Saphyr® System User Guide

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Revision History

Revision	Notes
A	Initial Release
B	Revised to reflect the changes associated with the release of Instrument Control Software v4.9
B1	Added missing image to page 17.
B2	Fixed the incorrect chip names
C	Updated to reflect increase in DNA output to 5 Tbp
D	Updated to reflect changes in ICS v5.1
E	Updated to reflect changes in ICS v5.2 • Updated Perform Run procedure section • Updated Pause Run procedure section Included Updating Software section • Updated Maintenance Section
F	Updated guidance on pipetting samples into chips and on loading two chips on the instrument
G	Fix errors in rev F

Chapter 1: Overview

Introduction

CAUTION: Prior to operating the Saphyr® Instrument, please review the Saphyr System Safety Guide (P/N 30144 or 30253) for all safety and compliance information.

The Saphyr System (Saphyr Instrument with Instrument Controller and Bionano Access® Server) together with the Saphyr Compute and Bionano Compute provides rapid, high-throughput, long-range optical genome mapping for *de novo* assembly of genome maps, rare variant analysis, hybrid scaffolding of NGS, and structural variation analysis.

Features

- **Easy load-and-go workflow** – The adaptive loading feature in the Instrument Control Software (ICS) ensures that the optimal conditions for DNA molecule loading are maintained throughout the scanning. The initial hands-on-time is approximately 5 minutes.

- **Convenient setup and flow** – Bionano Access allows users to design experiments, track run performance, perform downstream analysis, and share data.

- **Real-time analysis** – Bionano Access enables users to view real-time data and performance metrics from any internet browser with connection to your Access Server as the run progresses.

- **Saphyr Assure –** Optional automated system health monitoring service that continuously inspects data quality and instrument performance. Additionally, this provides a streamlined diagnostic workflow for customers to interact with Bionano Genomics Support.

Workflow

Prepare Sample → Set Up Experiment → Prepare Chip → Perform Run → Analyze Data

To perform a run on the Saphyr, use the following workflow example as a guide:

1. Perform DNA isolation and labeling of samples.
2. Set up an experiment and specify run parameters on Bionano Access.
3. Turn the Saphyr instrument on and sign into the Instrument Control Software.
4. Load the samples on a Saphyr Chip® and attach the Saphyr Clip to the chip.
5. Insert the Saphyr Chip into the instrument.
6. Setup a run.
7. Track run performance and analyze data on Bionano Access.
8. Remove the chip from the instrument.

Prepare Sample

For details on DNA isolation and labeling of samples, visit the Bionano Support page.

# Chapter 2: Getting Started

Start the Saphyr Instrument

1. Locate the power switch on the back of the instrument.

2. Toggle the power switch to turn the instrument on.

3. Boot the Saphyr Instrument Controller. Systems that are in "kiosk" mode will boot directly to the Instrument Control Software (ICS). These systems will not allow users to access anything on the Instrument Controller other than ICS. Systems that are not in kiosk mode will require the user to log into Windows, then double click on the ICS icon to launch the software. Use the username and password that were given to you by the Bionano Field Service Engineer (FSE) or Field Application Scientist (FAS). If you forget the username or password, please contact Bionano Support.

Laser Safety Light

⚠️ **IMPORTANT:** Before lifting the sample door to insert or remove the Saphyr Chip, ensure the laser safety light is blue (below, left). If the laser safety light is red (below, right), do not open the sample door and contact Bionano Genomics Support for assistance; users may be at risk for laser radiation exposure if the sample door is opened. For additional information, please refer to the Saphyr System Safety Guide (P/N 30144, 30253, or 30302).

Laser Safety Light   Instrument Status Light

Instrument Status Light

The instrument status light indicates the following:

Color	Description
Light off	**The instrument is powered off.**
Flashing yellow	**The instrument is initializing.**
Green	**The instrument is ready to use.**
Flashing green	**The instrument is running, or the workflow has been paused.**
Red	**The instrument encountered an error. Note: This does not present a safety issue to the user.**

Sign In to the Saphyr Instrument Control Software

1. When the software is launched, the Saphyr will initialize. This can be seen by clicking on the status icon. A description of all icons can be found in Appendix C and a description of the initialization screen can be found in Appendix D.

2. Enter the username and password. This is the username and password that has been set up on Bionano Access. See Chapter 3 for instructions.

3. Click **Logon**.

ICS Home Screen Options

The following menu appears on the Home Screen. Not all options are always available (depends on the state of the instrument).

Option	Description
Insert Chip	**Allows users to insert the Saphyr Chip into the Saphyr instrument.**
Configure Run	**Allows users to set up a run. This option is only available if a chip is loaded.**
Data Administration	**Allows users to manage data on the Saphyr Instrument Controller, such as managing disk space and deleting runs. This option is only needed for users who save their images during a run. See Appendix E for details.**
Remove Chip	**Allows users to remove the chip from the Saphyr instrument. This option is only available when a chip is loaded.**

Chapter 3: Configure Analysis Settings in Bionano Access

The Instrument Control Software and Bionano Access interact to allow users to track run progress and view results in real time. Prior to performing the run on the Saphyr instrument, an experiment must be set up in Bionano Access. For details on how to do this, as well as all Bionano Access analysis and visualization features, see the Bionano Access Software User Guide (P/N 30142).

Chapter 4: Prepare Chip

Load Sample on Saphyr Chip

- Equilibrate the labeled samples and Saphyr Chip (sealed in pouch) to room temperature for <u>at least 30 minutes</u> prior to use.

⚠ **Important:** Do not open the pouch containing the Saphyr Chip when the chip is cold. Wait until the pouch is at room temperature before removing the chip. Opening a chip while it is cold may damage the chip and cause performance degradation.

- When loading sample, ensure there is no airflow around the chip to avoid sample evaporation.

- Proceed through the following steps carefully, without interruption to minimize sample evaporation.

- Use gloves (powder free) for this procedure.

- The instructions below are applicable for either two or three flowcell chips.

⚠ **Important:** Make sure that you load the samples in the same flowcells that you set up in Access in the step above. Also, note the serial number of the chip so the chip can be associated with the correct experiment in Chapter 5 below (chip tracking table provided below).

| Chip SN	_____ - _____ - _____ - _____	_____ - _____ - _____ - _____
Flowcell 1 Sample :		
Flowcell 2 Sample :		
Flowcell 3 Sample :		

G2.2 or G2.3 Chips

Note: See video of loading the sample on the Saphyr Chip at https://bionanogenomics.com/videos/bionano-chip-20366-loading/

**Image:** Saphyr Chip (left) and Saphyr Clip (right) with removable plugs (bottom right)

1. Open the pouch containing the Saphyr Chip.

2. Place the chip on a clean and level lab bench.

3. With a 10 µl pipette, press the plunger down and hold at the first stop. Lower the pipette tip into the sample and gently stir the sample with the tip.

4. Position the pipette tip near the middle or bottom of the sample and slowly aspirate 8.5 µl of the sample.

5. Begin loading the sample in the inlet well of flowcell 1.

   Place the pipette tip into the flowcell 1 inlet well on the right of the chip, and then slowly dispense the sample until the bottom of the inlet well is coated with sample solution. Continue to dispense, adjusting the rate of dispensing as the DNA travels slowly into the flowcell fingers, until the entire volume of 8.5 µl is in the well.

   The fluid level in the inlet well should be flat. If the sample overflows out of the well and onto the top of the chip or onto the plastic, the flowcell is not usable. Always maintain positive pressure on the plunger to avoid bubble formation.

6. Repeat steps 3 through 5 for flowcell 2 and flowcell 3 inlet wells.

7. Set a timer for 2 minutes to allow the sample to wet through the nanochannels.

8. After two minutes, with a 20 µl pipette positioned near the middle or bottom of the sample, slowly aspirate 11 µl of the sample and slowly dispense the sample into the outlet well of flowcell 1. Repeat for flowcell 2 and flowcell 3.

   The fluid level in the outlet well should be flat. If the sample overflows out of the well and onto the top of the chip or onto the plastic, the flowcell is not usable. Always maintain positive pressure on the plunger to avoid bubble formation.

9. For flowcell 1, add 5 µl of nuclease-free water to the hydration reservoir on the <u>outlet</u> side of the chip (see "OUTLET" on the chip label. Adding additional water or adding water to the reservoir on the inlet side of the chip may lead to problems with the chip run.

10. Add the necessary volume of nuclease-free water to the inlet and outlet wells of both flowcells to replace sample solution that has evaporated. In order to lower the risk of evaporation, add water to make the fluid level slightly convex relative to the top of the chip. See the image below and the instructional video on the Bionano website.
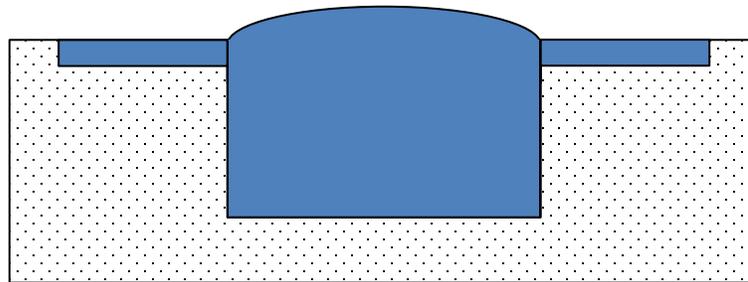
⚠️ **Important:** The inlet and outlet wells should be filled with sample solution during the initial load. Do not compensate for lack of sample solution by adding nuclease-free water or buffer. Nuclease-free water should only be added after all wells are loaded to replace volume lost by evaporation.
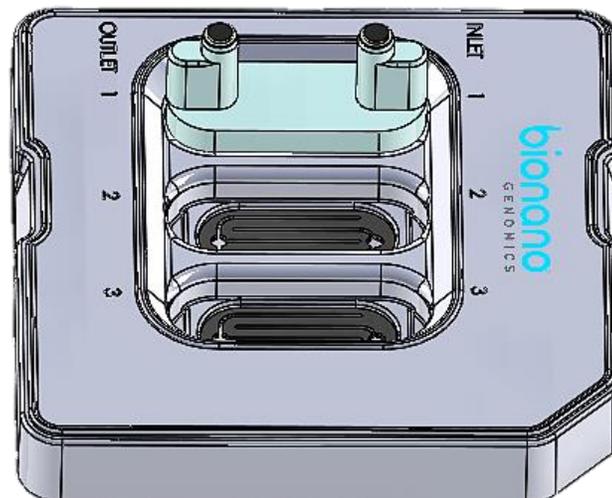
11. Align the plug (with the electrodes facing down) to flowcell 1. Use a slight to-and-away rocking motion on the plug to gently insert the plug fully into the chip.

12. Repeat steps 9-11 for flowcells 2 and 3.

    Note: It is highly recommended that all flowcells in the chip are used in the same run. Once the chip has been removed from the original sealed back, the performance will degrade over time.

13. Align the Saphyr Clip onto the Saphyr Chip (verify correct orientation) such that the electrode posts on the Teflon plugs fit in the opening in the clip. Using both hands, simultaneously apply an even downward force on the right and left side of the clip to attach. You will hear a click on each side of the clip when it is attached. Once the clip is attached to the chip, do not remove it unless necessary. If you remove any of the plugs, rehydrate the chip inlets and outlets with nuclease-free water to replace any sample solution that has evaporated so the fluid level in the wells is flat.

    ⚠️ **Important:** Even if all flowcells do not have samples, all 3 plugs must be inserted into the chip to ensure proper evaporation control from the clip.

14. The chip and sample can be left out for up to 1 hour at room temperature with the clip engaged before starting the run.

G1.2 Chips

Image: Saphyr Chip (left) and Saphyr Clip (right)

1. Open the pouch containing the Saphyr Chip.

2. Place the chip on a clean and level lab bench.

3. With a 10 µl pipette, press the plunger down and hold at the first stop. Lower the pipette tip into the sample and gently stir the sample with the tip.

4. Position the pipette tip near the middle or bottom of the sample and slowly aspirate 8.5 µl of the sample.

5. Begin loading the sample in the inlet well of flowcell 1.

   Place the pipette tip into the flowcell 1 inlet well on the right of the chip, and then slowly dispense the sample until the bottom of the inlet well is coated with sample solution. Continue to dispense, adjusting the rate of dispensing as the DNA travels slowly into the flowcell fingers, until the entire volume of 8.5 µl is in the well.

   The fluid level in the inlet well should be flat. If the sample overflows out of the well and onto the top of the chip or onto the plastic, the flowcell is not usable. Always maintain positive pressure on the plunger to avoid bubble formation.

