ODYSSEY® Sa

Odyssey Sa Infrared Imaging System

Operator's Manual

Version 2.0

LI-COR®
Biosciences

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Biosciences

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Declaration of Conformity

Manufacturer's Name: LI-COR, Inc.
Manufacturer's Address: 4647 Superior Street
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declares that the product

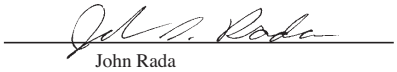
Product Name: Odyssey Sa Infrared Imaging System
Model Number(s): 9260
Product Options: All

conforms to the following Product Specifications:

Safety: UL 61010A-1: 2002
CAN/CSA C22.2 No. 1010.1: 1992
EN61010-1: 2001 (2nd Edition)
21 CFR Chapter 1 Subchapter J: Class 1 Laser Product
IEC 60825-1: 1993, A1: 1997, A2: 2001: Class 1 Laser Product

EMC: FCC 47 CFR Part 15.109 Radiated Emissions, Class A
FCC 47 CFR Part 15.107 Conducted Emissions, Class A
EN 55011: 2007: Radiated Emissions, Class A
EN 55011: 2007: Conducted Emissions, Class A
EN 61000-3-2: 2000, A1: 2001, A2: 2005: Harmonic Current Emissions
EN 61000-3-3: 1995, A1: 2001: Voltage Fluctuations and Flicker
EN 61326-1: 2006: Electrical equipment for measurement, control and laboratory use
IEC 61000-4-2: 1995: ESD, 4kV/4kV Contact/Air
IEC 61000-4-2: 1995, A1: 1998, A2: 2000: ESD, 4kV/4kV Contact/Air
IEC 61000-4-3: 2006: Radiated RF Immunity, 3V/m
IEC 61000-4-4: 2004: EFT - AC Mains Supply, 1kV
IEC 61000-4-5: 2005: Surge - AC Mains Supply, 0.5kV L-L, 1kV L-PE
IEC 61000-4-6: 2004: Conducted Immunity - AC Mains Supply, 3Vrms
IEC 61000-4-11: 2004: Voltage Interrupt, 250 cycles / 100%
IEC 61000-4-11: 2004: Voltage Dips, 0.5, 1, 25 cycles / 0, 0, 70%

Supplementary Information:
The product herewith complies with the requirements of the Low Voltage Directive 2006/95/EC (formerly 73/23/EEC) and the EMC Directive 2004/108/EC (formerly 89/336/EEC).



September 1, 2009
Document #53-11074

John Rada
Director of Engineering

Note on Safety

LI-COR products have been designed to be safe when operated in the manner described in this manual. The safety of this product can not be guaranteed if the product is used in any other way than is specified in this manual.

Equipment markings:

The product is marked with this symbol when it is necessary for you to refer to the manual or accompanying documents in order to protect against damage to the product.

The product is marked with this symbol when a hazardous voltage may be present.

The product is marked with this symbol for a Chassis Ground connection.

WARNING **Warnings** must be followed carefully to avoid bodily injury.

CAUTION **Cautions** must be observed to avoid damage to your equipment.

Manual markings:

WARNING **Warnings** must be followed carefully to avoid bodily injury.

CAUTION **Cautions** must be observed to avoid damage to your equipment.

NOTE **Notes** contain important information and useful tips on the operation of your equipment.

Notice

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Printing History

Publication Number: 977-11497
Version 2.0: September 2010

Federal Communications Commission
Radio Frequency Interference Statement

WARNING: This equipment generates, uses, and can radiate radio frequency energy and if not installed in accordance with the instruction manual, may cause interference to radio communications. It has been tested and found to comply with the limits for a Class A computing device pursuant to Subpart J of Part 15 of FCC rules, which are designed to provide a reasonable protection against such interference when operated in a commercial environment. Operation of this equipment in a residential area is likely to cause interference in which case the user, at his own expense, will be required to take whatever measures may be required to correct the interference.

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Chapter 6: Appendix

Chapter 1: Safety and Operational Information

In This Manual...

The following topics are discussed in this manual:

- Safety considerations.
- Instrument placement and power considerations.
- Description of detection optics and signal processing.
- Operation of the Odyssey® Sa Imager.

Safety Considerations

Laser Safety

The Center for Devices and Radiological Health (CDRH) was established in October, 1982, by the U.S. Food and Drug Administration (FDA) to protect the public health in the fields of medical devices and radiological health.

Manufacturers of products subject to performance standards under the Radiation Control for Health and Safety Act of 1968 are required to furnish various reports to the CDRH.

The Odyssey Sa Infrared Imaging System is certified as a Class I laser product, containing a Class IIIb laser. This means that hazardous laser radiation is *not* emitted outside the instrument. Radiation emitted

inside the instrument is confined within protective housings and external covers. The laser beam cannot escape during any phase of user operation.

The CDRH implemented regulations for laser products on August 1, 1976 (CDRH radiation performance standard 21, Code of Federal Regulations Chapter 1, Subchapter J). Compliance for products marketed in the United States is mandatory. The label that must be attached to laser products marketed in the United States is Figure 1-1 and is located on the rear panel of the Odyssey Sa Imager, indicating compliance with CDRH regulations.

CAUTION: *Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.*

The Odyssey® Sa Infrared Imaging System contains two lasers: one emitting at 785 nm and one at 685 nm. Each has a peak power rating of 30 milliwatts. The 685 nm laser emits visible laser radiation – **direct exposure to either beam may cause eye damage**. Laser radiation is emitted through apertures at the top of the microscope assembly, inside the instrument enclosure.

Safety Interlocks

There are no user serviceable parts inside the Odyssey Sa Imager and it is not recommended that users open the instrument enclosure. If the top panel of the instrument is opened, safety interlocks immediately turn off the lasers. Audible tones also sound when the top panel of the enclosure is opened and closed.

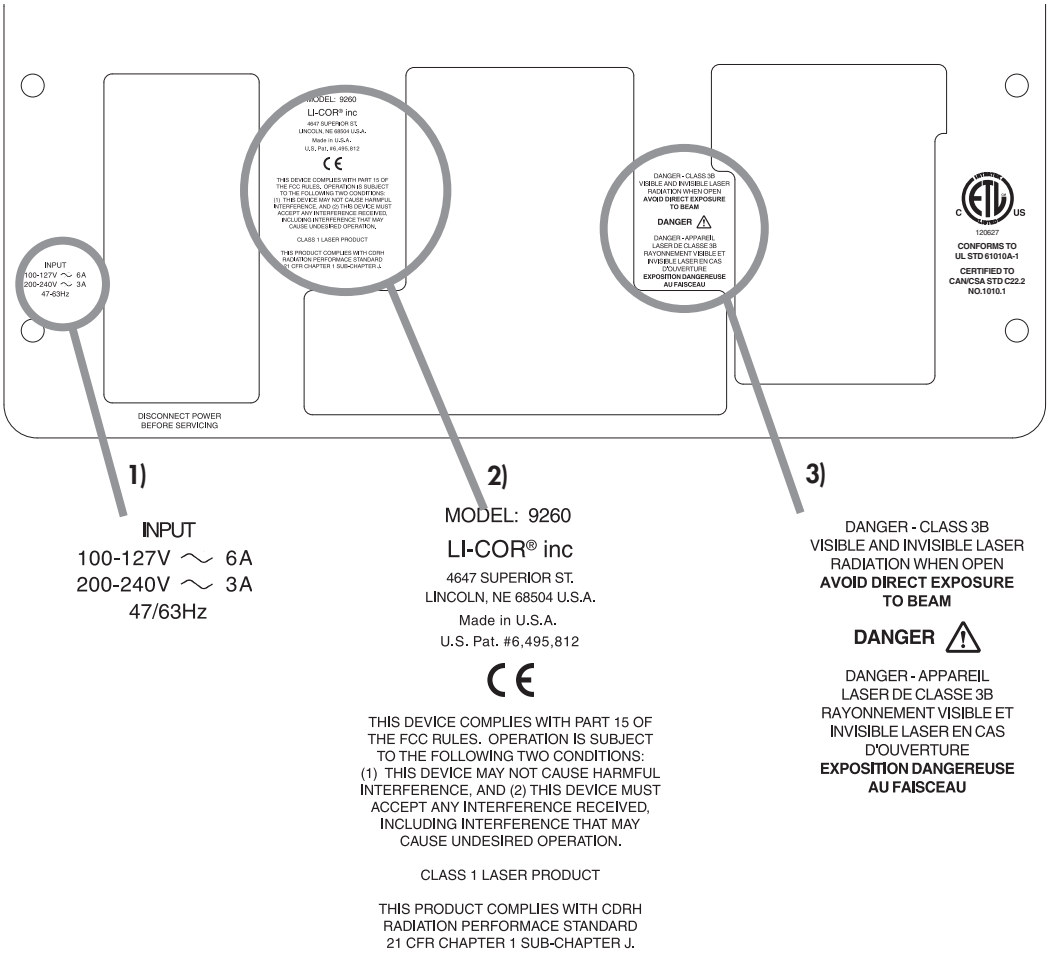


Figure 1-1. Back panel label of the Odyssey® Sa Imager showing the following: 1) Power requirements, 2) FCC and CDRH compliance notices, and 3) Laser radiation exposure warning.

