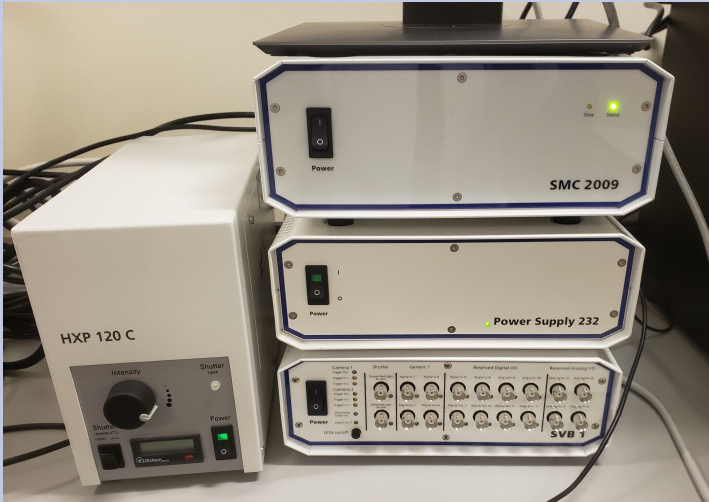
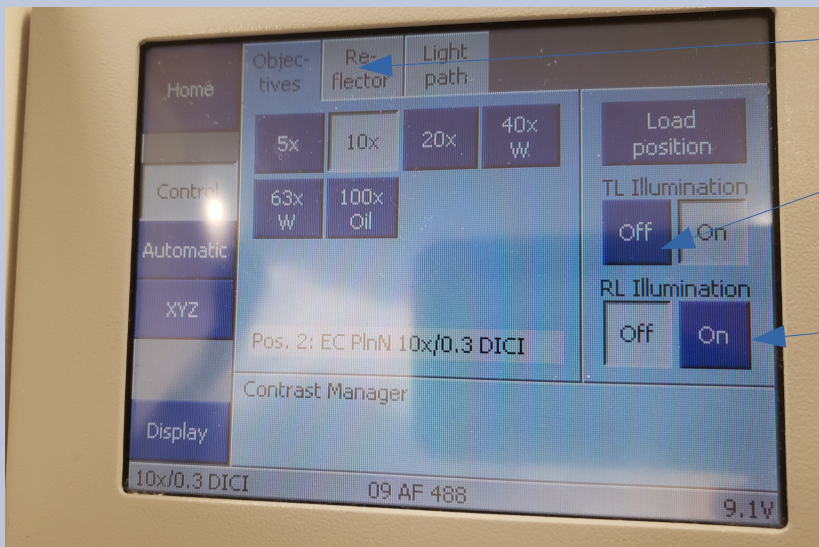


# Epifluorescence quick start guide

Start the PC first and let it fully boot before starting the microscope



Turn on the three power switches on the stack of boxes, then turn on the HXP lamp.

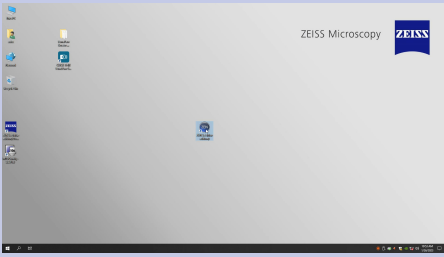


Epi filters for eyepiece

Transmitted light on

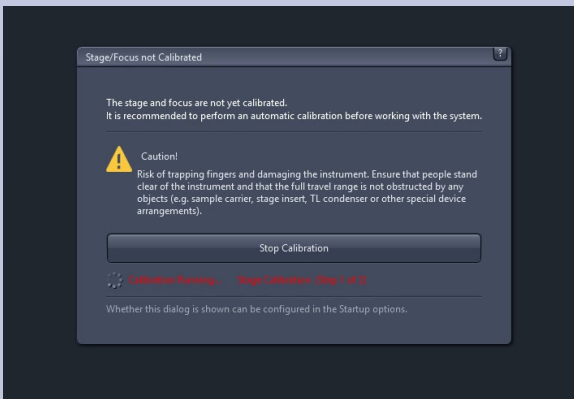
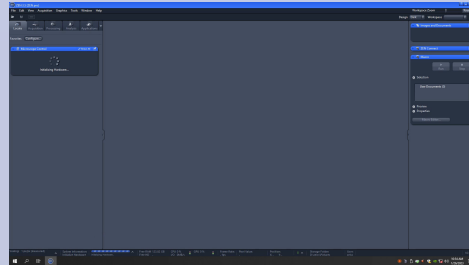
Fluorescence light on – “Reflected Light” - yes, I know...

The touch screen is not capacitive – you actually have to press...

