isolight®

System Guide

BRUKER

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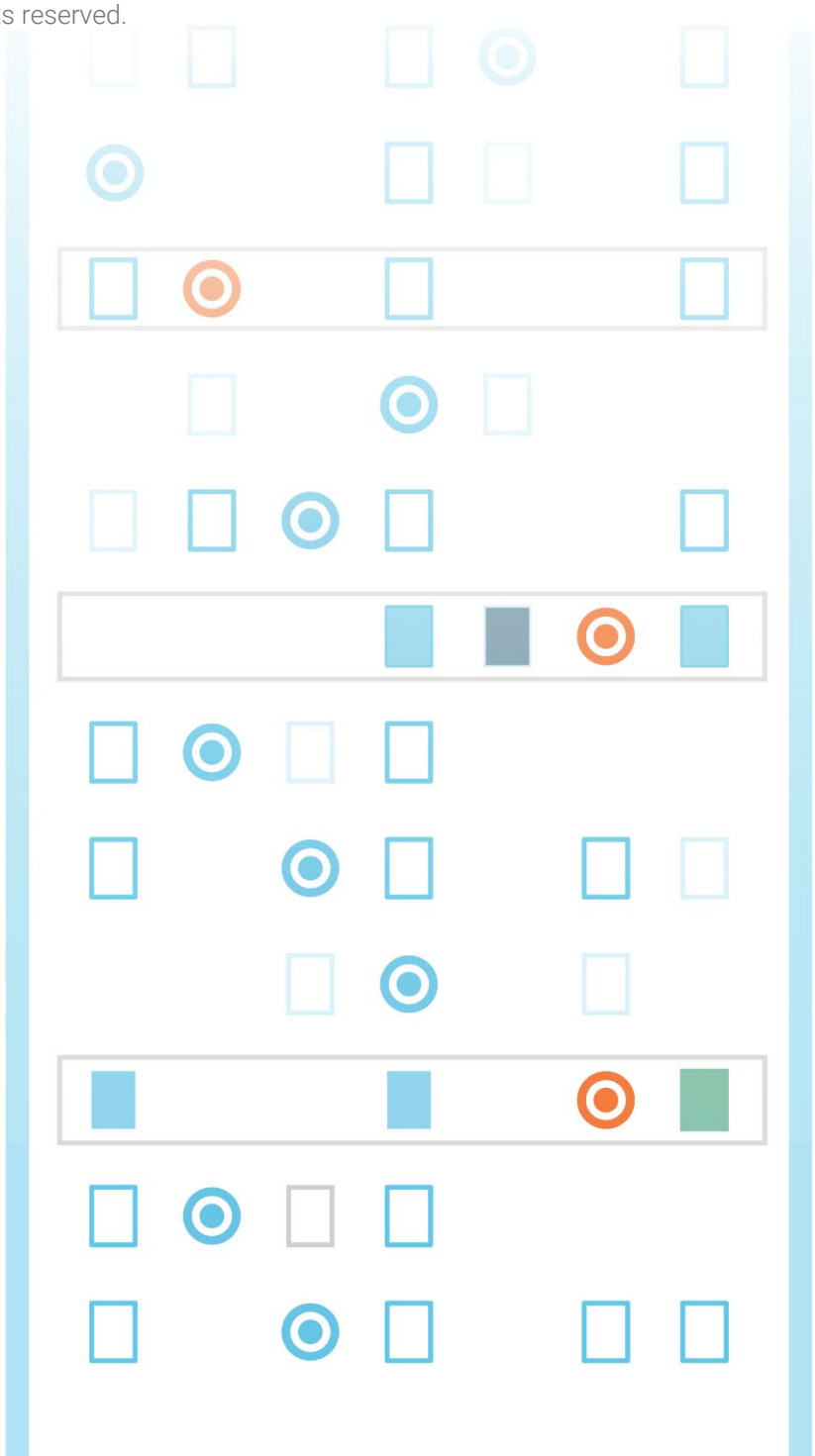

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Chapter 1: Overview

Introduction

The IsoLight platform's predictive immune response enables quality assessments of cell immunotherapies, as well as improved assessment of clinical response and mechanism of action in patient samples. The automated IsoLight platform, which runs the IsoCode and CodePlex Chips, is a versatile, precision automation platform designed to understand and characterize differences among immune cells, mapping thousands of cells per sample, to reveal full functional profiles and polyfunctionality among cell subsets.

Features

Interface controls	The IsoLight Software Interface provides controls to configure the instrument, set up and monitor runs, and perform maintenance procedures.
Convenient Chip Loading	A magnetic mechanism positions the chips during loading onto the instrument.
IsoSpeak Software	Integrated secondary analysis software processes data to perform functional protein analysis for each sample.

Additional Resources

The following documentation is available for download from the Bruker website.

Resource	Description
IsoLight Site Preparation Guide	Provides specifications for laboratory space, electrical requirements, and environmental considerations.
Bruker Cell stimulation and staining protocols	Various protocols providing instructions for Single cell suspension and stimulation preparation for different workflows and cell types.
IsoSpeak User Guide	Provides a comprehensive overview of analysis procedures, analysis workflows, and output files generated by IsoSpeak, as well as computing requirements, off-instrument installation instructions, and troubleshooting information.

Safety Information

The IsoLight instrument contains Class 1 lasers harmful to the human eye. The instrument is equipped with failsafe switches for the chip tray, turning off the lasers when the tray is opened.

If the IsoLight or associated equipment is used in a manner not specified by Bruker the built-in protection provided by the instrument may be impaired.

Only an authorized Field Service Engineer should move the instrument or remove any of the IsoLight instrument skins for maintenance and repair.

Earth grounding of the IsoLight is achieved through the ground terminal of the power cord. This ground potential is electrically connected to all conductive exposed surfaces to which a user has exposure.

WARNING

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

Components

The IsoLight has the following exterior components:

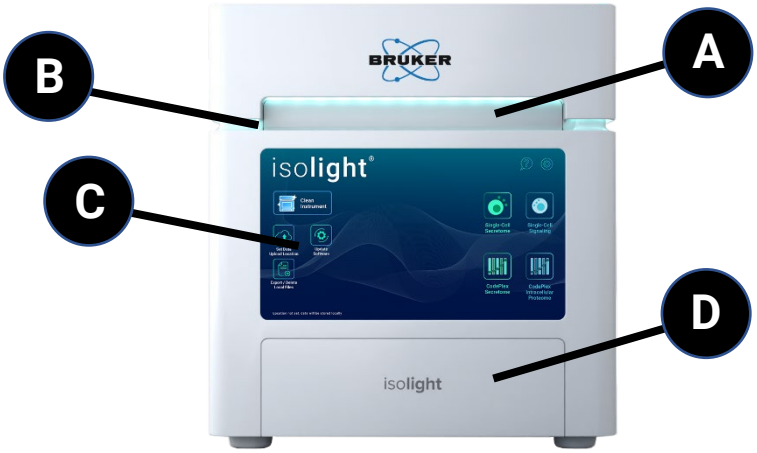


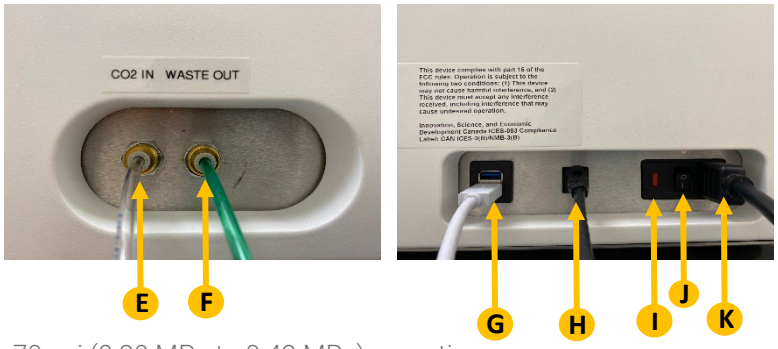
Figure 1

Control	Name	Description
A	Chip compartment	Contains the chip stage that houses the chips throughout the run. Chip stage motors move the stage out of the enclosed optical module for chip loading and return the stage when the run begins.
B	Status bar	Type of color represents the current step in the Run and is the same as shown on screen.

C	Touch screen monitor	Enables on-instrument configuration and run setup using the software interface.
D	Reagent compartment	Holds reagents used in IsoLight run. Must be accessible during IsoLight run setup.

Note: Safety switch turns off any lasers in use if the stage is moved out of the enclosed module

Back side of Instrument:



E – CO2 port – 30 to 70 psi (0.20 MPa to 0.48 MPa) operating range

F – Waste port – Connects to external waste bottle

G – Two External USB ports – Facilitates the transfer of files and data to the instrument computer from the touch screen monitor.

H – Ethernet port

I – Fuse

J – Power switch

K – Power supply

Electrical requirements for the IsoLight

Use an approved UL Listed detachable power supply cord, as supplied, to connect the system to the wall or UPS (uninterruptible power supply). Bruker recommends a UPS. Contact Bruker support for a UPS recommendation.

The IsoLight includes a 240 VAC, 6.3 A, 5 x 20 mm fuse within the power switch

Component	Input Voltage (VAC)	Frequency (Hz)	Rated Current (A)
IsoLight	100-240	50/60	6.3

Chip Loading Tray

Single-Cell Chips

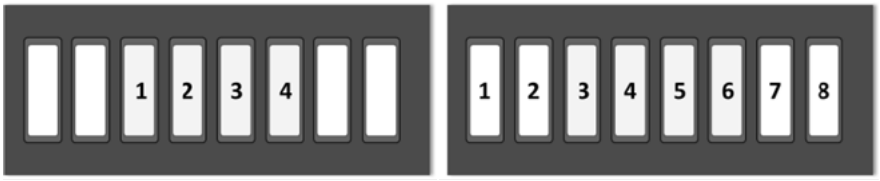


Figure 2 - Chip Tray and chip positions for loading of 4 or 8 chips

The chip compartment houses the chip tray, thermal station, and fluidics connections for each Single-Cell Chip. The tray is automatically moved out and retracted for loading and unloading of chips. Magnetic guides hold each chip in place to ensure correct positioning during a given run.

CodePlex Chips

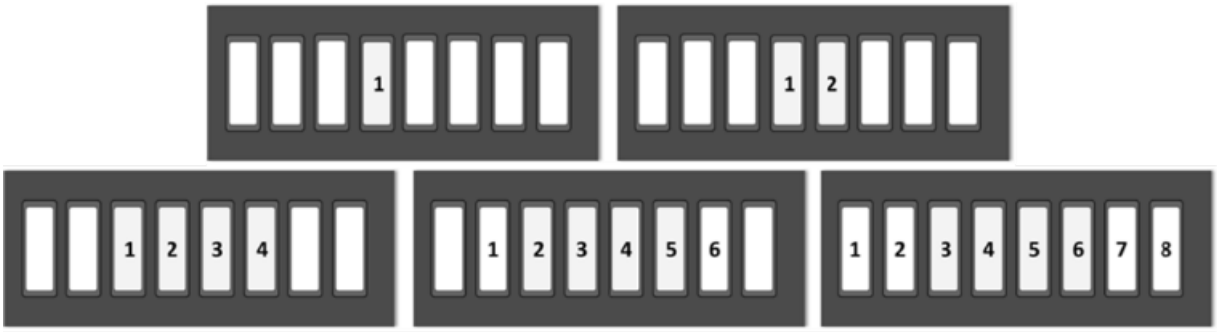


Figure 3 - Chip Tray and chip positions for loading of 1, 2, 4, 6 or 8 chips

The Chip compartment houses the chip tray and fluidics connections for each CodePlex Chip. The tray is automatically moved out and retracted for loading and unloading of chips. Magnetic guides hold each chip in place to ensure correct positioning during a given run.

Single-Cell Signaling Chips

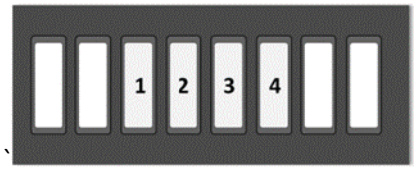


Figure 4 - Chip loading positions for 4 Single-Cell Signaling Chips

The chip compartment houses the chip tray, thermal station, and fluidics connections for each Single-Cell Signaling Chip. The tray is automatically moved out and retracted for loading and unloading of chips. Magnetic guides hold each chip in place to ensure correct positioning during a given run.

Reagent Compartment

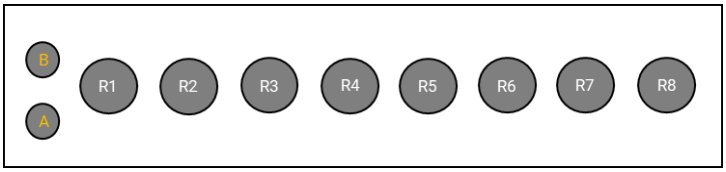


Figure 5 - Liquid Reagent positions

The reagent compartment houses the single-use reagents. It should only be opened and closed when instructed in order to maintain consistent temperature. Any liquid waste generated during the run and automated post-run cleaning step is being collected in the external waste bottle attached to the waste line.

IsoSpeak Concepts

The following concepts and terms are common to the run setup steps on the IsoLight.

Concept	Description
IsoSpeak Analysis File	A saved file and associated subfolder containing all the analysis for a single chip. This is first generated by IsoSpeak on the IsoSpeak Computer (see below) when planning out your experiment. Upon completion of a run of the IsoLight, the corresponding IsoSpeak analysis files are updated with the generated data on the IsoSpeak computer.
IsoSpeak Computer	A separate computer for initial experiment planning and for secondary data analysis, once a run of the IsoLight has been completed. Transfer of IsoLight Run Folder between the IsoSpeak Computer and the IsoLight happen either via provided USB flash drive or over ethernet if a connection is present.
IsoSpeak Analysis Folder	A specified folder containing IsoSpeak analysis files that is accessible on the IsoSpeak Computer.
IsoLight Run Folder	A specified folder on the D drive of the IsoLight (D:\Pictures) populated with run data by the IsoLight software each time a run is performed.

Please consult the IsoSpeak User Guide for more information on data processing and analysis.