Chemical Safety

LI-COR® Biosciences recommends that all biochemicals be handled carefully, and that safe laboratory procedures be followed at all times. Be aware of the hazards associated with any chemical before you begin work.

The Odyssey® Sa Imager should not be used with any radioactive materials.

Placement in the Laboratory

The Odyssey Sa Imager weighs approximately 26 kg (56 lb). When combined with the BioTek® BioStack™ Automated Microplate Stacking System, the combined weight is 34 kg (75 lb). The system should be placed on a level laboratory bench that is sufficiently sturdy to bear its weight.

Ambient Laboratory Conditions

Place the Odyssey Sa Imager away from external heat sources (furnaces, windows, etc.). Additional heating can cause high temperatures within the enclosure, resulting in instrument shut down. Place the instrument away from sinks or other sources of water that pose a shock hazard. Recommended operating conditions are 15-35°C and a dew point not greater than 19°C. Extended operation outside these conditions will cause damage to the instrument.

Ventilation

The instrument enclosure and circuit boards are cooled with two internal fans. There are no restrictions regarding placement of the instrument with respect to the fan cover; the fan shrouds are not filtered (requires no service), and the cover serves only as an exhaust outlet.

Space Requirements

The Odyssey® Sa Imager requires an area approximately 44 cm W (17") × 57 cm D (22"), and a vertical clearance of at least 37 cm (14"). When used with the BioTek® BioStack™ microplate stacking system, the area required is approximately 61 x 122 cm (24 x 48").

Moving the Odyssey Sa Imager

Be cautious when moving the instrument. Use a minimum of two people when moving the Odyssey Sa Imager, as it weighs about 26 kg (56 pounds). Lift under each side of the instrument, and keep the instrument level as it is moved. Gently set the Odyssey Sa Imager at its new position. If you intend to ship the instrument, contact LI-COR® Technical Support for instructions on how to prepare the instrument for shipment.

Each of the 4 legs on the bottom of the instrument case are threaded and can be turned in either direction to adjust its height. After moving the instrument, turn the feet all the way in and then back out one turn so that all the legs are the same height. Press downward on each of the four corners of the instrument to find out if the instrument rocks and whether all four legs contact the surface. Adjust any legs as necessary to prevent the instrument from rocking.

Electrical Considerations

Power Cords

The Odyssey® Sa Imager is equipped with a 3-wire grounding-type plug. This plug will only fit into a grounding-type outlet. This is a necessary safety feature. If you are unable to insert the plug into the outlet, you will need to replace the outlet. Do not defeat the purpose of the grounding-type plug.

Do not locate the Odyssey Sa Imager where the power cord will be walked on or exposed to water or chemical spills.

The Odyssey Sa Imager draws a maximum of 2.25 amperes at 120V. If an extension cord is used, make sure the total of the ampere ratings on the instruments plugged into the extension cord does not exceed the extension cord ampere rating. Also make sure the total amperage of instruments plugged into the wall outlet does not exceed the amperage capacity for the outlet. In the U.S., this is usually 15 or 20 amperes.

Fuse Replacement

There are no user serviceable fuses inside the Odyssey Sa Imager. If the instrument fails to power up, and you suspect that a fuse has blown, contact LI-COR® Technical Support.

Routine Maintenance

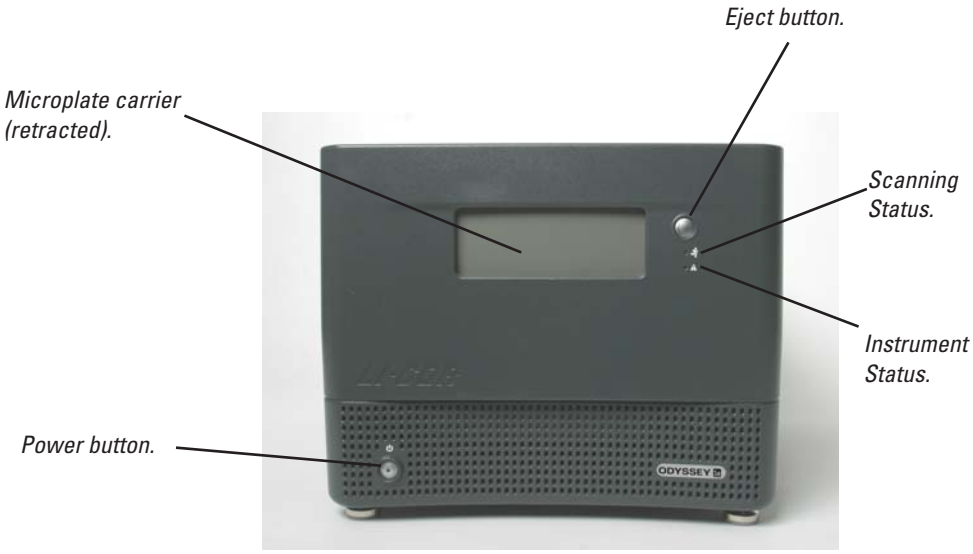
The Odyssey® Sa Imager requires only minimal maintenance. However, as with any equipment utilizing electrical voltages, there is a danger of fire or electrical shock if the equipment is not properly maintained.

LI-COR® Biosciences recommends that you routinely inspect the system and follow these general maintenance guidelines:

- Wipe all chemical spills from the exterior of the case.
- Clean the exterior case parts with warm water and a damp cloth. The exterior case parts are resistant to chemical spills, but do not use scouring compounds, solvents such as acetone, benzene, carbon tetrachlorides, lacquer thinner, or alcohol to clean the case.
- Inspect all cables and power cords for evidence of fraying, exposed wire, or loose connections.

Chapter 2: Overview and General Description

Buttons and Indicator Lights



Power Button

Briefly press the power button on the front panel to start up or shut down the Odyssey® Sa instrument. An indicator light in the power button blinks during the start-up or shut-down procedures and is continuously on when the instrument is ready to operate.

Using the Power Button to Reset the Odyssey Sa Imager

The power button has another function beyond turning the instrument on or off. In the event that the instrument becomes unresponsive, the instrument can be reset by pressing and holding in the power button for five seconds. This procedure cuts power to the instrument and internal computer, so it should only be used when communication cannot be established by any other means. Normal operation should resume after the power button is pressed again to turn the instrument back on, though start up may take several minutes.

Eject Button

Pressing the eject button extends or retracts the Odyssey Sa plate carrier. A protective door automatically opens and closes as the plate carrier extends or retracts. This door protects the user from exposure to laser radiation. Do not defeat the operation of this door.

Using the Eject Button to Reset Network Parameters

The eject button also has an alternate use. After pressing the power button, there is a brief period during the startup procedure when the green scanning status light blinks. While the scanning status light below the eject button is blinking, the network parameters (IP address, etc.) can be set to the factory defaults by pressing the eject button and holding it down until the scanning status light stops blinking.

Indicator Lights

Scanning Status: During normal operation, the scanning status light illuminates green when there is a scan in progress and is off at all other times.

Instrument Status: The instrument status light is normally off, but illuminates red when there is a malfunction in the instrument. Contact LI-COR® Technical Support if the instrument status light turns on.

Back Panel Connectors

Below the fan on the Odyssey® Sa back panel are a variety of connectors for the server hardware inside the instrument. *None of these connectors should be used except the network port*. The network port is used to connect the Odyssey Sa instrument to the computer via a Cat. 5 RJ-45 crossover cable (Chapter 4).

The receptacle for the power cable is also located on the back panel.

Note: The power switch for the power supply on the back panel should always be in the ON position. For normal operation, the instrument should always be powered on and off from the power switch on the instrument front panel.