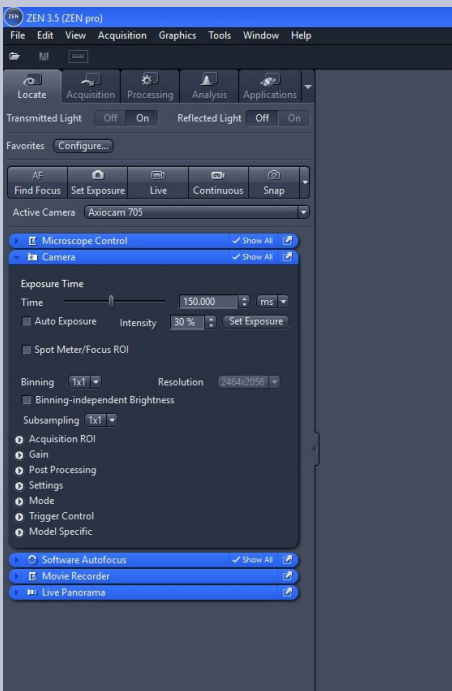


# Start Zen blue

Connecting to the scope takes about five minutes. Don't interrupt it.

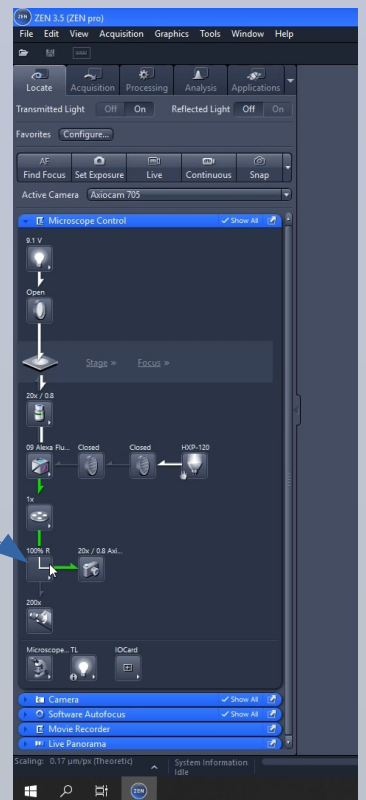


Calibrate the stage ( or you won't have stage control ). This moves things around a lot – remove any slides and make sure the stage won't hit anything

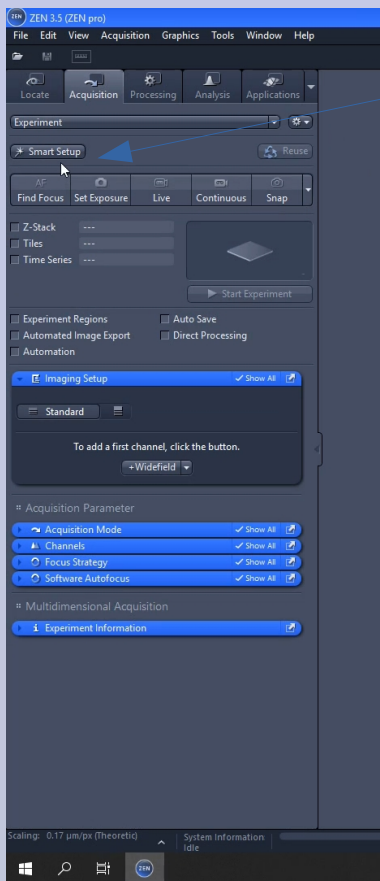


You're finally ready to start. Make sure you're on the 'Locate; tab or you won't have eyepiece access

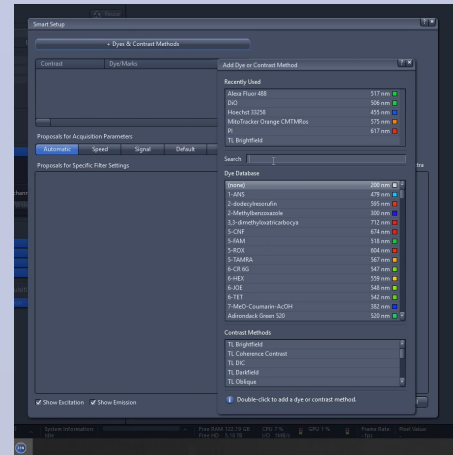
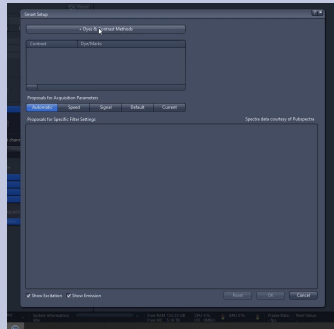
To activate the eye pieces open the microscope control tab and click here to change the path to 100% eyepiece



