

King's College London : Wohl Cellular Imaging Centre

Opera Phenix: Quick Start Guide

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Scope

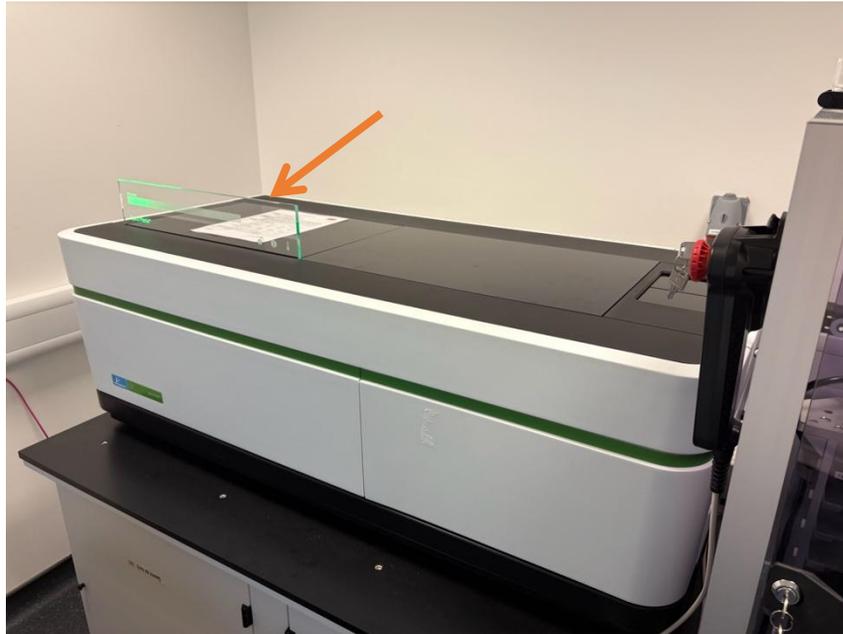
This document serves as a simple guide to setting up imaging on the Opera Phenix. This guide should be used as a reminder for users who have already received training and is not a substitute for training nor does it contain the same level of detail.

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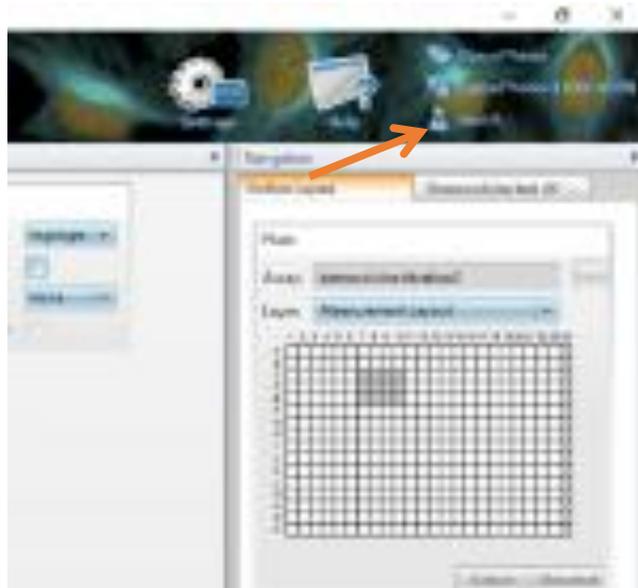
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1 – Introduction

- Opera should be switched on when you arrive – the glass progress bar on top of the microscope should be illuminated green as shown below



- Log in to PPMS. Harmony should be open already – you then need to log into your Harmony user profile by clicking on the name of the current user on the top right of the screen



2 – Plate Selection

- Select the plate that you will be using for imaging
- For successful imaging and to avoid damaging the microscope, it is essential that the exact make and model of plate you are using is selected
- Most plates will already be available as options in the drop-down menu – if you do not see an option for the plate that you are using, contact a technician
- **DO NOT** attempt to image with the wrong plate selected

Experiment: n.a. ...

Plate Type: 384 PerkinElmer CellCarni... ▼

Autofocus: Two Peak (Default) ▼

Objective: 40x Water, NA 1.1 ▼

Opt. Mode: Non-Confocal **Confocal**

Binning: 2 ▼

Live Preview:

