BD Pathway Tutorial

1. Turn on system and explain OH&S
2. Install Objective
3. Install Sample holder and slide/plate
4. Setup → Geometry → Assign Objective
5. Select existing Lightpath and press edit, explain lightpath and flatfield correction. (also show Lightpath menu under Setup menu)
6. Open Multiwell plate menu and select suitable single well.
7. Select Eyepiece Viewing mode
8. Select Lightpath from Main menu shortcut and open shutter
9. Focus then close shutter and eyepiece.
10. Open Dye Setup menu and explain signal optimisation and segmentation, optimise RGB dyes and setup segmentation for DAPI nucleus.
11. Setup → Auto-ROI menu, test nucleus segmentation.
12. Open probe cycle menu and create probe cycle using RGB dyes.
13. Open Macro menu, create autofocus and RGB macro and run.
14. Open Multiwell menu, select 2 wells, create compound macro using RGB and run
15. Open Z-stack menu and test with 5 level stack.
16. Create compound Z-stack macro using RGB multiwell macro and run
17. Open Montage menu and test with 2x2 montage
18. Create compound montage macro using RGB multiwell macro and run
19. Open Assay Launcher and explain use.
20. Setup → XYZ menu, explain custom position definition and use through macro menu.

Menu Explanations

LightPath menu	Wavelength or Filter Selection, as well as Confocal mode selection and Flatfield correction. Ie: create a filter combination required for DAPI fluorophore.
Dye Setup menu	Image collection and optimisation, including thresholding, auto-segmentation etc. ie: adjust exposure settings, adjust contrast and gain of camera, set threshold required to auto-segment the nucleus of a cell stained with DAPI fluorophore.
Probe Cycle menu	grouping of dye setups required for one stage of experiment, ie: capture Red, Green and Blue images, and auto-segment as per each dyes setup.
Macro menu	Combine probe cycles, multiwell plate movement, autofocus, liquid handling etc, to perform an experiment. Ie: Move to well A1, autofocus, capture RGB image, auto-segment nucleus from image, move to well A2, repeat.
Assay Launcher	Assign macros to a button, for quick editing, plate selection and running.