6. Repeat steps 3 through 5 for the flowcell 2 well.

7. Set a timer for 2 minutes to allow the sample to wet through the nanochannels.

8. After two minutes, with a 20 µl pipette positioned near the middle or bottom of the sample, slowly aspirate

11 µl of the sample and slowly dispense the sample into the outlet well of flowcell 1. Repeat for flowcell 2.

The fluid level in the outlet well should be flat. If the sample overflows out of the well and onto the top of the chip or onto the plastic, the flowcell is not usable. Always maintain positive pressure on the plunger to avoid bubble formation.

9. Add the necessary volume of nuclease-free water to the inlet and outlet wells of both flowcells to replace sample solution that has evaporated. In order to lower the risk of evaporation, add water to make the fluid level slightly convex relative to the top of the chip. See the image below and the instructional video on the Bionano website.
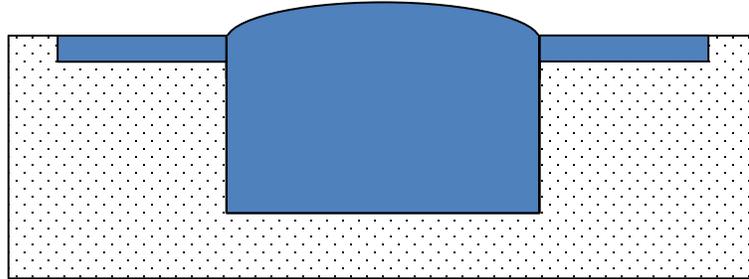
⚠️ **Important:** The inlet and outlet wells should be filled with sample solution during the initial load. Do not compensate for lack of sample solution by adding nuclease-free water or buffer. Nuclease-free water should only be added after all wells are loaded to replace volume lost by evaporation.

10. Align the clip on the chip by verifying the appropriate clip orientation.
    Make sure the clip is aligned correctly; putting the clip on backwards may break the chip.

11. Place the clip over the chip.

12. Using both hands, apply an even downward force on the right and left side of the clip to attach.
    Note: downward pressure should be applied to the clip, not the chip.
    Users will hear a click when the clip is attached. Once the clip is attached to the chip, do not remove it unless necessary. If users remove the clip, then rehydrate the chip inlets and outlets with nuclease-free water to replace any sample solution that has evaporated.

13. The chip can be left out for up to 1 hour at room temperature before starting the run.
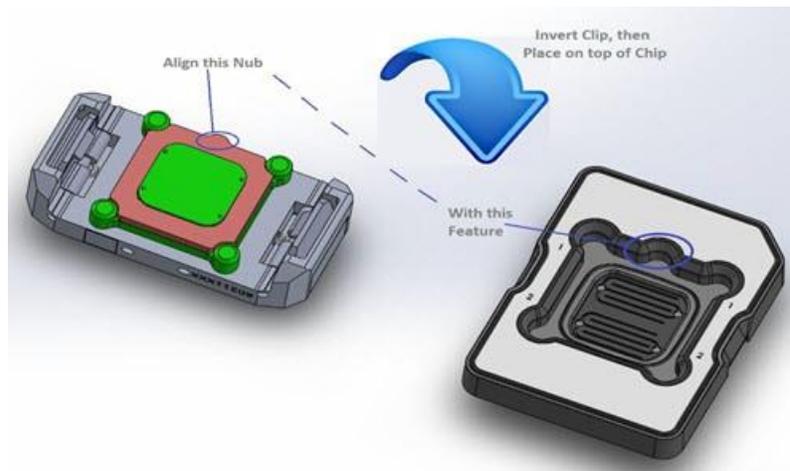
Saphyr Clip Care

Always position clip inserts on their side or upside down, as shown below. Do not place them on the bench with the electrodes down.

Correct Position
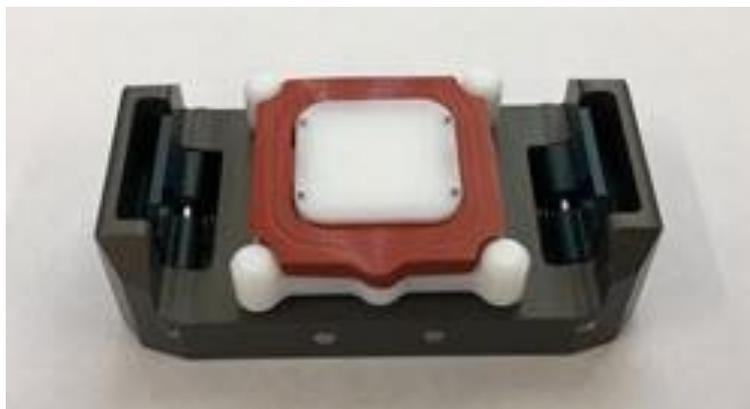
Chapter 5: Perform Run

Insert Saphyr Chip

Before inserting the Saphyr Chip into the Saphyr instrument, carefully check the underside of the chip for dust or debris without turning the chip upside down. If there is dust or debris, gently wipe the underside of the chip with lens paper. Avoid touching the underside of the chip with anything other than lens paper.

1. From the Home Screen, click **Insert Chip**.
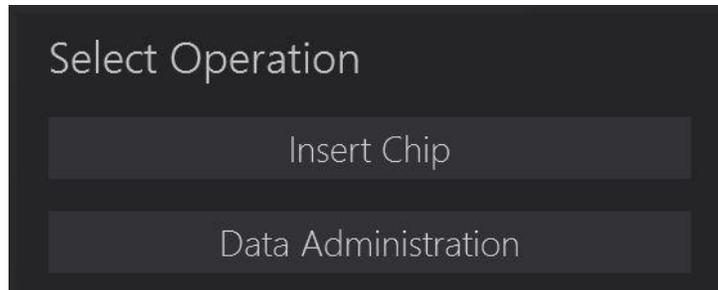
Users will hear the stage moving outward, which can take about 10 seconds. When the stage has reached the proper position to allow for insertion of the chip, the "Chip is Inserted" option will activate on the screen.

2. Manually lift the sample door of the instrument.
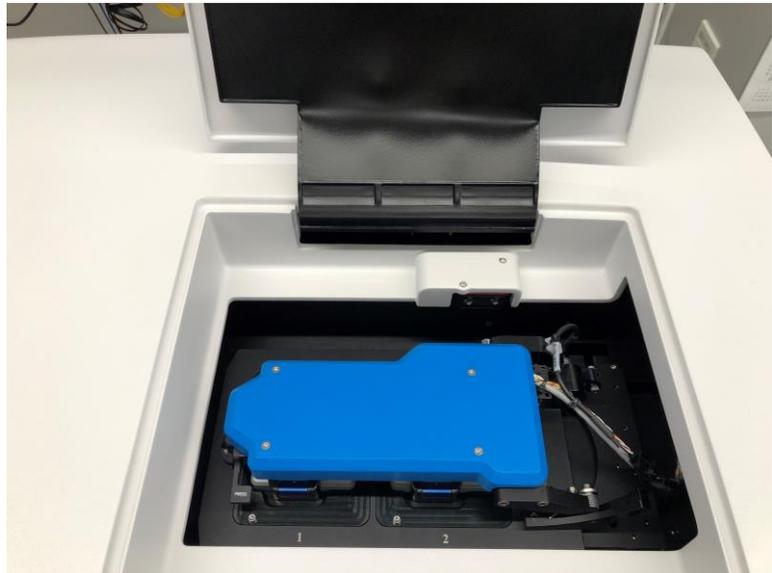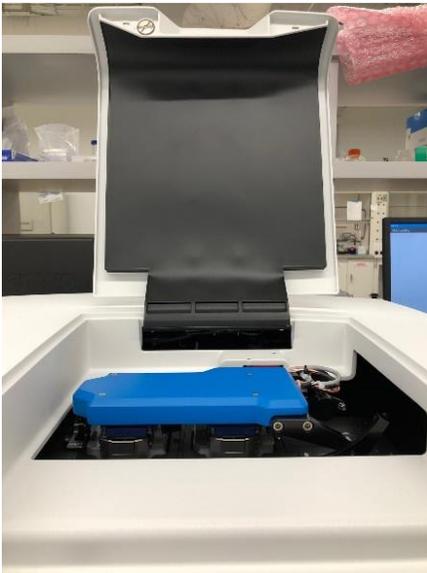
3. Lift the bundle arm:

    For dual chip instrument, press the button that says "Press" to release the bundle arm. The bundle gently rises to a vertical orientation, thereby presenting the two positions where the chip(s) can be inserted into the instrument.
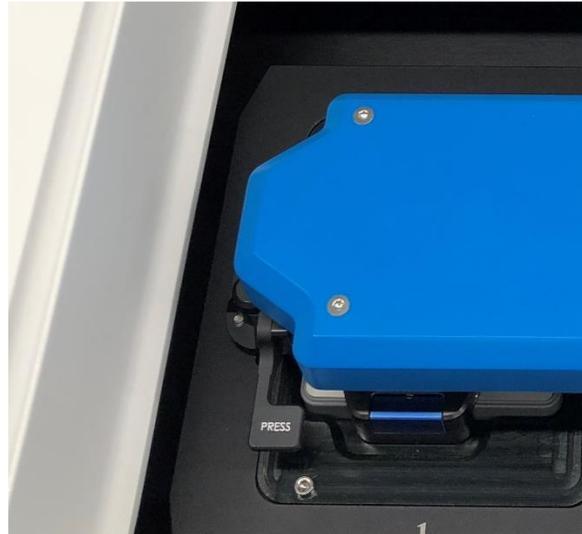
    For single chip instrument, manually lift the bundle arm.
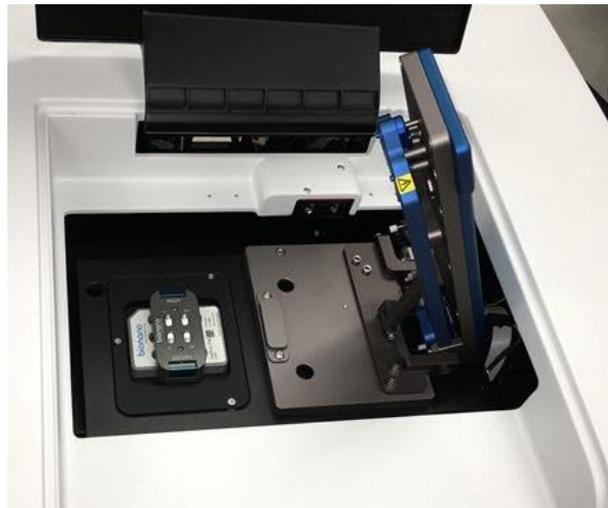
4. Insert one or two chips (if applicable) into the instrument and ensure that it rests snugly on the stage. If only inserting one chip, either platform can be used.

Note: The clip is designed to provide evaporation control for up to 65 hours. If planning on inserting two chips into the instrument, the second chip will need to be complete within 65 hours.

    Tip: If loading two chips, record which chip is on which platform. The ICS will ask you which experiment to associate with each chip (See Configure Run section below).

Stage Position	1 (left)	2 (right)

Chip SN :	_____ - _____ - _____ - _____	_____ - _____ - _____ - _____

5. Gently lower the bundle arm onto the Saphyr clip(s), and then close the sample door.

    - In the Home Screen, click **Chip is Inserted**. The instrument will read the barcode to identify the chip(s). If the instrument is unable to identify the chip barcode, an error message will appear. Remove the chip and inspect the barcode to see if it is damaged. If not, reseat the chip(s), making sure that it is centered on the stage mounts. If you have further issues, contact Bionano Support.

6. Proceed to the Configure Run section.

Configure Run

1. From the Home Screen, click **Configure Run**. The Configure Run screen appears. If two chips were inserted, you will be prompted to configure the chip on platform 1 first (Chip Location: P1). Platform 1 is on the left side of the stage.

For instruments connected to Access 1.7 and above
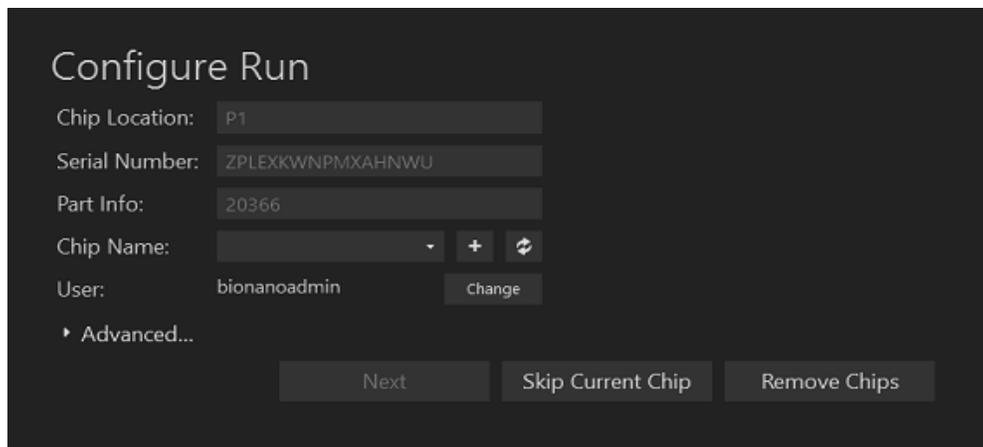
2. At the **Chip Name** field, select the chip setup to associate with the chip from the drop-down list. See Bionano Access User Guide (30142) for details on how to create an experiment.