Max Duration: 0h 05min

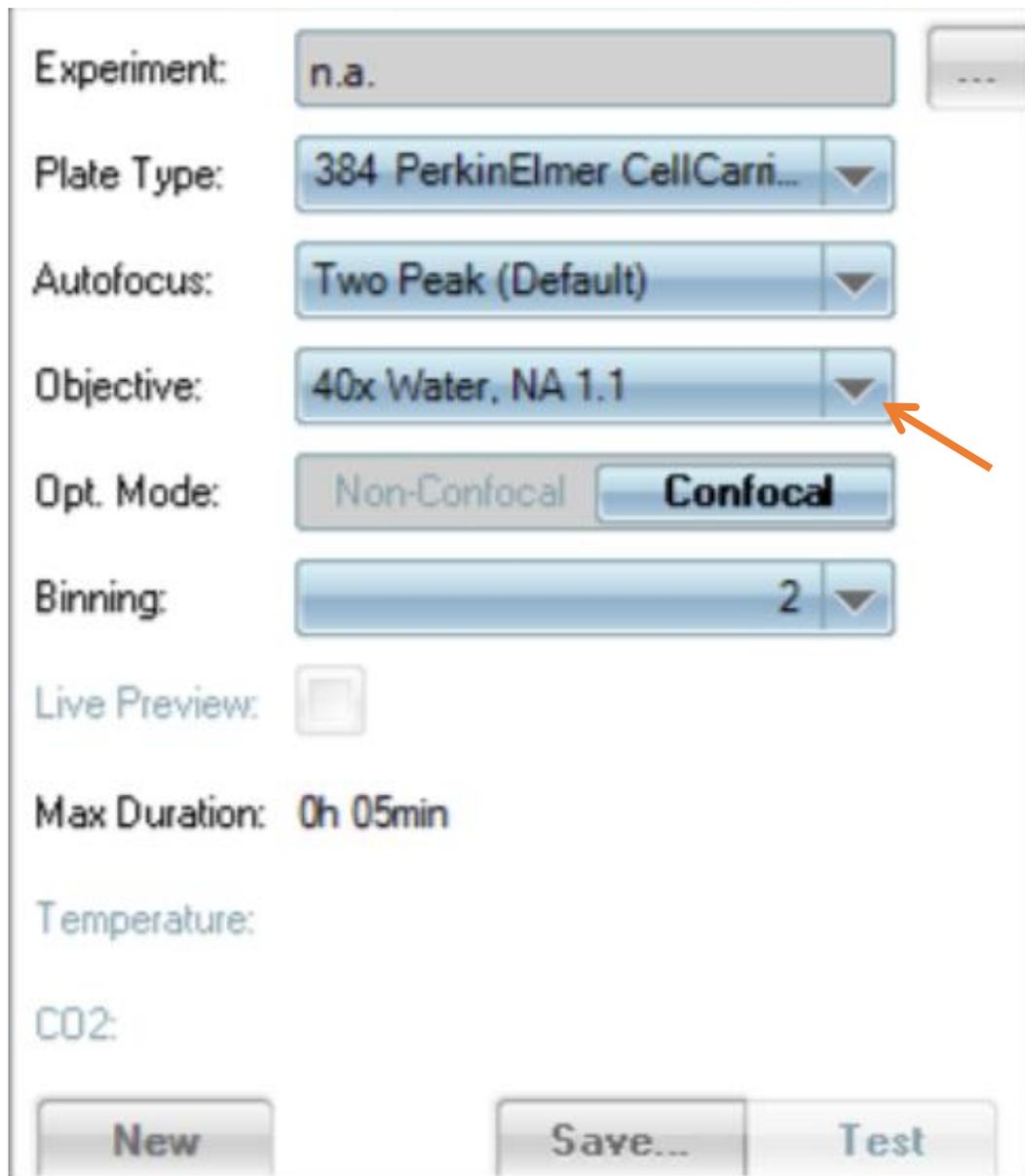
Temperature:

CO2:

New Save... Test

3 – Objective Selection

- The available objectives are listed on the laminated sheet next to the keyboard – objectives currently installed in the opera are marked with a red dash next to their name
- If you do not know which objective is suitable for your use, a technician can help you to decide
- Select the desired objective from the drop-down menu



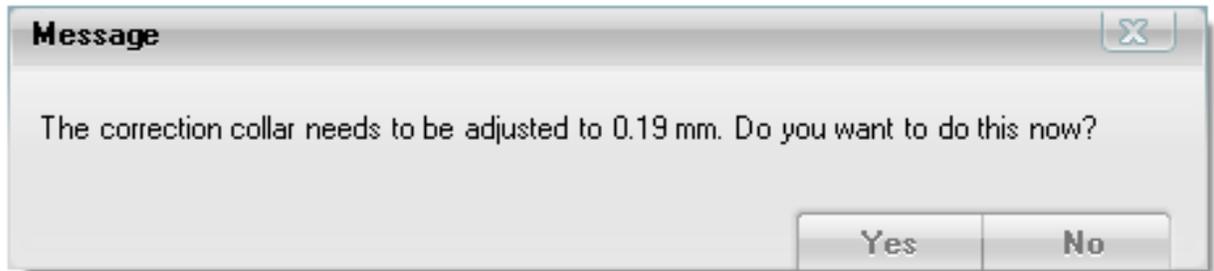
The screenshot shows a software interface with the following settings:

- Experiment: n.a.
- Plate Type: 384 PerkinElmer CellCarri...
- Autofocus: Two Peak (Default)
- Objective: 40x Water, NA 1.1 (indicated by an orange arrow pointing to the dropdown arrow)
- Opt. Mode: Non-Confocal (greyed out), Confocal (selected)
- Binning: 2
- Live Preview:
- Max Duration: 0h 05min
- Temperature:
- CO2:

