the lasers:** Laser
  - Under the Acquisition Tab, Go to Laser under Setup Manager
  - Turn on the Lasers that you need (including TiSaph if you are using it)
  - If you are using the Argon laser:
    - It will first go to standby for ~5 minute warm-up, then on
    - Under Laser Properties, increase the % Output until the Tube Current reaches 6.0A (~45% output)

- **Set up Tracks:** Imaging Setup
  - Either choose a saved Configuration Configuration RGB 2P
  - Or set-up manually:
    - Under Imaging Setup, Choose Channel Mode
    - Switch track every:
      - Frame – slower but better when you have different dichroics in each track
      - Line – faster but possibly less fluorophore spec.
    - ☑ Check the first Track and go to Light Path (below)

Acquisition, cont.

- **Set up Light Path (dichroics and filters):** 

  - Click on Laser and check the laser(s) you want to use 

    Start with the following % transmissions and adjust:
      TiSaph 3-5
      Argon 6-8
      HeNe's 15-20
  - Follow the laser path to the left to the first dichroic
    Choose an HFT that lists your lasers (see pg 7)
  - Follow the laser path up and right to the next two dichroics
    Either choose Mirror or an NFT dichroic
  - The next filters are band pass filters, where you will choose your emission wavelengths
  - Finally, check the detector channel for your fluorophore emission
    ChD will use the laser light to create a transmitted light image

- **Acquisition Mode:** 

  - Frame size – click Optimal for best resolution for your objective, or set your own lower resolution
  - Speed – Use the highest speed for best signal/noise or lower speed for longer dwell
  - Averaging – the fluorescence in each pixel will be averaged this number of times when you press Snap (not for Live or Continuous)
  - Scan Mode – Choose Line or Frame for averaging
    - Frame averaging reduces photo-bleaching
    - Line averaging gives smoother image

From the LSM 510 Manual

Filters and Dichroics (German)

KP= Low Pass Filter Kurz Pass = short
Example: KP 685 passes wavelengths less than 685 nm to the detector.

LP= High Pass Filter Lang Pass = long
Example: LP 505 passes wavelengths higher than 505 nm to the detector.

BP= Band Pass Filter
Example: BP 565-615 passes wavelengths 565 nm to 615 nm to the detector.

HFT= Main Dichroic Beam Splitter Haupt Farb Teiler = Main Color Splitter
Example: HFT 488/543 Excitation wavelengths (488 and 543 nm) sent to the sample. Passes all other wavelengths up to the detection channels.

Example: HFT KP 700/488 Works as both a dichroic and a low pass filter. Will pass all wavelengths less than 700 nm, except for 488 nm, which hits sample, but is not sent to detector.

NFT= Secondary Dichroic Beam Splitter Neben Farb Teiler = Secondary Color Splitter
Example: NFT 543 Wavelengths above 543 nm, pass straight through. Wavelengths less than this amount are reflected 90 degrees.

NT= Neutral Density Filter
Example: Works as an attenuation filter. NT 80/20 passes 80% of the light, cuts 20% of light.

Acquisition, cont.

- **Image Acquisition and Channels:** 

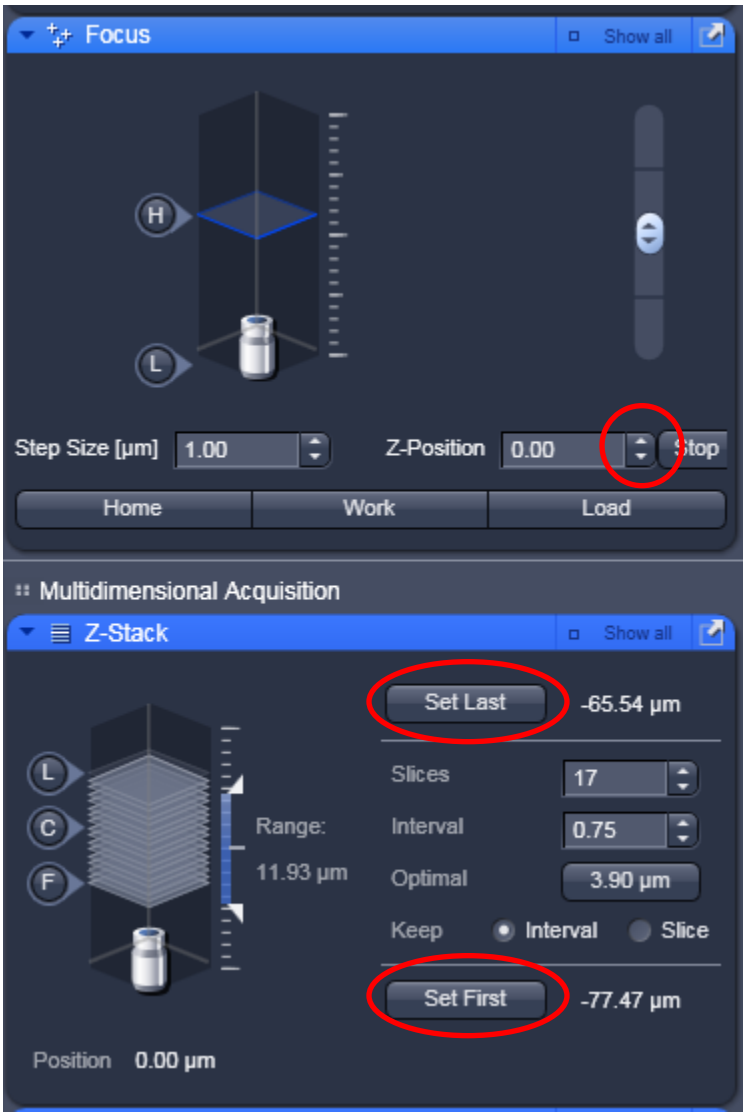
You will use Image acquisition buttons (Auto Exposure, Live, Snap) and Channels buttons in this section, so keep Channels button open (Under Online Acquisition).