    [Optional] Click ![+] to create a new chip setup. This will bring up Bionano Access within the Instrument Control Software. See the Bionano Access User Guide (30142) for instructions on setting up a chip.

    [Optional] Click ![refresh] to update the list.

For instruments connected to Access 1.6 and older

Configure Run

Chip Location: P1
Serial Number: RI5K2UWNPOAWNNWU
Part Info: 20366
Experiment: ▼ Refresh
Chip Setup: ▼
User: bionanoadmin
▸ Advanced...

Next   Skip Current Chip   Remove Chips
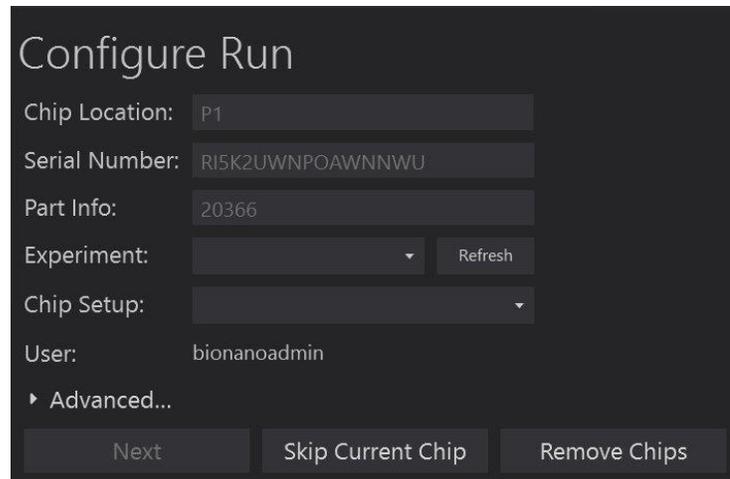
3. At the **Experiment** field, select the experiment to associate with the chip from the drop-down list. See Bionano Access User Guide (30142) for details on how to create an experiment.

   [Optional] Click **Refresh** to update the list.

4. If applicable, at the **Chip Setup** field, select the chip setup from the drop-down list.

   Note: This step is only applicable if there is more than one chip associated with the experiment. This will take you to the expanded Configure Run screen (below).

For all Access versions:
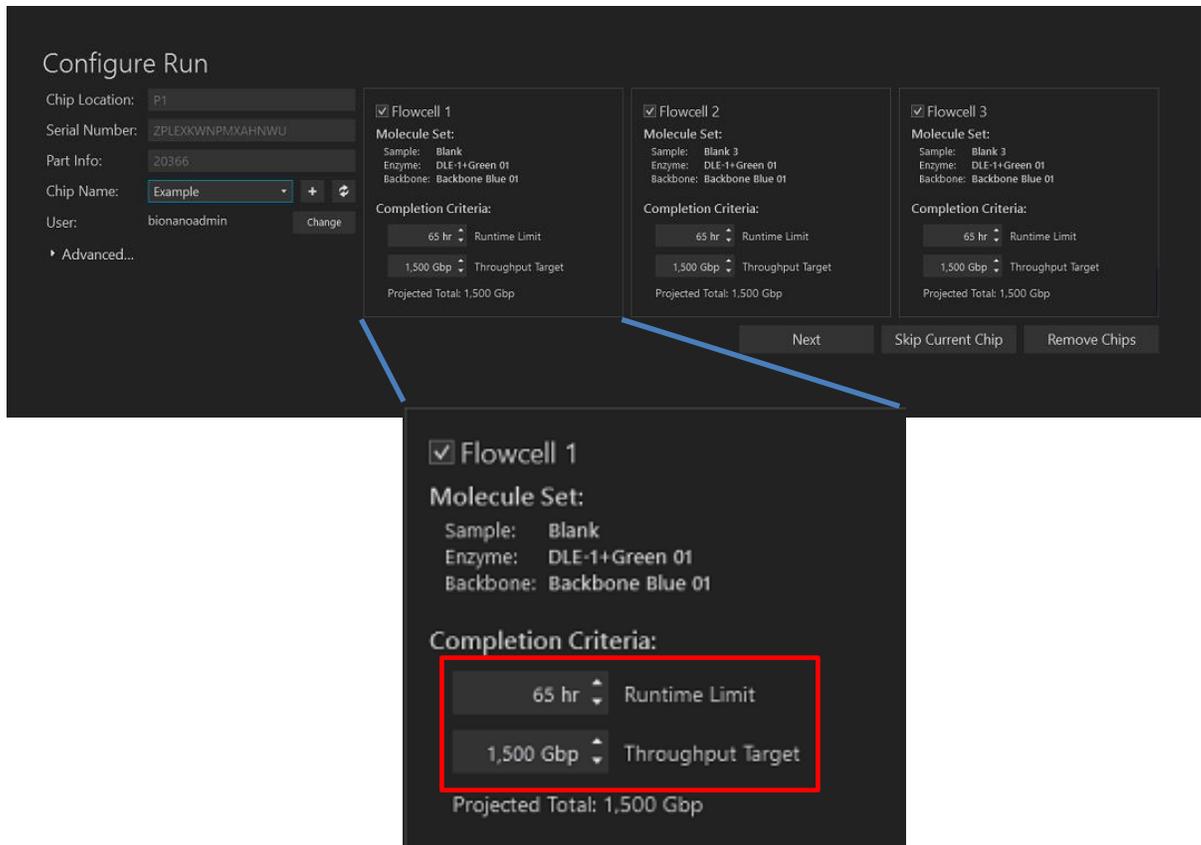
5. Review the runtime and throughput target parameters for each of the flowcells. These values will default to the values that were entered into Bionano Access during the setup. The user may decrease the runtime or throughput as desired.

   Note: If the chip has been run previously, the runtime limit and throughput target may be modified. See Appendix B for details.

6. [Optional] Prior to clicking **Accept** or **Next**, you can click on either **Skip Current Chip** or **Remove Chips.**

   a) **Skip Current Chip** – This will allow you to disregard the chip in this platform. The chip will not be run. See the Chip and Flowcell States section below for more details.

   b) **Remove Chips** – This will allow you to stop configuring runs and remove the chip(s), if needed. After chips are removed, you will return to the Home Screen.

7. [Optional] Clicking on the arrow next to **Advanced…** will give you the option to save the images that are captured during the run. By default, images are not saved. If you choose to save images, see Appendix E on managing that data. This option is only available for customers who are not running in kiosk mode.

8. If you have inserted two chips into the instrument, click **Next** and repeat steps 2-7.

   Note: If the second chip belongs to a different user, change users before starting the second chip configuration. Individuals with "User" privileges can only run experiments that they have created

9. Click **Accept**.

10. If you have inserted two chips, you will have the option of which chip to run first. Click **Start Run** on the chip you want to run first.

Select First Run

LOCATION	LOCATION
P1	P2
SERIAL NUMBER	SERIAL NUMBER
RI5K2UWNPOAWNNWU	NM53CHWNPPMO6HHG
CHIP NAME	CHIP NAME
Demo Test	Demo Test 2
Start Run	Start Run

Cancel
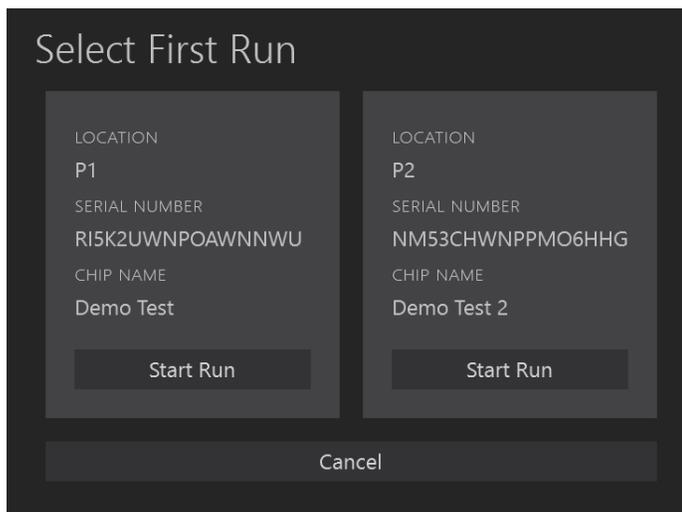
11. Saphyr starts the following process:

a) Chip Registration (Approximately 5 minutes)

b) DNA Loading Optimization (Approximately 1 hour)

c) Scanning

12. During scanning, the user can view the run metrics and most recent images on the Run Dashboard. Run metrics are also available to view in Bionano Access.
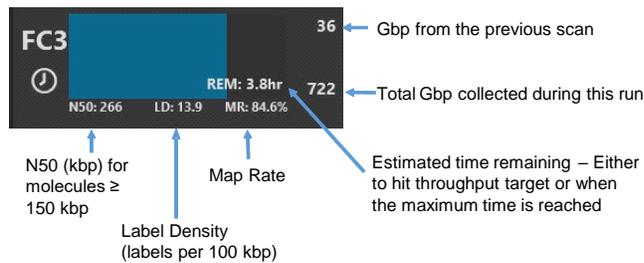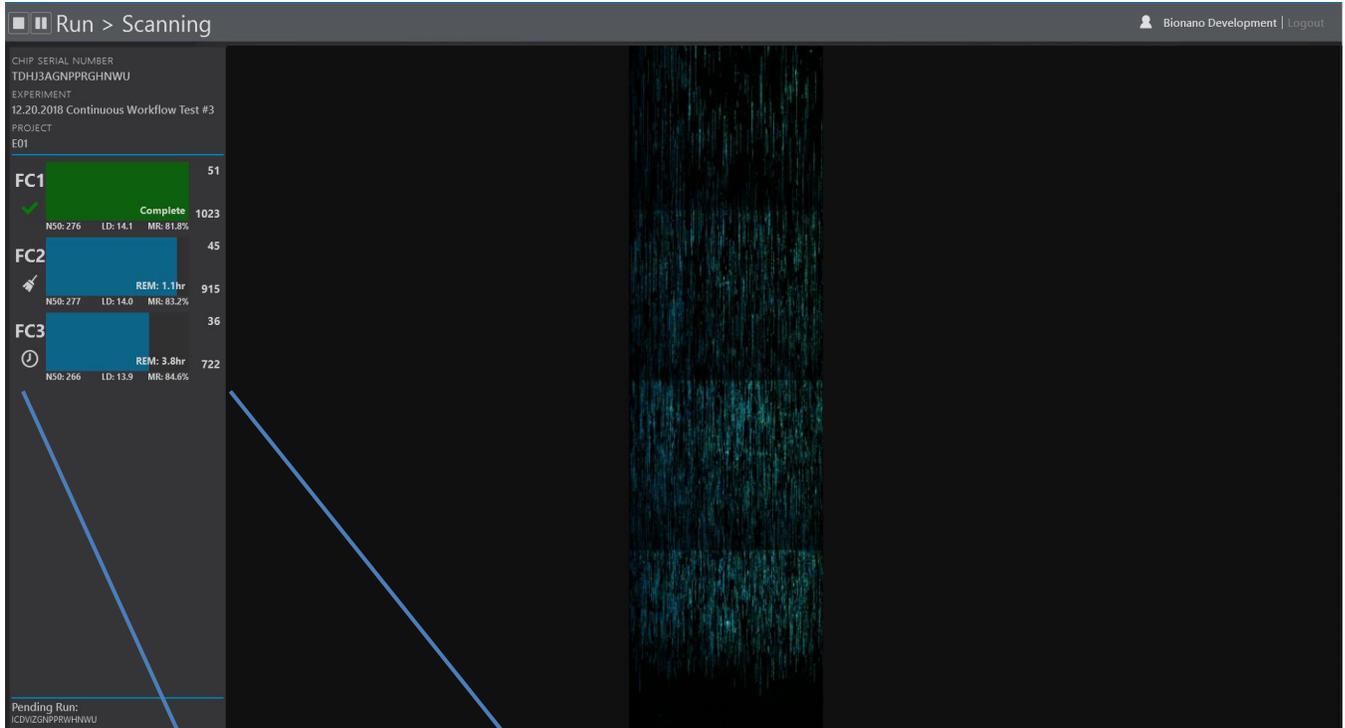
Note: The color of the progress bar indicates the following:

Color	Description
Blue	**Flowcell is running**
Green	**Flowcell has completed running**
Yellow	**Flowcell has been cancelled and is stopping**
Red	**Flowcell has stopped due to an error**

13. Clicking on one of the flowcells expands the details of the run metrics for that flowcell. In the expanded view, you can see the per-scan throughput and map rate metrics:

When the chip run is complete, if there is another chip ("pending") in the instrument, it will automatically start running. The pending chip will be shown on the bottom left of the screen.
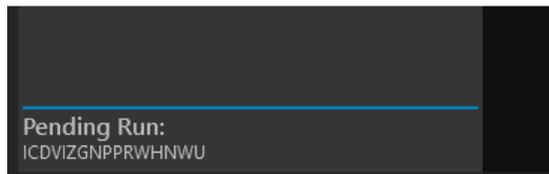
Once all chips on the instrument have been completed ICS will return to Home Screen. Check the Run Info Icon for run details.