Buttons at the bottom: New, Save..., Test

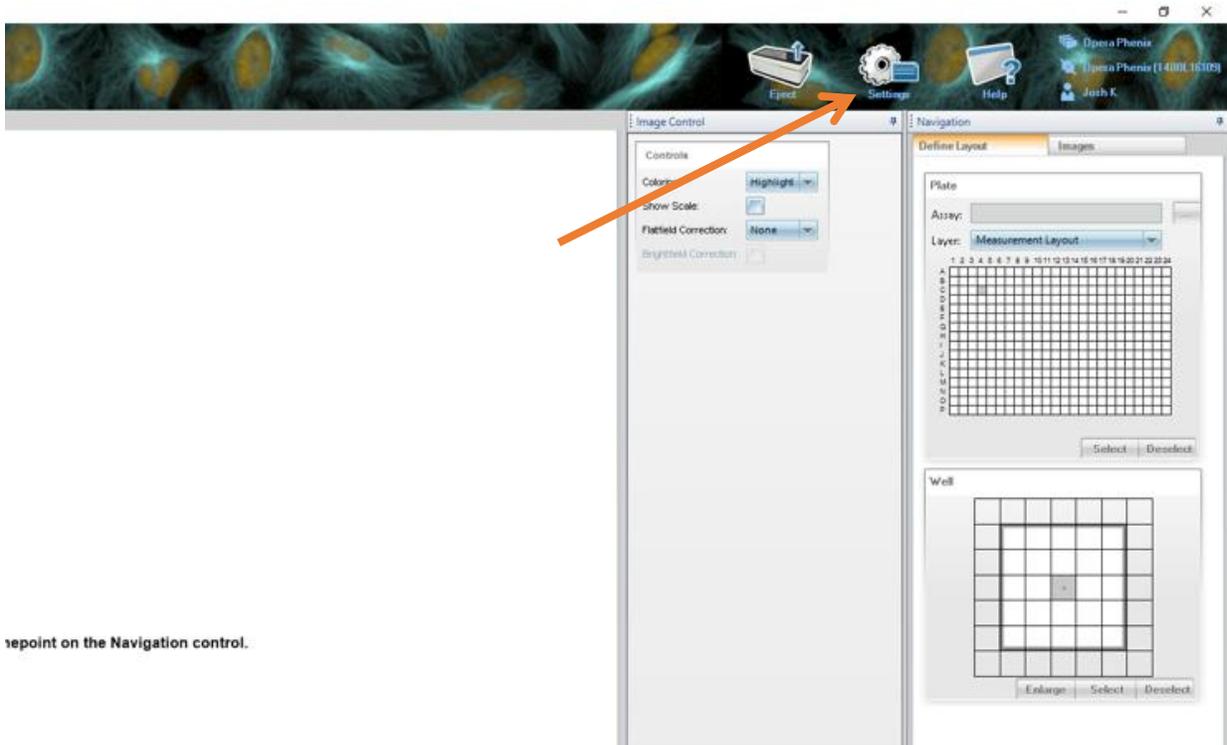
If using 20x air lens with a correction collar

- Remember to open the lid and adjust the correction collar as you were shown in training
- Assuming you have the correct plate type selected, when you select the lens a pop-up will appear telling you what the correction collar should be set to
- Select “Yes” to open the lid

