Takara Bio USA

Shasta™ Single Cell Advanced Features User Manual

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I. Introduction

Shasta CELLSTUDIO Software is the main application software for instrument controls, including cell/reagent dispensing and cell imaging. While basic operations are covered in the Shasta Single Cell User Manual, this manual covers features of interest to advanced users, especially those wanting to synthesize their own experiments using the power and flexibility of the Shasta Single Cell System.

The sections of this document can be applied for the purposes summarized in Table 1, provided to help you navigate this document more easily based on your goals.

Table 1. Guide to using this manual for your experimental goals.

User manual section	Tool	Purpose
Sections II & III	App Manager	Defining a custom application workflow (dispense and imaging steps) in CELLSTUDIO software.
Section IV	Barcode Manager	Creation and management of the barcode options available when setting up an experiment.
Section V	Visualizer	Tool to help you with decisions involving dispense patterns and volumes.
Section VI	Microscope user utility	Tool to help you determine the ideal imaging step configuration when creating your custom application, especially when using novel stains or sample types.
Appendix A	--	Points to consider when beginning to design a custom application or scaling up from a plate-based experiment to a low input, high-throughput automated protocol. The advice in this section is provided by the Takara Bio scientists who have first-hand experience designing our prevalidated Shasta applications.

II. App Manager: Available Actions

The prevalidated applications defined by Takara Bio—Shasta whole-genome amplification (WGA), total RNA-seq, and mRNA-seq—are configured within CELLSTUDIO software by default.

The app manager (application manager) is a tool in CELLSTUDIO software that allows for the creation, modification, and management of application workflows for use with the Shasta Single Cell System. By using the app manager, additional workflow options become available for selection and use on the main CELLSTUDIO software interface.

The application manager can be accessed from within the CELLSTUDIO software under the Options menu (hamburger ☰ icon) in the title bar and selecting the **App Manager** menu option.

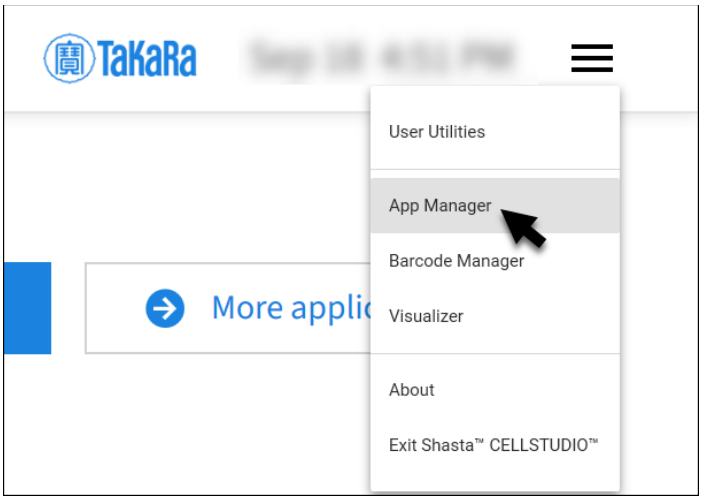

Figure 1. How to bring up the app manager.

This will bring up the *App manager* view.

Figure 2. *App manager* view.

The sections below describe the functions available in the app manager and correspond to the letters labeling areas of the *App manager* view in Figure 3.