Chip and Flowcell States

Depending on the situation, the chip(s) and their respective flowcells may be in various different states.

Chip State	Description
Running	**A chip in the instrument is currently running (data is being collected on one or more flowcells)**
Complete	**A chip has finished its run. This means that all of the associated flowcells have either reached their throughput or time limit, an issue has occurred (see flowcell states), or the user has stopped the run.**

Pending	A chip is ready to run (loaded into the instrument, and the user has configured a run), but is awaiting the completion of a currently running chip.
Unscheduled	A chip is loaded into the instrument, but the user has not configured a run.

When a chip is running, the flowcells associated with the chip can be in any of the following states:

Flowcell State	Description	Icon
Loading	Sample is being loaded in preparation for scanning.	
Scanning	The flowcell is being imaged.	
Cleaning	Post-scanning cleaning is taking place.	
Complete	Flowcell has achieved its throughput target or runtime limit as defined in the Run Configuration.	
Evaporated	The instrument is unable to load DNA in the flowcell due to loss of contact. See the Pausing a Run to Rehydrate the Chip section below for more information.	
Error	An error has occurred that has prevented the instrument from collecting data.	
Cancelled	The flowcell was cancelled by the user and will stop when possible.	
Paused	The flowcell is temporarily paused by user request.	
Waiting	The flowcell is waiting for instrument resources to become available to load or scan.	

Note: When all flowcells cease to be in a Loading, Scanning, Cleaning, or Waiting state, the chip state then changes from "Running" to "Complete".

Pausing a Run to Insert a New Chip and/or Remove a Completed Chip – For Instruments with Dual Chip Capability Only

In order to support continuous data collection, a run may be paused in order to swap out a chip that has already completed with a new one.

1. Press the pause icon at the top left of the user interface.
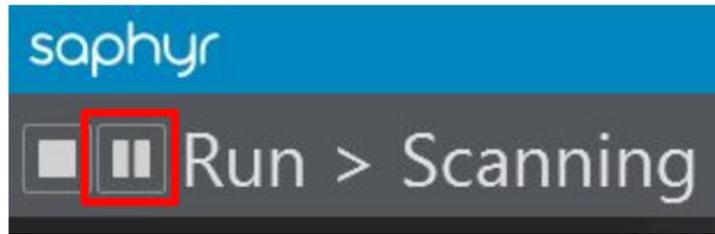
2. When you are asked whether you want to pause the run, click **yes**. The current scan will complete before the run is paused. This may take up to 15 minutes.
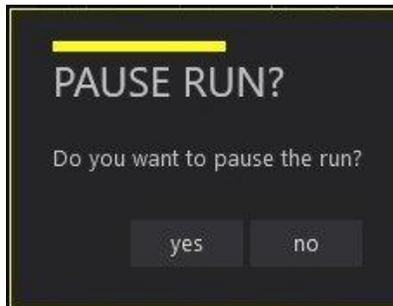

3. You will be prompted to insert and remove chips. Open the sample door to add and/or remove chips as described in steps 2-7 of the Insert Saphyr Chip section above. Be sure <u>not</u> to remove the chip that you are currently running if you want it to continue running. Press **Resume** when you have the new chip inserted.
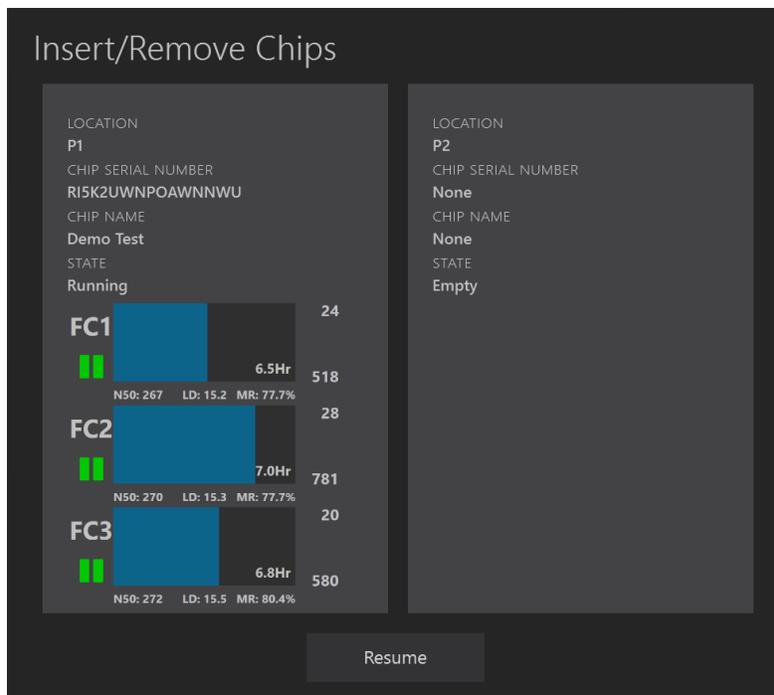
a. If the Active Chip is Removed (from the instrument or moved to another platform) ICS will detect the move and will prompt a warning indicating that the current run will be stopped if the user continues.

The following chip run will be stopped:
ASOW6TWNPNSWLNWU

Are you sure you want to continue?

ok   cancel
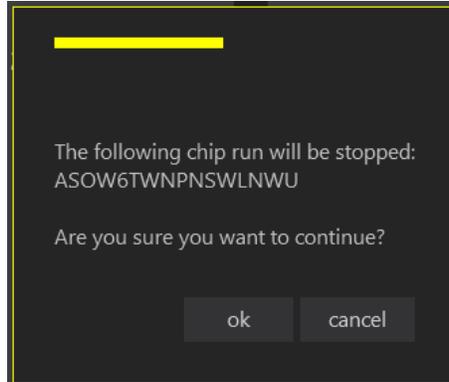
4. In the Configure Run screen, confirm that you are the correct user in the User field. If you are not, update the username. Individuals with "User" privileges can only run experiments that they have created. To update the username, click on **Change** next to the current user. Then enter your username and password. This is the same username and password that you use in Bionano Access.

5. For systems connected to Access 1.7 or above, select the Chip Name that you wish to run from the dropdown list.

Configure Run

Chip Location: P2
Serial Number: 6PNJFBWNPMXQHNWU
Part Info: 20366
Chip Name: ▾ + ⟳
User: bionanoadmin   Change
▸ Advanced...

                  Accept   Skip Current Chip   Remove Chips
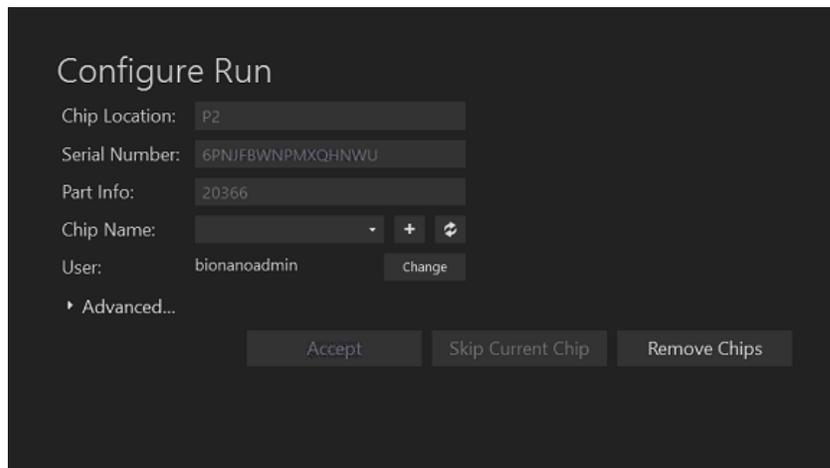
6. For systems connected to Access 1.6 and older, select the experiment that you wish to run from the dropdown list.

Configure Run

Chip Location: P2
Serial Number: UKGDR4GNPP7WHNWU
Part Info: 20366
Experiment: ▾   Refresh
Chip Setup: ▾
User: bionanoadmin   Change
▸ Advanced...

        Accept   Skip Current Chip   Remove Chips
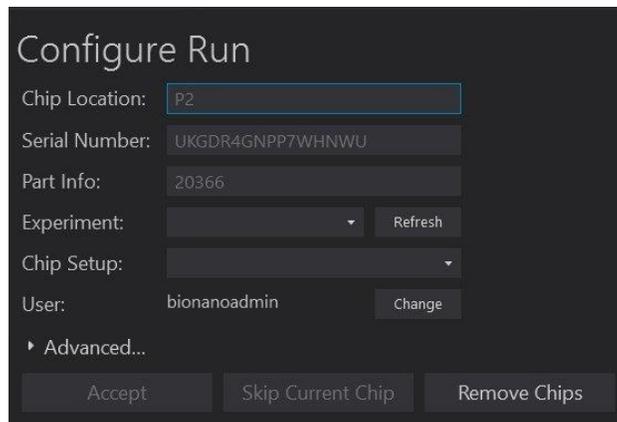
7. Review the runtime and throughput target parameters for each of the flowcells. These values will default to the values that were entered into Bionano Access during the experiment setup. The user may decrease the runtime or throughput as desired.

    Note: If the chip has been run previously, the runtime limit and throughput target may be modified. See Appendix B for details.
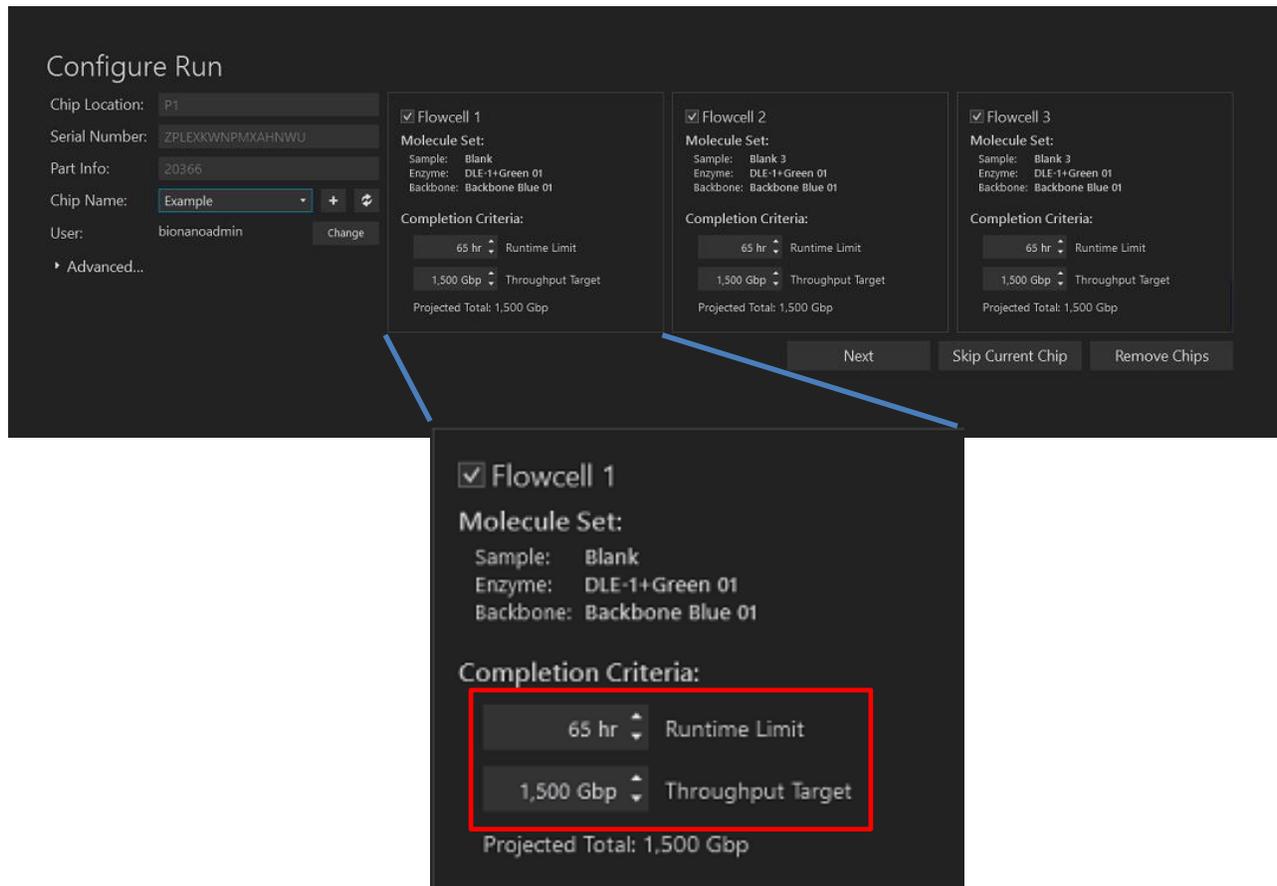

8. Click **Accept**

9. The current run will resume, first verifying chip alignment of the chip that was previously running. The newly placed chip will be put in a pending state.

