SOP#: 006	Date Issued: 8/03/2021	Date Revised: 7/08/2021
TITLE:	**LI-COR Odyssey CLx Imager**	
SCOPE:	Research Personnel	
RESPONSIBILITY:	BORC staff	
PURPOSE:	To outline the proper procedures for use and maintenance of the LI-COR Odyssey CLx Imager.	

1 PURPOSE

This SOP explains how to apply LI-COR Odyssey CLx Imager in biomedical research such as western blots, cell-based assay, ex vivo imaging etc.

2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3 BEFORE CONDUCTING YOUR EXPERIMENT

3.1 The following procedure is recommended before every scan:

3.1.1 Thoroughly clean the glass scanning surface with ultrapure water and wipe with a lint-free tissue.

3.1.2 Repeat the wash with isopropanol to remove any visible smears. Use ethanol to remove any remaining residues. If dye contamination continues to be a problem, slightly wet a cloth with acetone and wipe the glass.

Important: Do not allow the acetone to contact anything other than the glass. The paint can be damaged by acetone.

3.1.3 If the silicone mat is used, rinse it under warm water. Gentle lab soap may be used, but the soap must be completely rinsed away before use. The silicone mat may also be rinsed with isopropanol if needed. Dry the silicone mat with a lint-free tissue.

3.2 Tips for different application

3.2.1 Using Membranes

For Western blotting methods, nitrocellulose or PVDF membranes may be used (see www.licor.com/bio for the latest membranes and kits). Pure-cast nitrocellulose membranes are recommended for best performance. Detailed blotting protocols can be found in the Odyssey CLx Application Protocols manual. There are some general tips, however, for using membranes with the Odyssey CLx Imager.

- Membranes are placed on the scanning surface with the sample surface down and the top of the membrane facing the front of the instrument.
- Do not touch the membrane – handle only with a clean, smooth-edged forceps. Lift the membrane only by the corners. Fingerprints, even from a glove, will show clearly when imaged.

- Use the silicone mat included to cover the membrane(s) before scanning. Use the 4" soft roller included to remove any air bubbles that may be present. These optional steps help keep the membrane flat against the scanning surface for optimum imaging.
- Protect the membrane from light until it has been scanned.
- Keep the membrane wet if it is to be stripped and re-used. For Western blots store dry or in PBS buffer at 4°C.
- Use clean containers to avoid cross-contamination and reduce background.
- Multiple membranes can be washed together, provided there is ample volume so each membrane moves freely.
- If the signal on the membrane is too strong, the saturated pixels will appear cyan (blue) in the image. Re-scan at a lower intensity setting in Manual mode, or use the Auto mode.
- The fluorescent signal on a dried membrane will remain stable for several months or longer if protected from light.

3.2.2 Using Gels

A protocol for In-Gel Westerns is provided in the Odyssey® CLx Imager Application Protocols Manual. Coomassie-stained gels can also be scanned since Coomassie Blue dye can be seen clearly in the 700 nm channel, and faintly in the 800 nm channel (see the Western Blot Analysis protocol for details). Nucleic acids stained with Syto® 60 and separated in a gel can be imaged in the 700 nm channel (see the Syto 60 Staining of Nucleic Acids in Gels protocol for more information). To scan a gel, follow these procedures:

1) Thoroughly rinse the gel with destaining solution or water to remove dye particulates.
2) When placing the gel on the scanning surface, take care not to trap air bubbles underneath. Cover the gel with plastic wrap to prevent drying, if desired.
3) Scan the gel in the 700 nm channel.
4) Adjust the focus offset for the gel thickness. The correct focus offset is 1/2 the thickness of the gel; for a 1 mm gel, set the focus offset to 0.5 mm. The maximum offset is about 4 mm in the Odyssey CLx Imaging system, allowing gels of up to 8 mm to be scanned.
5) After removing the gel, clean the glass surface to remove any residual dye by following the instructions in the section ***3.1***.

3.2.3 Using Microplates

Microplates that meet certain physical characteristics can be scanned directly on the Odyssey CLx Imager scanning surface. Proper selection of microplates significantly affects the results of your analysis as each plate has its own characteristics including well depth, plate auto-fluorescence, and well-to-well signal crossover. Some general considerations for microplate selection are provided here.

- The plastic microplate alignment guide (P/N 9891-080) should be placed on the scan surface so the corner of the guide contacts the front, left corner of the bezel surrounding the scan surface. Push the guide into the corner until it contacts the bezel on both the front and left sides. Put the microplate on the scanning surface

and slide it into place until it contacts both the front and left side of the alignment guide. The first well in the first row (A1) should be toward the back and left side of the alignment guide as shown

