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Title: **Use and Maintenance of the Licor Odyssey Scanner**

Version: **v2**

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SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
V2	01/01/2018	Update

1.0 Purpose –
The purpose of this SOP is to outline the principles of the routine use and maintenance of the Odyssey in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to routine use and maintenance of the Odyssey within the SASoM.

3.0 Responsibilities –
It is the responsibility of all users of the Odyssey within the SASoM to comply with this SOP.

4.0 Procedure –
Principles of Operation:
The Li-Cor Odyssey is used to scan for immuno-reactivity by infrared imaging.

For Western blot scanning:
Turn on the instrument power switch, and turn on computer.

Open the lid of the Odyssey scanner by lifting up the front edge of the lid.

Wipe the scanning surface with soft tissue with 70% ethanol followed by distilled water.

Put the membrane on the glass plate with sample surface down and the top of the membrane facing the front of the scanner.

Flatten the membrane by placing the soft square pad on it.

Remember the area on which the membrane is according to the alignment guide at the left and bottom edge of the scanning surface.

Close the lid by pulling down gently.

UV light can harm eyes & skin. Do not try to take measurements with the sample chamber open.

Log in to the linked computer with the user's name.

Double click on the 'Odyssey V3.0' icon on the desktop to open the software.

Choose File>New to create a new project.

Enter the directory pathway and project name in the New Project dialog box.

Click the blue arrow icon on toolbar to start a scan. Both the user name and the password are 'Breakthrough'. Click 'OK'.

The scanner console window opens after log in.

Parameter settings.

Group: Breakthrough
Preset: membrane
Resolution: 169µm
Quality: medium
Focus offset: 0.0 mm
Selection of the channel(s) depends on which fluorophore is conjugated to the secondary antibodies. If IRDye 680 is used, tick the channel of 700. Tick 800 for IRDye 800CW. Tick both channels if both secondary antibodies are used. Scan area (cm): it can be defined by setting the values of 'X cord' & 'Y cord' in origin and 'width' & 'height' in Size. Or press the left mouse button and drag the cursor on the area definition grids. The settings of 'Intensity' are flexible. The bigger the values, the higher the intensity of both the bands and background. Try different values and click preview to see what values can cause best signal-to-noise ratio. Click 'start scan' once the optimal intensity values are decided.

Click 'save…' to save the scan after the scan is complete.

Enter a scan name or leave the default scan name as it is.

Enter a name for the first analysis of the images and click 'OK'. If you need to flip or rotate the images before saving them, Click 'Advanced'.

DO NOT do any modification to the folders containing the scan. If users want to analyze the images with other softwares (e.g. Microvigeine), please copy and paste the folders to another drive.

For Reverse phase Protein array (RPPA) scanning:
The operation is the same as Western Blot scanning except the parameter settings.

Parameter settings for RPPA:

Group: Breakthrough
Preset: membrane
Resolution: 21µm
Quality: medium
Focus offset: 0.0 mm
Selection of the channel(s) depends on which fluorophore is conjugated to the secondary antibodies. If IRDye 680 is used, tick the channel of 700. Tick 800 for IRDye 800CW. Tick both channels if both secondary antibodies are used. Scan area (cm): it can be defined by setting the values of 'X cord' & 'Y cord' in origin and 'width' & 'height' in Size. Or press the left mouse button and drag the cursor on the area definition grids. The settings of 'Intensity' are flexible. The bigger the values, the higher the intensity of both the bands and background. Try different values and click preview to see what values can cause best signal-to-noise ratio. Click 'start scan' once the optimal intensity values are decided.

For In-cell Western scanning:
The operation is the same as Western Blot scanning except on how to place the black/clear 96-well plates in step 3 and the parameter settings.

Place the 96-well plates on the scanning surface with bottom down and the edge with column number'1,2,3…..12' facing the rear of the scanner. There is no need to use the soft flattening pad.

Parameter settings for RPPA:

Group: Breakthrough
Preset: membrane
Resolution: 169µm
Quality: medium
Focus offset: 4.0 mm
Selection of the channel(s) depends on which fluorophore is conjugated to the secondary antibodies. If IRDye 680 is used, tick the channel of 700. Tick 800 for IRDye 800CW. Tick both channels if both secondary antibodies are used. Scan area (cm): it can be defined by setting the values of 'X cord' & 'Y cord' in origin and 'width' & 'height' in Size. Or press the left mouse button and drag the cursor on the area definition grids. The settings of 'Intensity' are flexible. The bigger the values, the higher the intensity of both the bands and background. Try different values and click preview to see what values can cause best signal-to-noise ratio. Click 'start scan' once the optimal intensity values are decided.

5.0 Personal protection -
Howie coat must be worn at all times.

6.0 General maintenance -
Clean surfaces of the apparatus with soft cloth and mild detergent**.**

Glass surface should be cleaned after use.

7.0 Training -
All users have to be trained before using the Instrument by a designated person.

8.0 Related documents –

8.1 Equipment manual

8.2 Equipment Maintenance Information sheet

8.3 Risk assessments – RA/GEN/030, COSHH/013

9.0 Approval and sign off –

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