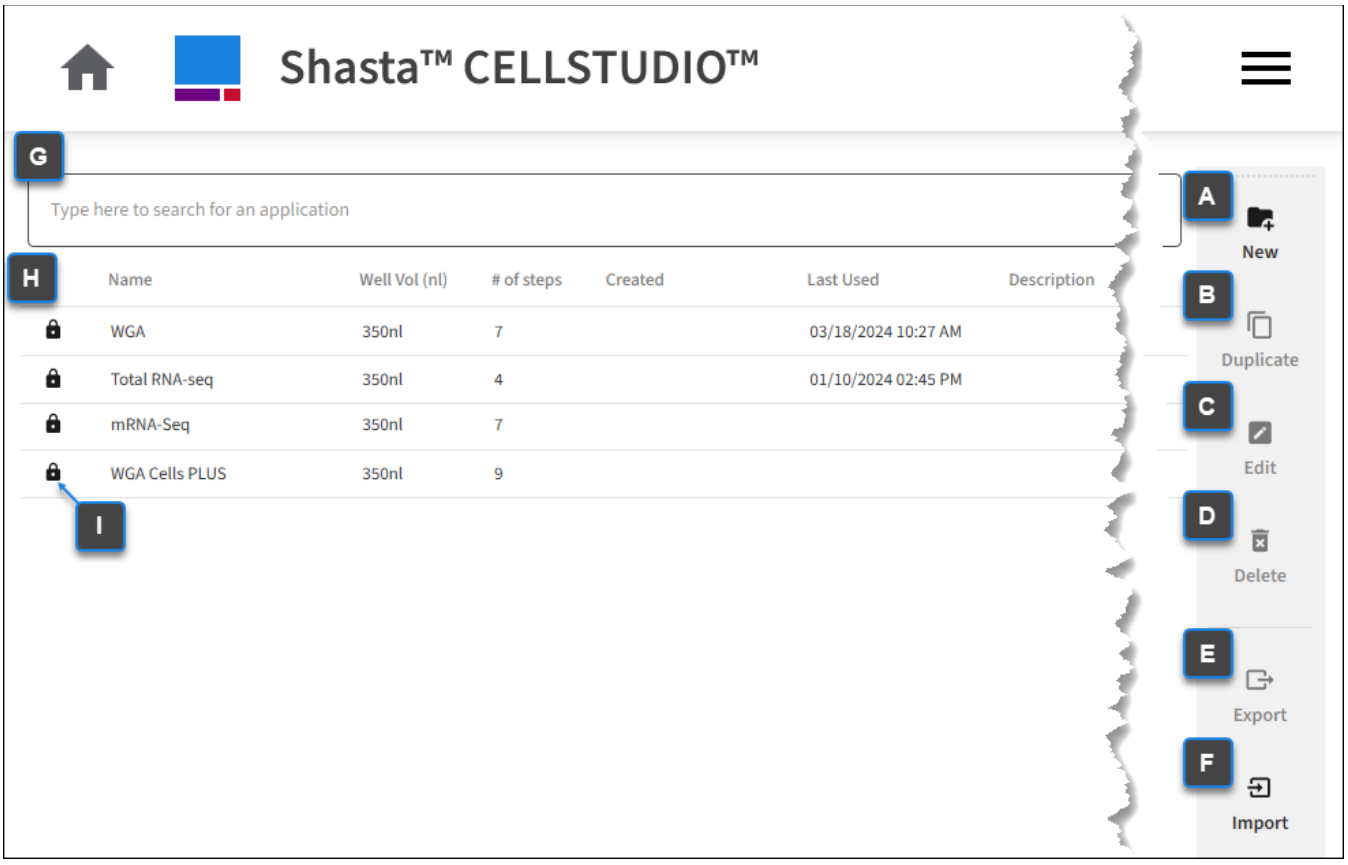

Figure 3. *App manager* elements. For more information about the labeled options, see Sections A–I below.

A. Create a New Application

Clicking the [New] icon will bring up the *App details* page (Section III.A).

Refer to Section III for details about using the *App details* page to configure the parameters of the application.

B. Duplicate an Existing Application

The [Duplicate] button can be used to create a copy of an existing application. This can be useful when synthesizing a new workflow. For example, when doing optimization experiments, you can easily recreate a previously defined application to modify it without affecting the original one.

To duplicate an application:

1. Click on the name of the application you want to copy. In the example in Figure 4 (below), the 'Total RNA-seq' application was selected.
2. Click the [Duplicate] button on the right-sidebar menu.

The duplicated application is added to the end of the list with "(Copy)" appended to the name. The name can be changed (customized) by editing the application. Refer to Section II.C, the next section, for how to edit the application.