- Plate dimensions must be such that the distance from the Odyssey CLx Imager scanning surface to the target detection area of the plate is 4.0 mm or less.
- In order to avoid well-to-well signal spread, black-walled, clear bottom plates should be used for assays that involve imaging of a liquid. Since the In-Cell Western™ Assay uses detection at the well surface with no liquid present, both clear and black-walled plates can be used. Consult protocols in the Application Protocols manual or on the LI-COR® Bio Technical Resources Library (http://www.licor.com/bio/support) for specific recommendations.
- Do not use plates with white walls because the auto-fluorescence from the white surface will create significant noise.
- For an In-Cell Western™ Assay requiring sterile plates for tissue culture growth, the following
  plates are recommended by LI-COR Biosciences.
  96-well format Nunc® (P/N 161093) Clear
  96-well format Nunc (P/N 165305) Black
  96-well format Falcon™ (P/N 353075) Clear
  96-well format Falcon (P/N 353948) Black
  384-well format Nunc (P/N 164688) Clear
  384-well format Nunc (P/N 164730) Black
  384-well format Falcon (P/N 353961) Clear
  384-well format Falcon (P/N 353962) Black
  - Before plate scanning, clean the bottom plate surface with a moist, lint-free paper to remove any obstructions. Additionally, the Odyssey CLx Imager scanning surface should be thoroughly cleaned using the procedures described earlier in this chapter.
  - Protect plates from light before imaging to ensure highest sensitivity. When storing plates after imaging, protect plates from light at room temperature.
  - A **Focus Offset** of 3.0 mm should be entered in the scan parameters when using the plates specified above.
  - Plates other than those recommended above may require lower or higher focus offsets for optimal resolution and detection. If alternative plates are used, an initial optimization scan will be necessary. Scan a plate containing experimental and control samples at 0.5, 1.0, 2.0, 3.0, and 4.0 mm focus offsets. Use the same intensity settings for each scan. After reviewing the collected scans, use the focus offset with the highest signal-to-noise ratio as the focus offset for experiments with the alternate plates.
  - Select the Automatic Channel Intensities for a wide dynamic range. If using the Manual Channel Intensities mode, the **Intensity** scan parameter for both 700 and 800 nm channels should be set to 5 for initial scanning. If the image signal is saturated or too high, re-scan using a lower intensity setting (i.e. 2.5). Likewise, if the image signal is too low, re-scan using a higher intensity setting (i.e. 7.5).
- l For satisfactory images with minimal scan time, the **Quality** scan parameter should be lowest, with **Resolution** set to 169 µm. Higher scan quality and resolution may be used, but scan time will increase.

3.2.4 Using the MousePOD Accessory

The optional Odyssey® MousePOD® Accessory fits over the Odyssey CLx Imager scanning surface for ***in vivo*** imaging of up to three mice or one rat in a temperature-controlled enclosure. Operation of the MousePOD Accessory is described in the Odyssey ***In vivo*** Imaging Guide available at: http://www.licor.com/bio/support.

4 PROCEDURES

4.1 Start Image Studio Software

Double-click the Image Studio icon on the desktop to start Image Studio Software.

4.2 Set Up a Work Area

The first time you start Image Studio, you will need to create a Work Area. A Work Area contains related images, analyses, and software settings.

4.2.1 Add a Work Area

1) Click Create New... in the Set Active Work Area dialog.
2) In the Name field, type a name for your Work Area. Browse to the location of the Overall folder where you want to save the Work Area, and click Save

4.2.2 Remove a Work Area

To remove a Work Area from the Set Active Work Area list, select the Work Area to remove and click Remove from List.

- l Removing the Work Area from the list does not delete the Work Area folder or its contents from your hard drive.
- l You can add the Work Area back to the list using the Add Existing option.

4.3 Connect to the Instrument

The first time you connect, power on the instrument and wait several minutes to allow the instrument to establish a network address. The software will discover the instrument automatically.

4.3.1 Select the correct instrument in the Instrument Model Selection dialog.

4.3.2 Click OK. Image Studio Software will open your Work Area and search for the instrument.

4.4 Acquire an Image

4.4.1 Place a sample on the scan bed (see **3.2** for tips for different applications). Click the Acquire tab and choose the following settings：

- Setup group.
  - Select a Membrane Scan Preset from the Scan Preset list .
  - Select No Analysis in the Analysis Type list.
- Channels group
  - Click Auto .
  - Select the check box next to the desired channel(s) (700, 800, or both) to designate which channels to image.
- Scan Area group

- Select No Image from the list.
- Click Draw New and drag a new rectangle onto the scan grid. Any previously drawn scan areas will be erased.
- Scanner group

4.4.2 Click **Start** to start the scan.

4.5 Adjust Image Display

4.5.1 Choose Display

After acquiring an image, the Choose Display dialog will open. Click the view with the best display.

4.5.2 Adjust Display

The Adjust Display dialog will open to allow for further adjustments. Click Dimmer or Brighter to optimize the image display.

4.5.3 Finer Adjustments

For finer adjustments, brightness and contrast adjustments can be made using the adjustable sliders on the Display panel on the right side of the window.

1) Click Curves to toggle the view to the Curves Image Display Adjustments.
2) Adjust the three points on the histogram. To view the entire histogram, click the Show All icon.

4.6 Export Image for Publication or Presentation

4.6.1 Click the Images tab above the table and then click on the image to be exported.

4.6.2 Click the Image Studio Application button and hover over Export.

- To export an image suitable for use in a slide presentation, click Image for Digital Media.
- To export an image for use in a journal or other print media, click Image for Print.
- Ensure the image size is set appropriately for your needs. Click Reset Inches (or Reset Pixels) to set the exported image size to the original size of the image.

4.6.3 Click Save

5 MAINTENANCE

The Odyssey CLx Imager requires only minimal maintenance as routinely inspecting the system and the scanning surface. However, as with any equipment utilizing electrical voltages, there is a danger of fire or electrical shock if the equipment is not properly maintained.

- Wipe all chemical spills from the case and/or scanning surface to prevent damage to the surface coating.
- Inspect all cables and power cords for evidence of fraying, exposed wire, or loose connections.
- Keep the scanning surface free of organic solvents and other combustibles.
- Clean the exterior case parts with warm water and a damp cloth. The exterior case parts are
- painted with a durable urethane coating that is resistant to chemical spills. Do not use scouring
- compounds or solvents (e.g., acetone, benzene, carbon tetrachlorides, lacquer thinner, or alcohol) to clean the case.

6 REFERENCES

Refer to the manufacturer's manual for additional information.

END OF DOCUMENT