2 - Using the Old Fluorescence Microscope

Introduction

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The old fluorescence microscope is temperamental and makes no sense. If something goes wrong try turning the programme off and on again, yelling at it, or, in extreme cases, physical violence. It is advised that when setting up you have someone else there, as failing by yourself is infinitely more depressing than failing with someone else. If in doubt ask Nick / George.

Materials

- Slides
- Fluorescence Microscope
- Microscope oil (probably not its technical name but you know what I mean)
- Patience mixed with an ability to laugh when things go wrong

Procedure

Setting Up the Microscope

1. When entering the microscopy room knock first as if people are visualising you can mess things up by entering suddenly. Also if people are running experiments dont turn on the lights even if they are not there because you can mess up time lapses.

2. Log onto the computer under the user 'Lab' with the password 'lab'. If the computer asks you to change the password, change it from 'lab' to 'lab' and the computer will be satisfied for another month.

3. Turn on the four machines around the microscope. With some of the machines 'on' is with the button on the line, and on some machines it is when it is on the circle. The only reliable way to tell if the machine is on is if it has a light on. Two machines are to the left and two on a shelf to the right. The mercury lamp to your left needs the ignition turned on. Press the ignition button until you hear two clicks. Apparently the on button isn't enough.

4. Put a small blob of microscope oil on the cover slip over the agarose, and put it face down over the lens. Using the joystick on the right and the focus knobs, manouver the lens into the drop of oil. You can lift the camera and light out of the way by pushing back on the **grey** part of the microscope (don't push the black part or you will push it out of alignment).

5. Open the ironically named 'Simple PCI 6' programme. Click on the camera to start the imaging. Set the blue channel to 'Newdic' with an Exposure Time of 0.2s if visualising GFP and about 0.03s if visualising mCherry, and set the gain to 0. It is normally best to fiddle with it a bit to get the right brightness. If visualising GFP set the green channel to 'GFP', if visualising mCherry set the green channel to 'RFP', and the gain of this channel to 255. At the bottom of the microscope you can move a sliding filter set to 'GFP' if looking at 'GFP', or 'YFP' if looking at mCherry. Clearly when all this was made, mCherry wasn't a thing.

6. If you are looking at mCherry (and probably to be safe, GFP as well), you now need to go thorough a pointless and complicated ritual to the Gods of the Microscope. Go into 'file' in the PCI window, and go to 'Current Profile'. Go to the 'Device Control' tab and click on PRIOR: COM4, Double and then click on the 'Properties' button. Go to 'Filter Set Up', click 'Apply' and then click Test. At this point the microscope will cycle through all the different different coloured light it can make, providing you with a 3 second disco. The Gods of the Old Microscope will now be satisfied and you can close the windows and go back to your day to day life.

Actually looking at the Effing Cells

7. Make sure the last Channel you have clicked on was the blue channel, and so this tab should be in front of whatever fluorescence tab you are using. Click live (on the other window). If it asks you to put filters in place, you didn't do what I told you to do in the first place, and you only have yourself to blame. Once you are live you can focus the microscope using the focus knobs on the right, and move around the slide using the joystick.

8. When you see cells (congratulations on getting this far by the way) you can see if you have any fluorescence. Click 'Capture1' which is above the live button. It will ask you to put filters in place. Use a thick piece of card to block out the light going onto the slide and then click ok. If you are looking at GFP you should see a blue/white light, and if you are looking at mCherry you should see a very green light. If you don't see a green light, its probably because you didn't do the ritual, and now you are being punished.

9. You should have an image with the cells on the screen. To see just the fluorescence Channel, click the red, green and blue bubble icon at the bar at the top and select the red or green channel (depending on which one you used). You can then play with the contrast by clicking the button to the left of this, which should open the 'Image Display Contrast' window and playing with the Maximum and Minimum sliders. Once set, from then on this button will then apply the same settings when pushed. Remember to unclick the channel and this button when going back to the live view. Sometimes this button doesn't work, probably because it's not feeling too well and needs a bit of a break. If this is the case, click some of the buttons to the left of it, and one of them sometimes does the same thing.

10. To save the image right click, go to Save, and save all components.

11. To take a repeat reading move the view a little way away, because the UV light photobleaches slightly outside of the field of view as well. Then press Capture1 as before.

12. Each new slide needs a little bit of microscope oil, but less than the first one.

Putting away the Microscope

13. Take off the slides and throw them into the yellow bin in the microscope room.

14. Take a slip of microscope tissue paper (again not the technical term) and wipe down the lens. only touch the edges of the tissue paper to avoid getting finger grease on the lens.

15. Put a clean slide over the lens to stop dust falling onto it

16. Turn off all the machines and log off

17. Walk out of the room and realise that it is much later than you thought it was going to be when you finished and collapse into the nearest available chair.