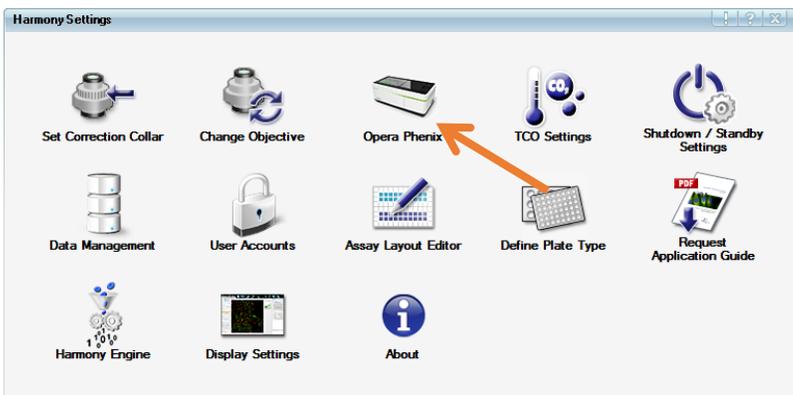


If using a Water Immersion Lens

- Don't forget to flush the lens twice as demonstrated during training

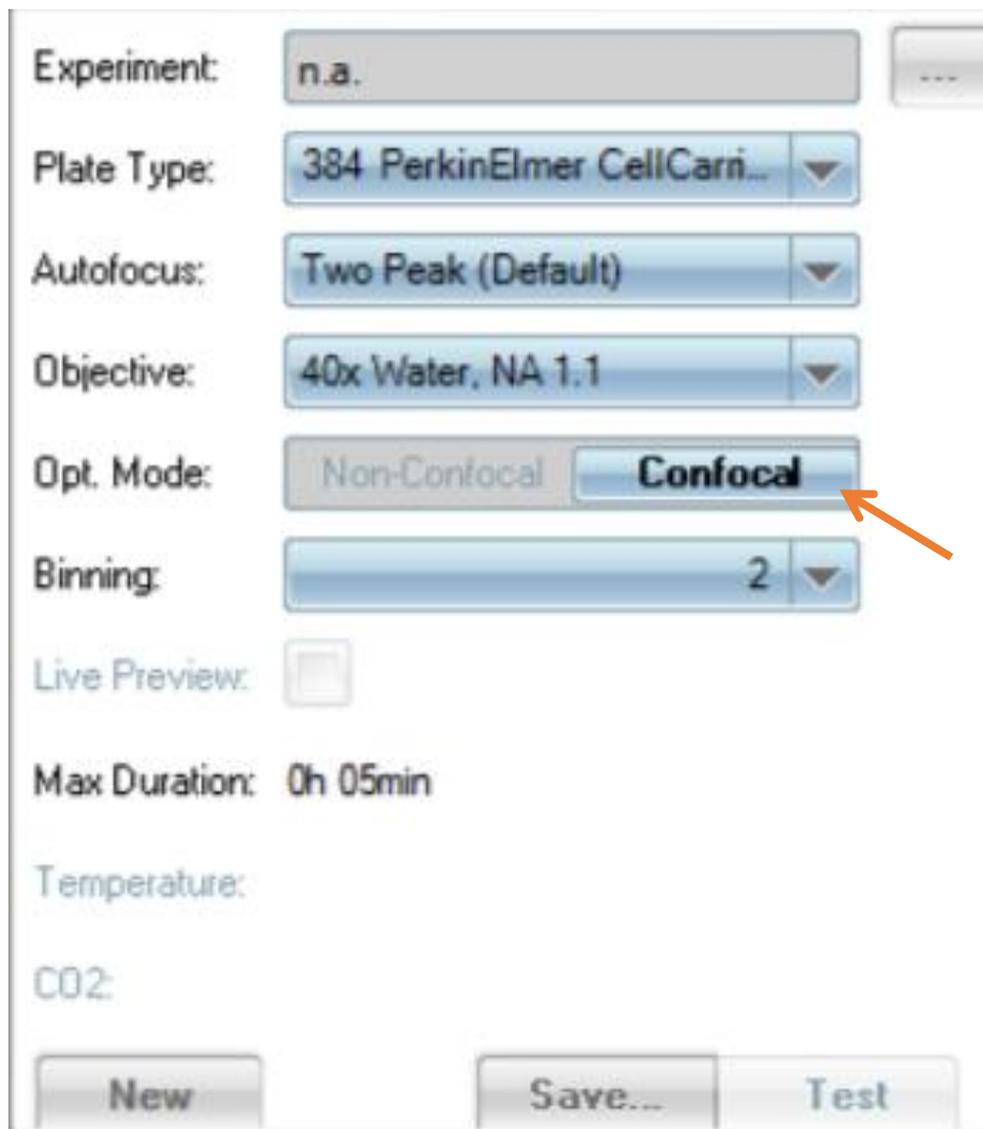


repoint on the Navigation control.



4 - Confocal vs Non-confocal Imaging

- Confocal gives higher resolution but lower signal intensity due to the blockage of out-of-focus light
- Non-confocal gives lower resolution but higher signal intensity – a good option for boosting your signal without increasing run time or laser power if your signal to background ratio is too small
- Selected option is highlighted in blue



Experiment: n.a.

Plate Type: 384 PerkinElmer CellCarni...

Autofocus: Two Peak (Default)

Objective: 40x Water, NA 1.1

Opt. Mode: Non-Confocal **Confocal**

Binning: 2

Live Preview:

Max Duration: 0h 05min

Temperature:

CO2:

New Save... Test

5 - Remaining Left-Hand Panel Options

- Autofocus – should nearly always be left on “Two Peak (Default)”
- Binning – a method of averaging pixels. Binning 2 will provide lower detail than binning 1 but increases signal intensity and reduces file size – 2 is the best option for most use cases
- Temperature and CO₂ options – if you need incubation options and do not know how to use them, please contact a technician to arrange training
- Live preview – should be ticked
- Max duration – gives an upper limit for how long your imaging could take with your current settings. Will adjust automatically as you adjust your experiment.

Experiment: n.a.

Plate Type: 384 PerkinElmer CellCarn...

Autofocus: Two Peak (Default)

Objective: 40x Water, NA 1.1

Opt. Mode: Non-Confocal **Confocal**

Binning: 2

Live Preview:

Max Duration: 0h 05min

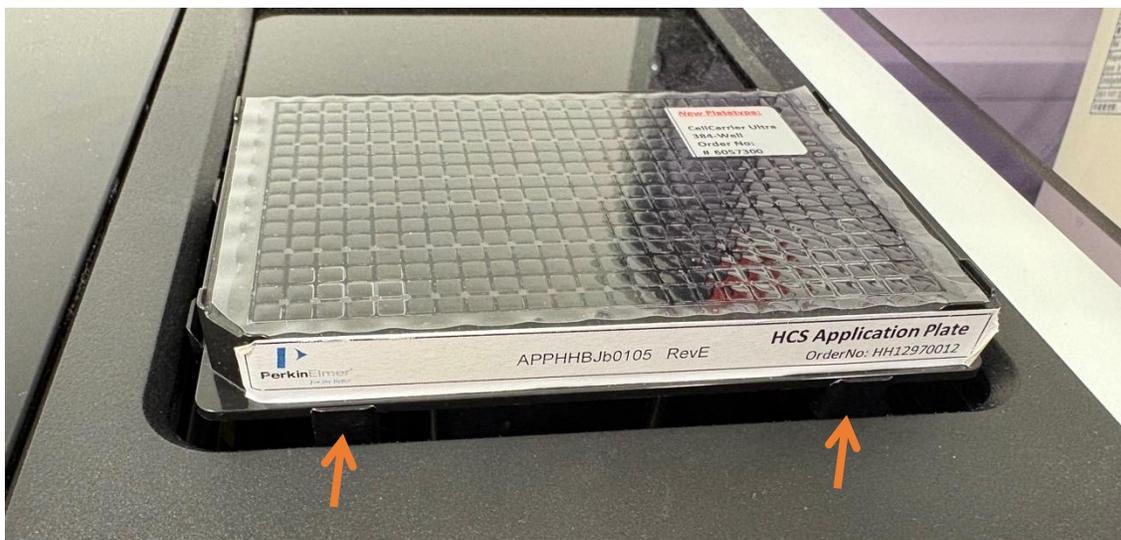
Temperature:

CO₂:

New Save... Test

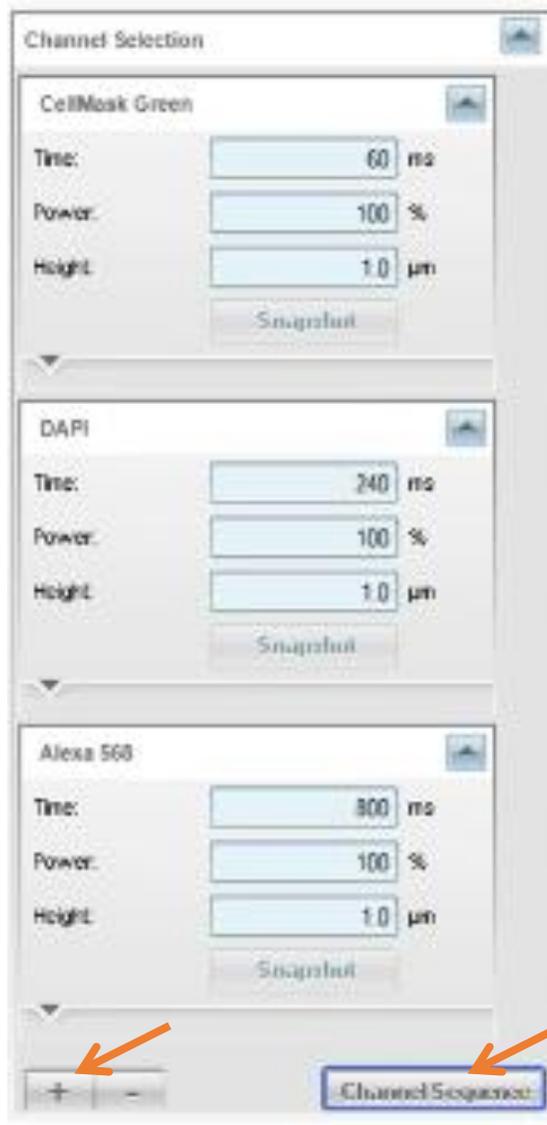
6 – Inserting your Plate

- Click on “Eject” on the top right of the page in Harmony to open the plate loader
- Clean the bottom of your plate with ethanol
- Insert your plate into the loader – **ALL 8 PRONGS SHOULD BE VISIBLE AROUND THE EDGES OF THE PLATE** – FEEL around the edge of your plate to check. This is very important, if the plate is not properly aligned in the holder it could damage the microscope. See below for example
- Once you have checked that your plate is positioned properly, click “Load”