Pausing the Run to Rehydrate the Chip

If the sample level becomes too low during a run, the ICS will notify the user that there is a sample contact problem and will not continue to run that flowcell.
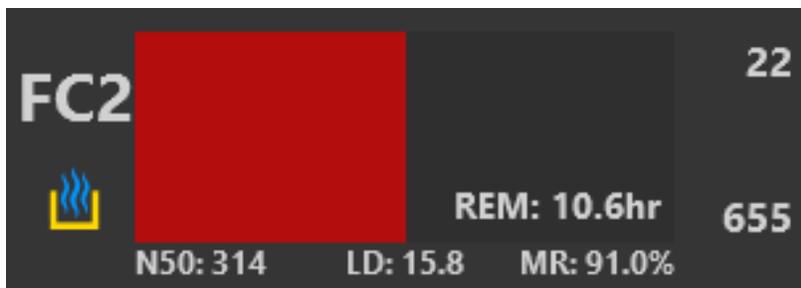
1. To pause a run that has flowcell(s) with a sample contact problem, follow the instructions above in the Pausing a Run to Insert a New Chip and/or Remove a Completed Chip Section.

2. Remove the chip from the instrument and top off **ALL** flowcells with nuclease-free water as described in Chapter 4 above. This includes adding water to the hydration reservoir if the water there has evaporated completely.

    ⚠ **Note:** Make sure to keep track of which plugs are in which flowcells to prevent cross-contamination.

3. Resume the run by placing the chip back into the same position in the instrument and following the instructions in the Pausing a Run to Insert a New Chip and/or Remove a Completed Chip Section above.

    Note: If all of the flowcells have a sample contact issue, then the instrument will move on to the next chip if one is available. If no chips are pending, the instrument will return to the start menu. Once the chip has been re-hydrated, the chip can be rerun up to the runtime limit by starting a new run. A new experiment in Access does not need to be defined; however, in Access a new chip run will be created that is associated with that experiment. This may require the user to merge the molecules files. See Bionano Access User Guide (P/N 30142) for more information.

Stopping the Run

1. To stop the instrument run before it reaches the limits that were set at the beginning of the run, press the stop icon at the top left of the user interface. Otherwise, the run will continue until the throughput or time limit is met.
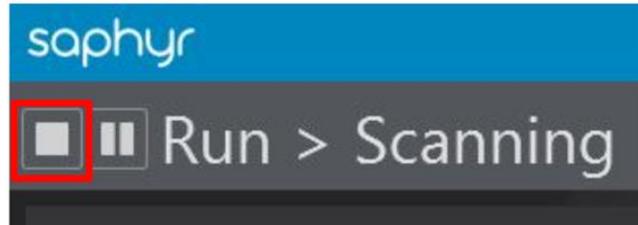
2. When you press this button, you will be asked to confirm you want to stop.
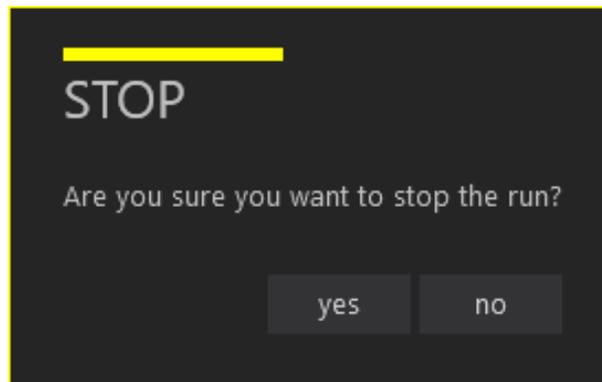
3. If there is no pending run, then selecting yes will stop the run on that chip. If there is a pending run, you will be asked to confirm if you want to stop all chips or just the one currently running.
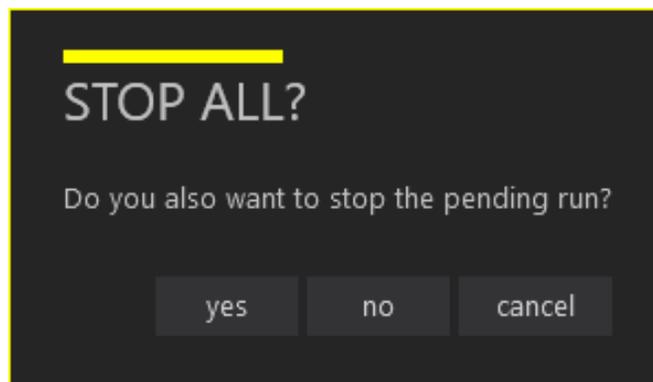
4. Select **yes** to stop both chips. Select **no** to stop the current chip but allow the pending chip to begin. Select **cancel** to return to the current chip run.

Remove Chip(s)

In addition to the *Pausing a Run* section above, users can remove the Saphyr Chip from the Saphyr instrument when the run is successfully complete or when the run is stopped.

1. From the Home Screen, click **Remove Chip**.

2. Once the wait icon is no longer showing, manually lift the sample door of the instrument.

3. Press the Press button to release bundle arm.

4. Remove the chip with clip attached.

5. Lower the bundle arm and close the sample door after removing the chip.

6. From the Main Menu, click **Chip is Removed**.

7. Close the Instrument Control Software program.

8. [Optional] Turn off the instrument.

    The Saphyr instrument is designed to run continuously. To set up another chip run, repeat these tasks:

    - *Create an Experiment*

    - *Insert Saphyr Chip*

    - *Configure Run*

    After removing the chip, detach and clean the Saphyr clip and plugs as soon as possible. See the *Maintenance* section for details.

Chapter 6: Analyze Data

Data Transfer for Analysis

After the instrument scans the chip for about 2-3 minutes, users can monitor the run progress and view quality metrics in real time on Bionano Access.

Following the completion of a run, users can perform various analyses. For more details on Bionano Access analysis and visualization features, see the Bionano Access Software User Guide (P/N 30142).

Chapter 7: Saphyr Assure

Saphyr Assure is a remote service provided by Bionano Genomics that enables the Instrument Control Software (ICS) to transmit instrument health metrics, run diagnostics and configuration information to enable Bionano to proactively provide support to customers. Saphyr Assure enables users to install software updates for Saphyr ICS, allowing for quick and easy updates. Saphyr Assure also manages windows update checking to ensure the system is always running with the latest validated updates.

To enable the instrument health monitoring feature of Saphyr Assure, the Bionano Access administrator is required to opt-in. The administrator has the option to enable the Saphyr Assure & Compute on Demand service, or if the user is not using Compute On Demand, they can opt-in to just the Saphyr Assure service. To opt-in, log into the Bionano Access server as an administrator, click settings and then click system settings.
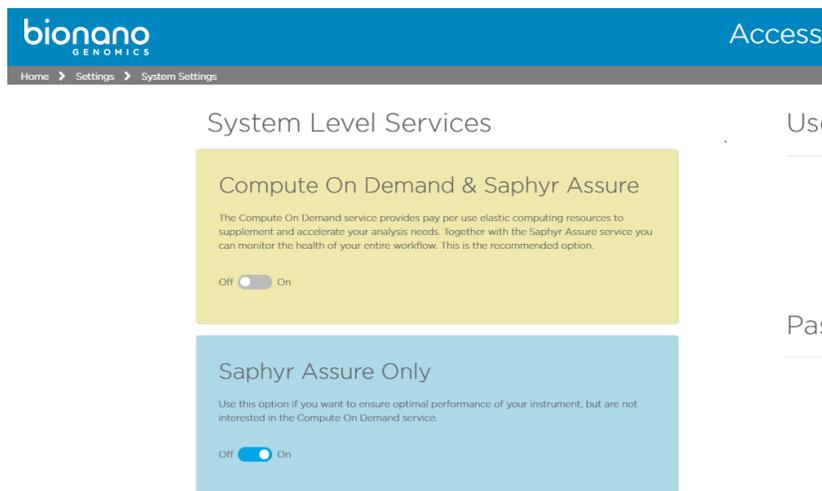

Chapter 8: Updating Software

For systems that are on ICS 5.1 or above, software can be updated through the ICS UI. When a software update is available, the Saphyr Assure icon will turn orange .

1. Click on the Saphyr Assure icon .

2. Select **Saphyr ICS Updates.**
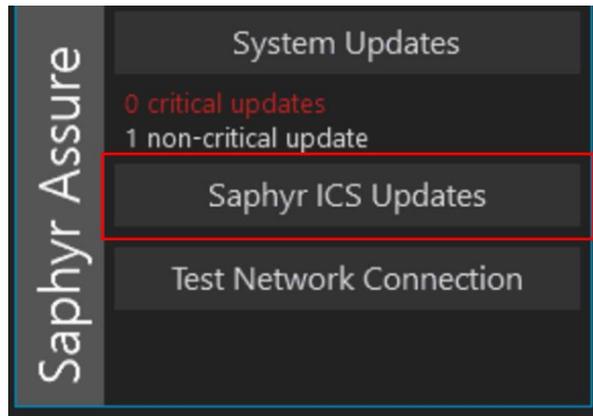
3. Select **Install**.

4. The system will alert the user that a restart is needed to complete the installation. Select **Yes** to proceed.

Chapter 9: Logging off and System Administration

To either restart the Instrument Controller or to turn off the Instrument Controller , click the applicable

icon at the top right of the screen. Users with System Administrator credentials will also see an icon that will allow them access to the System Settings menu as well as the Windows login.

Chapter 10: Maintenance

Clean Saphyr Clip Plugs

Clean the clip plugs before and after each use. Use gloves when cleaning clips.

1. Use a lint-free wipe saturated with 70% alcohol to remove any visible dirt or debris on all sides of the clip plugs.

2. Air dry the clip plugs before using them again. Do not use canned air.

3. Store the clip in a clean case with a cover when not in use.

Clean the Objective Lens

We recommend that users clean the objective lens every 3 months or whenever a spill occurred on the instrument stage.

1. Launch the Instrument Control Software.

2. Click the HELP icon at the top right of the screen

3. In the Help screen, click **Clean Objective**, then click the **Clean Objective** button that appears below
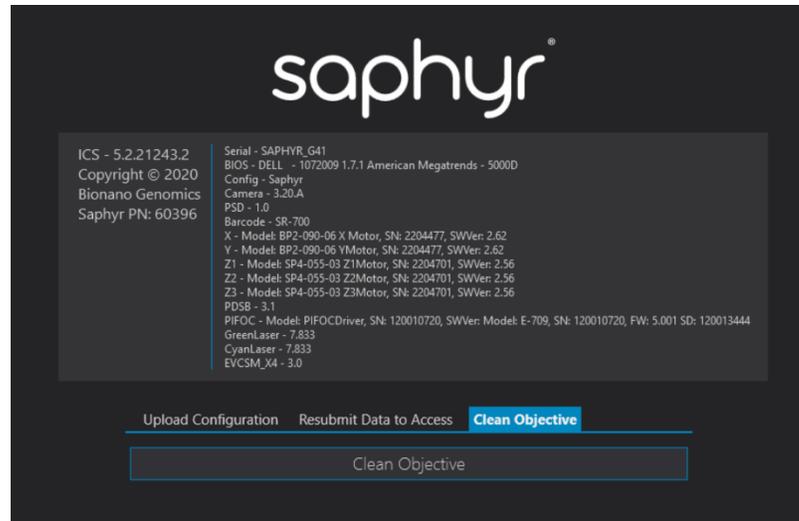
4. The instrument will move the stage into a position that will allow the objective to be accessible. Once this has completed, click **Begin Cleaning**. You'll get the following message. Click OK.