Get in focus then move to the Acquisition tab

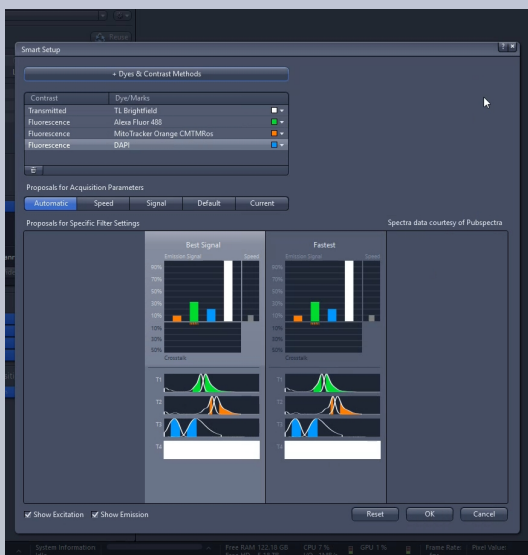


Choose 'Smart Setup' then Dyes and Contrast Methods

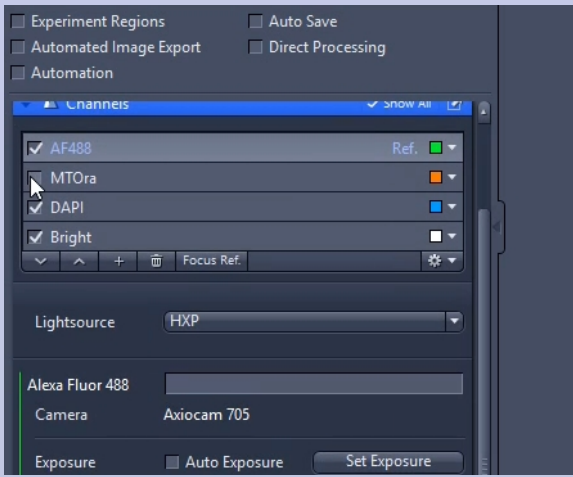


Add your channels using the pulldown list. A new channel is added automatically just by double clicking a menu item.

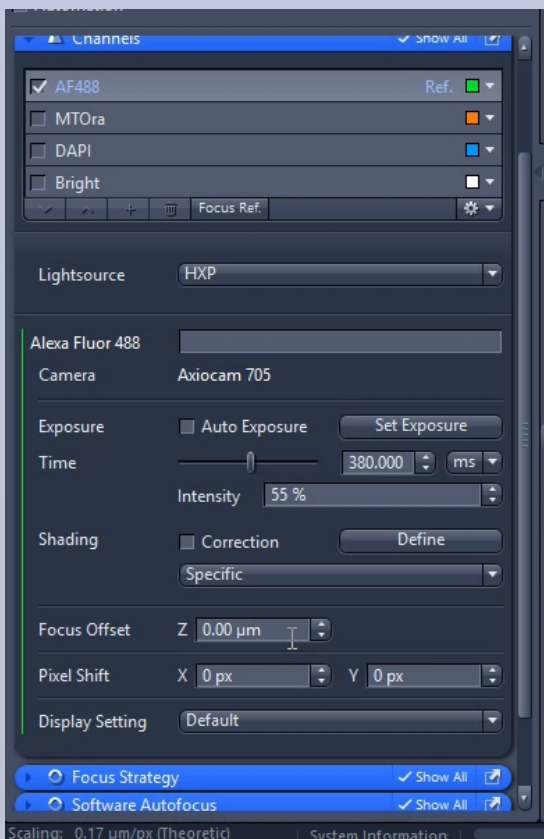
Remember this is not a full on confocal – you have three filters, red, green and blue so don't use close together fluorophores ( GFP + YFP big no no )



There's rarely any difference between the two on an Epi set up so just hit 'Smartest' then OK



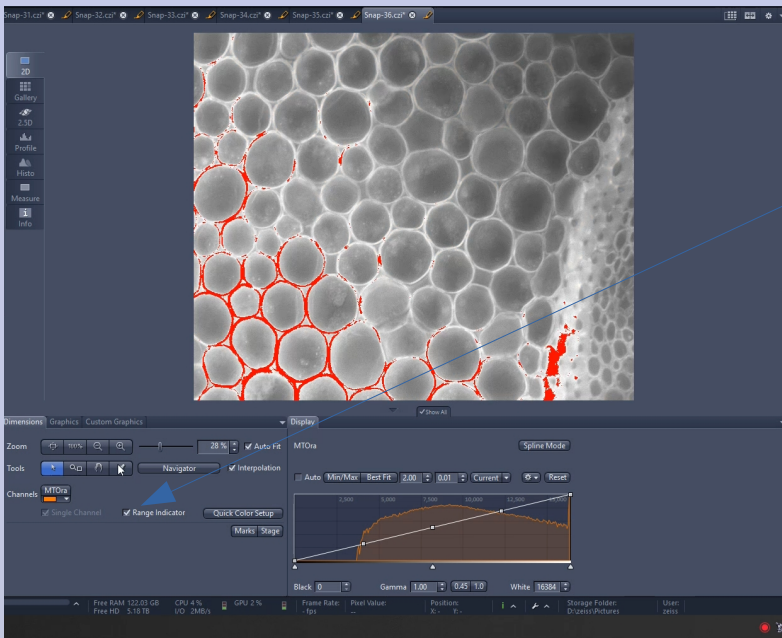
Open the Channels window. It's much much easier to set your channels one at a time so untick all but one channel.