Hardware Description

Scanning and Detection Hardware

The Odyssey Sa Imager uses two solid-state **diode lasers** to simultaneously provide light excitation at 685 and 785 nm. **Collimating lenses**, **optical bandpass filters**, and a **focusing lens** focus and tune the laser beams to produce an excitation spot on the target detection area of the microplate. The microscope electronics modulate the laser beams to discriminate the infrared (IR) dye signal fluorescence from background fluorescence. The scanner **detection optics** focus on the excitation spot and collect light from the fluorescing IR dyes.

After collection by the **microscope objective**, the light is passed to a **dichroic mirror**. The mirror splits the light and essentially sorts the fluorescent signals by transmitting the light from one IR dye and reflecting light from the other IR dye. The transmitted and reflected lights travel two independent paths through optics designed to remove scattered and stray light. The light is ultimately focused onto one of two **avalanche photodiodes** that converts the light to an electrical signal for processing by the microscope detection electronics.

In the microscope electronics, the signal is amplified, filtered, and finally converted to a digital value by an analog-to-digital converter. The digital signal is demodulated, filtered again, and coordinated with the microscope position by a **Digital Signal Processor** (DSP) to produce the image file.

The entire compact laser/microscope assembly travels on a platform that moves beneath the microplate along both the X- and Y-axis. The microscope can also be adjusted along the Z-axis to change the height of the microscope's focal point. Changing the focus height makes it possible for the instrument to accommodate variations in

the height of the target detection area for different brands of microplates. The focus height can be varied from 1.71 to 3.95 mm, though each instrument has unique maximum and minimum values. The actual values for your particular instrument can be found in the System Administration settings in the Odyssey® Sa Software (see Chapter 13 of the Odyssey Sa User Guide).

Odyssey Sa Server and Server Software

The Odyssey Sa Imager contains a server that controls scanning, data storage, and communication with Odyssey Sa Application Software. The Odyssey Sa server has a pre-installed copy of Odyssey Sa Server software. This software is periodically updated with new releases that are posted on LI-COR® Biosciences' web site. Users will be contacted with download instructions when new releases are available. Instructions for installing server software updates can be found in Chapter 13 of the Odyssey Sa User Guide.

The Odyssey Sa server communicates with the computer via TCP/IP in a stand-alone configuration. When a scan is started from the Odyssey Sa Application Software, scan parameters are sent to the Odyssey Sa server, which starts the scan, collects the data, stores the data in its internal hard disk, and sends a copy of the scanned images to Odyssey Sa Application Software for analysis and storage on a hard disk in the computer.

Note: *The Odyssey Sa Imager should be used for temporary storage only*; when the hard disk is full, the oldest files are overwritten to make space.

Files on the internal hard disk are stored in scan groups. Scan groups are like folders, but access is restricted to users that have been given permission to use the scan group. The Odyssey Sa Imager uses password protected user accounts to establish the access rights for each user.

Chapter 3: Operation

Selecting Microplates

Microplates must meet certain physical characteristics to be scanned by the Odyssey® Sa Imager. Proper selection of microplates significantly affects the results of your analysis as each plate has its own characteristics including well depth, plate autofluorescence, and well-to-well signal crossover. Some general considerations for microplate selection are provided below and some of the Odyssey Sa application protocols give more specific microplate recommendations.

Microplate Compatibility

In-Cell Western™ analyses use detection at the well surface with no liquid present. This results in minimal well-to-well signal spread, allowing the use of both clear as well as black-sided plates with optically clear bottoms. For assays that involve imaging of a liquid, plates that have black walls and clear bottoms should be used to avoid well-to-well signal spread. **Do not use plates with white walls since the auto-fluorescence from the white surface will create significant noise**.

The Odyssey Sa microplate carrier supports both normal height and deep-well microplates conforming to the following standards:

ANSI/SBS 1-2004 Microplates - Footprint Dimensions
ANSI/SBS 2-2004 Microplates - Footprint Dimensions
ANSI/SBS 3-2004 Microplates - Bottom Outside Flange Dimensions

ANSI/SBS 4-2004 Microplates - Well Positions

Note: *The ANSI/SBS standards can be found on the Internet at http://www.sbsonline.org.*

The ANSI/SBS standards specify standard dimensions for plates, but some plates conforming to these dimensions may still not be compatible. Plates vary in the location and thickness of the well bottoms, which can result in the Odyssey® Sa Imager not being able to focus in the correct position. Some plate materials are also not sufficiently transparent or may produce high background fluorescence. When choosing a brand of plate, test it to make sure you have sufficient fluorescent intensity from the samples and that the background fluorescence from the plate is low.

The following plates are recommended by LI-COR® Biosciences:

96-well format	Nunc® (P/N 161093 or165305)
96-well format	Falcon™ (P/N/ 353075 or 353948)
384-well format	Nunc (P/N 164688 or 164730)
384-well format	Falcon (P/N 353961 or 353962)

Note: *The Odyssey Sa Imager does support deep-well microplates.*

Determining Focus Offset

The Odyssey Sa Imager requires that microplates have a maximum of 4.0 mm distance from the base of the microplate to the target detection area of the plate. When using the plates specified above, the recommended starting point for the focus offset is 3.0 mm.

The optimal focus offset for these plates or other plates can be determined by scanning a plate containing experimental and control samples at 1.7, 2.0, 3.0, and 4.0 mm focus offsets (actual maximum (4.0) and minimum (1.7) values vary with each instrument and are found by choosing **Settings > System Administration** in Odyssey Sa Software and then clicking **Scanner Information**). Use the same intensity settings for each scan. After reviewing the collected scans, use the focus offset with the highest signal-to-noise for experiments.

Scanning Microplates

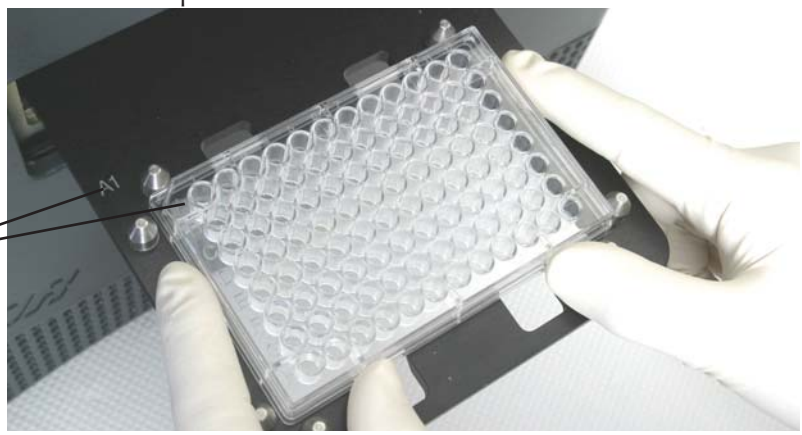
Loading Microplates for Standard Scans

Before scanning, clean the bottom plate surface with a moist, lint-free paper to remove any obstructions during scanning.

Protect plates from light before scanning to ensure highest sensitivity. When storing plates after scanning, the plates should remain protected from light at room temperature.

1) Press the eject button on the Odyssey® Sa front panel to open the microplate carrier.

2) Orient the microplate with well A1 aligned with the A1 marker on the microplate carrier.



Align well A1 with A1 on the microplate carrier.

3) Lower the microplate between the alignment pins until it rests on the microplate carrier and press the eject button again to retract the microplate carrier.

Starting Microplate Scans

Scans are started by choosing **File > Scan** in Odyssey® Sa Application Software. The complete scanning procedure is discussed in Chapter 2 of the *Odyssey Sa User Guide*. Below are the typical scan parameters that should be used for initial runs with microplates. These values are the default values in the microplate templates supplied with the Odyssey Sa Application Software.

The **Focus Offset** can be set at 3.0 mm when using the plates specified by LI-COR® Biosciences.

The **Intensity** for both 700 and 800 nm channels should be set to 7 for initial scanning. If the image signal is saturated or too high, re-scan using a lower intensity setting (i.e., 4). Likewise, If the image signal is too low, re-scan using a higher intensity setting (i.e., 8).

For satisfactory images with minimal scan time, use 200 µm for the **Resolution**. Higher resolution may be used, but scan time will increase.

More specific scanning guidelines may also be given in the various protocols in the Odyssey® Sa Application Protocols manual.

Scanning Membranes in the Membrane Carrier

The Membrane Carrier allows for scanning blots or gels up to 2-13/16" x 4-1/2" (7.2 x 11.4 cm) in size. The Membrane Carrier has the same outer dimensions as a standard microplate, but with a ridge around the edges to prevent liquid from spilling into the Odyssey Sa Imager. The physical thickness of the Membrane Carrier surface is 3/16" (4.8 mm); however, due to the angle of incidence of the excitation lasers, the focus offset values suggested below are corrected values to account for the refractive index of the glass.