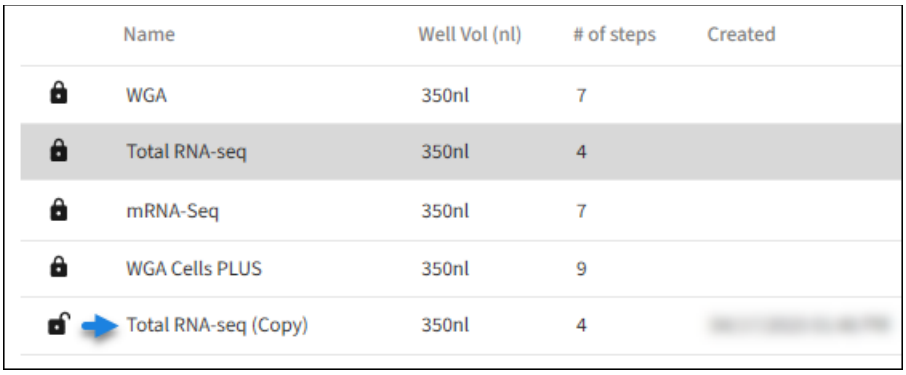

Figure 4. Example of duplicating an application in App Manager. The application highlighted in gray (`Total RNA-seq`) was the application duplicated; the blue arrow indicates the newly created duplicate application [`Total RNA-seq (Copy)`].

C. Edit an Existing Application

The application edit function can be used to modify an existing custom application. Select a custom application, then click the [Edit] icon to bring up the *App details* page with the current configuration options for the application.

Changes can then be made to the configuration, such as editing the experiment details, adding or removing steps, etc. Refer to Section III for more information.

NOTE: The prevalidated applications installed as part of CELLSTUDIO software cannot be directly edited. Refer to Section II.I for a method to derive a custom, editable application from one of the prevalidated apps.

D. Delete a Custom Application from the App Manager

1. Select the desired app on the list view.
2. Click on [Delete] to delete the selected app.
3. Confirm the deletion on the confirmation dialog.

NOTE: The prevalidated applications installed as part of CELLSTUDIO software cannot be deleted.

E. Export an Application

Any application defined in CELLSTUDIO software can have its configuration exported to a JSON-formatted file. This is useful for sharing user-defined applications or for deriving an application from a preexisting one, such as one of the prevalidated applications.

To perform an export:

1. On the list view, select the desired application to export.
2. Click [Export] on the right menu bar to bring up the *Save file* dialog.
3. Navigate in the dialog to the location where the exported app file should be saved.
4. Provide a name for the exported file.
5. Click [Save] to complete the export.

F. Import an Application

An exported application can also be imported into the app manager. This is useful for sharing user-defined applications or for deriving an application from a preexisting one, such as one of the prevalidated applications.

To perform an import:

1. Click [Import] on the right menu bar to bring up a File Explorer selection window.
2. Select a previously exported application file (`*.json`).

Figure 5. Selecting an application JSON file for import into CELLSTUDIO software. For more information about exporting an application, see Section II.E, above.

3. Click [Open].

The app will display in the list of applications in the app manager upon a successful import (Figure 6).

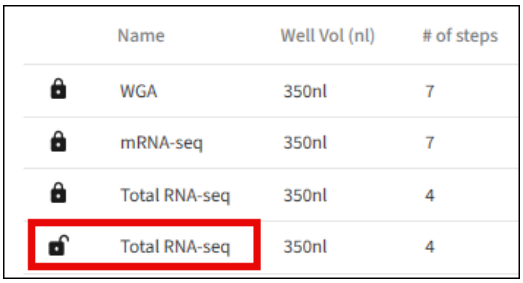

Figure 6. The *App manager* list after import of an application. A key indicator that this is a custom or imported application is the unlocked icon next to the application name.

NOTE: The application name will display (Figure 6) with the assigned name at the time the application was exported—NOT the file name (Figure 5). To modify the name displayed in the list, edit the newly imported app (Section II.C and change the "Name" field to be how you want the application to display in the list (Figure 7 & Figure 8).

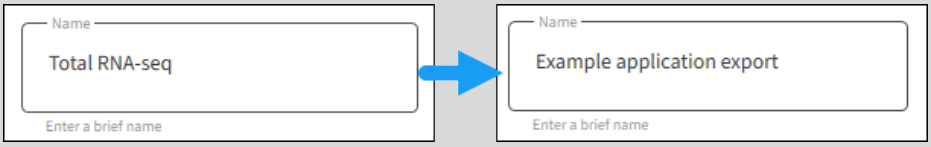

Figure 7. Editing the application "Name" field.