**Auto exposure** - constantly updates the exposure settings – useful for consistent looking images but useless if you need to compare fluorescence. A better option is to use **Set Exposure** ( runs Autoexposure once ) then tweak the settings and leave them alone for the session.

Use 'Continuous' or 'Snap' to adjust settings – 'Live' is a reduced resolution for navigation.





For the best possible quality image, use the Range Indicator tool. Any areas highlighted in red are overexposed reducing the resolution of the image and obscuring detail.

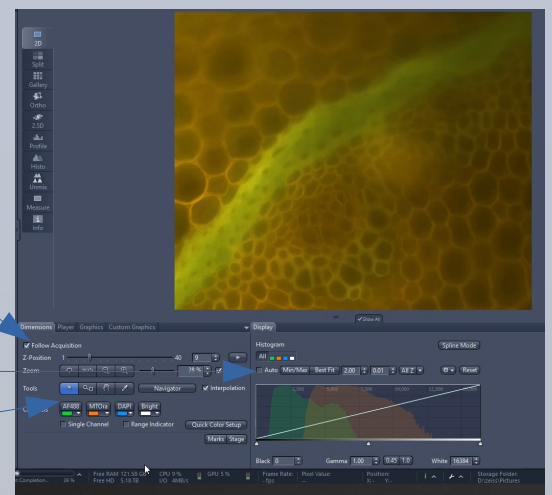
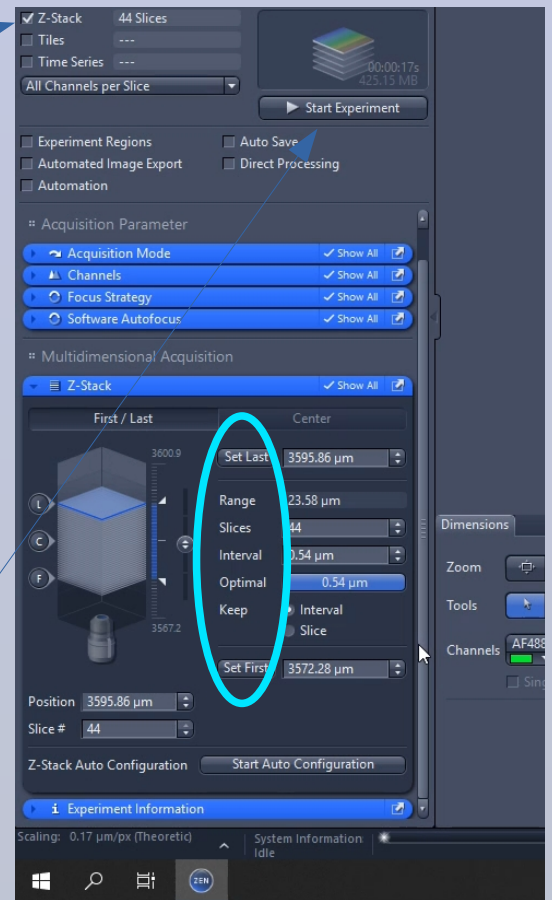
## Z stacks

Tick the box and a new window becomes available. Use Live or Continuous with one channel selected to set your focus ( Z ) range. Use “Set First” and “Set Last” to mark the focus positions ( it doesn’t matter which order ). It’s essential that you use the ‘Optimal’ button after you’re done to set the correct Z spacing ( or you might have huge gaps ).

Remember to reselect all your channels then hit “Start Experiment”

“Follow Acquisition” updates the screen with every new frame

The histogram “Auto” adjusts on screen brightness – remember to turn it off when setting exposure. Clicking this bar toggles each channel on and off.



## **Best practice -**

The microscope is sensitive to ambient light – turn the room lights off

If your sample is photosensitive, avoid using 'live' and 'continuous' with fluorescence – navigate with the transmitted light if you can and set your exposure by making adjustments blind and hitting 'snap' to check.

The 100x lens is meant to be used with 518f oil, not W – for routine imaging it's no big deal but it's worth cleaning the slide and using the correct oil if you need the best possible resolution

These oils set over time and trap dust, Never leave oil on the lens and if you like your slides, clean the coverslip with ethanol straight after use