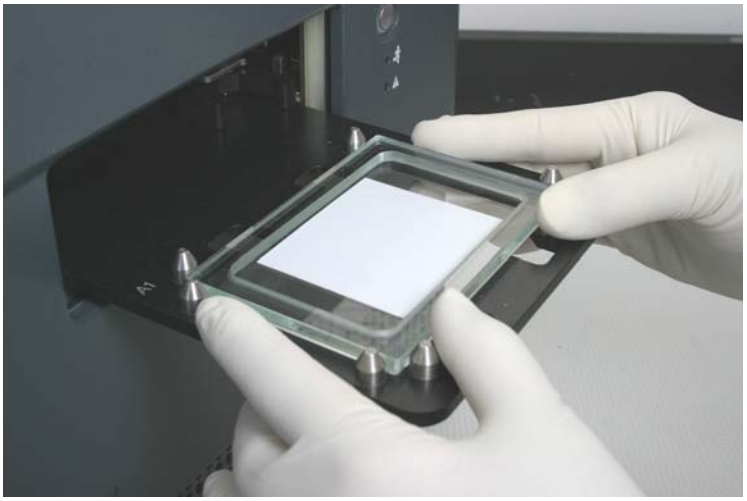
Loading a Membrane

1) Before each use, clean the Membrane Carrier with a mild laboratory detergent and rinse thoroughly with ultrapure water. Do not use abrasive brushes or cleaning materials on the Membrane Carrier, as they may scratch the Carrier surface. Allow the Membrane Carrier to air dry, or blot dry with lint-free paper towels.
2) Place the membrane face-down onto the surface of the Membrane Carrier. Avoid introducing bubbles, and ensure that the membrane lies flat and completely within the edge boundaries of the Carrier. Membranes may be scanned wet or

dry. When placing dry membranes onto the Membrane Carrier, make sure that the membrane lies flat on the glass surface.

⚠ **Caution:** When placing wet membranes or gels onto the Membrane Carrier, avoid excessive liquid in the Carrier, as liquid could inadvertently spill over the edges and into the instrument.

3) Press the eject button on the instrument front panel to open the microplate carrier. Place the Membrane Carrier into the microplate carrier and press the eject button again to close the microplate carrier.



Scanning starts on the side where the "A1" marker is located on the Odyssey® Sa plate carrier. If the membrane carrier is placed with the top of the membrane toward the "A1" marker you will get an image on the display that is oriented correctly. If you don't do this, the image may be upside down, but you can rotate it in the software before analysis.

Scanning Membranes

4) With a project open in the Odyssey® Sa software, choose **File > Scan > Scan** to open the scanner console window (see Chapter 2 of the Odyssey Sa User Guide for a complete description of the scanning software).

5) In the Scanner Console window, enter a **Focus Offset** value of 3.0 mm. **Note**: **Focus Offset** must be between 1.71 and 3.95 mm.

6) Change any other scan parameters as needed and click Start Scan in the Scanner Console window to begin the scan.

7) After the scan is completed, immediately eject the Membrane Carrier and remove it from the instrument. Clean and rinse the Membrane Carrier thoroughly as described above.

The default "SingleWellGlassPlate" preset contains typical scan parameters for scanning membranes. Use 3.0 for the focus offset and 7 for intensity.

Scanning Gels in the Membrane Carrier

The Odyssey Sa Imager can also be used to document Coomassie-stained gels, provided the gels fit in the Odyssey Sa Membrane Carrier (10.5 x 6.5 cm maximum) and are thin enough (< 2 mm) that the laser/microscope has enough focus offset to focus in the gel. Maximum focus offset is 3.95 mm and the focus offset for a given gel is 3.0 plus half the thickness of the gel. Scanning Coomassie-stained protein gels is described in the Western Blot Analysis protocol found in the Odyssey Sa Application Protocols manual.

Other protocols developed for the Odyssey Infrared Imaging System, such as In-Gel Westerns, may be adaptable to the Odyssey Sa Imager, though not currently supported. Factors

limiting the adaptability of these protocols include the size of the gel or membrane versus the Odyssey Sa Membrane Carrier and the focus offset of the Odyssey Sa Imager (there may not be enough focus offset to focus in the center of some gels). If you would like to try to adapt one of these protocols, they can be found at http://biosupport.licor.com.

Chapter 4: Assembling the Odyssey® Sa System

Introduction

Though not intended to be an installation guide, this chapter gives basic information on assembling the Odyssey Sa system. The Odyssey Sa Imager is a server device that connects to the computer via TCP/IP using Rendezvous – an open, standards-based networking technology that automatically connects electronic devices. Rendezvous technology allows the Odyssey Sa Application Software running on the computer to establish communication with the Odyssey Sa Imager without any user configuration.

Installing the Odyssey Sa Application Software

Odyssey Sa Application software is normally installed during instrument installation. The following instructions can be used to re-install the software. Any future upgrade will include installation instructions that supersede those given below.

1) Close all other programs.

2) Insert the Odyssey Sa Application Software CD into the CD-ROM drive. The installation application will start automatically if AutoPlay is turned on. If the application does not start automatically, do one of the following:

- **Vista**: Choose “Run Setup.exe” when the Auto Play window opens. If the Auto Play window does not open automatically, follow the instructions for Windows XP below.

- **XP**: Choose **Run** on the Windows **Start** menu and click **Browse** in the Run window. In the file selection window, switch to the CD, select *Setup.exe* and click **Open**. Click **OK** in the Run window.

3) Click **Next** in the installation window and read the license information. Click **Yes** to continue the installation, if you accept the terms of the license.

4) Choose whether to make the Odyssey Sa application available to all users of the computer or just the current user, and click **Next** (this refers to user accounts in the Windows operating system, not Odyssey Sa accounts).

5) Click **Next** to install in the default directory on drive C: (recommended) or browse for a different drive.

6) Click **Finish** when installation is complete.

Assembling the Odyssey Sa System

Since this chapter is not an installation guide, it is assumed the computer is setup according the manufacturer's instructions. Use the Windows® control panels to make sure the **Date and Time** control panel is set accurately, and that the **Power Options** control panel has all power management options set to **Never**. The Odyssey Sa Imager and network switch are also assumed to be powered on but not connected.

1) Start with both the computer and instrument turned off.

2) Connect the included Cat. 5 RJ-45 crossover cable to the Odyssey Sa network port. The network port is identified in Chapter 2.

3) Connect the other end of the Cat. 5 RJ-45 cable to the network port on the computer.

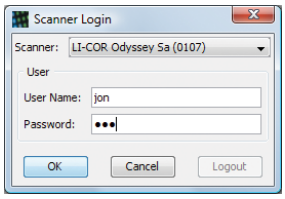
4) Turn on the computer and the Odyssey Sa instrument, and wait for their start-up procedures to be completed.

5) Start the Odyssey Sa Application Software on your computer.

When Odyssey Sa software starts, it goes through an autodiscovery process that finds the Odyssey Sa instrument (can take several minutes).

6) To confirm that Odyssey Sa software can communicate with the Odyssey Sa Imager, choosing **Settings > System Administration**.

7) In the Scanner Login window, choose your scanner, enter the name and password of an account with *Administrator* access rights, and click **OK**.



The default administrator account shipped with the Odyssey Sa instrument is named “admin” and the password is also “admin” (case sensitive). For best security, you should create a new account with *Administrator* rights and delete the default “admin” account. If you can log in, you are successfully connected.

Adding Users

User account information is stored on the Odyssey Sa Imager rather than on the computer. If you do not have a user account, you will need to create one. Chapter 12 in the Odyssey Sa User Guide describes how to use the System Administration window for creating user accounts.

8) Close the System Administration window when you are through adding user accounts.

Setup is now complete.