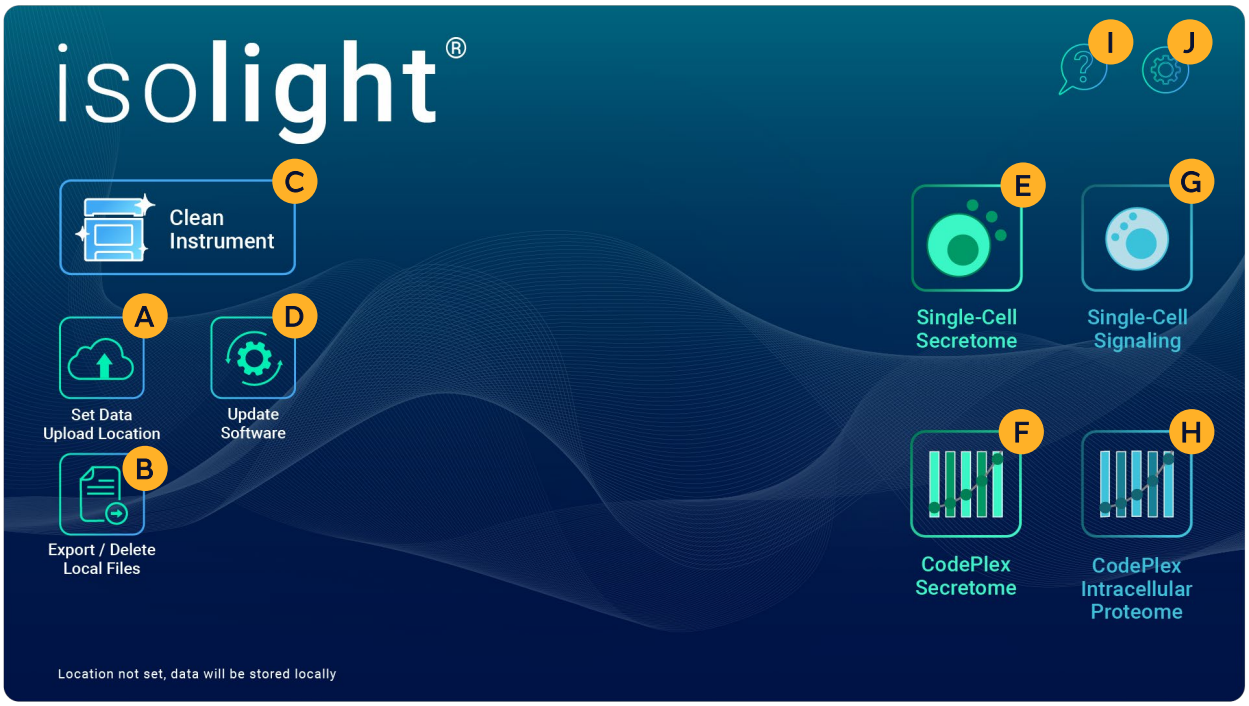
IsoLight Software

- IsoLight software — Controls instrument operation and guides user through the run setup and post-run data retrieval

- During the run, the IsoLight software operates the chip stage, dispenses reagents, controls chip temperatures, image capture and initiates the post-run wash

- Software used off-instrument includes the IsoSpeak data analysis software. For more information, see IsoSpeak Overview on page 9

The IsoLight home screen is automatically displayed after boot-up and between runs allowing the user to execute several tasks (please see Appendix for details for each feature).



Control	Name	Description
A	Set Data Upload Location	Select external data upload destination (USB drive or network location)
B	Export / Delete Assay Files	Export or delete assay data from the IsoLight
C	Clean Instrument	Perform maintenance cleaning
D	Update Software	Update IsoLight software using USB drive
E	Single-Cell Secretome	Start a Single-Cell Secretome run
F	CodePlex Secretome	Start a CodePlex Secretome run
G	Single-Cell Signaling	Start a Single-Cell Signaling run
H	CodePlex Intracellular Proteome	Start a CodePlex Intracellular Proteome run
I	Help Button	Displays the user guide
J	Settings	Misc. instrument settings

Run Information Screen

Single-Cell Secretome Chips

Step Indicators

Real-time information of run progression is displayed on screen (Figure 66) for each of the six major steps:

(A): Data Upload Status (B) Cell Loading, Cell Imaging, Incubation, Signal Capture, Signal Imaging, and Cleaning steps using color-coded progress bars displayed within each chip icon (C) and in the overview's bar underneath (D). Overall remaining run time (E) and time of completion for the run (F) are provided. The status bar on the IsoLight will change colors accordingly.

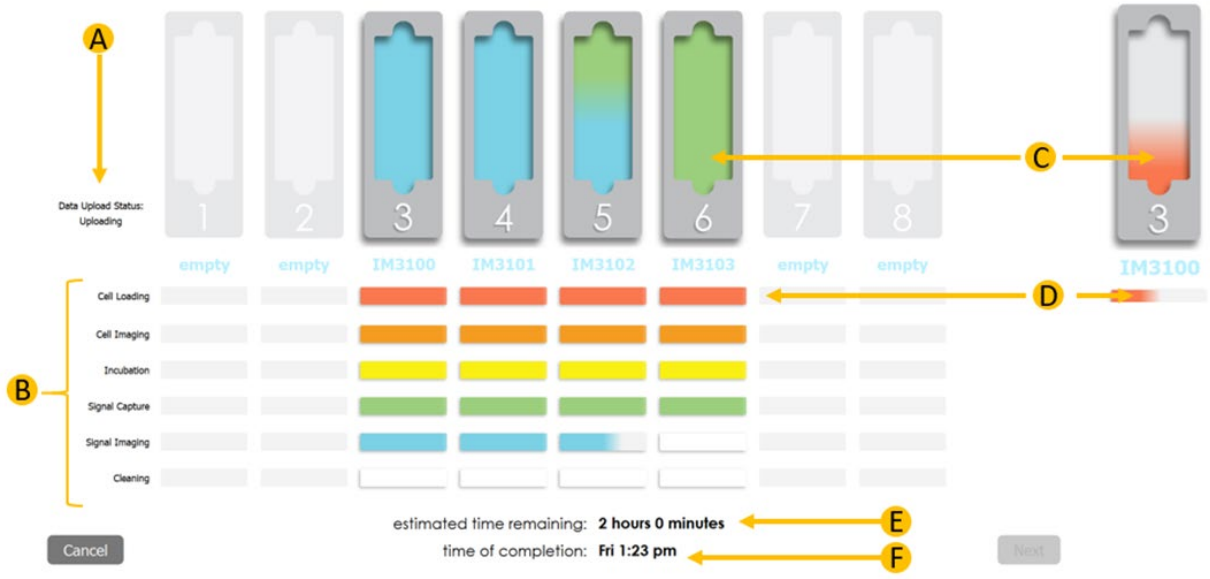


Figure 6 - User interface providing color-coded progress bars, steps, and runtime information for Single-Cell Secretome Chip runs

Message Windows

An interactive step-by-step guide is displayed during experiment setup assisting with chip loading, display status of IsoLight analysis, and run completion information.

CodePlex Chips

Step Indicators

Real-time information of run progression is displayed on screen (Figure 77) for each of the four major steps:

(A): Data Upload Status (B) Sample Incubation, Signal Capture, Signal Imaging, and Cleaning steps using color-coded progress bars displayed within each CodePlex chip icon (C) and in the overview's bar underneath (D). Overall remaining run time (E) and time of completion for the run (F) are provided. The status bar on the IsoLight will change colors accordingly.

Figure 7 - User interface providing color-coded progress bars, steps, and runtime information for CodePlex Chip runs

Message Windows

An interactive step-by-step guide is displayed during experiment setup assisting with chip loading, display status of IsoLight analysis, and run completion information.

Single-Cell Signaling Chips

Step Indicators

Real-time information of run progression is displayed on screen (Figure 88) for each of the six major steps:

(A): Data Upload Status (B) Cell Loading, Cell Lysis, Cell Imaging, Incubation, Signal Capture, Signal Imaging, and Cleaning steps using color-coded progress bars displayed within each chip icon (C) and in the overview's bar underneath (D). Overall remaining run time (E) and time of completion for the run (F) are provided. The status bar on the IsoLight will change colors accordingly.

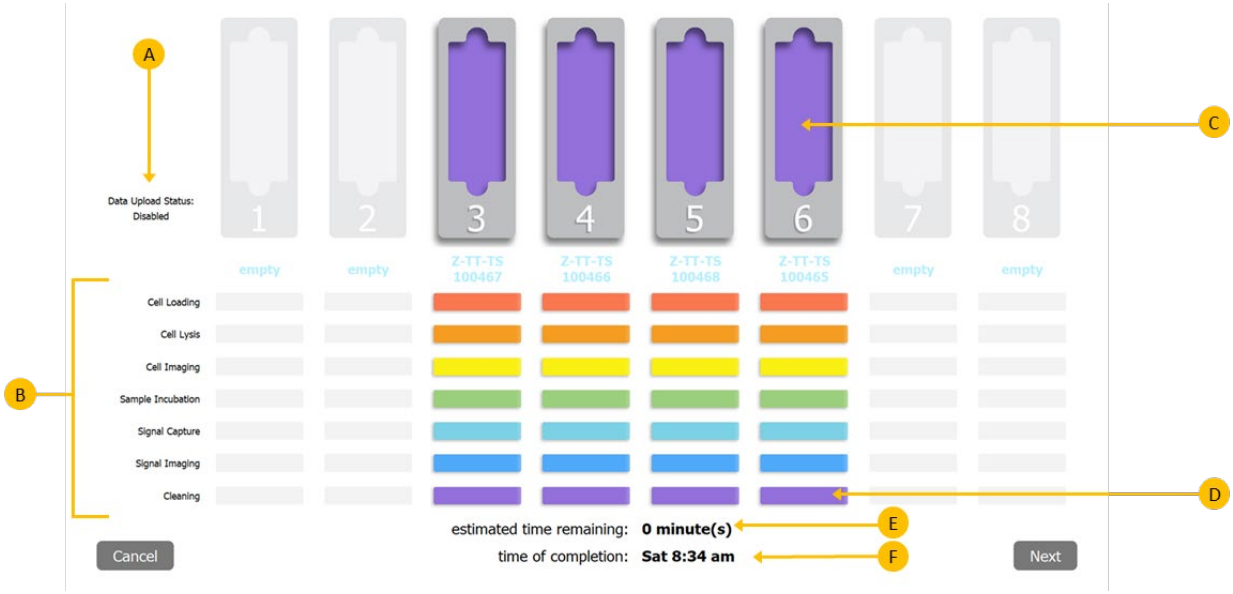


Figure 8 - User interface providing color-coded progress bars, steps, and runtime information for Single-Cell Signaling Chip runs

Message Windows

An interactive step-by-step guide is displayed during experiment setup assisting with chip loading, display status of IsoLight analysis, and run completion information.

IsoSpeak Data Analysis

Upon completion of the IsoLight run, analysis is continued using the IsoSpeak data analysis software, which comes pre-installed on the IsoSpeak analysis laptop.

IsoSpeak Overview

IsoSpeak is a data analysis application that processes data generated by the IsoLight software and provides a suite of bioinformatics tools for subsequent analysis. Data needs to be transferred from the IsoLight instrument to the IsoSpeak Analysis Laptop using the provided USB drive or over ethernet (if available).

There are several steps involved in the IsoSpeak analysis process. These are described in the IsoSpeak Software Guide.

Required Disk Space and File Management

The integrated instrument computer has approximately 450 GB of data storage capacity. Each Single-Cell chip requires approximately 15 GB of data and each CodePlex chip requires approximately 7 GB of data. The software prompts the user if insufficient disk space is available to initiate the run. It is recommended to transfer data from previous runs to a backup server and delete old run data on the IsoLight on a regular basis. Please see instructions on page 46 in the Appendix on how to clear disk space on the IsoLight.

Consumable Kit Overview

Each run requires the use of a consumable kit. The kit contains all the necessary reagents to perform a run on the IsoLight. Please refer to our website for ordering details and available Single-Cell and CodePlex chip types.

Note: The consumable kits that are shipped contain components that need to be stored at different temperatures (RT, 4°C, -20°C). Please see product insert for details.

Single-Cell Chip

The Single-Cell chip is at the core of the IsoLight single-cell technology.

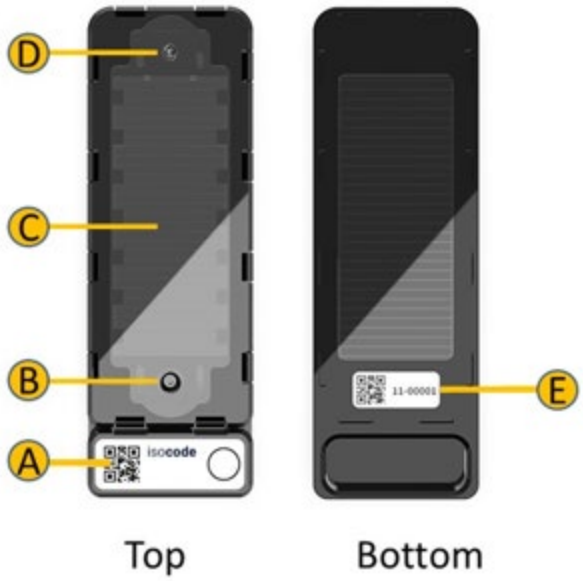


Figure 9 – Single-Cell Chip features

Note: *For illustration purposes, the capture chip shown appears clear whereas production chips are tinted in black*

A – Chip identifier

B – Inlet port/Sample loading port

C – Imaging area

D – Outlet port

E – Barcode scanned by barcode scanner

The single-use Single-Cell Chips have an inlet port and outlet port (Figure 9B and Figure 9D, respectively). The inlet port is greater in diameter and used for sample loading. Reagents enter the Single-Cell Chip through the inlet port, pass through the single-lane imaging area, and then exit the Single-Cell Chip through the outlet port. Waste exiting the Single-Cell Chip is delivered to the provided waste bottle residing next to the instrument. The barcode (Figure 9E) is required for sample tracking and "flow- patterning" information for the IsoSpeak software (see IsoSpeak section for details).

CodePlex Chip

The CodePlex Chip is a bulk cytokine population assay that targets 30+ cytokines in bulk and automated on the IsoLight system.

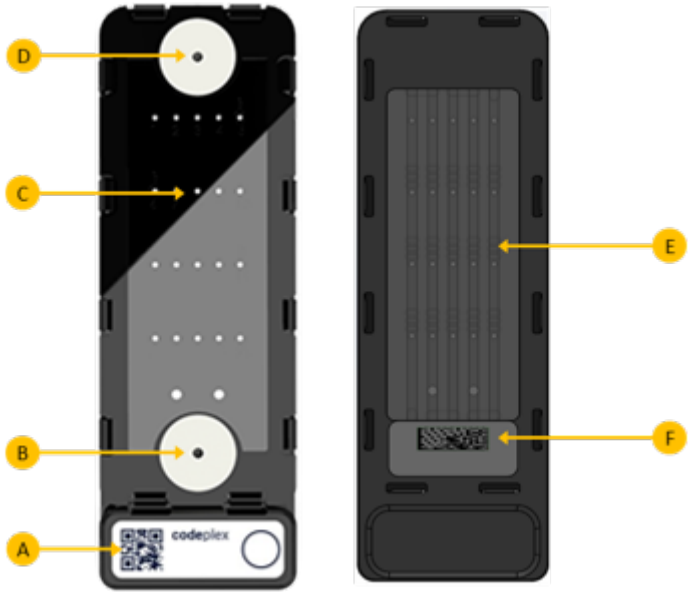


Figure 10 - CodePlex chip features

Note: *For illustration purposes, the capture chip shown appears clear whereas production chips are tinted in black*

A - Chip identifier

B – Inlet port

C – Sample ports with numerical identifiers

D – Outlet port

E – Imaging area

F – Barcode scanned by barcode scanner

The CodePlex chips have an inlet port and outlet port (Figure 1010B and Figure 1010D, respectively). Reagents enter the CodePlex Chip through the inlet port, pass through the flow cell, and then exit the CodePlex Chip through the outlet port. Waste exiting the CodePlex Chip is delivered to the provided waste bottle residing next to the instrument. The barcode (Figure 1010F) is required for transferring production data specific to each chip serial number to the IsoSpeak software (see IsoSpeak section for details).