.
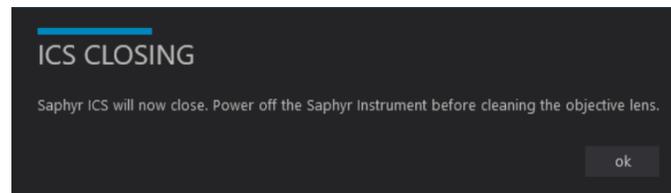
5. Manually lift the sample door.

   Users may need to apply some force to lift the sample door.

6. Fold a lens tissue into fourths to create a soft-point

7. Clamp the tissue with a flat hemostat or flat forceps, and then add 2 to 3 drops of ≥ 98% methanol on the tissue.

8. For a dual chip system, press the Press button to release the bundle arm. For a one chip system, lift the bundle arm. Clean the surface of the objective lens by moving the tissue in a circular motion.

9. Replace the bundle arm, close the sample door, then power on the instrument and control software.
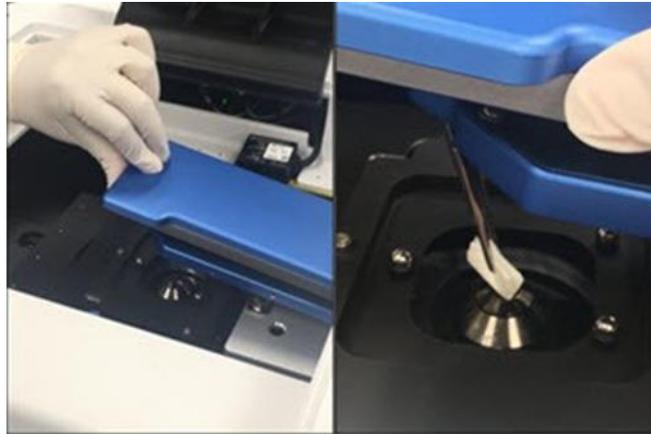
Replace Air Filter

The air filter should be replaced every 3 months. Air filters are included in the Accessory Kit that came with the Saphyr instrument. Additional air filters are supplied to the customer when the annual preventative maintenance is performed.

1. Turn off the instrument.

2. On the back of the instrument, pull the air filter cover firmly away from the instrument to release the magnets that hold the cover in place.

3. Remove the air filter from its cover.

4. Insert a new air filter. Make sure the Air Flow arrow symbols are facing up (air flows into instrument).

5. Attach the cover with the new air filter to the instrument.

Appendix A

Troubleshooting

1. **Issue: Unable to log into ICS**

   Possible causes:

   - Incorrect password was entered.

   - The Access server is not active.

   Solution:

   - Check if you can log into Access.

   - Refer to page 8 of the Bionano Access Software User Guide if you forgot your password.

   - Check whether the Access service status is active. If you can browse the Access webserver, the service is active.

   - Reboot the Instrument Controller.

2. **Issue: Data service is unable to connect to Bionano Access.**

   Solution: Check if the Access webserver is active. Confirm that the cable connection between the instrument controller and Access is secure. If the issue is not resolved, contact Bionano Support at support@bionanogenomics.com.

3. **Issue: Instrument Does Not Initialize**
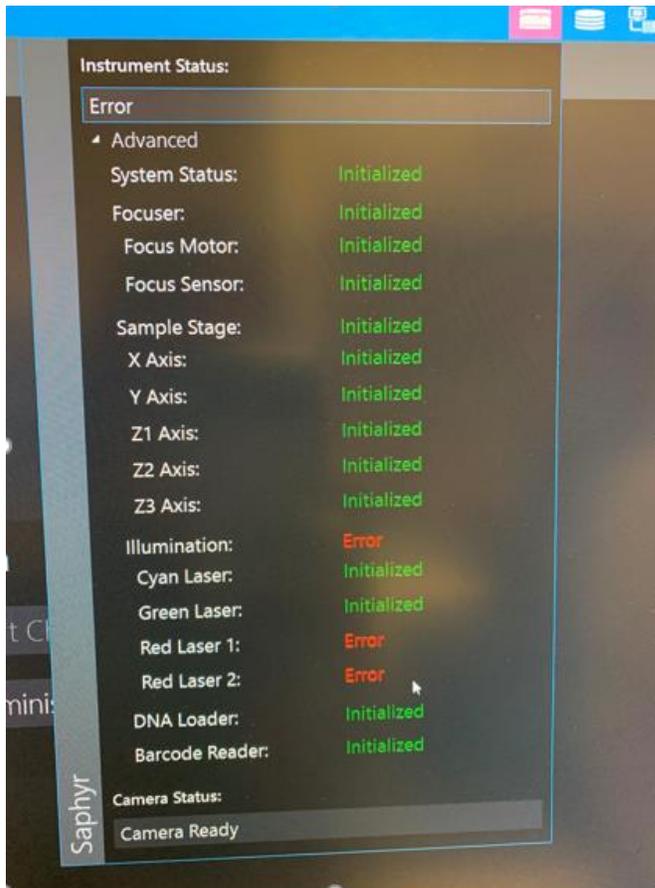

Fig. 1. Image of initialization status of each instrument component.

Possible cause: This is more likely a hardware issue.

Solution:

- Make sure to close ICS first.

- Switch off the instrument and wait for at least 15 sec before switching back on.

- Wait approximately 30 seconds. Restart ICS and wait for system to re-initialize.

- If this does not resolve the issue, contact Bionano at support@bionanogenomics.com.

4. **Issue: Barcode Read Error (Barcode Cannot be Read)**

Possible causes:

- Chip not well seated.

- Barcode (Q-code) is damaged.

- Barcode reader failure

- Barcode reader is misaligned

Solution:

- Reseat the chip.

- Ensure there is no physical damage to the chip that may prevent it from fitting well into the chip slot.

- Retry Barcode scan. If it fails again, then try the following:

  - Remove the chip.   Ensure there is no physical damage to the chip that may prevent it from fitting well into the chip slot.

  - Ensure that the chip sits flat and fits within the groove on the platform. Restart the run.

- Contact Bionano at support@bionanogenomics.com if this happens with more than 1 chip.

5. **Issue: Disk Full (Not enough disk space)**
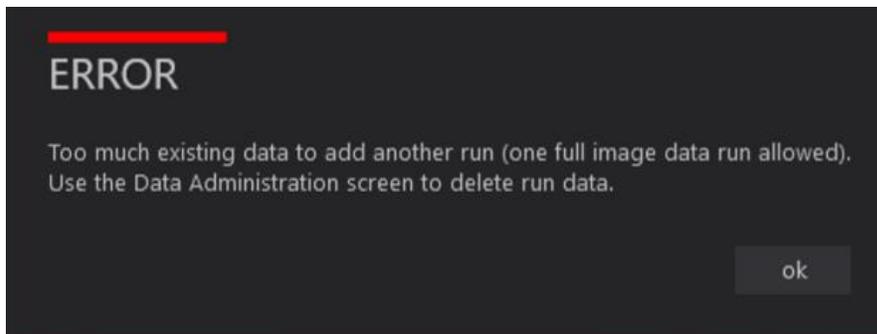
Fig. 2. Image of error indicating that the disk space is full.

Possible cause: The instrument control PC hard drive only holds one run. If you try to save images on a run and have not transferred or removed the images from the previous run, you will see this error message (See Fig. 2).

Solution: The software will prompt you to delete the previously saved run if you start a new run.

1.   Go to the main menu of ICS and select 'Data administration.'

2.   Use the tick box (right-hand side) to select run(s) to delete from the run list and click the 'Delete' button.

6. **Issue: No electrical contact could be established. ICS message: "Check sample fluid level."**

Possible cause: Sample Evaporation

Solution :

- Pause the run; Remove the chip; Remove the clip from the chip.

- Rehydrate the chip with nuclease-free water.

- Attach the clip to the chip; Reinsert the chip into the instrument; Resume the run.

7. **Issue: Bionano Access dashboard is not being updated in real time as the run proceeds**

   Possible cause:

   • Connection is lost between Bionano Access Webserver and the Saphyr Instrument Controller.

   Solution:

   • Confirm that the cable linking both components is plugged in at both ends.

   • Refresh Bionano Access webpage (allow some time for the run data to populate in Access).

   • **Note**: When loss of connection occurs, the instrument keeps running and data is stored locally on the Instrument Controller. Transfer will restart when the connection is restored.

8. **Issue: Map rate is zero**

   Possible causes:

   • Reference not provided

   • Wrong reference provided

   • Wrong labelling enzyme selected

   Solution:

   • Wait for the run to complete and for the sample's molecules object to appear in the corresponding project (do not stop the run).

   • Make sure the correct reference is loaded in Bionano Access. If it is not, refer to the "Edit Job" section in the Bionano Access Software User Guide (30142) to change the reference.

9. **Issue: No throughput**

   Possible causes:

   • Evaporation might have occurred.

   Solution:

   • Remove the chip.

   • If there is no liquid, re-hydrate the chip and re-start the run.

   • If the issue is not resolved, contact Bionano at support@bionanogenomics.com.

10. Issue: Chip Throughput Max Reached

Fig. 4. Error message showing flowcell 3 has reached maximum throughput.

Solution:

- Uncheck the box next to the flowcell that reached max throughput.

- Continue to run the other flowcells to collect more throughput, if desired.

Export Tier 1 Run Diagnostics

If there is an issue with a flowcell run that needs to be addressed by Bionano Genomics Support for troubleshooting, a small diagnostic package as Tier 1 Run Diagnostics can be generated. If you are connected to the Saphyr Assure service, these diagnostic files can be transmitted directly to Bionano Support through the ICS.

1. At the top of the window, click on the [icon] icon.

2. A list of recent chip runs will be displayed. Select the serial number of the chip associated with the run containing the diagnostic information you would like to export.
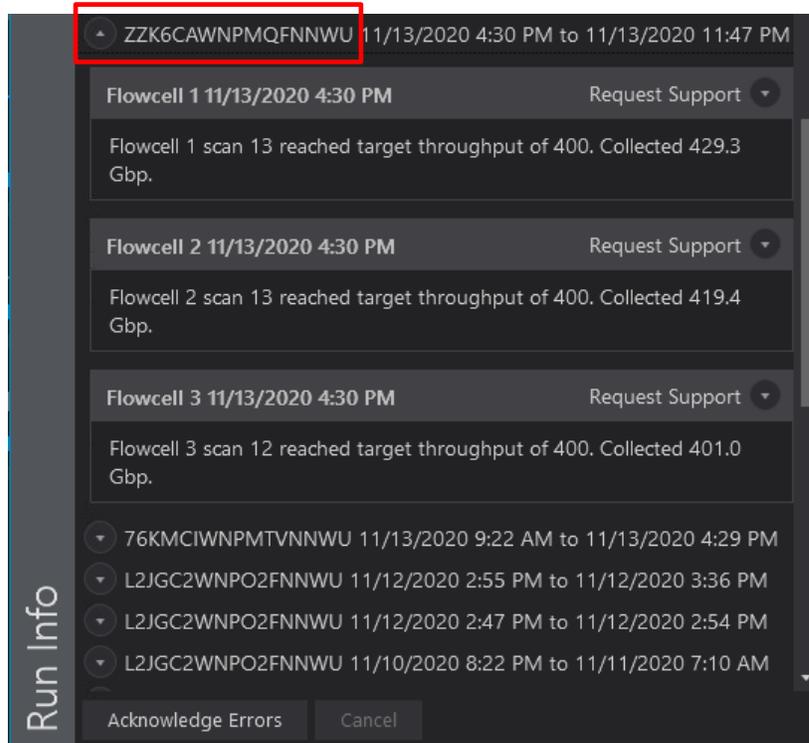
3. Click on the arrow to the right of "Request Support" for the flowcell that you'd like to troubleshoot.
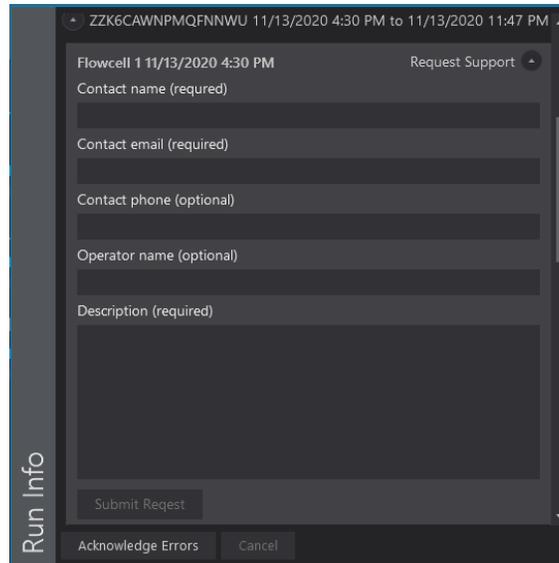
4. In the dropdown that appears, fill in the applicable information and press Submit Request. This will transmit the diagnostic information to Bionano Support. The Contact Name, Contact Email, and Description fields are required for submission. The Operator Name will be automatically populated with the User ID of the person logged into ICS.