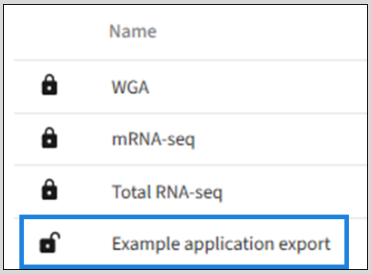

Figure 8. The *App manager list* after application "Name" editing.

G. Search the Applications

If you have a long list of applications and you want to find one or a group of applications with basic keyword matching, you can use the search input box to filter the list.

To begin a search, click on the box and begin typing. The search is case-insensitive and will do partial matching on any part of the application name. Figure 9 shows an example of searching the list for the phrase 'rna'; the two results, 'mRNA-seq' and 'Total RNA-seq', demonstrate the pattern matching does not require that the search phrase start at the beginning of the name, nor does it need to match the case (lower or upper case) of the phrase.

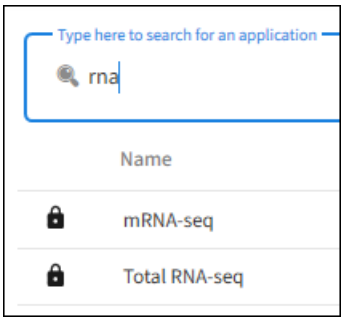

Figure 9. Example of the search bar results in the app manager.

H. Sort the Application List

Each column can be sorted in either ascending or descending order. To do so, click on the text of the column headers.

Figure 10. Pointing to the text of a column header in the *App manager* application list. Moving the mouse cursor over the text and clicking will cause the selected column to sort based on the given value.

The initial click will sort the column in ascending order (Figure 11). An arrow pointing up (highlighted by the blue box) shows that the order is ascending.

Figure 11. Application list sorting, ascending order. The arrow (outlined in blue) indicates ascending order.

A second click will toggle to descending order (Figure 12), with the arrow pointing down.

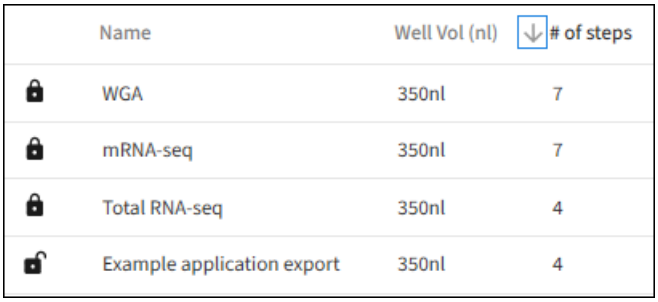

Figure 12. Application list sorting, descending order.

Re-sorting on a given column or a different column can be repeated as often desired.

NOTE: Sorting can also be applied to a filtered subset of applications returned by the search function (Section II.G)

I. Prevalidated Applications

The apps developed by Takara Bio are denoted by a lock icon next to the app name, highlighted by the blue box in Figure 13.

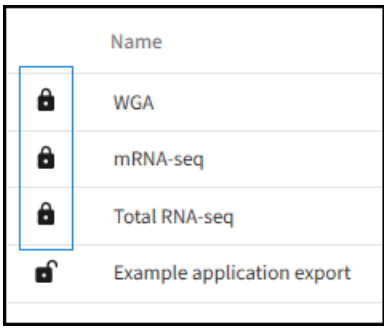

Figure 13. The lock icon next to the name of the Takara Bio prevalidated applications.

Unlike custom applications, denoted by an unlocked icon, prevalidated apps cannot be modified or deleted. However, prevalidated applications can be used as a starting point to derive a new custom app, by the following steps:

1. Duplicate the application (Section II.B).
2. Edit the newly imported application (Section II.C).

J. Exit App Manager

To exit the app manager, either click on the home icon or select another option from the hamburger menu.

III. App Manager: Creating or Editing a Custom Application

This section specifically covers creating an application within the CELLSTUDIO software app manager. Refer to Appendix A, "Designing an Application" for more guidance on designing an experiment that will use the application.

Basic application definition overview

- Fill out the experiment information fields (Section III.A.1)
- Add or edit a desired workflow step
  - Add a workflow step (Section III.C)
  - Configure the step options (Section III.B)
  - Edit an existing step (Section III.H)
  - Change the order of the steps (Section III.E)
  - Remove a step (Section III.D)
  - Undo changes (Section III.G)
- Save the application configuration (Section III.E)
- Exit from *App details* (Section III.I)

A. App Details view

Most of the available actions in this section take place in the *App details* view page. A brief description of the parts of the view can be found below.