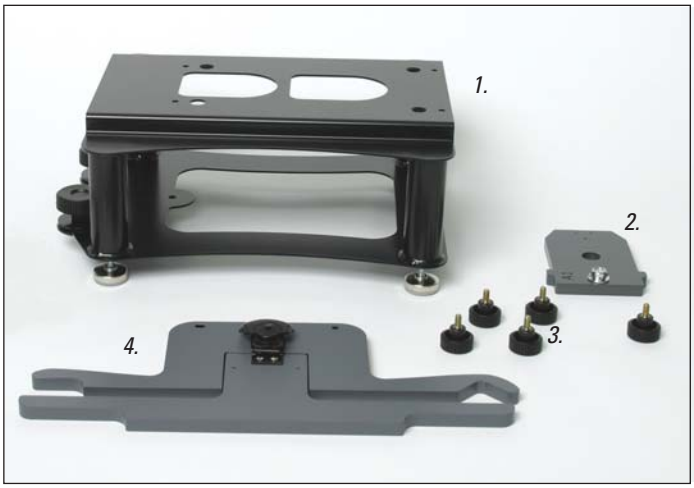
Chapter 5: Installing a BioTek® BioStack™ Plate Stacker

Introduction

This chapter describes how to install a BioTek BioStack Automated Plate Stacking System. When connected to the Odyssey® Sa Imager, the plate stacker is controlled by the Odyssey Sa Express software, allowing up to 50 microplates to be scanned during an express run. Installation requires the Plate Stacker Install Kit (LI-COR®, P/N 9250-14), which is used to lock the BioStack Plate Stacker in position at the correct height and distance from the Odyssey Sa Imager. It also includes communication cables to connect the two instruments. The components of the installation kit are shown below.

Components:

1. *BioStack stand.*
2. *Leveling and alignment fixture with storage thumbscrew.*
3. *Thumbscrews to connect the BioStack Plate Stacker to the stand.*
4. *Locator clamp.*
5. *USB cable and calibration ruler (not pictured).*



Installation Summary

1) Connect the BioStack Plate Stacker to the stand.

2) Connect the power cables and communication cables.

3) Level the Odyssey® Sa Imager.

4) Install the BioStack locator clamp around the front legs of the Odyssey Sa Imager.

5) Adjust the height of the BioStack Plate Stacker.

6) Level the BioStack Plate Stacker.

7) Orient the BioStack Plate Stacker so that microplates are correctly positioned on the Odyssey Sa plate carrier.

8) Use the Odyssey Sa software to adjust the vertical travel of the BioStack plate gripper to match the height of the Odyssey Sa plate carrier.

Odyssey Sa Imager and the BioStack Plate Stacker after installation.

Initial Odyssey® Sa Setup

Chapter 1 describes placement of the Odyssey Sa Imager. The addition of the BioStack™ Plate Stacker adds some other requirements. Chapter 1 describes the additional space requirements and the weight of the combined systems. A sturdy surface is also a requirement. Both the Odyssey Sa Imager and the BioStack Plate Stacker must be level during operation. Any surface that is susceptible to movement during operation is not a good choice for the Odyssey Sa/BioStack combination.

1) Before starting installation, each leveling foot on the Odyssey Sa Imager should be turned out one turn.

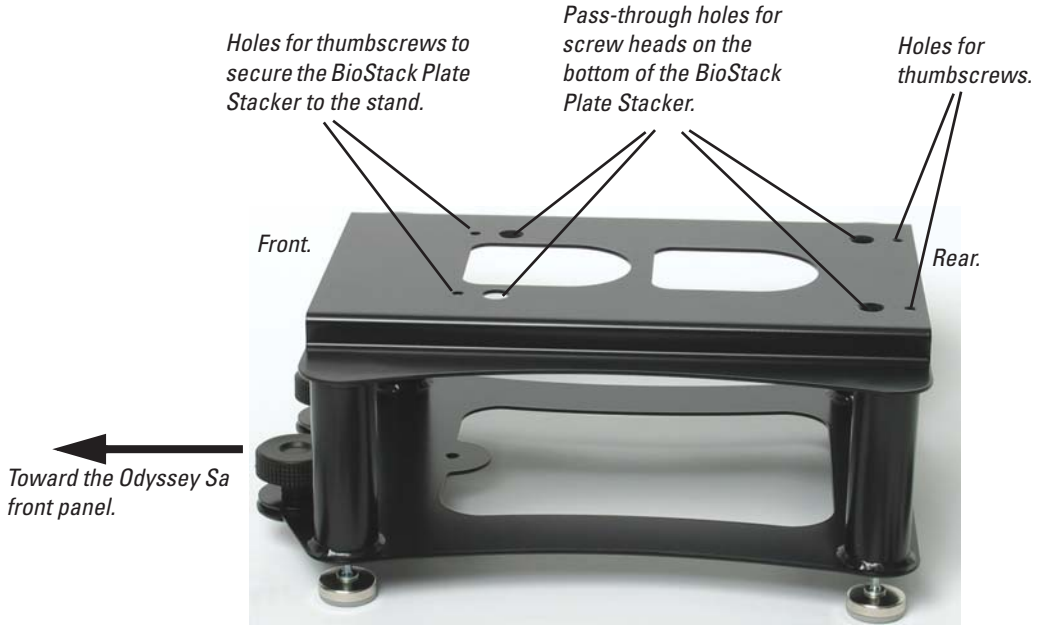
Note: *The feet are normally turned all the way in for shipment. If necessary, turn any loose foot all the way in (finger tight) before turning it out one turn. Turning the feet out one turn will make it easier to level the instrument in later steps.*

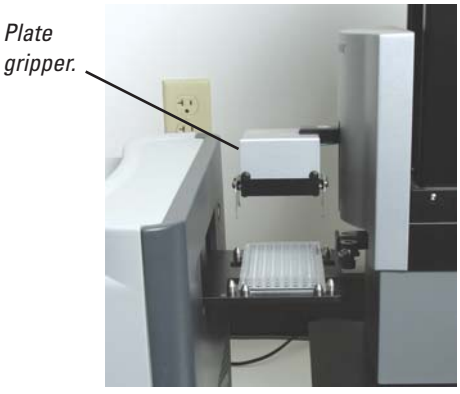
Initial BioStack Setup

2) Unpack the BioStack Plate Stacker and remove its shipping locks using the instructions in the BioStack Operator's Manual supplied by BioTek®.

⚠ **Important:** Do not apply power to the BioStack Plate Stacker or Odyssey Sa Imager until directed to do so later in this procedure. Do not install the input/output stacks until installation is complete.

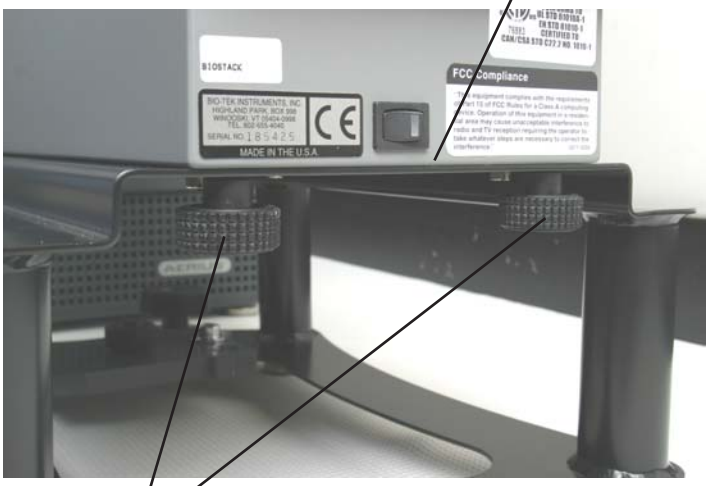
3) Orient the BioStack stand so the end with the two large knobs (front) faces toward the front of the Odyssey® Sa Imager. Orient the BioStack instrument so the side with the plate gripper also faces toward the Odyssey Sa Imager.





4) Place the BioStack™ Plate Stacker on the stand with the plate gripper facing the front of the stand and the back of the BioStack Plate Stacker flush with the rear edge of the stand. When the back of the BioStack Plate Stacker is flush with the rear of the stand, the screw heads on the bottom of the BioStack Plate Stacker should pass through the holes on the stand.

The back of the BioStack Plate Stacker should be flush with the back of the stand.



The thumbscrews pass through the bottom of the stand and into threaded holes in the bottom of the BioStack Plate Stacker.

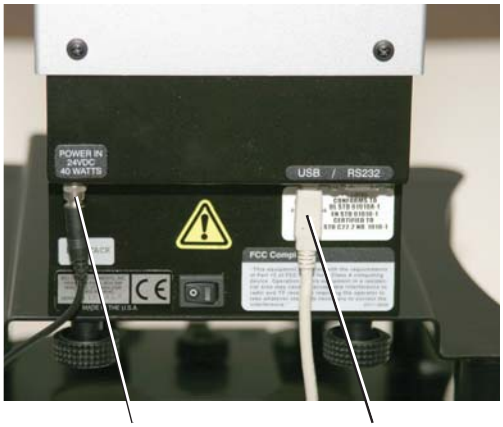
5) Secure the BioStack Plate Stacker to the stand using four thumbscrews.