Chip storage and handling

Chips are supplied in vacuum-sealed pouches, which must be stored at -20°C upon receival.

> **WARNING**
>
> This set of reagents contains bleach, which is corrosive. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, powder-free gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region.

Liquid Consumables Overview

Each run requires the user to attach 10 sippers and 10 conical tubes containing single-use IsoLight reagents.

Tubes A and B require the addition of detection antibodies prior to use (Step 3: Prepare and load single-use Reagent tubes).

Note: Only use provided tubes A and B as tubes from other manufacturers may not be compatible with the IsoLight.

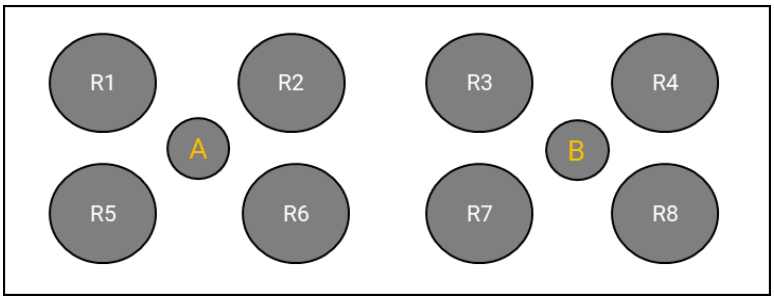


Figure 11 - Consumable box containing labeled consumables and empty tubes A and B

Table 1: Kit Reagent Tubes

Label	Reagent Name	Description
Cocktail A Mixing Tube	Cocktail A mixing tube	15 mL tube prefilled with buffer to dilute detection Cocktail A
Cocktail B Mixing Tube	Cocktail B mixing tube	15 mL tube prefilled with buffer to dilute detection Cocktail B
R1, R2, R3, R4, R5, R6, R8	Run reagents	Various ready-to-use single-use reagents required for run
R7	Run reagent 7	Contains bleach
Cocktail A	Concentrated detection cocktail A	Microcentrifuge tube “A” containing concentrated detection cocktail
Cocktail B*	Concentrated detection cocktail B	Microcentrifuge tube “B” containing concentrated detection cocktail

*Cocktail B not used with Single-Cell Signaling kits

Table 2: Provided Non-Liquid Consumables

Position	Item	Description
A, B, 1-8	Sippers	Single-use sippers to be attached to reagent tray

Waste Bottle

The waste bottle can be placed on the same surface as the IsoLight. It can also be positioned such that the bottom of the waste bottle rests no more than 30.5 cm below the surface that the IsoLight rests on.

Starting the IsoLight

Note: For best performance, leave the instrument on continuously. However, if needed, the instrument can be turned off using the power switch at the back of the instrument (Figure 412). Wait a minimum of 60 seconds before turning the power switch back to the ON position.

1. If the IsoLight is not already on, reach around the left side of the instrument to locate the power switch on the back panel.
2. Turn the power switch to the ON position. The integrated instrument computer starts.

Figure 42

Wait until the operating system has finished loading. When the system is ready, the IsoLight Software launches and initializes the system automatically. After the initialization step is complete, the home screen appears.

Set Data Upload Location

The user can specify the external automatic data upload location of IsoLight image files for IsoSpeak analysis. The IsoLight software will copy the raw image files to this location during the IsoLight run. This folder can be on either the provided USB flash drive or the local network.

1. From the home screen, press “Set data upload location.”
2. Find the USB drive or network location.
3. The file location on the home screen will turn black if connected.

See page 45 in the Appendix for more details on setting upload location. See Site Prep Guide for more info on available data transfer options.

Shut Down the Instrument

If the instrument needs to be shut down, please turn off the power switch at the rear left side of the instrument.

Chapter 3: Performing an IsoLight Run

Introduction

To perform a run using a Single-Cell experiment kit on the IsoLight, follow the setup steps described in this chapter.

Important IsoLight Notes Before Starting the Run

It is important to perform an 8-chip experiment at least once every two weeks. Within a two-week time period, if the instrument will be idle or if experiments with less than 8 chips are run, it is necessary to complete a maintenance cleaning. If at least one 8-chip experiment is run within a two-week time period, it is not necessary to perform a maintenance cleaning. Deep cleans should be performed monthly.

Required cleaning products:

1. Reusable Cleaning Chips (ISOCODE-1800-8)
2. Reagents: Reusable Cleaning Tube Set (ISOCODE-1701-8) or Prefilled Single-Use Cleaning Tube Set (ISOCODE-1700-8)

Maintenance cleaning will be complete in approximately 1.5 hours and deep cleaning in approximately 3 hours. Please see the Appendix for instructions.

WARNING

The IsoLight is sensitive to vibration. Touching the instrument after starting a run could adversely affect IsoLight results.

After selecting Start Run, do not open the chip compartment or the reagent compartment doors, or touch the instrument monitor except to exit the run.

WARNING

Do not plug in or remove external media via USB while the IsoLight run is in progress. Also make sure to close all files on the IsoLight before starting a run, and do not open files during a run.

Pre-run check CO_2, last clean and waste level

1. When using CO_2 tanks: Make sure that the CO_2 tank valve is open and there are no leaks on any fittings. An automatic switchover system with at least two tanks is recommended to ensure CO_2 delivery throughout the run. Contact Bruker support for an automatic switchover system recommendation. If using only one tank, ensure that the pressure of the tank is at least 750 psi (5 MPa). Exchange the tank for a new tank if the pressure is below 750 psi (5 MPa).

   The system can stay pressurized even when the instrument is not running, there is no need to close the CO_2 valve between runs. Ensure that CO_2 is supplied at 30 to 70 psi (0.20 MPa to 0.48 MPa) into the instrument.

2. Check that external 1L waste bottle is at least half empty and attached to the waste line (labeled as “Waste”).

Note: Only use the provided waste bottle and vented cap for waste collection. If replacement is necessary, please make sure that a vented cap is being used.

Performing a Single-Cell Secretome Run

Workflow Overview

This IsoLight User Guide covers Steps 3 – 4 of the overall workflow depicted in Figure 53 below. Please refer to the sample preparation protocols for cell stimulation and staining steps (Figure 53, step 1). For details on how to use the IsoSpeak software, please refer to the IsoSpeak User Guide (Figure 53, step 5).

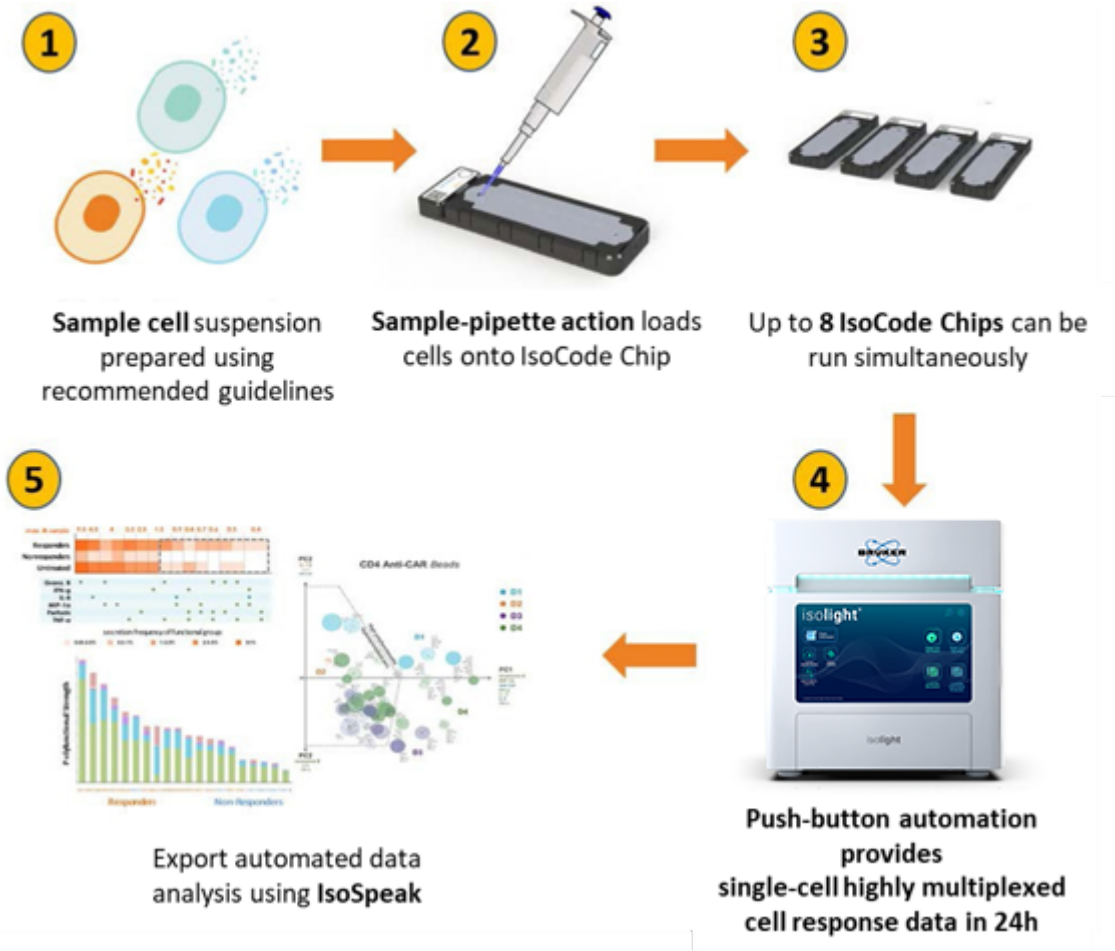


Figure 53

Run Duration – Single-Cell Secretome Chips

# of Single-Cell Chips	4	8
Duration [h]	20	24.25

Step 1: Pre-run check CO_2, last clean and waste level

See the Introduction section in this chapter for details on this step.

A Single-Cell chip run will consume approximately 17 lbs (7.7 kg) of CO_2.

Step 2: Prepare and load single-use reagent tubes

1. Remove the reagent box from 4°C storage.
2. Invert tubes R1 to R8 several times to mix and inspect contents. R2-R8 should contain approximately 45 mL of solution.
3. Dilute kit reagents A and B:
    a. Briefly spin down Cocktail A and Cocktail B microcentrifuge tubes.
    b. Pipette volume of Cocktail A specified on the microcentrifuge tube label into the solution in Cocktail A Mixing Tube.
    c. Pipette solution in Cocktail A Mixing tube 3-5 times with a p1000 set to 1000 uL to ensure solution is well-mixed. Avoid generating bubbles.
    d. Pipette volume of Cocktail B specified on the microcentrifuge tube label into the solution in Cocktail B Mixing Tube.
    e. Pipette solution in Cocktail B Mixing tube 3-5 times with a p1000 set to 1000 uL to ensure solution is well-mixed. Avoid generating bubbles.

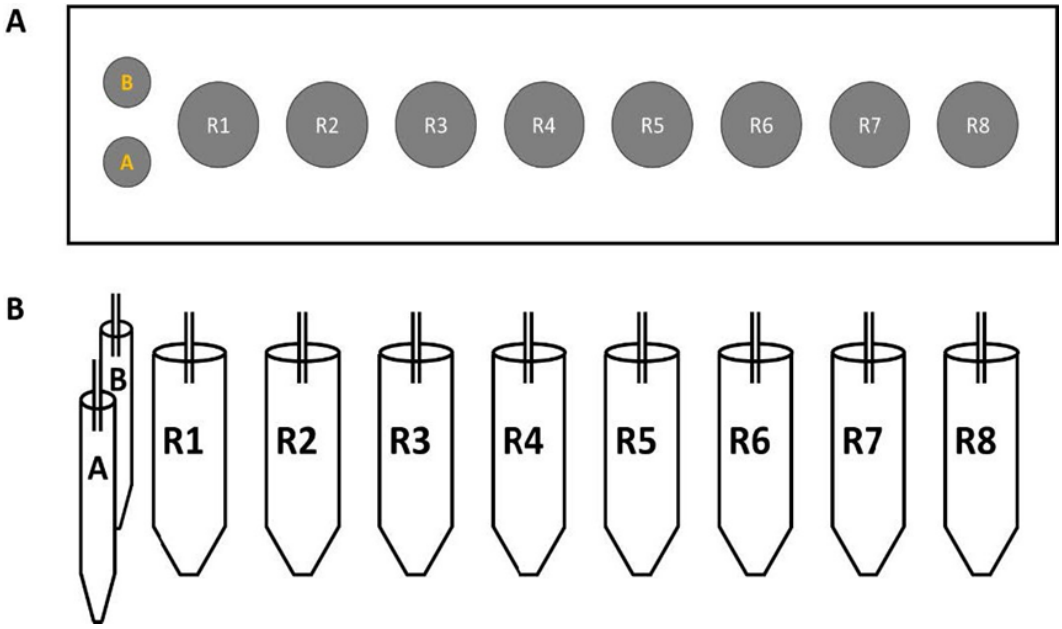


Figure 64 - Reagent loading positions

4. Attach the sippers to each position in the loading bay (Figure 64) by pushing them firmly onto the nozzle. Put on new gloves immediately before handling the sippers.

WARNING

Wear new, powder-free gloves, immediately before handling and attaching the sippers

5. Tubes and reagent bay positions are labeled. Attach each tube by firmly screwing the tube into the corresponding position until they come to a full stop. For example, tube R1 is attached to position R1 on the instrument.

Step 3: Load and Run Chips

Ensure there is enough free space on the IsoLight

If automatic upload is desired, ensure that the data upload location is set (see page 18). External location must have enough space for all IsoLight images (14 GB for each Single-Cell chip).

Note: The IsoLight itself requires 200 GB of free space locally to start and complete a given run.

Select an assay

Select the desired Single-Cell Secretome assay to run from the home screen. Selecting the wrong assay for the type of consumable chip inserted into the IsoLight will prevent the user from starting their run after the consumable barcodes are scanned.