5. If the submission begins but does not complete due to a network connection problem, it will automatically be re-submitted at a later time.

   For users who have not opted in to Saphyr Assure and do not have network access to enable connection to Saphyr Assure, the diagnostic package must be saved and emailed to Bionano support. Follow steps 1 and 2 above, then do the following:

6. For systems in kiosk mode:

   a. Click on the arrow to the right of "Export Diagnostics" for the flowcell that you would like to troubleshoot.
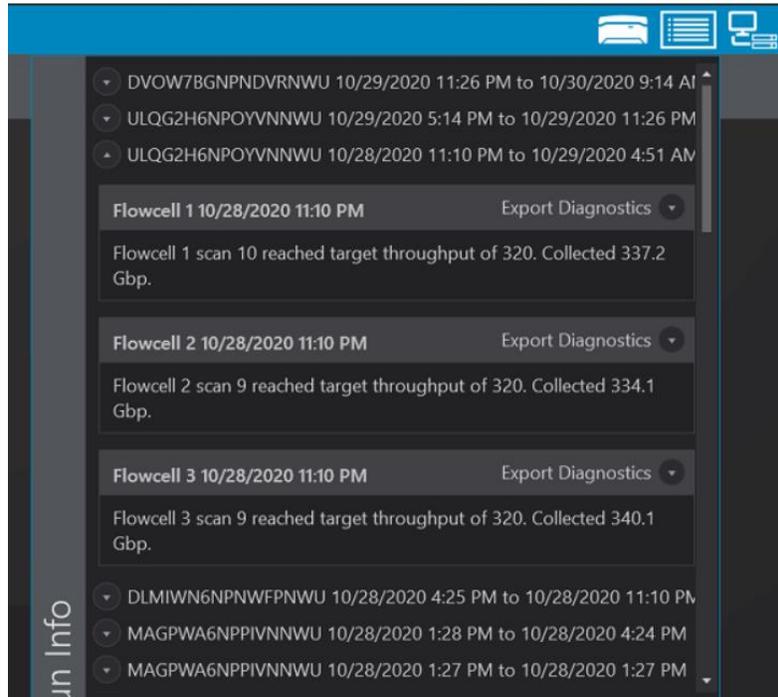
a. Insert exactly one USB flash drive into the Instrument Controller and wait 30 seconds for the device to be recognized.

b. In the dropdown that appears, fill in the applicable information and press Export.
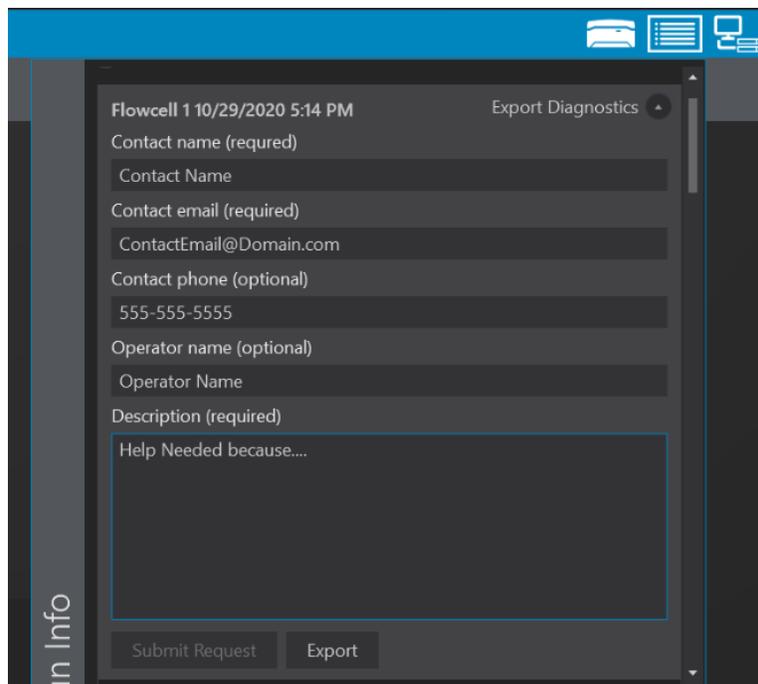
b. ICS will generate the diagnostic file and save it into the root of the USB drive. ICS indicates that the export is complete by displaying a green checkbox.
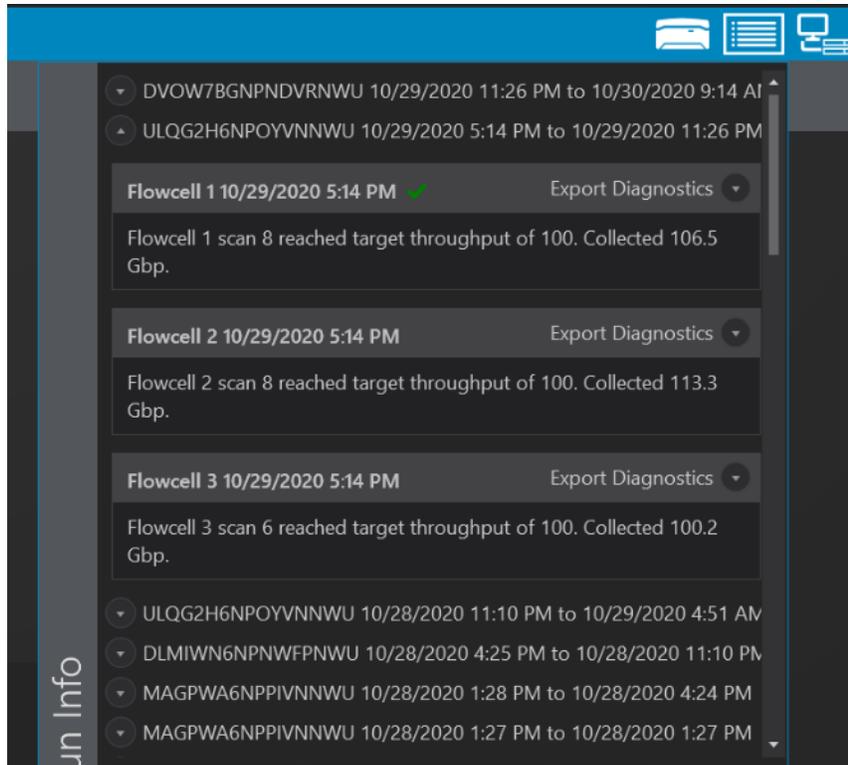
c. Remove the USB drive and take it to another computer and email to Bionano Genomics Support (support@bionanogenomics.com). The file size is intended to be small enough to email as an attachment using most standard email service providers.

7. For systems not in kiosk mode:

a. Click on the arrow to the right of "Export Diagnostics" for the flowcell that you would like to troubleshoot.

b. Select the location to save the Saphyr Archive (.sar) file, and then click OK.

c. In the dropdown that appears, fill in the applicable information and press Export.

d. When ICS completes compressing the files, navigate to the location of the file, then send it to Bionano Genomics Support (support@bionanogenomics.com). The file size is intended to be small enough to email as an attachment using most standard email service providers.

e. Once the file has been transmitted, delete the exported file to prevent filling the hard drive.

Export Tier 2 Run Diagnostics

If the Tier 1 diagnostic package is insufficient to address the issue, Bionano will need to collect additional data from the run. For users connected to Saphyr Assure, this will occur without any intervention from the user.

Users not connected to Saphyr Assure will be instructed by Bionano Genomics Support to assist in providing the additional information. The user will be provided a Diagnostic Request File (.drf) from Bionano Support.

For systems in kiosk mode:

1. Save the .drf file on a USB stick at the top level (root) of the directory structure. The USB stick may only have one .drf file in this location. Attach only one USB stick at a time to the Instrument Controller.

2. Click on the Run Info icon. ⬛ Select Open Diagnostic Request to import a .drf file.

3. Once the file is imported, ICS will generate a .sar file and save it to the USB stick. When the export is complete, remove the USB stick from the ICS Controller and transfer the .sar file to Bionano Genomics Support

For systems not in kiosk mode:

1. Save the .drf file in the instrument hard drive, and double click the .drf file. This will open ICS (if it is not already open) and prompt the user to save a .sar file.

2. Choose a location on the Instrument Controller and save the file.

3. Once the file is saved, transfer it to Bionano Genomics Support.

4. Once the file is transmitted, delete the .sar file to ensure that enough hard drive space remains on the Instrument Controller for normal operation.

Export / Upload Data Configuration

Users can export the instrument data configuration to their computer, such as log and calibration data from the instrument, and then send to Bionano Genomics Support for troubleshooting. For customers that are connected to Saphyr Assure, the configuration can up upload directly to Saphyr Assure.

1. From the Home Screen, click the **Help** icon at the top-right corner of the screen. A dialog box appears.

2. For users not connected to Assure, click **Export Configuration**. For users connected to Assure, skip to number 9.

3. [Optional] Select the **Full configuration** checkbox to collect additional system information. This option increases the export file size and the time it takes to compress the files.

4. Click **Compress and Export Configuration**.

5. Type the file name.

6. Select the location to save the ZIP file. If your system is in kiosk mode, you will not be prompted to select the location, the file will be saved directly to a USB device that has been inserted into the Instrument Controller. Only one USB drive may be inserted into the Instrument Controller.

7. Click **Save**.

8. When ICS completes compressing the files, navigate to location of the ZIP file, and then send it to Bionano Genomics Support.

9. For users connected to Assure, click **Upload Configuration**. Fill out the optional and required information and click **Compress and Upload Configuration**.

Appendix B

Rerunning a Chip

ICS allows for user flexibility in collecting data to accommodate various workflow issues and scenarios. Each flowcell in the chip has an associated maximum accumulated throughput and maximum accumulated runtime. The chip can be re-run as many times as desired to collect data up until either of these limits are reached. See the Chip Specification Document (P/N 30256 and 30264) for the maximum accumulated throughput and accumulated runtime associated for a particular chip part number.

There are a few situations where a chip may need to be re-run. These include failure to successfully achieve the desired throughput target in the initial run due to:

- Loss of power

- Instrument Control Software or operating system crash.

- Loss of electrode contact to all flowcells from evaporation, resulting in ICS stopping the chip run prematurely.

- Data collection rate insufficient to achieve target within the allocated time.

In addition, if the desired throughput was collected, the user can return the chip to collect more data on the desired flowcells provided the maximum accumulated throughput and maximum accumulated runtime have not yet been achieved.

Notes on safe handling the chip for re-running:

- Re-hydrate the flowcells prior to re-running chip (see steps 9-13 of the appropriate section in Chapter 4: Prepare Chip).

- It's recommended that the chip be re-run immediately after the initial run, within 8 hours of the previous run being completed.

  Note: If the same chip is run multiple times, it will have multiple chip runs and multiple molecules objects in Bionano Access. See Bionano Access User Guide (P/N 30142) for instructions.

Example scenarios for re-running a chip

Note: Actual Saphyr Chip specifications are provided on the product specifications for your chip. The following numbers are for example only.

Scenario 1: Desired throughput targets not achieved in the initial run due to insufficient data generation rate.

In this example, only flowcell 3 was able to achieve its desired throughput during the allocated runtime:

Flowcell	Run Conditions Set by the User in the First Run		What Actually Happened During First Run	
	Desired Throughput Target	**Desired Runtime Limit**	**Throughput Collected During the Run**	**Total Flowcell Runtime**
1	5000 Gbp	65 hr	4000 Gbp	65 hr
2	5000 Gbp	65 hr	4500 Gbp	65 hr
3	5000 Gbp	65 hr	5000 Gbp	65 hr

If the user desires to re-run the chip to collect more data on flowcells 1 & 2, the configure run screen will appear as below. Here, the user can re-run the chip to collect an additional 1000 Gbp and 500 Gbp in flowcells 1 & 2 respectively. Since flowcell 3 has achieved the maximum accumulated throughput limit of 5000 Gbp, it can no longer be run.

Scenario 2: Desired throughput targets not achieved in the initial run due to loss of contact on all flowcells.

In this example, all three flowcells independently lost contact, resulting in ICS stopping the chip run prematurely. (ICS will only stop the chip run when all flowcells have lost contact.)