Connecting Power and Communication Cables

6) Connect one end of the USB cable into one of the Odyssey® Sa USB ports.

7) Connect the other end of the USB cable to the USB port on the BioStack Plate Stacker.

BioStack Back Panel.



8) Connect the power supply to the BioStack Plate Stacker and tighten the threaded retainer ring until it is finger tight. Plug the power cord into a wall outlet.

9) Turn on the BioStack Plate Stacker. Initialization takes about 20 seconds.

10) If necessary, connect the power and network cables to the Odyssey® Sa as described earlier in this manual.

⚠ **Important:** The BioStack™ must always be turned on before the Odyssey Sa Imager.

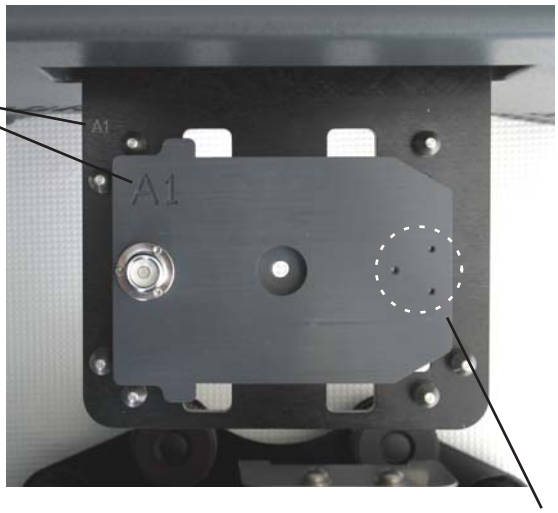
11) Turn on the Odyssey Sa Imager. The Odyssey Sa Imager should be turned on for all remaining steps.

Leveling the Odyssey Sa Imager

12) If necessary, move the BioStack Plate Stacker so there is at least 18 cm (7 in.) clearance between the Odyssey Sa and the BioStack plate gripper. (If there is insufficient clearance, the extended Odyssey Sa plate carrier will collide with the BioStack accessory.)

13) Push the eject button on the Odyssey Sa front panel to extend the plate carrier. Place the leveling and alignment fixture into the plate carrier as shown below.

"A1" on the fixture should align with "A1" on the plate carrier.



Note: *If the bubble level is difficult to see, it can be moved to the alternate position as shown above.*

14) Turn the leveling feet in or out on the Odyssey® Sa Imager until the bubble falls within the circle on the bubble level, indicating that the instrument is level.

⚠ **Important:** Do not extend the legs over 3 cm (1.2 in.). The instrument may become unstable or potentially damage the Odyssey Sa enclosure. If necessary, turn the legs back in as you level the instrument rather than continuing to extend the legs.

15) Press downward on each of the four corners of the Odyssey Sa Imager to make sure the instrument does not rock and that all four legs are contacting the surface. If necessary, adjust any legs as needed and level the instrument again.

16) Remove the leveling and alignment fixture from the plate carrier and push the eject button to retract the plate carrier.

Installing the Locator Clamp

A locator clamp is provided that locks around the front legs of the Odyssey Sa Imager. In later steps, the BioStack stand will be connected to the locator clamp, which locks both the Odyssey Sa and the BioStack Plate Stacker in position.

17) Make sure the clamp is open before starting. Flip the latch cover up and rotate the latch until it disengages from the hook, if necessary.

18) Place the clamp on the surface in front of the Odyssey Sa Imager with the jaws open. Slide the left side of the clamp under the instrument in such a way that the left-front leg passes between the jaws of the clamp.

19) Push the right side of the clamp under the instrument and move the clamp to the right so the right-front leg passes between the jaws of the clamp. When properly positioned, the legs of the Odyssey® Sa Imager will rest in the indentations in the front of the clamp.

20) Close the clamp by placing the latch over the hook and turning the latch. After the latch is rotated, it can be pressed down into its locked position.

Front view showing the locator clamp locked in position around the two front legs of the Odyssey Sa Imager.

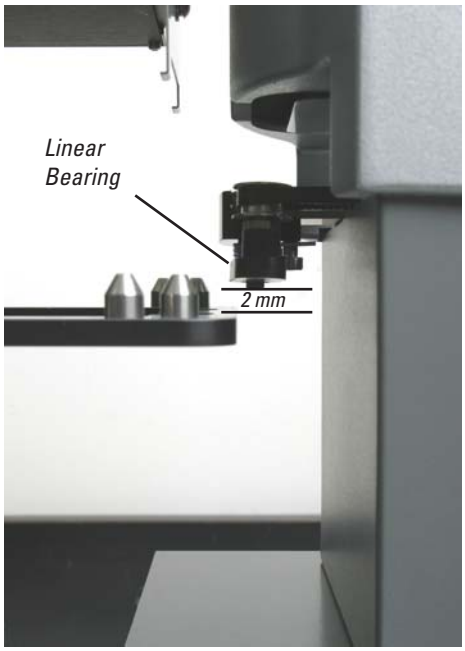
Side view showing the locator clamp locked in position with the left front leg clamped in the indentation in the clamp.

Leveling the BioStack™ Plate Stacker

21) Push the eject button on the Odyssey Sa front panel to extend the plate carrier.

22) Move the BioStack Plate Stacker directly in front of the Odyssey® Sa Imager. Be careful not to strike the Odyssey Sa plate carrier.

23) Look between the instruments with your line of sight level with the Odyssey Sa plate carrier. Adjust the leveling feet on the BioStack stand so the Odyssey Sa plate carrier is about 2 mm below the linear bearing on the BioStack Plate Stacker.



⚠ Be careful during the height adjustments. Do not let the Odyssey Sa plate carrier collide with the linear bearing on the BioStack Plate Stacker.

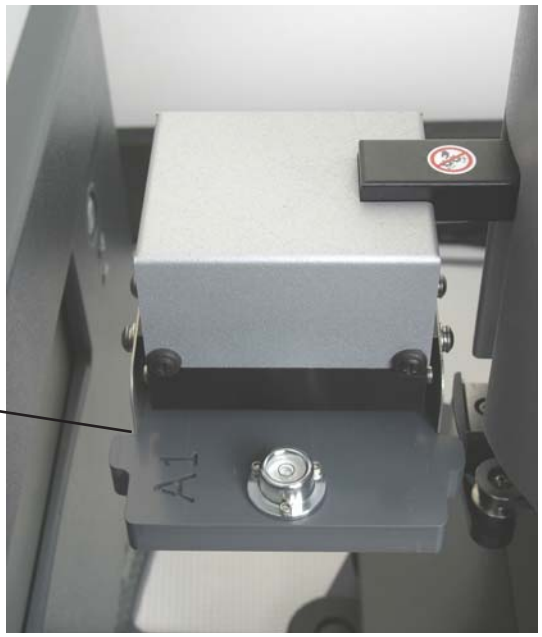
24) Push the eject button on the Odyssey Sa front panel to retract the plate carrier.

25) Turn off the BioStack Plate Stacker.

26) Slide the leveling and alignment fixture into the BioStack™ plate gripper and make sure it rests flat on the gripper arms. The "A1" corner of the fixture should be in the same orientation that it was in the Odyssey® Sa plate carrier. Push the fixture all the way in until it contacts the gripper arms.

Leveling and alignment fixture shown in the BioStack plate gripper.

The fixture should contact the gripper arms.



27) Adjust the leveling feet on the BioStack stand until the bubble level indicates the plate gripper is level. Adjust the feet only enough to level the instrument, while trying to maintain the same height and clearance from the Odyssey Sa plate carrier.

28) Move the plate gripper to its highest position and press the eject button on the Odyssey Sa to extend the plate carrier. Verify that the clearance between the BioStack linear bearing and the Odyssey Sa plate carrier is still about 2 mm. Adjust the height and level the BioStack Plate Stacker again as needed.

29) Push the Odyssey® Sa eject button to retract the plate carrier. Remove the leveling and alignment fixture from the plate gripper.