Instrument Initialization

The system initializes and checks for sufficient disk space and will notify user to clear space if disk space is insufficient (see pages 46-47).

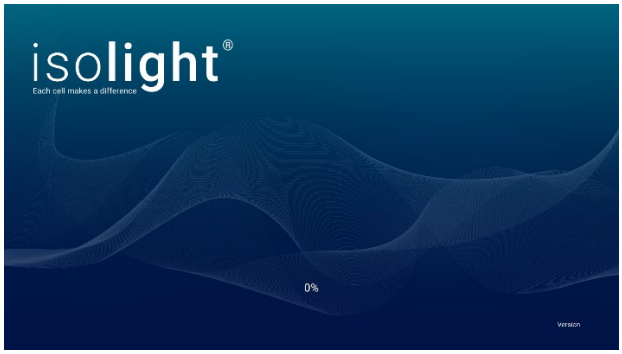


Figure 75 - IsoLight software initialization splash screen

Prepare and load chips

1. Once the screen looks like Figure 86, follow the appropriate Sample Preparation Protocol to prepare and load the chips with samples.

Figure 86 - Chip loading screen

2. Click “Open Chip Tray” to move the tray out (Figure 97).

Figure 97 - Open chip tray

3. Chips need to be loaded from the ”inside out” (not “left-to-right”) as shown in Figure 108:
    a. Remove the protective blue tape on the bottom of the chip. Failure to remove will interfere with imaging and invalidate results.
    b. Holding the chip on the labeled side and with the ports facing up, slide chip into the tray until it clicks into position by engaging with the magnet.
    c. Verify that each chip is pushed all the way to its stop and that all chips are loaded in the proper orientation.
4. Press “Close Chip Tray” and the instrument will start scanning the chip barcodes.

Note: Wear gloves at all times when handling chips. Do not touch the bottom and top surface of the chip.

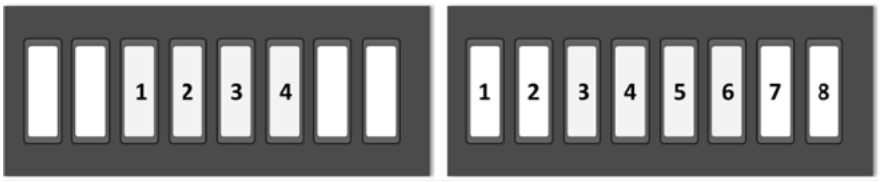


Figure 108 - Chip loading positions for 4,and 8 Single-Cell Secretome Chips

Note: If the barcode does not scan correctly, press the “INVALID” text label and enter the appropriate number for that chip. See page 41 for troubleshooting details.

Note: If the IsoLight detects consumables that do not match the type of assay selected at the home screen, the IsoLight will display an error to the user and the user will not be able to proceed. Press “Exit” to return to the home screen and select the appropriate assay for consumables loaded.

5. Press ”Start Assay” after all tray positions were scanned and the chips displayed (Figure 119).

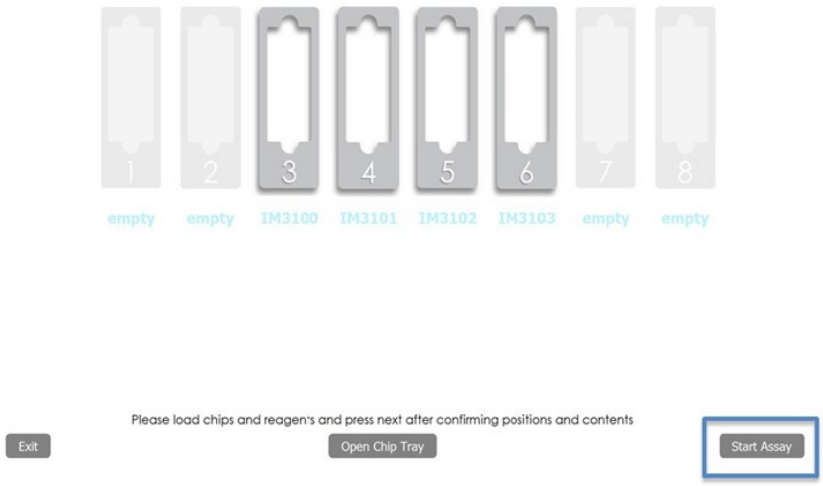


Figure 119

During the run

The instrument now executes all the required steps from cell loading to post-run cleaning and informs the user of the run status using color coded progress bars as described in Figure 66.

Finishing the run

1. Press "Next" button which activates after the run completed (Figure).

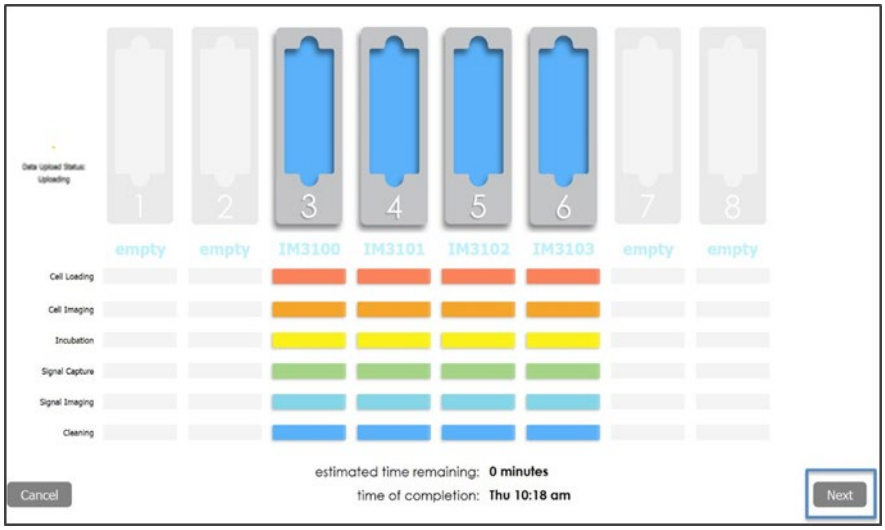


Figure 20 – User interface at end of run

2. Remove the chips by pressing "Remove Chips" (Figure 121) and close chip tray.
3. If not configured to transfer automatically, use provided USB drive to transfer data. See IsoSpeak overview on page 9 for downstream analysis.
    a. Green text indicates all upload occurred during the IsoLight run and no other upload is necessary.
    b. Black text indicates the data is still being uploaded.

c. Red text indicates automatic upload did not occur. USB must be connected and manually uploaded.

4. Remove liquid consumable conical tubes and sippers from the reagent bay and dispose of any waste in the external waste bottle.

5. Press “Finish” to conclude the run and to return to the home screen.

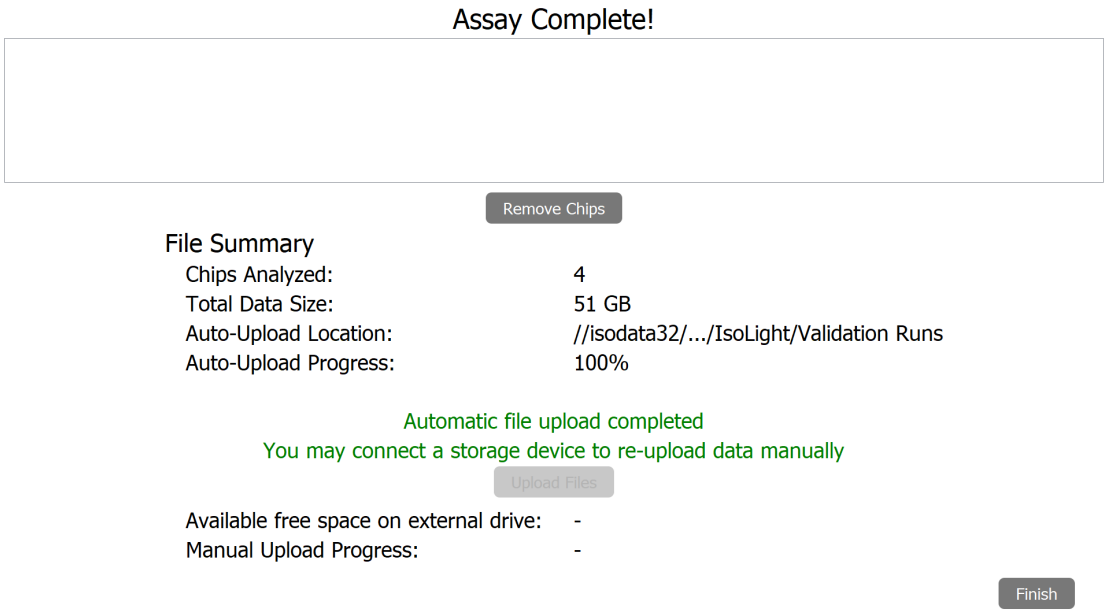


Figure 121 – “Assay Complete!” screen provides run summary information and data export options

Performing a CodePlex Run

Workflow Overview

This User Guide covers Steps 3 – 4 of the overall workflow depicted in Figure 132 below. Please refer to the sample preparation protocols for preparing and loading samples (Figure 132, step 1). For details on how to use the IsoSpeak software, please refer to the IsoSpeak User Guide (Figure 132, step 5).

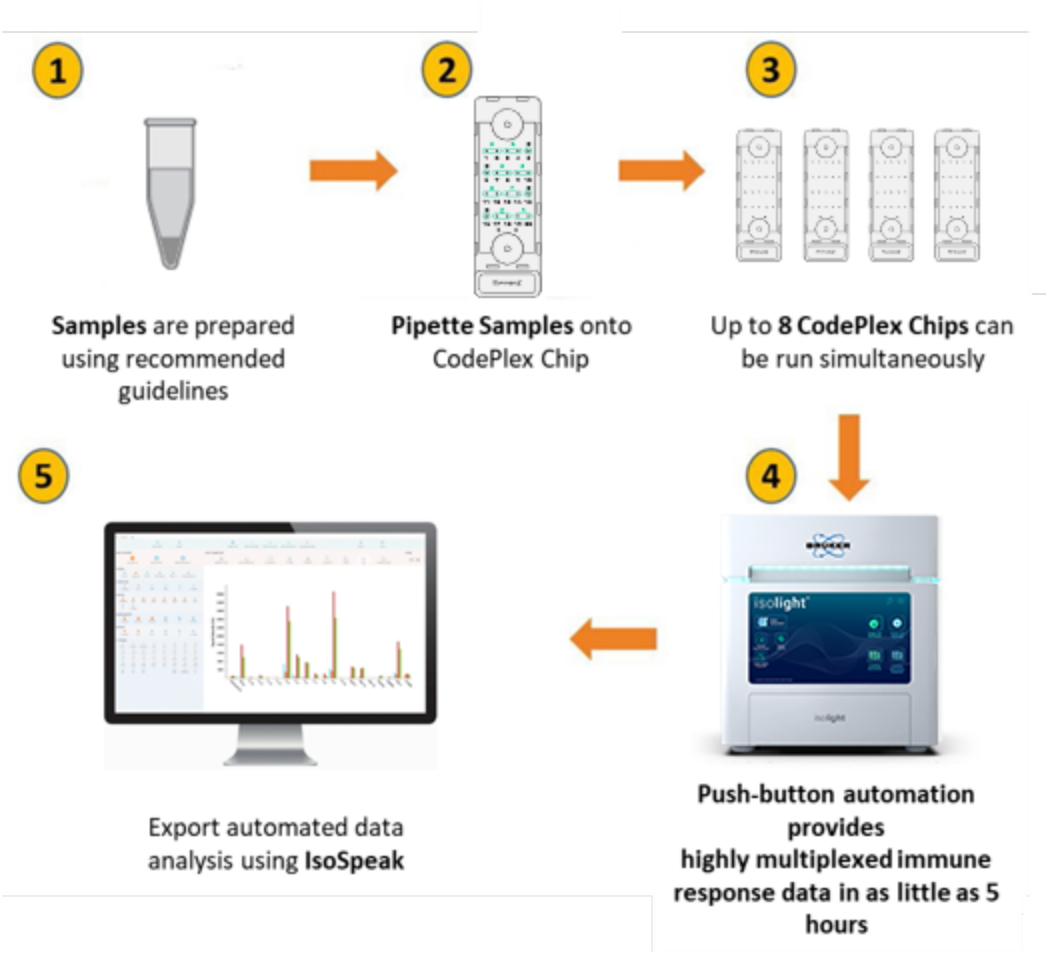


Figure 132

Run Duration – CodePlex Chips

# of CodePlex Chips	1	2	4	6	8
Duration [h]	4.5	5.5	7.75	10	12

Step 1: Pre-run check CO_2, last clean and waste level

See the Introduction section in this chapter for details on this step.

A CodePlex Chip run will consume less than 1 lb (0.45 kg) of CO_2.

Step 2: Prepare and load single-use reagent tubes

1. Remove the reagent box from 4°C storage.
2. Invert tubes R1 to R8 several times to mix and inspect content. R2-R8 should contain approximately 45 mL of solution.
3. Dilute kit reagents A and B:
    a. Briefly spin down Cocktail A and Cocktail B microcentrifuge tubes.
    b. Pipette volume of Cocktail A specified on the microcentrifuge tube label into the solution in Cocktail A Mixing Tube.
    c. Pipette solution in Cocktail A Mixing tube 3-5 times with a p1000 set to 1000 uL to ensure solution is well-mixed. Avoid generating bubbles.
    d. Pipette volume of Cocktail B specified on the microcentrifuge tube label into the solution in Cocktail B Mixing Tube.
    e. Pipette solution in Cocktail B Mixing tube 3-5 times with a p1000 set to 1000 uL to ensure solution is well-mixed. Avoid generating bubbles.