Flowcell	Run Conditions Set by the User in the First Run		What Actually Happened During First Run	
	Desired Throughput Target	**Desired Runtime Limit**	**Throughput Collected During the Run**	**Total Flowcell Runtime**
1	1300 Gbp	65 hr	1200 Gbp	28 hr (Loss of contact)
2	1300 Gbp	65 hr	1220 Gbp	32 hr (Loss of contact)
3	1300 Gbp	65 hr	1190 Gbp	25 hr (Loss of contact)

If the user desires to re-run the chip to collect more data, the configure run screen will appear as below. Here the user can re-run the chip to collect additional data on all three flowcells.

Scenario 3: Desired throughput target insufficient.

In this example, the user determines that the amount of data collected was insufficient and wants to collect more. This is allowed provided the total accumulated throughput is within the allowed limits. For example, the initial run completed with the following:

Flowcell	Run Conditions Set by the User in the First Run		What Actually Happened During First Run	
	Desired Throughput Target	**Desired Runtime Limit**	**Throughput Collected During the Run**	**Total Flowcell Runtime**
1	320 Gbp	65 hr	320 Gbp	12 hr
2	320 Gbp	65 hr	320 Gbp	13 hr
3	320 Gbp	65 hr	320 Gbp	11 hr

However, after the run, the user desires to collect a total of 400 Gbp on each flowcell. In this case the user would fill out the configure run screen as follows (note that ICS provides a projected total of 400 Gbp for each flowcell):

☑ Flowcell 1
Molecule Set:
Sample: Sample Green
Enzyme: DLE-1+Green 01
Backbone: Backbone Blue 01

Completion Criteria:
65 hr ⬍ Runtime Limit
80 Gbp ⬍ Throughput Target
Projected Total: 400 Gbp

☑ Flowcell 2
Molecule Set:
Sample: Sample Green
Enzyme: DLE-1+Green 01
Backbone: Backbone Blue 01

Completion Criteria:
65 hr ⬍ Runtime Limit
80 Gbp ⬍ Throughput Target
Projected Total: 400 Gbp

☑ Flowcell 3
Molecule Set:
Sample: Sample Green
Enzyme: DLE-1+Green 01
Backbone: Backbone Blue 01

Completion Criteria:
65 hr ⬍ Runtime Limit
80 Gbp ⬍ Throughput Target
Projected Total: 400 Gbp

Appendix C

Instrument Control Software (ICS) Icons

The icons are at the top right-corner of the ICS screen.

Saphyr Instrument Status

The Saphyr instrument icon indicates the status of the Saphyr instrument.

Icon	Color	Description
	Blue	**The instrument is working normally.**
	Flashing Green	**The instrument is initializing.**
	Flashing Red	**The instrument encountered an error. Contact Bionano Genomics Support for assistance.**
	Flashing Bright Blue	**The instrument door is open. If the instrument door is closed, there is an issue with the safety interlocks, please contact Bionano Support.**
	Flashing Amber	**The instrument is not receiving power or is not turned on.**
	Purple	**The instrument hardware is not correctly configured. Contact Bionano Genomics Support for assistance.**
	Flashing Dark Orange	**There is an interlock fault. Contact Bionano Genomics Support before attempting to start the run.**

Run Info

The run info icon indicates the status of recent runs.

Icon	Color	Description
	Blue	**There are no run errors, or any previous run errors have been acknowledged.**
	Red	**There is run error that has not yet been acknowledged.**

Bionano Access Connection Status

The Bionano Access Connection icon indicates that there is a network connection between the Saphyr Instrument Controller and Bionano Access.

The instrument must be connected to Bionano Access before it can begin a run. If connection fails while the instrument is scanning, ICS will cache run data locally and will automatically resume once the connection is reestablished.

Icon	Color	Description
	Blue	**The instrument control software is connected to Bionano Access.**
	Amber	**The instrument control software is unable to connect to Bionano Access.**

Saphyr Assure Status

The Saphyr Assure Status icon indicates the status of the Saphyr Assure connection and any available updates.

Icon	Color	Description
	Green	**Saphyr Assure is connected and health monitoring is enabled.**
	Blue	**Saphyr Assure is connected and health monitoring is disabled.**
	Red	**There is a connection error between ICS and Saphyr Assure.**
	Amber	**Update(s) are available for either ICS or Windows**
	Blue	**Disabled / Establishing Connection**

Remote Support

The Remote Support icon indicates whether the system is connected to remote support.

Icon	Color	Description
	Green	**Remote support is enabled.**
	Blue	**Remote support is disabled.**

Appendix D

Instrument Initialization

The hardware components of the Saphyr instrument initialize upon startup of the Instrument Control Software. During initialization, the instrument status icon will flash green. Once initialization is complete, the icon will turn blue. If there is an issue with the initialization of any component, the icon will turn red. Clicking on the icon will show the initialization status of each component, as shown below.
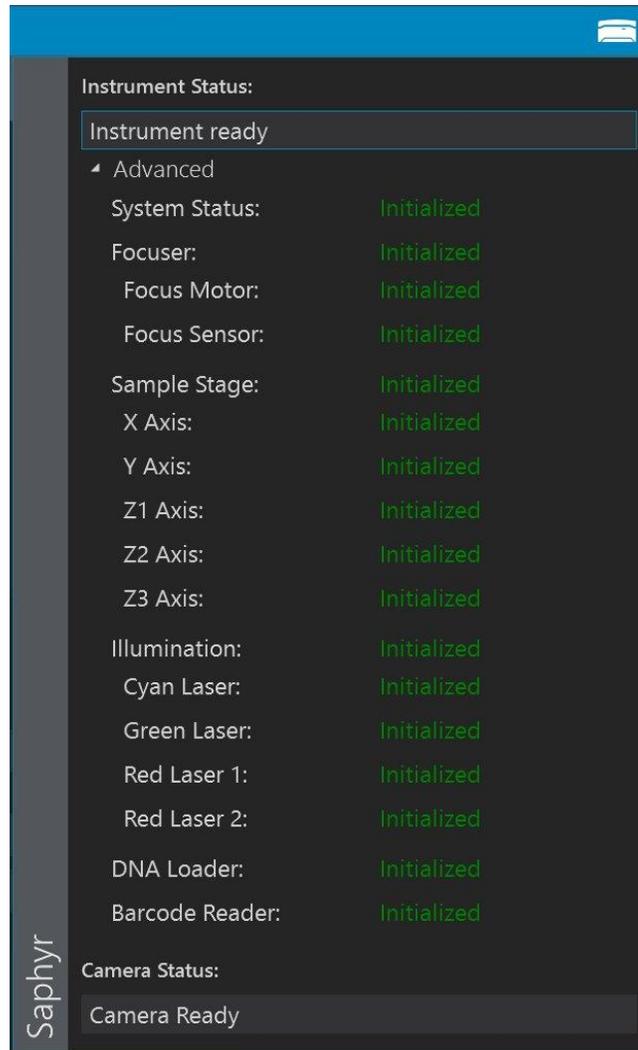
Appendix E

Data Administration

The Data Administration option from the Home Screen allows users to manage run data that are on the Saphyr Instrument Controller. This is applicable only if you are using the option to save images.

Insufficient Disk Space

If you are using the option to save images, the data needs to be removed from the Instrument Controller before starting a new run. The Instrument Controller will only save one run at a time before prompting the user that there is not enough space remaining. When clicking **Accept** in the *Configure Run* screen, if there is a run that has not been removed, a message will appear ("Too much existing data to add another run") prompting you that you cannot proceed until data is removed.

Follow these steps:

1. Click **OK**.

2. At the *Configure Run* screen, click **Cancel**.

3. Copy off any images that you want to retain to a separate server.

   Note: Bionano Genomics is not responsible for the integrity of the data transfer

   Note: Do not cut or delete images from the Instrument Controller. Use the copy function only. Removing images from this directory will inhibit the ability to archive diagnostic information that may be useful for troubleshooting issues in the future. Remove images by following the instructions in the Delete Runs section below

4. Once you have confirmed that your images have been transferred, proceed to the Delete Runs section below.

Delete Runs

After data analysis is complete, delete runs to clear space for a subsequent run. Runs that are currently in progress cannot be deleted.

Warning: Once images have been deleted, they cannot be recovered.

1. From the Home Screen, select **Data Administration**.

2. If there are images saved, the serial number of that chip will appear. Click **Delete**.

Saved Image Data

SERIAL NUMBER
KOB7XH6NPPJGLNWX

SIZE
39.50 GB
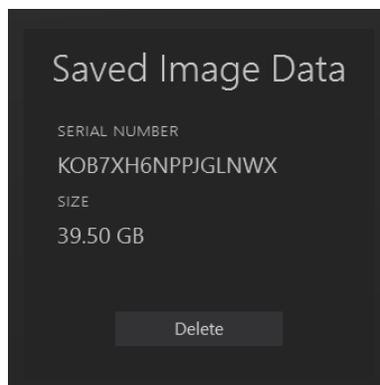
Delete

3. When the process is complete, the screen will show no additional data that can be deleted.

4. Click the **Home** icon to return to the Home Screen.

Appendix F

Upload Run to Access

The Resubmit Run to Access option from the Help Screen allows users to resubmit a dataset for reanalysis. This may be used if an error in the initial analysis of the run data has occurred.

Note: If ICS and Access disconnect during a run, the run will continue. Access will automatically resume analysis once the connection is reestablished. In this case, there is no need to resubmit the data. The status of the data transfer to Access can be monitored using the Data Service Status icon.

1. Click on "**Help**" in the upper right corner of the screen.

2. Select **Resubmit Data to Access**.

3. Select **Upload Run to Access.**
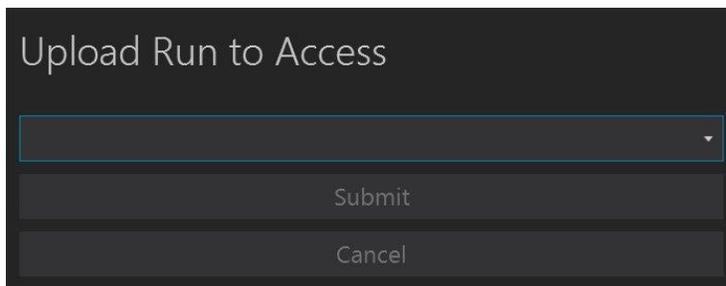
4. Select the run you wish to reanalyze from the dropdown list.

5. Click **Submit**.

6. **The existing Chip Run will be deleted from the Access database,** and a new run will be created in Access.

   Note: The Chip Run ID will remain the same.

Appendix G

Instrument Controller Operations

- The Instrument Controller runs Windows Defender for anti-virus protection. It's recommended that no third-party anti-virus software (AVS) is installed. However, if a customer chooses to install a different AVS, it must only be run manually when the instrument is not running a chip. The stability of the Instrument Controller cannot be guaranteed if additional software is installed. Systems that have additional software added will not be supported.

- Customers should not delete any files from the Instrument Controller using Windows, as this may inhibit Saphyr's ability to archive diagnostics and may result in data loss. The only exception to this rule is the diagnostics packages generated by ICS after the packages have been safely copied off the instrument and sent to Bionano Genomics customer support.

- It is strongly recommended that the Instrument Controller is not added to the customer's Windows domain. This may cause the Windows security settings, power states, or other settings critical to the function of the instrument to be modified. Systems that have been added to a domain will not be supported

- It is strongly recommended that user only use the provided Windows User login.

- Please do not make any changes to the Instrument Controller system, including:

    o   Remove or modify any files in C:\Installs

    o   Uninstall or install any programs

    o   Uninstall or install any drivers

    o   Change any PC power settings

    o   Change any BIOS settings

    o   Change any Local Group Policy settings

    o   Add or remove user accounts

    o   Change user account passwords

    o   Change Ethernet adapter settings

    o   Modify the Windows hosts file

   o   Change any Windows Update settings

Appendix H

Instrument Disposal

This label indicates that the instrument meets the 2012/19/EU Directive on waste electrical and electronic equipment (WEEE).

Email support@bionanogenomics.com for instrument disposal in the EU.

WEEE Label

Additional Resources

The following documentation is available for download from the Bionano Support page.

Resource	Description
Saphyr System Safety Guides (P/N 30144, 30253, 30302, 30467)	Provides information about the instrument safety considerations.
Saphyr Site Preparation Guide (P/N 30145)	Provides specifications for laboratory space, electrical requirements, and environmental considerations.
Bionano Access Software Guide (P/N 30142)	Provides an overview of data analysis.
Saphyr Networking and Setup Guide (P/N 30251)	Provides networking information for all Bionano Genomics computers.
Bionano Genomics Data Security Guidelines (P/N 30292)	Provides information about how Bionano Genomics data is handled.

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

Type	Contact
Email	support@bionanogenomics.com
Phone	**Hours of Operation:** **Monday through Friday, 9:00 a.m. to 5:00 p.m., PST** **US: +1 (858) 888-7663**
Website	**www.bionanogenomics.com/support**

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