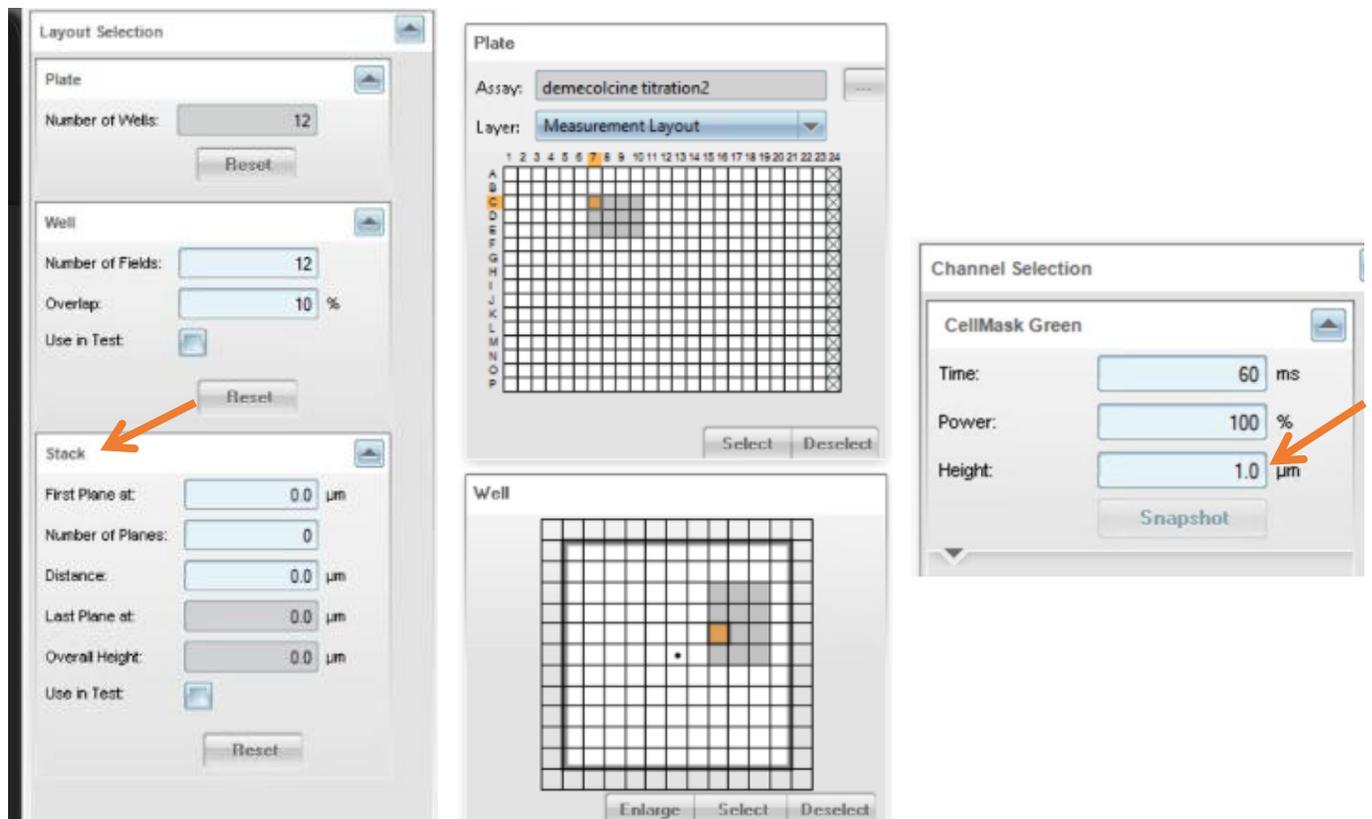
7 – Channel Selection

- The channels you need are most likely already available under PKI service
- Hit the + icon to add channels – this will bring up a database of folders, select “PKI Service” and look for the channel you want (unless you have your own channel setups saved somewhere)
 - The laser/filter combinations don’t allow you to label more than 4 channels – Alexa Fluor 546 and Alexa Fluor 568 use the exact same settings and you cannot image both!
- Selecting “Channel Sequence” will open a menu that allows you to separate channels during imaging to prevent crosstalk between overlapping channels



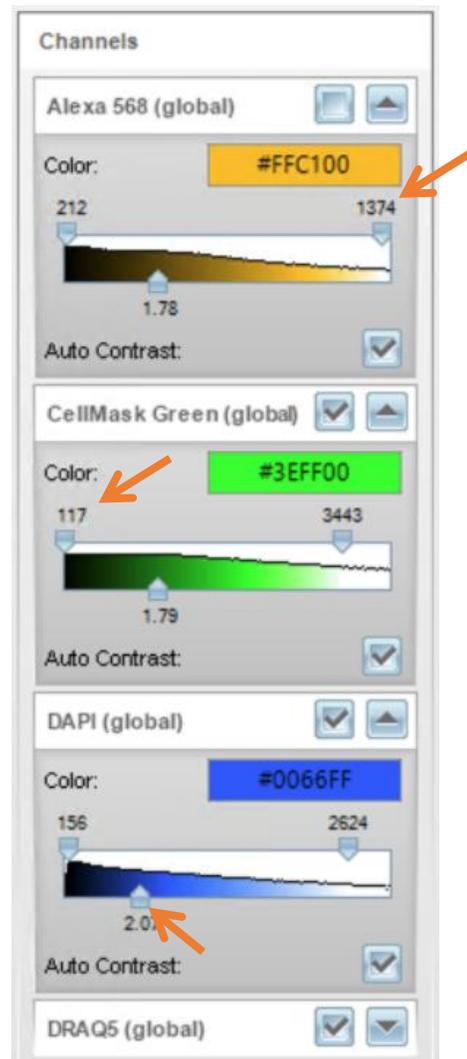
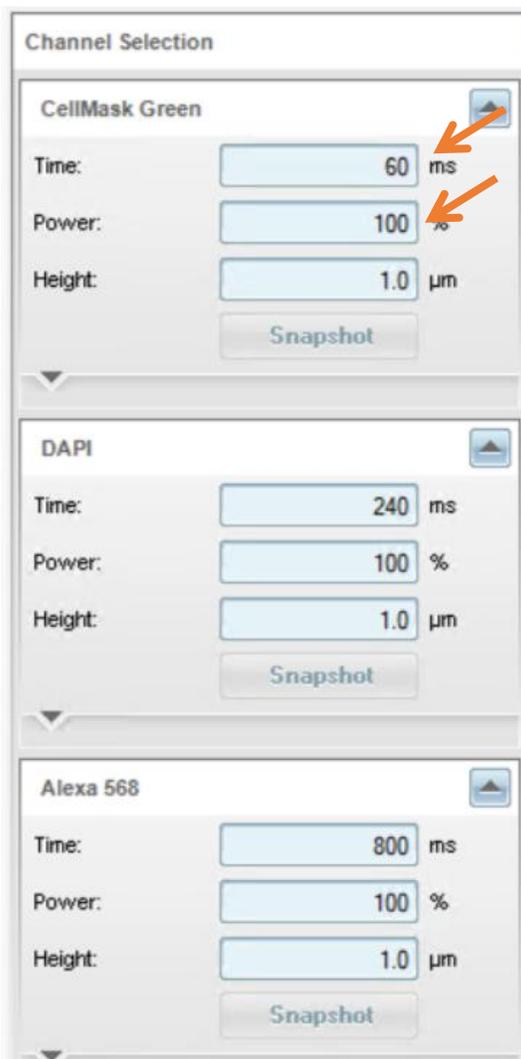
8 – Layout Selection

- Click on a well or field to highlight it in orange, click “select” to highlight currently orange wells in grey
- Grey wells and fields are used for tests and experiments, orange ones are used for snapshot. To avoid confusion, highlight the wells you wish to use in both colours
- Set up a coarse Z-stack to find your focal plane
- Run a test. Look through your test images to determine your focal plane
- If you wish to image in 3D, set up a new Z stack with your focal plane in the middle. To take a single 2D image, press “reset” on your Z-stack and set the height of each individual channel under channel selection as your focal plane height.