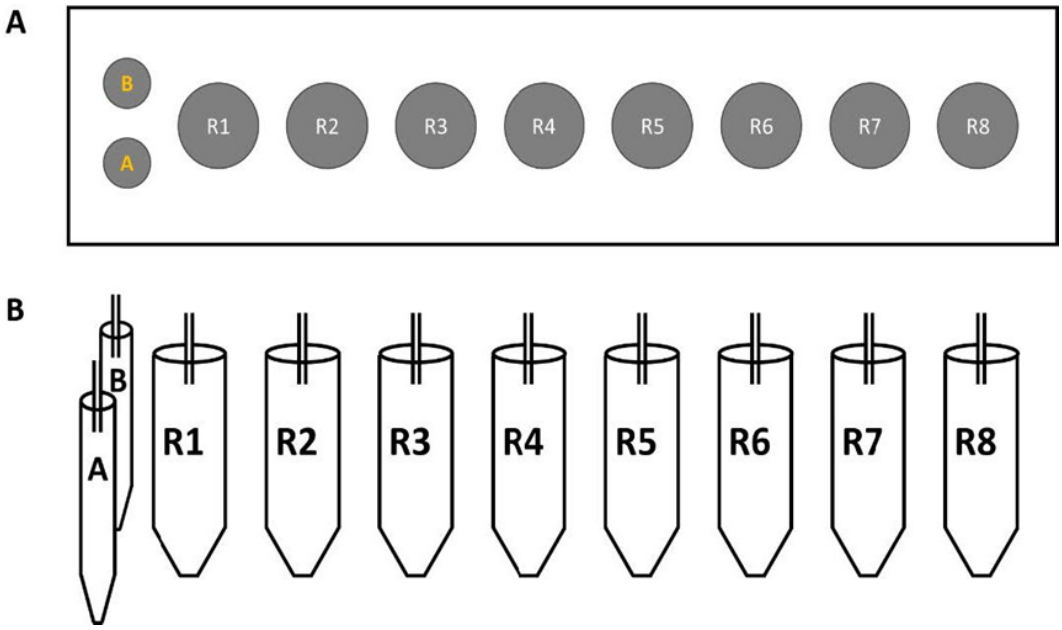


Figure 143 – Reagent loading positions

4. Attach the sippers to each position in the loading bay (Figure 143) by pushing them firmly onto the nozzle. Put on new gloves immediately before handling the sippers.

WARNING

Wear new, powder-free gloves, immediately before handling and attaching the sippers

5. Tubes and reagent bay positions are labeled. Attach each tube by firmly screwing the tube into the corresponding position until they come to a full stop. For example, tube R1 is attached to position R1 on the instrument.

Step 3: Load and Run Chips

Ensure there is enough free space on the IsoLight

If automatic upload is desired, ensure that the data upload location is set (see page 18). External location must have enough space for all IsoLight images (7 GB for each CodePlex chip).

Note: The Isolight itself requires 200 GB of free space locally to start and complete a given run.

Select an assay

Press “CodePlex Secretome”. Selecting the wrong assay for the type of consumable chip inserted into the IsoLight will prevent the user from starting the assay after the consumable barcodes are scanned.

Instrument Initialization

The system initializes and checks for sufficient disk space and will notify user to clear space if disk space is insufficient (see pages 46-47).

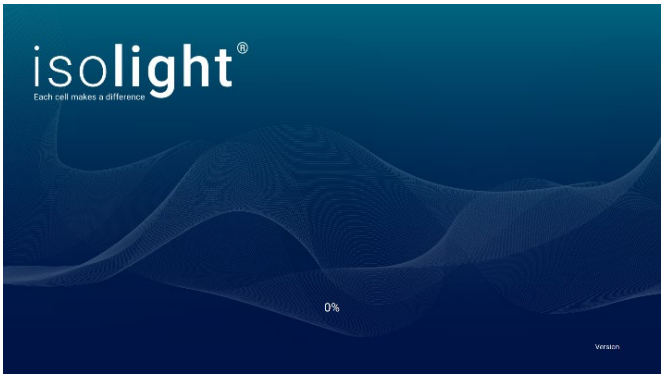


Figure 24 - IsoLight software initialization splash screen

Scan Calibration Chip

The calibration chip window (Figure 155) will be displayed when CodePlex is selected.

1. Press “Open Chip Tray”

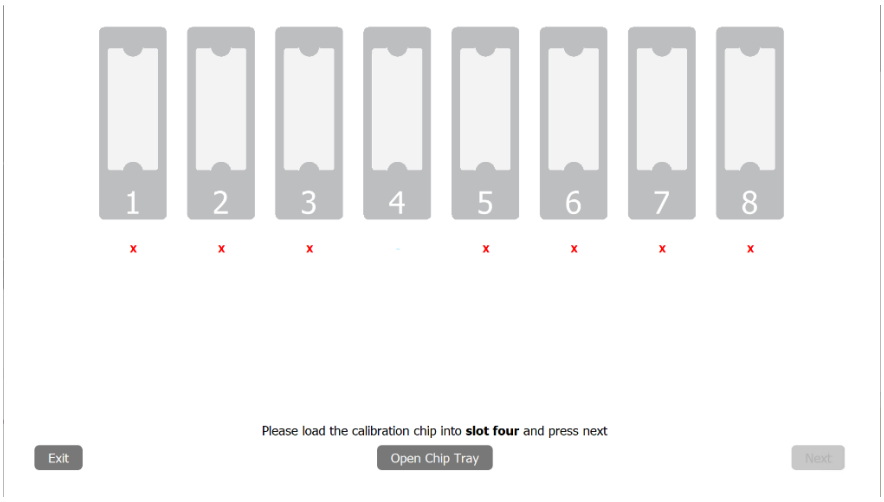


Figure 155 - Calibration Window

2. Load CodePlex Calibration Chip provided with the CodePlex Kit into tray position 4 once the tray is open. Load the chip with the small screw facing the IsoLight and "CAL" label facing up as shown in Figure 166.

   The Calibration Chip contains standard curve data and enables the IsoLight system and IsoSpeak software to display concentrations in pg/mL.

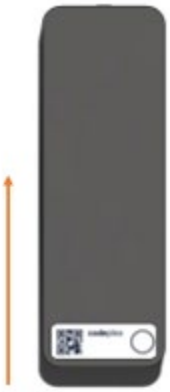


Figure 166 - Proper Orientation for Loading Calibration Chip into IsoLight

3. Press "Close Chip Tray" once the Calibration Chip is loaded. After the Calibration Chip barcode scans (takes a moment), the display will appear as in Figure 177.

   Note: If the barcode does not scan correctly it will display "INVALID". Press "Open Chip Tray". Check chip orientation and compare with Figure 166. Verify the chip is fully inserted into the tray. Press "Close Chip Tray" and the IsoLight will scan the Calibration Chip barcode again. If "INVALID" is displayed again, select "Next" to complete the CodePlex assay. See Chapter 4 for troubleshooting details.

Figure 177 – Calibration Chip Scanned Successfully

4. Press "Next" to continue. The tray will automatically open with a message stating to remove the Calibration Chip.

Prepare and load chips

1. Once IsoLight display matches Figure 188, follow the appropriate Sample Preparation Protocol to prepare and load the chips with samples. Apply provided cover tape to top of consumable. Ensure even application with provided tape application tool.

Figure 188 - Chip loading screen

2. Click "Open Chip Tray" to move the tray out (Figure 199)

Figure 199 - Open chip tray

3. Chips need to be loaded from the "inside out" (not "left-to-right") as shown in Figure 30:
    a. Remove the protective blue tape on the bottom of the chip. Failure to remove will interfere with imaging and invalidate results.
    b. Holding the chip on the labeled side and with the ports facing up, slide chip into the tray until it clicks into position by engaging with the magnet.

c. Verify that each chip is pushed all the way to its stop and that all chips are loaded in the proper orientation.

4. Press “Close Chip Tray” and the instrument will start scanning the chip barcodes.

   The instrument will also verify that the Cover Tape over the Sample Wells has been properly applied. If the cover tape is not detected, the Software will display an error (see Chapter 4 on page 41) and allow the user to open the tray to fix tape application.

   See CodePlex Sample Protocol for full details of proper application.

Note: Wear gloves at all times when handling chips. Do not touch the bottom surface of the chip or the white rubber inlet and outlet seals.

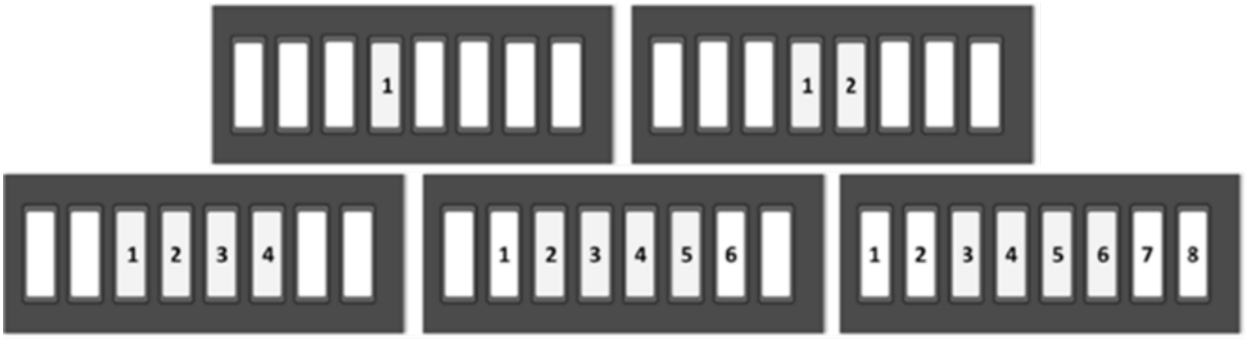


Figure 30 - Chip loading positions for 1, 2, 4, 6 and 8 CodePlex chips

Note: If the barcode does not scan correctly press the “INVALID” text label and enter the appropriate number for that chip. See page 41 for troubleshooting details.

Note: If the barcode read does not correspond to a CodePlex chip, exit out of the application, and select the appropriate assay at the home screen.

5. Press “Start Assay” after all tray positions were scanned and the chips displayed (Figure 201).

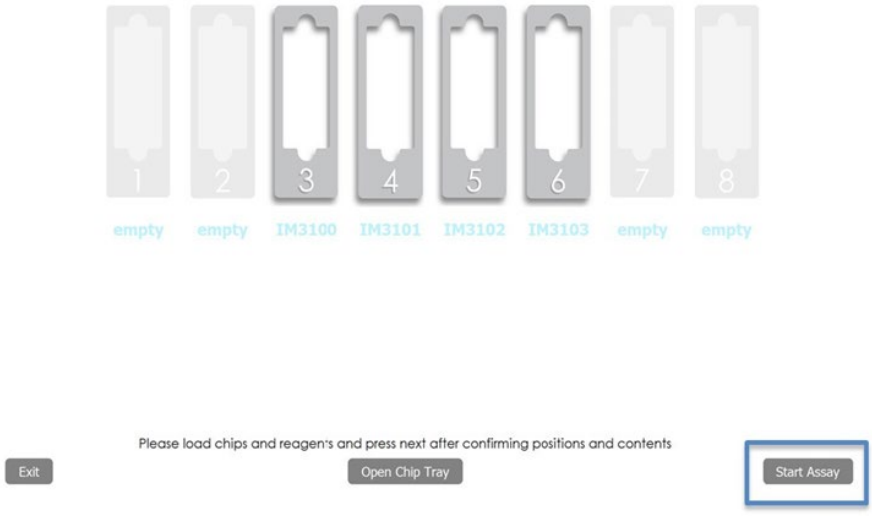


Figure 201

During the run

The instrument now executes all the required steps from sample incubation to post-run cleaning and informs the user of the run status using color coded progress bars as described in Figure 77.

Finishing the run

1. Press “Next” button which activates after the run completed (Figure 212).

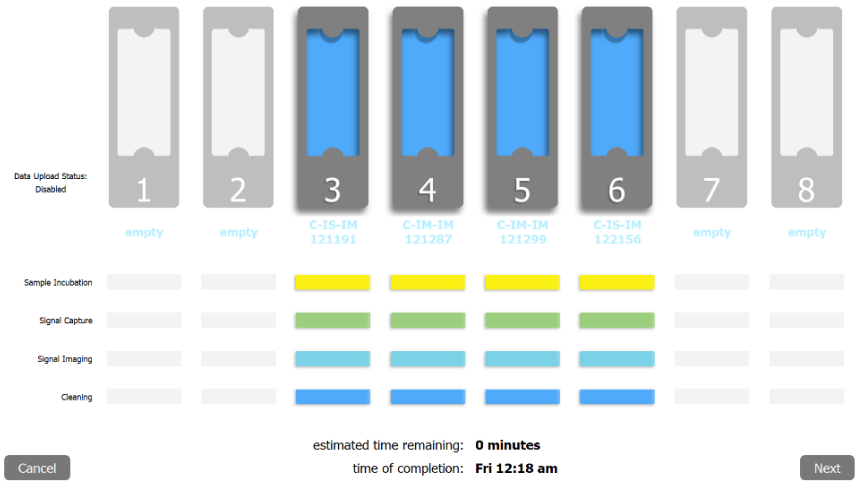


Figure 212 - User interface at end of run

2. Remove the chips by pressing “Remove Chips” (Figure 223) and close chip tray.
3. If not configured to transfer automatically, use provided USB drive to transfer data. See IsoSpeak overview on page 14 for downstream analysis.
    a. Green text indicates all upload occurred during the IsoLight run and no other upload is necessary
    b. Black text indicates the data is still being uploaded
    c. Red text indicates automatic upload did not occur. USB must be connected and manually uploaded
4. Remove liquid consumable conical tubes and sippers from the reagent bay and dispose of any waste in the external waste bottle.
5. Press “Finish” to conclude the run and to return to the home screen.

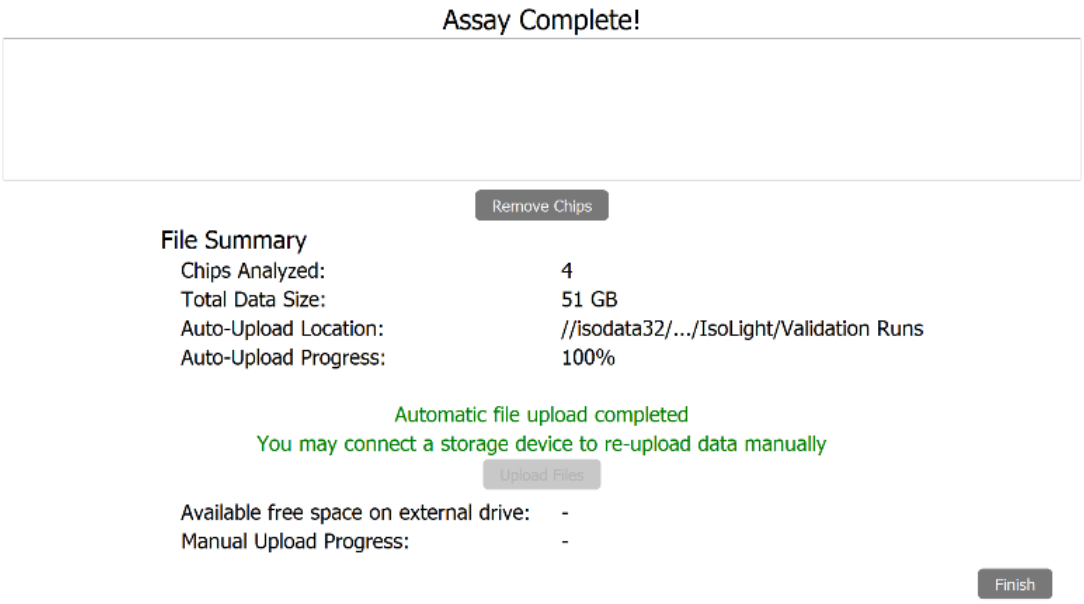


Figure 223 - "Assay Complete!' screen provides run summary information and data export options

Performing a Single-Cell Signaling Run

Workflow Overview

This IsoLight User Guide covers Steps 3 – 4 of the overall workflow depicted in Figure 5 below. Please refer to the sample preparation protocols for cell stimulation and staining steps (Figure 534, step 1). For details on how to use the IsoSpeak software, please refer to the IsoSpeak User Guide (Figure 5, step 5).