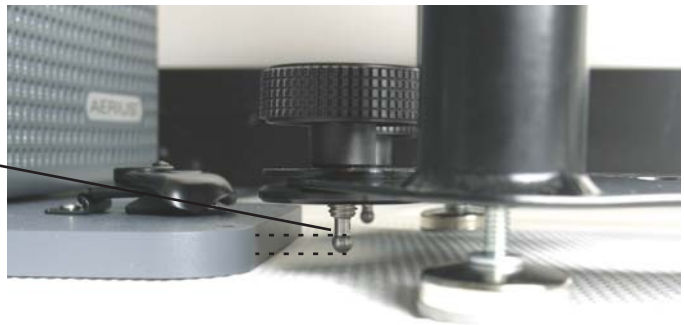
Connecting the BioStack Plate Stacker to the Locator

The BioStack stand has two large knobs on the front of the stand that tighten two alignment pins. These alignment pins fit into the two holes on the locator clamp. The steps below can be used to adjust the height of the alignment pins and to connect the BioStack stand to the locator clamp.

30) Loosen the large knobs on the bottom of the BioStack stand just enough so the alignment pins move freely.

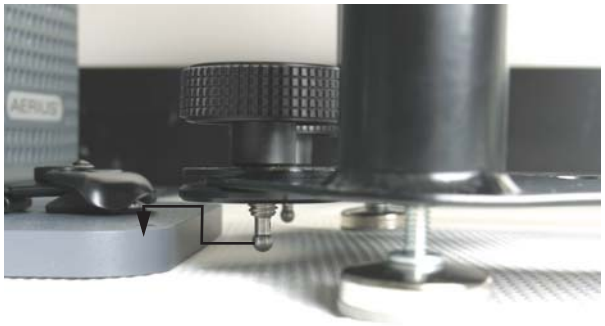
31) Observe the alignment pins below the BioStack stand. When they are adjusted properly the ball on the end of the pins should be centered within the thickness of the locator clamp. If the pins are too high or low, follow step 32 for height adjustment. Skip to step 33 if the pins are properly adjusted.

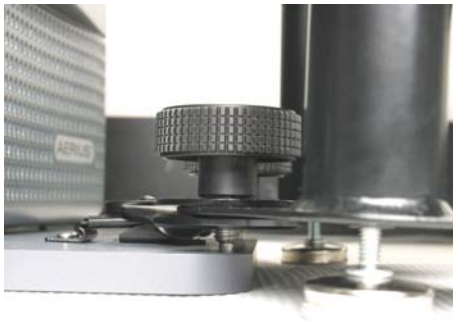
The balls on the alignment pins should be centered within the thickness of the locator clamp.



32) If you need to adjust the height of the alignment pins, hold the threaded washer below the stand and unscrew the knob until it comes off. While holding the threaded washer, screw the alignment pin in or out as needed to adjust the height and then replace the knob, but don't tighten it.

33) Lift the front of the BioStack™ stand and guide the alignment pins into the holes in the locator clamp.





Alignment pins correctly installed.

34) Press the eject button on the Odyssey® Sa to extend the plate carrier and verify there is enough clearance between it and the BioStack Plate Stacker.

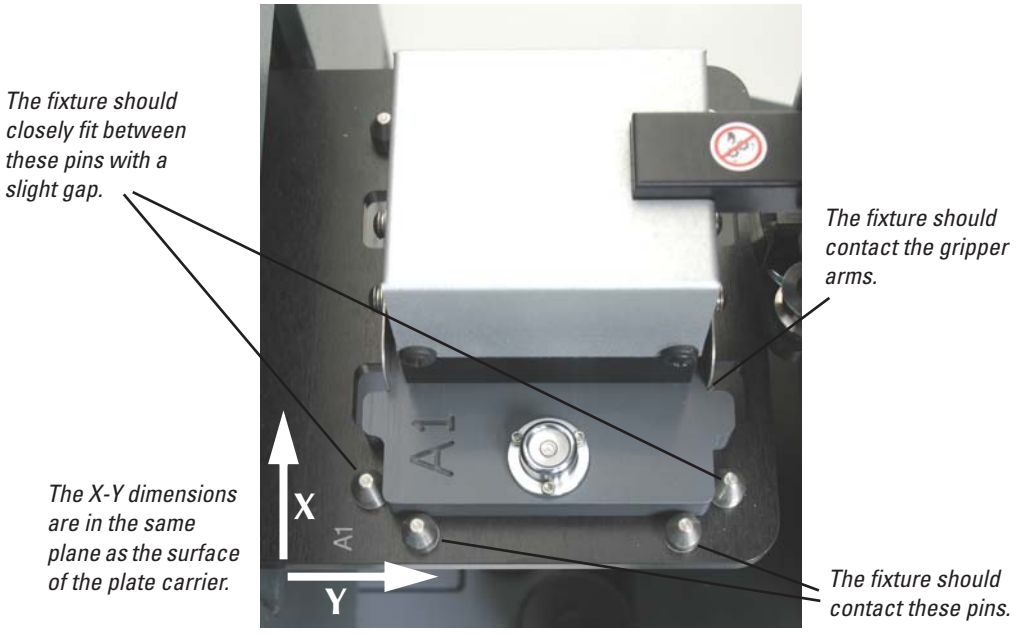
35) Slide the leveling and alignment fixture back in the plate gripper and verify that the BioStack accessory is level. Adjust as needed.

BioStack X-Y Alignment

The BioStack Plate Stacker is now close to the final position, but some small movements will be needed in the X-Y dimensions to ensure that microplates are placed squarely on the plate carrier.

36) With the leveling and alignment fixture still in the BioStack plate gripper, manually lower the plate gripper until it is just above the Odyssey® Sa plate carrier. (Make sure the BioStack stacker is off.)

37) Observe the position of the fixture relative to the four alignment pins on the plate carrier. When the X-Y alignment is correct, the fixture will fit as shown below.



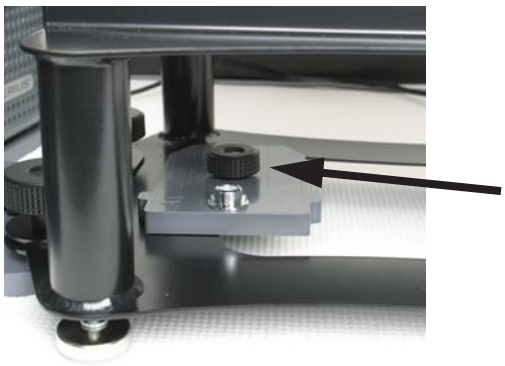
38) If you observe that the fixture will contact one or more of the pins as it is lowered, carefully move the BioStack™ Plate Stacker in small increments until the fixture drops onto the surface of the plate carrier without hitting any of the pins and binding.

⚠ **Important:** Move the BioStack Plate Stacker carefully and only in small increments. Excessive movement can stress the plate gripper assembly, potentially causing damage.

39) Observe the bubble level on the fixture and make any necessary adjustments to level the BioStack Plate Stacker.

40) Tighten the knob on both alignment pins. You may need to hold the threaded washer until the knob begins to tighten.

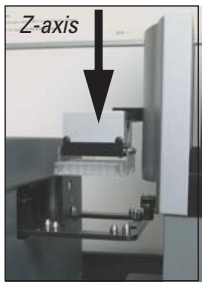
41) Manually raise the BioStack plate gripper and remove the leveling and alignment fixture. Use the fifth thumbscrew to secure the fixture to the BioStack stand for storage.



42) Press the eject button on the Odyssey® Sa to retract the plate carrier.

43) Turn on the BioStack instrument and wait for it to initialize.

BioStack Z-Axis Training

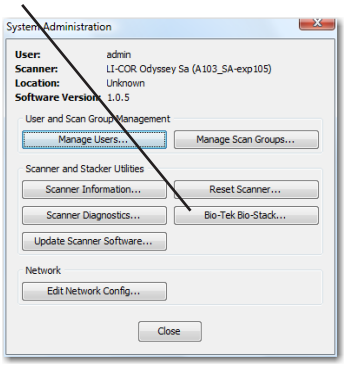


The final procedure is to adjust the vertical (Z-axis) travel of the BioStack plate gripper. The goal is for microplates to be placed on the Odyssey Sa plate carrier from the optimal height. If the value of the vertical travel distance on the Z-axis is too small, the plate will drop onto the Odyssey Sa plate carrier rather than being placed on it. If the value of the vertical travel distance is too large, the plate will hit the Odyssey Sa plate carrier and the plate gripper arms will continue to travel downward, which could result in the bottom of the gripper assembly impacting the microplate as it rests on the Odyssey Sa plate carrier.