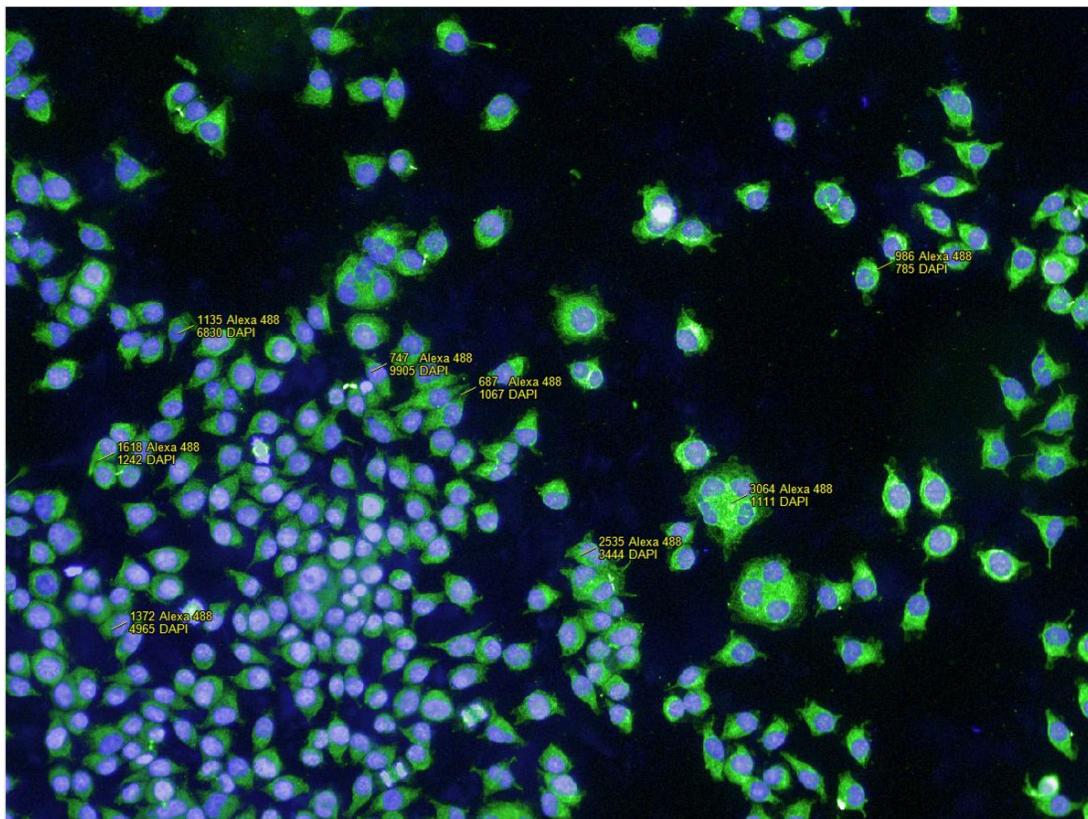
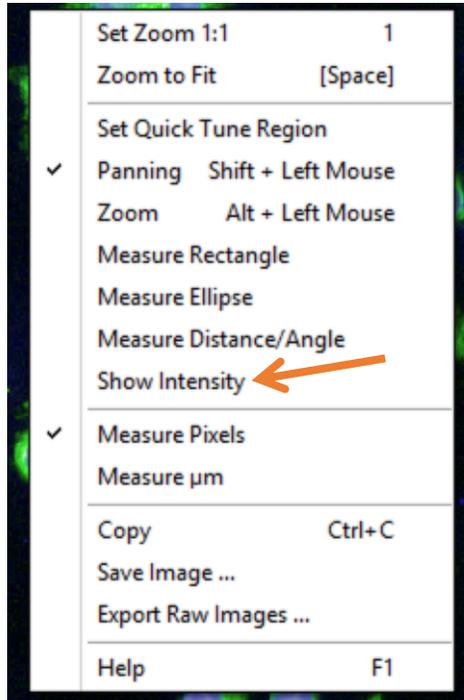


9 – Channel Optimisation

- 2 parameters to optimise – time (detector exposure time) and power (laser)
- If you have a low signal / background intensity ratio, increase either power or time for that channel in small increments, taking snapshots after each change to test its effects. Increasing these increases risk of bleaching.
- The LUTs can be used to view your current maximum and minimum intensities for each channel, as well as to adjust contrast
- You should aim for your signal to be at least 1000 units higher than your background for automated analysis
- Do not oversaturate your sample (max intensity is 50 000, anything above 40 000 and you should decrease your power or exposure)



- Right click on your live image and select “Show Intensity Values” to allow you to click and view local intensity anywhere on your image – do this for a couple of background areas and a couple of target areas to determine your average signal to background ratio