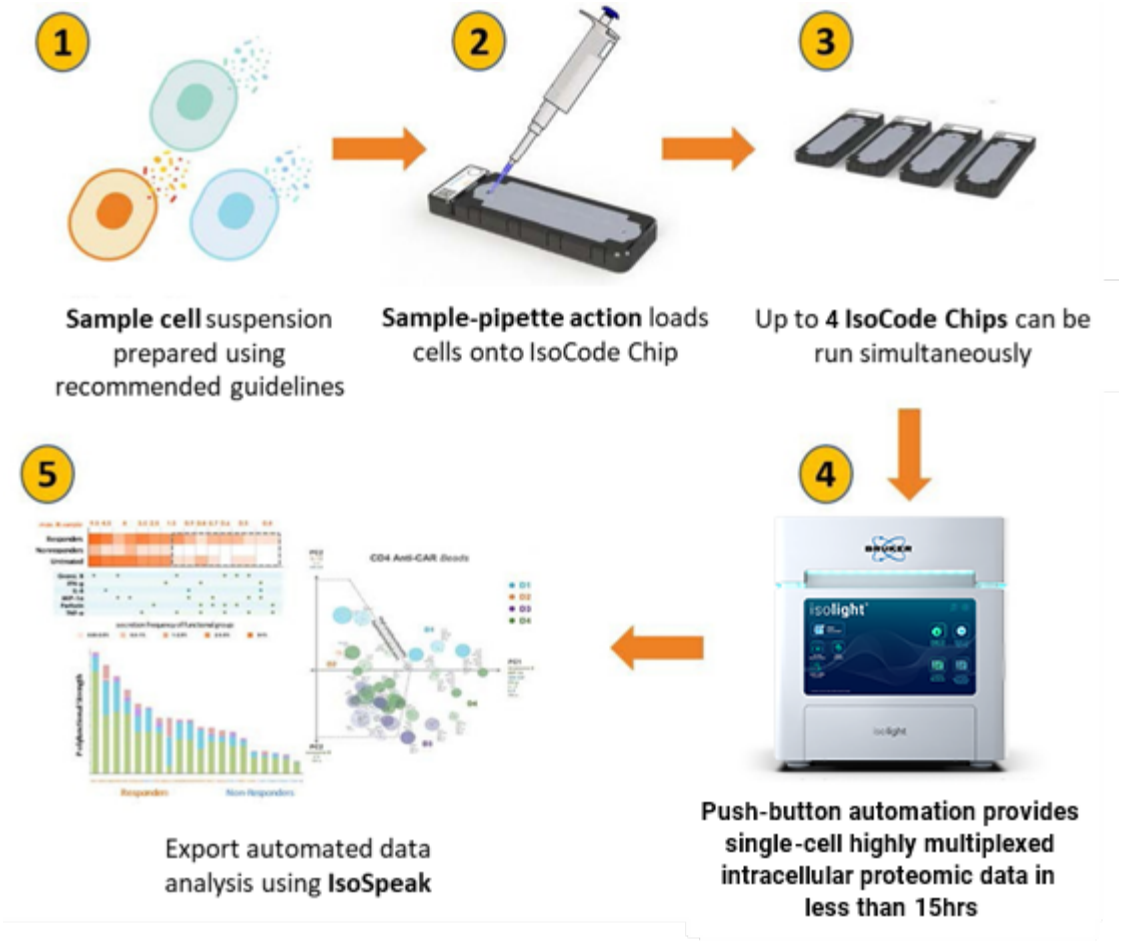


Figure 234

Run Duration – Single-Cell Signaling Chips

# of Single-Cell Signaling Chips	4
Duration [h]	15

Step 1: Pre-run check CO_2, last clean and waste level

See the Introduction section in this chapter for details on this step.

A Single-Cell Signaling chip run consumes less than 1 lb (0.45 kg) of CO_2.

Step 2: Prepare and load single-use reagent tubes

6. Remove the reagent box from 4°C storage and R2 from -20°C storage.
7. Invert tubes R1-R8 several times to mix and inspect contents. R3-R8 should contain approximately 45 mL of solution and R2 should contain approximately 15 mL.
8. Dilute kit reagents A:
    a. Briefly spin down the Cocktail A microcentrifuge tube.
    b. Pipette volume of Cocktail A specified on the microcentrifuge tube label into the solution in Cocktail A Mixing Tube.
    c. Pipette solution in Cocktail A Mixing tube 3-5 times with a p1000 set to 1000 uL to ensure solution is well-mixed. Avoid generating bubbles.
    d. The reagent B tube is used as supplied.

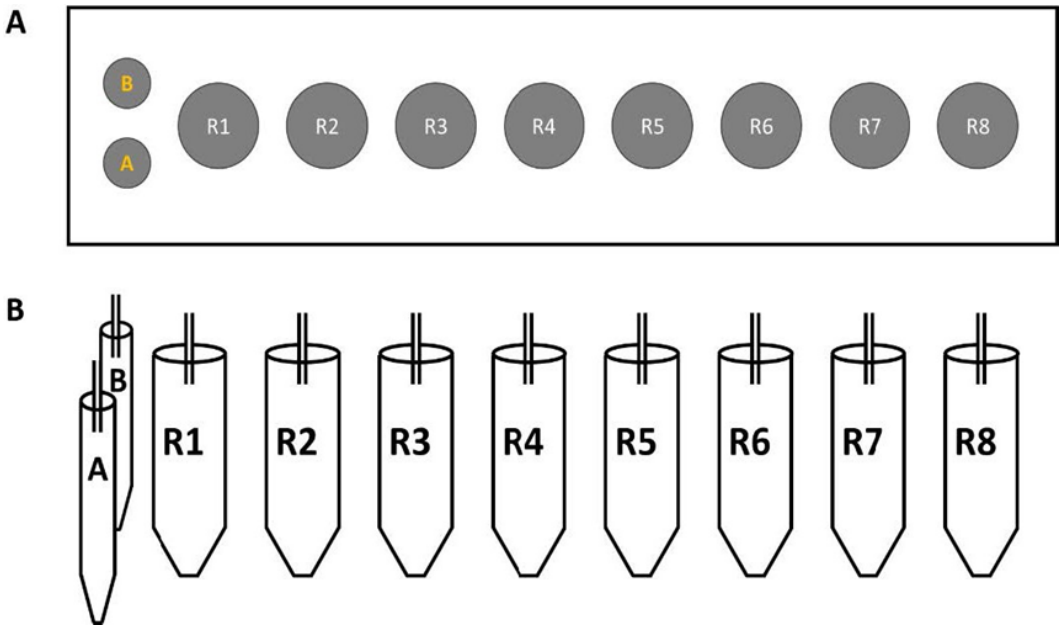


Figure 245 - Reagent loading positions

9. Attach the sippers to each position in the loading bay (Figure 635) by pushing them firmly onto the nozzle. Put on new gloves immediately before handling the sippers.

> **WARNING**
>
> Wear new, powder-free gloves, immediately before handling and attaching the sippers

10. Tubes and reagent bay positions are labeled. Attach each tube by firmly screwing the tube into the corresponding position until they come to a full stop. For example, tube R1 is attached to position R1 on the instrument.

Step 3: Load and Run Chips

Ensure there is enough free space on the IsoLight

If automatic upload is desired, ensure that the data upload location is set (see page 18). External location must have enough space for all IsoLight images (14 GB for each Single-Cell Signaling chip).

Note: The IsoLight itself requires 200 GB of free space locally to start and complete a given run.

Select an assay

Select the desired Single-Cell Signaling assay to run from the home screen. Selecting the wrong assay for the type of consumable chip inserted into the IsoLight will prevent the user from starting their run after the consumable barcodes are scanned.

Instrument Initialization

The system initializes and checks for sufficient disk space and will notify user to clear space if disk space is insufficient (see pages 46-47).

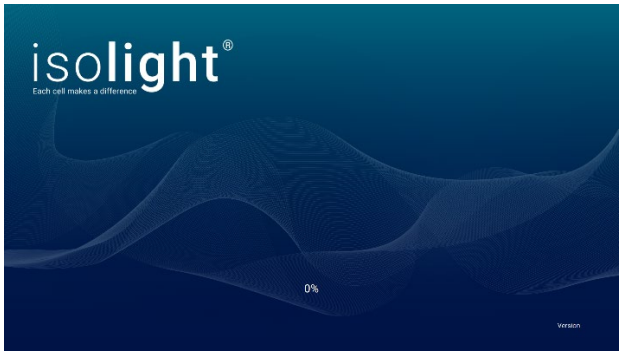


Figure 256 - IsoLight software initialization splash screen

Prepare and load chips

6. Once the screen looks like Figure 37, follow the appropriate Sample Preparation Protocol to prepare and load the chips with samples.

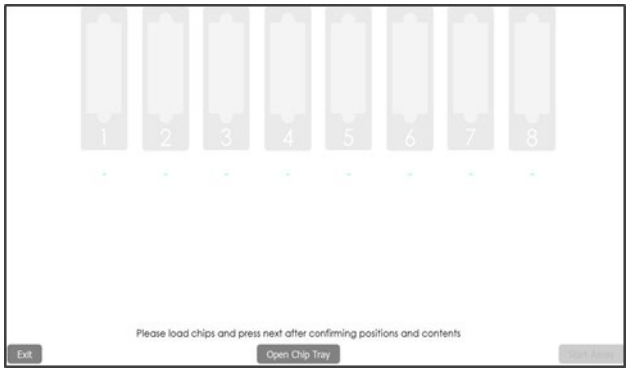


Figure 267 - Chip loading screen

7. Click “Open Chip Tray” to move the tray out (Figure 938).

Figure 278 - Open chip tray

8. Chips need to be loaded from the ”inside out” (not “left-to-right”) as shown in Figure 1039:
    a. Remove the protective blue tape on the bottom of the chip. Failure to remove will interfere with imaging and invalidate results.
    b. Holding the chip on the labeled side and with the ports facing up, slide chip into the tray until it clicks into position by engaging with the magnet.
    c. Verify that each chip is pushed all the way to its stop and that all chips are loaded in the proper orientation.
9. Press “Close Chip Tray” and the instrument will start scanning the chip barcodes.

Note: Wear gloves at all times when handling chips. Do not touch the bottom and top surface of the chip.

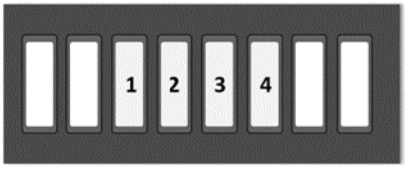


Figure 289 - Chip loading positions for 4 Single-Cell Signaling chip

Note: If the barcode does not scan correctly, press the “INVALID” text label and enter the appropriate number for that chip. See page 40 for troubleshooting details.

Note: If the IsoLight detects consumables that do not match the type of assay selected at the home screen, the IsoLight will display an error to the user and the user will not be able to proceed. Press “Exit” to return to the home screen and select the appropriate assay for consumables loaded.

10. Press ”Start Assay” after all tray positions were scanned and the chips displayed (Figure 40).

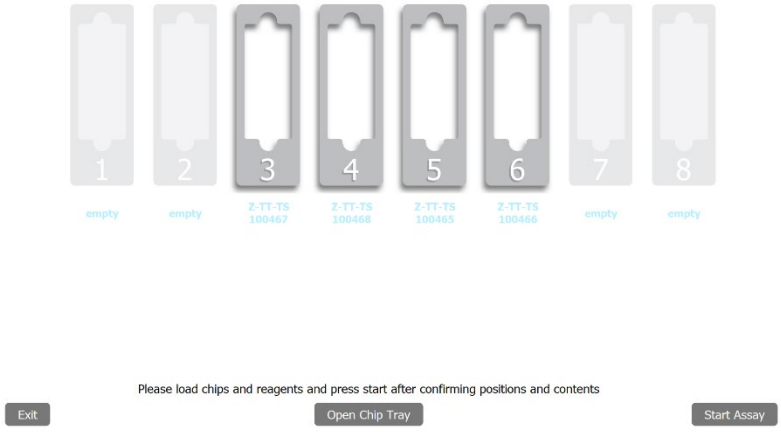


Figure 40

During the run

The instrument now executes all the required steps from cell loading to post-run cleaning and informs the user of the run status using color coded progress bars as described in Figure 68.

Finishing the run

6. Press “Next” button which activates after the run completed (Figure 41).

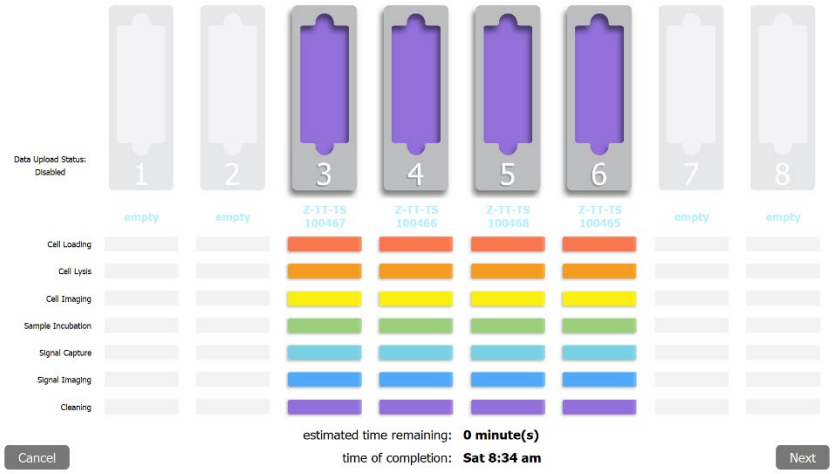


Figure 291 - User interface at end of run

7. Remove the chips by pressing “Remove Chips” (Figure 1242) and close chip tray.
8. If not configured to transfer automatically, use provided USB drive to transfer data. See IsoSpeak overview on page 9 for downstream analysis.
    a. Green text indicates all upload occurred during the IsoLight run and no other upload is necessary.
    b. Black text indicates the data is still being uploaded.
    c. Red text indicates automatic upload did not occur. USB must be connected and manually uploaded.
9. Remove liquid consumable conical tubes and sippers from the reagent bay and dispose of any waste in the external waste bottle.
10. Press “Finish” to conclude the run and to return to the home screen.