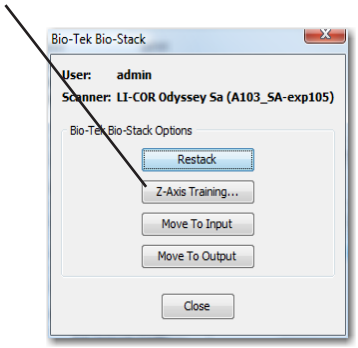
44) Start the Odyssey Sa Express software on your computer and choose **Settings > System Administration**.

45) Log in using an account with *Administrator* access rights.

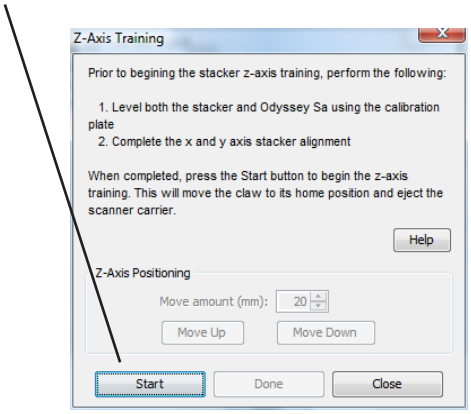
46) Click **BioTek BioStack** in the System Administration window.



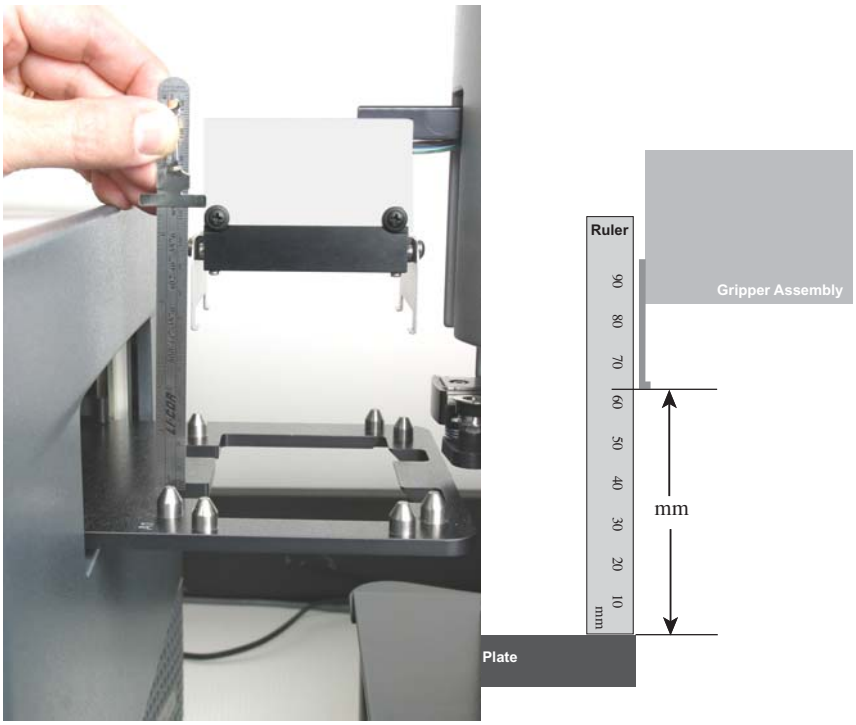
47) Click **Z-Axis Training** in the BioTek® BioStack™ window.



48) Click **Start** in the Z-axis training window. This moves the BioStack plate gripper to its highest vertical position and opens the Odyssey® Sa plate carrier.

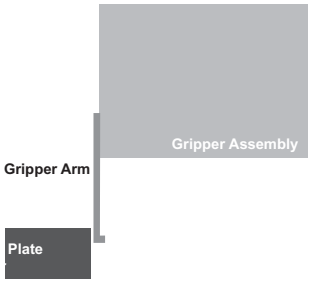


49) Use the supplied calibration ruler to measure, in millimeters, from the bottom tip of the plate gripper to the top surface of the Odyssey® Sa plate carrier.



50) Enter the measured Z-axis distance plus three millimeters (integers only) in the **Move Amount (mm)** field, or use the arrows to set a value. Three millimeters are added because the gripper arms should travel just below the upper surface of the Odyssey Sa plate carrier.

51) Click **Move Down**. The bottom edge of the gripper should travel slightly below the top surface of the Odyssey® Sa plate carrier and the bend in the gripper arm that a plate normally rests on should be slightly below the top surface of the plate carrier as shown below.



52) If the gripper stops above the Odyssey Sa plate carrier, put one millimeter in the **Move Amount** and click **Move Down** again. Conversely, if the gripper arms travel downward more than half way through the thickness of the Odyssey Sa plate carrier, put one millimeter in the **Move Amount** and click **Move Up**. Repeat the corrections until gripper arm stops just in the correct position.

Note: *Always enter a "move amount" that is relative to the current position. For example, if your initial "move amount" is 60 and you need to add 1 millimeter, you should enter 1 rather than 61.*

53) Click **Done** in the Z-axis Training window and close all of the System Administration windows.

The BioTek® BioStack™ Plate Stacker is now ready for operation. Consult the BioStack Operator's Manual supplied by BioTek for instructions on loading the input and output stacks. Note, however, that the Odyssey Sa Express software expects microplates on the plate carrier to be in a certain orientation. If the input stacks are loaded randomly, some images will be right side up and others upside down.

Note: *The Odyssey Sa membrane carrier is not compatible with the BioStack Plate Stacker.*

In the Odyssey® Sa Express software, you must start an express run to use the BioStack Plate Stacker. A suggested method to learn how to use express runs would be to read the Appendix of the Odyssey Sa User Guide and then do an express run with a few empty microplates.

Express Run Errors Related to the BioStack Plate Stacker

If there is a problem during an express run, you should check the log file and read the log for any error messages. Messages about the BioStack Plate Stacker usually begin with "Scanner reported the following error" appended with a message from the BioStack Plate Stacker that may be difficult to interpret.

The most common problems are jams or failure to pick-up or place plates properly. Jams are commonly caused by non-standard plates, leaving a plate top on, or by barcode labels that may come loose and stick to something. If the instruments have been moved out of alignment, you may also get errors if the BioStack Plate Stacker can't pickup a plate or if it drops the plate incorrectly on the Odyssey Sa plate carrier.

In general, the software remedy for jams and other errors is to start the express run again. If you are using express run templates, you can re-stack the plates, exclude the plates that have already been successfully scanned and start the express run again. If you were running an express run that was not saved as a tempate, you will need to define a new express run for the plates remaining in the input stack.

In general, you will have the least difficulty with jams and other problems related to the BioStack Plate Stacker if you use the recom-

mended plates (Chapter 3) and don't mix different types of plates in the input stack.

Another category of errors that can occur are communication errors between the Odyssey® Sa Imager and the BioStack™ Plate Stacker. These errors are generally caused by unplugging the USB cable and later plugging it back in. Communication is not always immediately established. The remedy is to turn off both instruments, turn on the BioStack Plate Stacker, and then turn on the Odyssey Sa Imager after the BioStack Plate Stacker has finished its startup routine.

Chapter 6: Appendix

Specifications

Laser/Microscope

Laser Lifetime: 40,000 hours typical.
700 Channel Laser Source: Solid-state diode laser at 685 nm.
800 Channel Laser Source: Solid-state diode laser at 785 nm.
Detectors: Silicon avalanche photodiodes.
Scanning Speed: Automatic.
Resolution: 20-500 µm.
Focusing Range: 2.25 mm (suitable for most microtiter plates with transparent, flat-bottom wells).

Operating Specifications

Operating Conditions: For Indoor use only; operating temperature 15-35°C and dew point not greater than 19°C, non-condensing; altitude not to exceed 2000 m.
Environmental Conditions: Pollution Degree 1.
Fuse Ratings: Fuses are non-user replaceable. A 5A fast blow fuse protects the Digital Signal Processor (DSP).
Power Requirements: Switched voltage selection between 100-127 VAC and 200-240 VAC (voltage fluctuations not to exceed 10% of the nominal voltage); 47-63 Hz; 1.1 A at 120V typical; 2.25A at 120V maximum (270W). Insulation Category II.
Dimensions: 37H × 44W × 57D cm (14 × 17 × 22 inches). With addition of BioTek® BioStack™ Plate Stacker, 61H x 44W x 122D cm (24 x 17 x 48 inches).
Weight: 26 kg (56 lb.). With BioTek BioStack Plate Stacker, 34 kg (75 lb.).
Data Storage Capacity: >25 GB.
Network Protocol: TCP/IP.
Network Connection: Cat. 5 RJ-45, 10Base-T/100Base-TX.