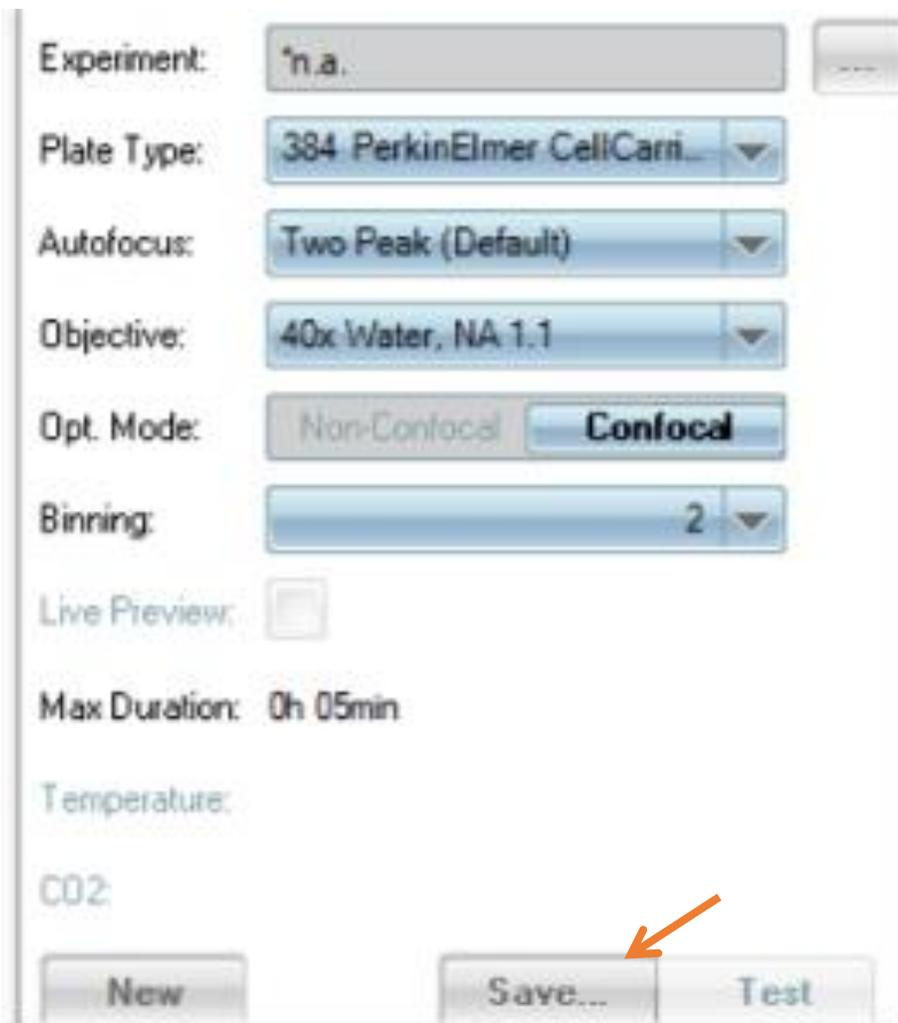
10 – Colouring Options

- Harmony comes with several built-in tools for visually adjusting your images
- Note that none of these tools change your raw data
- Under colouring options you can choose between standard, enhanced or highlight. Standard shows an image similar to what you would see under a normal confocal microscope. Enhanced and highlight can make it easier to see your targets within your sample but should be used cautiously as they can exaggerate features beyond the reality of what the data shows.
- Look up tables (LUTs) allow you to control the maximum and minimum intensity values that you want to display via the upper sliders, as well as controlling gamma with the lower slider
- There is also an “Auto Contrast” option that will allow the harmony software to automatically determine suitable contrast settings for your channels



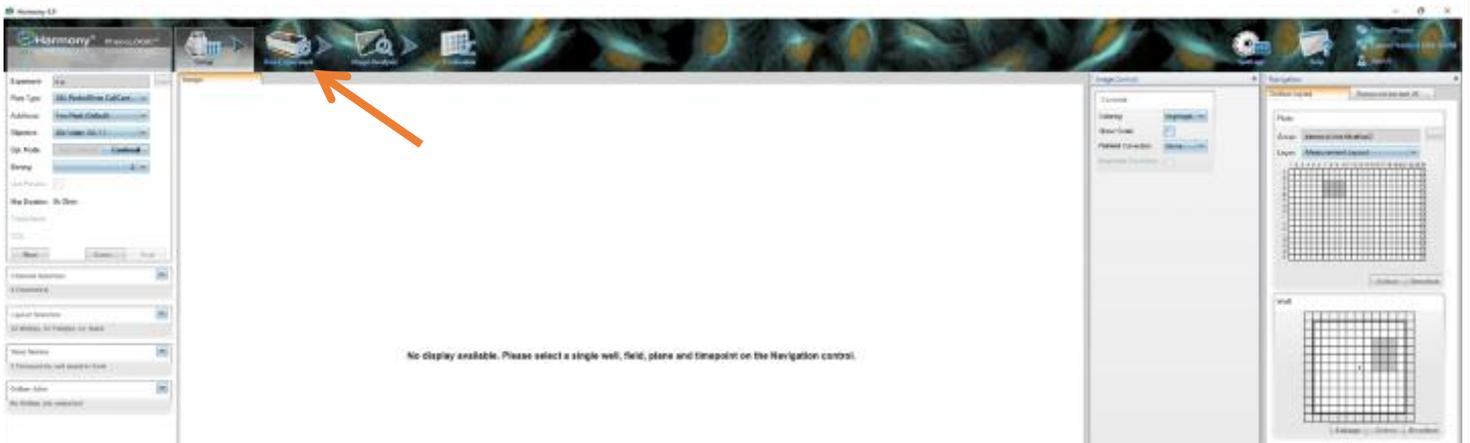
11 - Saving your Experiment

- “Experiment” in Harmony refers to all the imaging settings we have set up in this guide (excluding LUT settings)
- To save your experiment, click “Save” in the upper left panel
- This will automatically open your experiments folder within the harmony database
- Make sure you give your experiment a name



12 – Run Experiment

- Once you are happy with your settings and have saved them as an experiment, click on the “Run Experiment” tab
- Ensure that the details in the left-hand panel of this tab (plate, objective, environment settings, experiment selected etc.) are correct
- Give your measurement a name – this will be the name assigned to the file containing your images
- Click “Run”



13 – Data Management

- Don't forget to write and export your data within 48 hours of imaging to prevent it from being deleted
- For a detailed guide on data management in Harmony, as well as guides to Harmony image analysis, please refer to the WCIC website

<https://www.kclwcic.co.uk/operaphenix>

14 – Contacts

If you require assistance from an imaging technician, please contact:

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Chen Liang – +44 7469 356576

Josh Kellett -