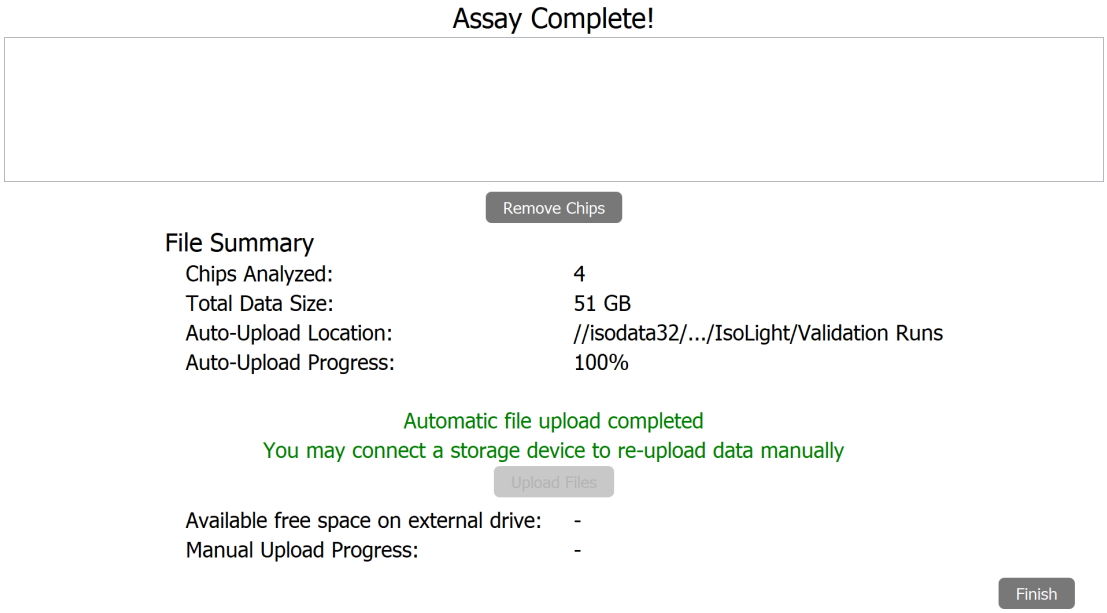


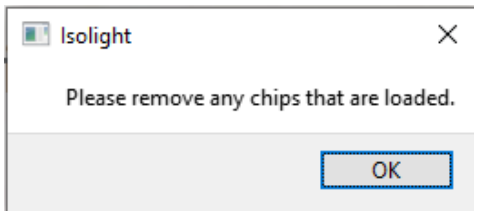
Figure 302 - "Assay Complete!" screen provides run summary information and data export options

Chapter 4: Troubleshooting

This chapter lists error messages can be encountered during an IsoLight run.

Initialization

1. If chips were detected during initialization:
    a. Tray will move out and pop up will appear.
    b. Remove all chips and click "OK" after chips were removed.
    c. The tray will close after pressing "OK." The instrument will continue initialization.



Note: Please contact Technical Support if no chips were loaded.

Chip Entry & Scanning

Calibration Chip failed to scan

During a CodePlex run the Calibration Chip failed to scan and "INVALID" was displayed.

Cause: Wrong chip orientation, damage to barcode etc.

1. Check the orientation of the chip as shown in Figure 166.
2. If the chip still failed to scan you may continue with the assay.
    a. Calibration chip data is not required to complete a CodePlex assay on IsoLight, but will be required to analyze data in IsoSpeak and view concentrations in pg/mL.
    b. Prior to IsoSpeak data analysis, contact Technical Support to obtain the required Calibration Chip standard curve information.

Invalid chip order detected - please check chip positions

Cause: The chips were not loaded from the center out and need to be reloaded.

1. Press "Open Chip Tray" and wait for tray to extend all the way.
2. Reposition chips according to Figure 108 (Single-Cell) or Figure 30 (CodePlex).
3. Press "Close Chip Tray" and the instrument will start scanning the chip barcodes again.

Invalid number of chips detected - please load a valid number of chips for this assay

Cause: There is an unsupported number of chips detected for this assay.

1. Press "Open Chip Tray" and wait for tray to extend all the way.

a. Ensure that the correct amount of chips are loaded into the IsoLight.

2. See Figure 108 (Single-Cell) or Figure 30 (CodePlex) for supported chip counts.
    a. E.g. Single-Cell – 4 or 8 chips
3. Press ”Close Chip Tray” and the instrument will start scanning the chip barcodes again.

Wrong chip type detected for assay selected - remove incorrect chip or exit application

Cause: The type of chip being used is unsupported for this assay.

1. Press “Open Chip Tray” and wait for tray to extend all the way.
2. Please check the first letter (prefix) of the chip ID. They determine which assay that chip should be used for.
    a. E.g. Take “C-SA-SA12345” as an example chip ID. It has ‘C’ as the prefix. This means it can only be run in a CodePlex assay
3. Press ”Close Chip Tray” and the instrument will start scanning the chip barcodes again.

Invalid barcode detected - please touch INVALID to manually enter the chip ID

Cause: Barcode did not scan correctly.

1. If a chip barcode could not be scanned, “INVALID” will appear under the chip instead of the chip number.
2. Press the “INVALID” text to enter in the chip number manually. See Figure 313.

    Note: It must be 6 digits. It can start with 0.

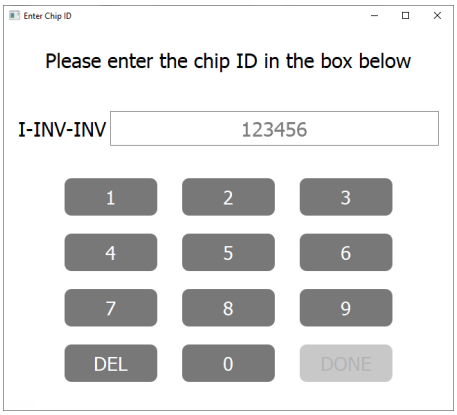


Figure 313 - Chip ID Popup after clicking "INVALID" text

CodePlex cover tape was not detected

Cause: Tape was not correctly applied or is not applied at all.

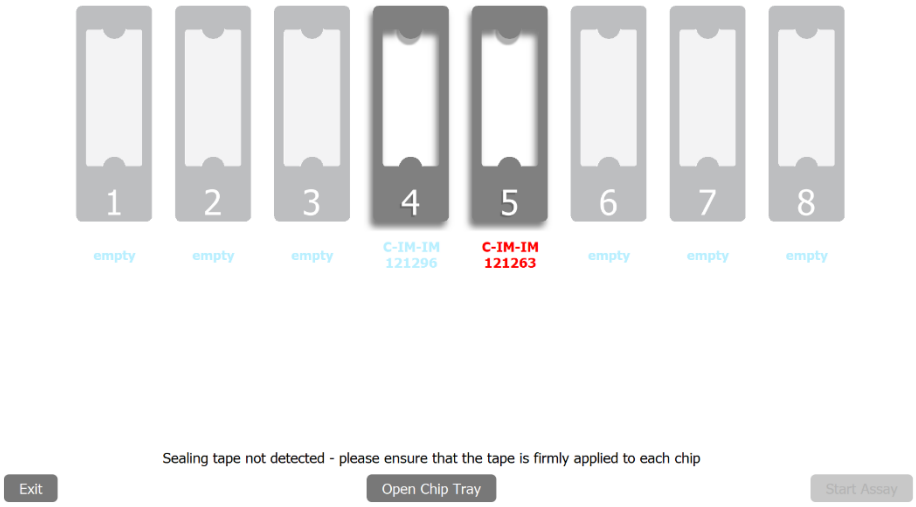


Figure 324 - Chip screen after failing to detect tape on CodePlex chip

1. Press “Open Chip Tray” and wait for tray to extend all the way.
2. Ensure cover tape is properly applied to CodePlex consumable(s), completely covering all sample holes.

   Note: See CodePlex Sample Protocol for full details of proper application.

Leak or block detected

1. If leak is detected: Open the liquid consumables bay and check for proper fitting of 15 mL and 50 mL tubes and try again.
2. If block is detected: Check that CO_2 is flowing and is supplied at ≥ 30 PSI (0.2 MPa).


1
2
3
4
5
6
7
8
empty
empty
IM3100
IM3101
IM3102
IM3103
empty
empty
Airflow leak detected. Please ensure that the reagent cartridge is properly aligned
Open Chip Tray
Exit
Start Assay

Manually Update IsoLight Software

1. Download latest IsoLight software version from your Bruker ShareFile account to a USB drive.
2. Plug USB drive containing the IsoLight software update into the USB port of IsoLight.
3. Select "Check for updates."
4. Using the file navigator, navigate to the attached USB drive and select the installer. Click "Open."
5. The software installation window will pop up.
6. Select "Next" on pop up screen.
7. Then check box agreeing to terms and select "Install."
8. Once the install is complete select "Finish."
9. Another pop up will appear asking user to restart the IsoLight. Select "Yes."
10. Once restarted, the IsoLight home screen will appear and the correct version should be displayed.

Set Data Upload Location

The IsoLight stores all data from a given run locally; the user application will not allow the starting of an assay if there is a problem with saving assay data locally.

However, given the large amount of data needed to transfer, to expedite experiment throughput the IsoLight can be configured to transfer data to a remote location while running (See Site Prep Guide for more information on available options)

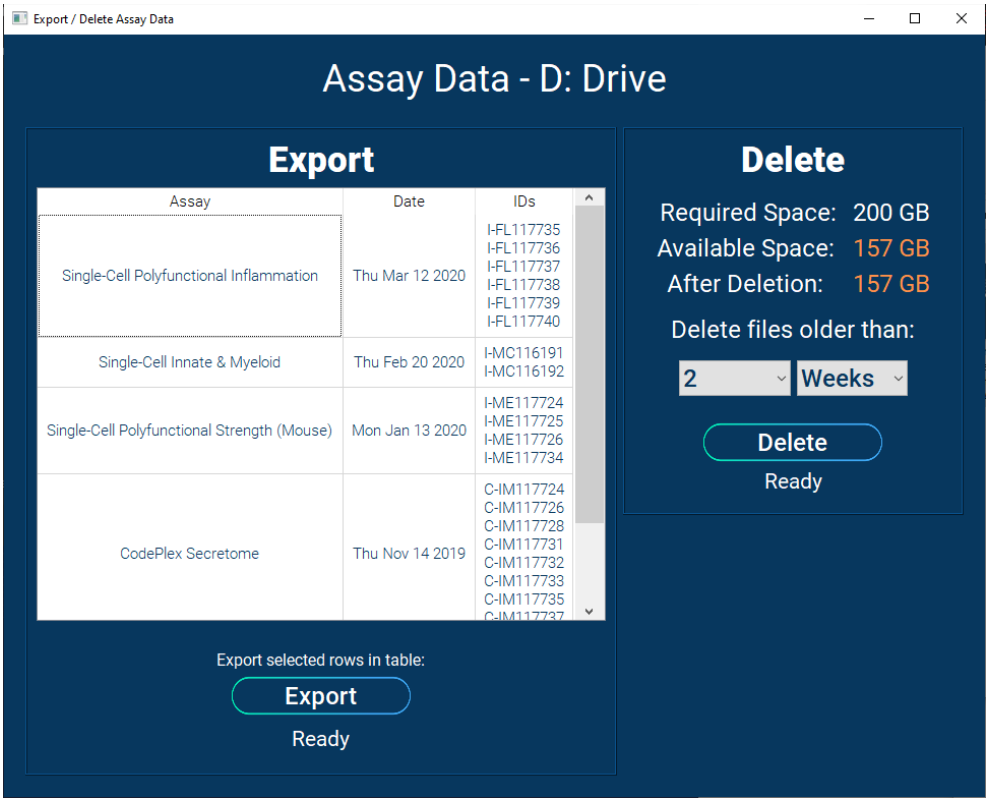
1. Press "Set Data Upload Location."
2. Using the directory navigator, select the desired destination folder.
3. The pop up will close and the data upload location will appear on the IsoLight home screen. If the text for the location is red, that means the folder location can no longer be connected.

Note: For support with issues connecting the IsoLight to network locations, Bruker requests the customer have an authorized representative from the organization's IT department with appropriate administrative access present and available throughout the support process.

Exporting and Deleting Assay Data

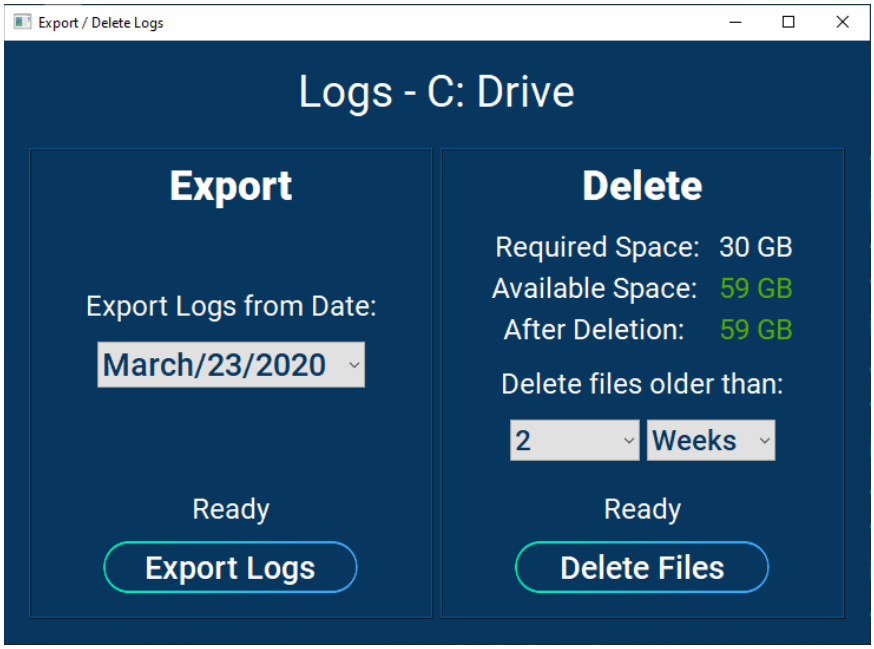
1. To export assay data from the IsoLight home screen, press “Export/Delete Assay Data.” The above dialog will appear.
2. To export files, select the desired experiments in the table view. Selected rows will appear highlighted. Press export after confirming selection.
3. A new file dialog will pop up, specify the export location.
4. Deleting assay data from the IsoLight happens from the same screen.
5. The “Available Space” (200 GB) must be greater than the “Required Space” for the IsoLight to start a run.
6. Select a time period to delete assay data from the two drop down selections. Updating the time period will update the "After Deletion” value.
7. Press “Delete.”