- Pinhole - Choose **1AU** for Argon and HeNe lasers
  This will set the pinhole size based on λ and obj.
  - Choose **Max** for 2-photon
- Auto Exposure  - Do this first for automatic adjustment of detector gain/ offset
  - **Gain**: Upper limit depending on Fluor
  - **Offset**: Lower limit depending on bkgd
- Live  - Select Fast and change detector color to 'Range Indicator' This can be found under the image in View/Dimensions, under Channels. Click once on a channel color to change to 'Range Indicator'
  - Manually adjust **Gain** until you see just a little red (upper limit/ saturation), and **offset** until your background is mostly blue.
- Snap  - Select Snap to record a single image, including averaging

Acquisition, cont.

- **Z-stacks:**
  - Check the Z-stack button

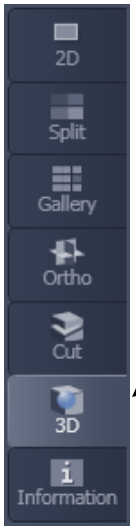
  - Open the Focus and Z-stack windows
  - Press the Live button for continuous scanning
  - Focus on the **upper** position & **Set First**
  - Then focus on the **lower** position & **Set Last**
  - Stop the Live Scanning



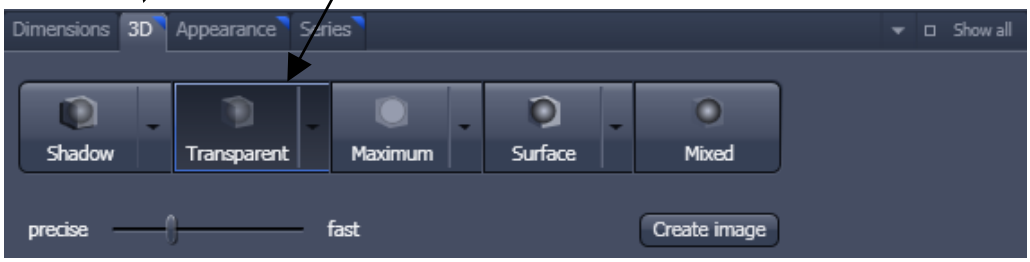
  - Start Experiment

3D Confocal Videos

- Use **ZEN 2009 Light Edition** on your personal computer (free PC download at www.zeiss.de)



- Open your Z-stack
- Go to **3D** and **Transparent**



- You should be able to select the **Rotation** button now

- Go to **Series** (if you can't see it, check the 'Show all' button)

Spin around **x** or **y**

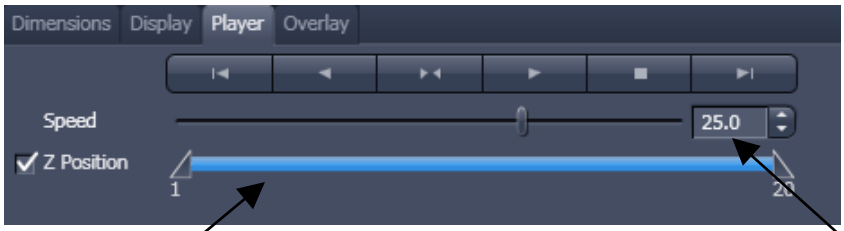
Choose the number of **frames**

I like to rotate it completely

- When you are satisfied, press **Apply**

  A new image window will appear called ‘Render Series’

- In the Render Series, go to **Player**



You can refine the **Z slices** that you want to see

Hit play and determine what **speed** you want. I like a slow speed (ex. 2)

- Go to File > Export

  Format: Video for Windows

  Data: Contents of image window – series

  Frames per second: 2

- Save and try it out

Shutdown

1. Check the online calendar to see if someone is after you

   - Always shut off 2-photon: Press and hold Off until Emission disappears & close shutter

   - If someone is on after you (5 hours or less): Leave other lasers on
   - If you are the last person: Turn off lasers

2. Close Zen Software

   It will prompt you again about leaving lasers on

3. Shut down Computer
4. Turn off the humidity and CO2, and shut off regulator
5. Turn off both switches on the Remote Control
6. Clean off objective

   Use Sparkle

7. Cover the scope
8. **Clean up after yourselves!!**

Startup Procedure- Upright

- **Starting the Upright Microscope:**
  1. Start the Mercury Bulb first –ONLY IF YOU NEED IT
     Turn on the FluoArc power supply for Hg bulb about 30mins before using the scope
  2. Turn on the Main Power Switch on the Remote Control (this gives power to both the computer and microscope)
  3. Turn on the Computer (button on the tower)
  4. Start Zen 2009 or AIM.exe software (We've found the Zen software doesn't' work well on this older scope.
     Select 'Scan New Images/ Start in expert mode'
  5. If you need the 2-photon laser, the Mai Tai control software is only on the Inverted Confocal computer, so start it there.

- **Setting up your Sample on Upright:**
- Microscope Buttons:
  - Pull the bar on the top of the scope to switch from LSM to Ocular
  - Vertical dials: x/y stage position
  - Right side - **bottom/right**: objectives
    - **far right**: HAL halogen, FL mercury bulb
    - **front**: diaphragm for halogen bulb brightness
  - Left side - **front up/down buttons**: z stage position
    - **bottom/left**: emission filters for mercury bulb
      1=no filter, 2-4= blue, green, red emission
  - Condenser - the wheel on the front of stage
    - matches the emission filter and is obj. spec.

☆ Remember to add water or oil to the objectives (check the text on the objective) ☆