Note: The IsoLight is not intended for use as a long-term storage device. Bruker recommends exporting assay data off the instrument.



Exporting and Deleting Log Files

1. Users will typically not need to export log files unless requested by the support team. Users will need to periodically delete old logs to perform new instrument runs.
2. To export and delete log files off the IsoLight press “Export/Delete Log Files” on the home screen. The above dialog will appear.
3. To export logs, select which date to export from. Logs are dated from the day an experiment was started.
4. To delete logs, select the files to be deleted from the drop-down menu. Files only need to be deleted if the available space value is in red. Logs older than 2 weeks may be freely deleted; more recent logs should be retained if possible.
5. Once enough data has been deleted the available space value will turn green.



Maintenance Cleaning of the IsoLight

It is important to perform an 8-chip experiment at least once every two weeks. Within a two-week time period, if the instrument will be idle or if experiments with less than 8 chips are run, it is necessary to complete a maintenance cleaning. If at least one 8-chip experiment is run within a two-week time period, it is not necessary to perform a maintenance cleaning. Deep cleans should be performed monthly.

Required cleaning products:

1. Reusable Cleaning Chips (ISOCODE-1800-8)
2. Reagents: Reusable Cleaning Tube Set (ISOCODE-1701-8) or Prefilled Single-Use Cleaning Tube Set (ISOCODE-1700-8)

Maintenance cleaning will be complete in approximately 1.5 hours.

To initiate maintenance clean

1. On the home screen, press “Clean Instrument”. See below.

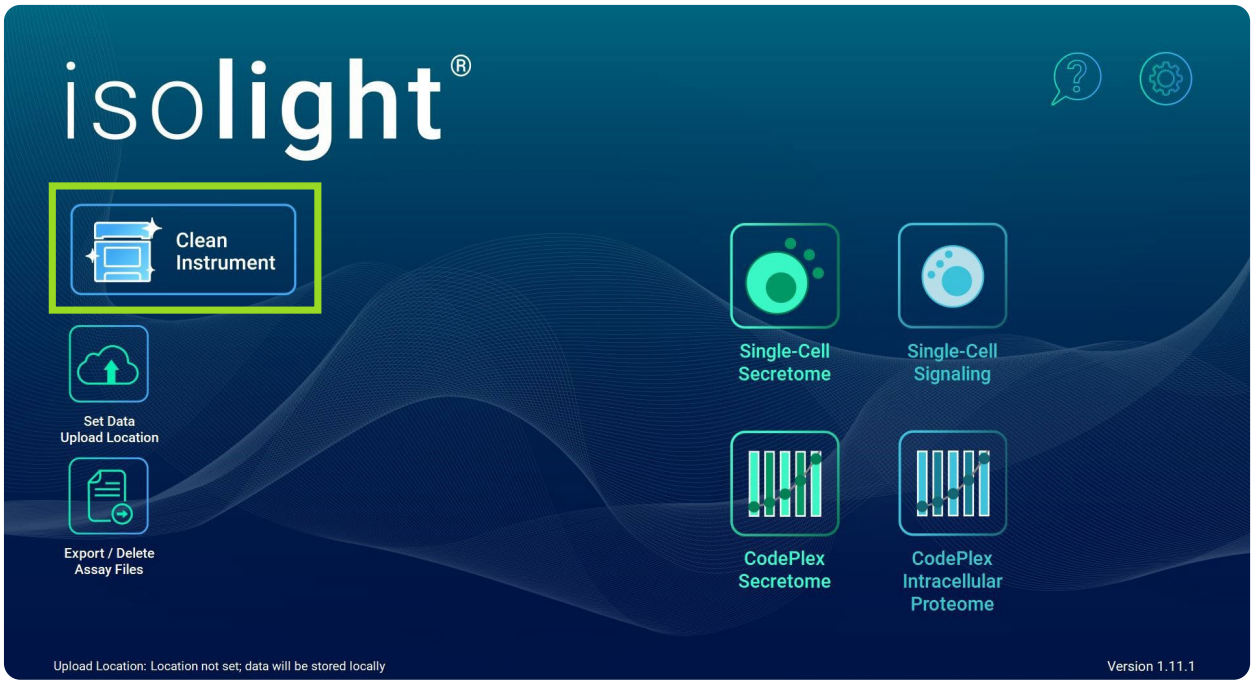


Figure 335 - Home screen showing location of “Clean Instrument” button

2. Select “Maintenance Clean” to access the maintenance clean guided prompts.
3. Follow the on-screen prompts to insert reagents and Reusable Cleaning Chips and to start the maintenance clean run.
4. The maintenance cleaning run takes about 1.5 hours.
5. Upon successful completion, remove all reagents and Reusable Cleaning Chips.

Deep Cleaning of the IsoLight

The instrument deep clean must be performed monthly and is available with IsoLight Software version 1.7.2 and later.

Required cleaning products:

1. Reusable Cleaning Chips (ISOCODE-1800-8)
2. Reagents: Reusable Cleaning Tube Set (ISOCODE-1701-8) or Prefilled Single-Use Cleaning Tube Set (ISOCODE-1700-8)

WARNING

Do not use single-use cleaning chips to perform an instrument deep clean. **Only Reusable Cleaning Chips can be used**. Using single-use cleaning chips to perform a deep clean may damage the instrument. Reusable cleaning chips, ISOCODE-1800-8 can be used with IsoLight software version 1.7.2 or later.

Deep cleaning will be complete in approximately 3.5 hours.

To initiate deep clean

1. On the home screen, press “Clean Instrument”. See .
2. Select “Deep Clean” to access the deep clean guided prompts.
3. Follow the on-screen prompts to insert reagents and Reusable Cleaning Chips and to start the maintenance clean run.
4. The cleaning step takes about 3.5 hours.
5. Upon successful completion, remove all reagents and Reusable Cleaning Chips.

Flow Diagnostic Check

The instrument flow diagnostic check is optional and can be performed prior to sample prep to ensure optimal performance of the fluidic system. The flow diagnostic check is available with IsoLight Software version 1.9.0 and later.

Only the Reusable Cleaning Chip can be used for instrument flow diagnostic check:

> The Reusable Cleaning Chips, ISOCODE-1800-8, Deionized Water (DI Water), reusable ISLFDC-1000-1 Flow Diagnostic Check Tubes, and replacement ISLFDC-1001-1 Flow Diagnostic Check Multipack Sippers

WARNING

Do not use single-use cleaning chips to perform an instrument flow diagnostic check. **Only Reusable Cleaning Chips can be used**. Using single-use cleaning chips to perform a flow diagnostic check may damage the instrument. Reusable cleaning chips, ISOCODE-1800-8 can be used with IsoLight software version 1.7.2 or later.

Flow Diagnostic Check will be complete in approximately 30 minutes.

To initiate flow diagnostic check:

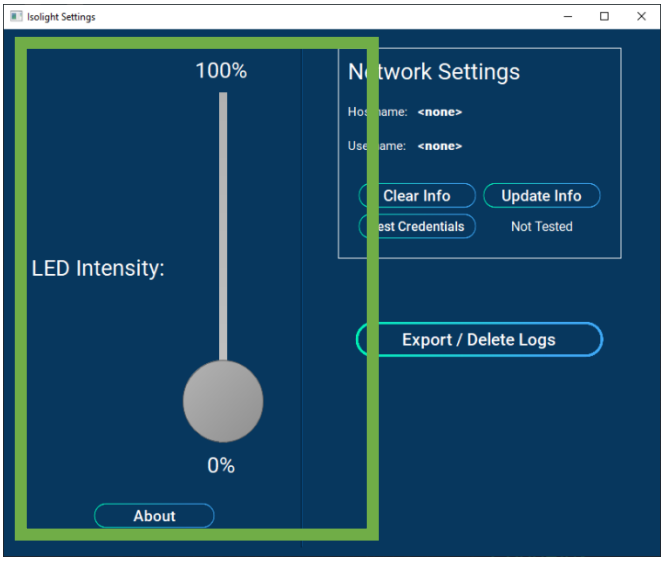
1. On the home screen, press “Clean Instrument”. See .
2. Select “Flow Diagnostic Check” to access the flow diagnostic check guided prompts.
3. Follow the on-screen prompts to insert reusable cleaning chips, fill tubes with DI water, attach tubes and sippers, and to start the flow diagnostic check run.
4. The diagnostic check step takes about 30 minutes.
5. Upon successful completion, remove reusable cleaning chips, empty DI water, and recap tubes for storage.

Settings Window

The settings window can be accessed by pressing the gear icon in the top right of the IsoLight home screen.

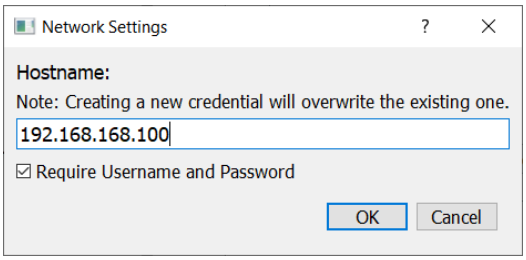
Adjusting the Brightness of the Status Lights

Light brightness can be adjusted by moving the LED Intensity slider up or down. This only changes the brightness of the LED's on the IsoLight and not the screen intensity. Light brightness will be updated once cell loading has started.

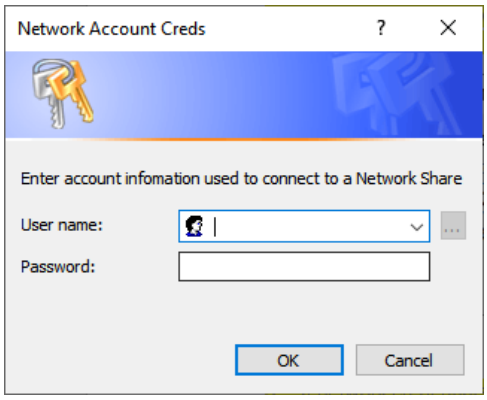


Configure Network Destination and Credentials

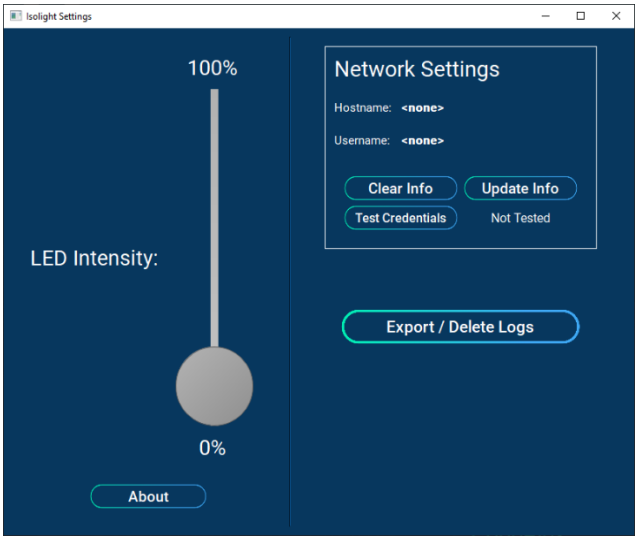
1. In the settings window, press “Update Info”.
2. Enter the destination hostname without leading or trailing slashes.



3. If network credentials are required to access the given destination, check the box labeled “Require Username and Password”. If checked, a credential management dialog will open. Enter details as required.



4. The IsoLight can only be configured for one network location at a time. If a new hostname is configured, credential information must be provided again (even if the credential information is the same).



Note: Bruker does not and will never store credential information.

Decontamination of Instrument

The exterior of the instrument can be wiped down with 1% bleach in order to decontaminate its surfaces.

Delivery and Installation

An Bruker-authorized service provider delivers the instrument, uncrates the system, and places it on the lab bench. The space and bench must be ready in advance of delivery.

CAUTION

Only Bruker-authorized personnel can uncrate, install, or move the instrument. Mishandling of the instrument can affect the alignment or damage instrument components. A Bruker representative installs and prepares the instrument.

CAUTION

After your Bruker representative has installed and prepared the instrument, do not relocate the instrument. Moving the instrument improperly can cause injury and affect the optical alignment and compromise data integrity. Contact your Bruker representative for assistance with relocating the instrument.

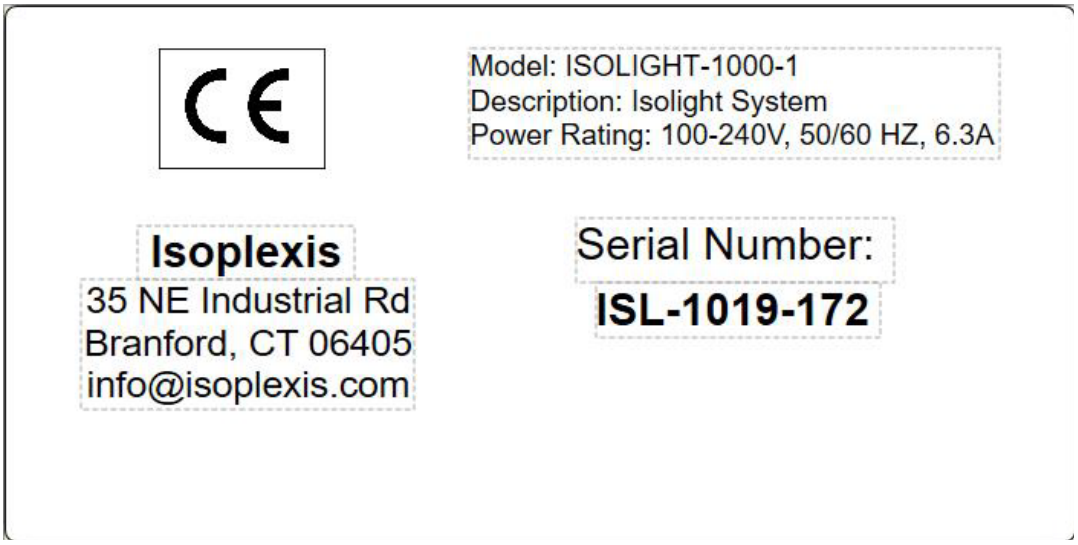
Regulatory, Certification and Labeling

1. Publication IEC 60825-1: 2014 was followed for the safety of laser products used in the IsoLight.

2. The IsoLight contains Class 1 lasers with the specifications in the table below.

Wavelength	Beam Divergence Parallel-TYP (°)	Beam Divergence Perpendicular-TYP (°)	Max Power Output (mW)
473	8	24	300
638	8	13	185
405	14	41	350

3. These are the typical markings of the IsoLight instrument when certification has been completed. These labels are located on the back, left side of the IsoLight above the power cord and ethernet cable ports.



This device complies with part 15 of the FCC rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) This device must accept any interference received, including interference that may cause undesired operation.

Innovation, Science, and Economic Development Canada ICES-003 Compliance Label: CAN ICES-3(B)/NMB-3(B)

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