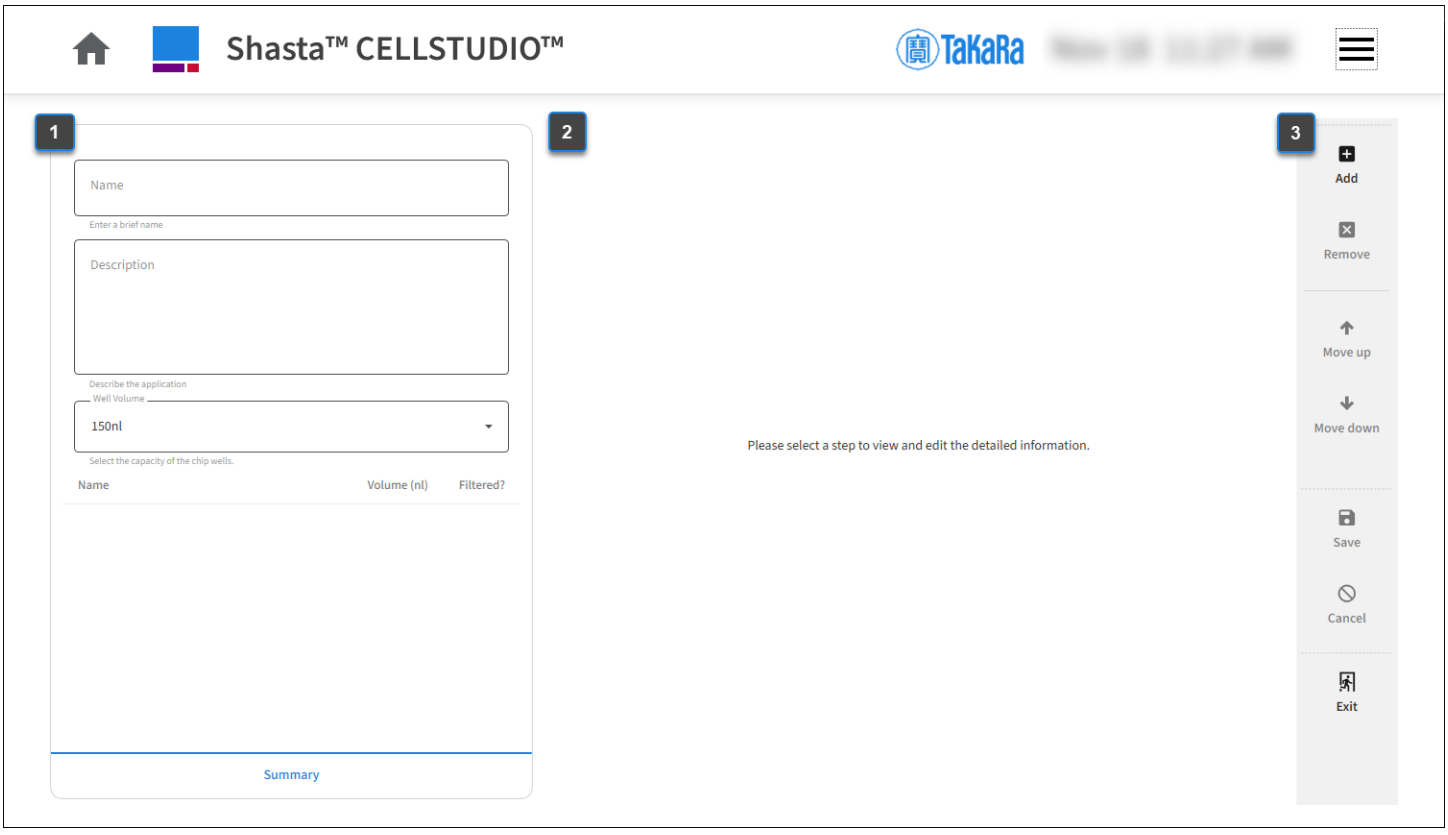

Figure 14. The full *App details* view.

1. Experiment information

The left side of the page contains the fields for the overall experiment configuration.

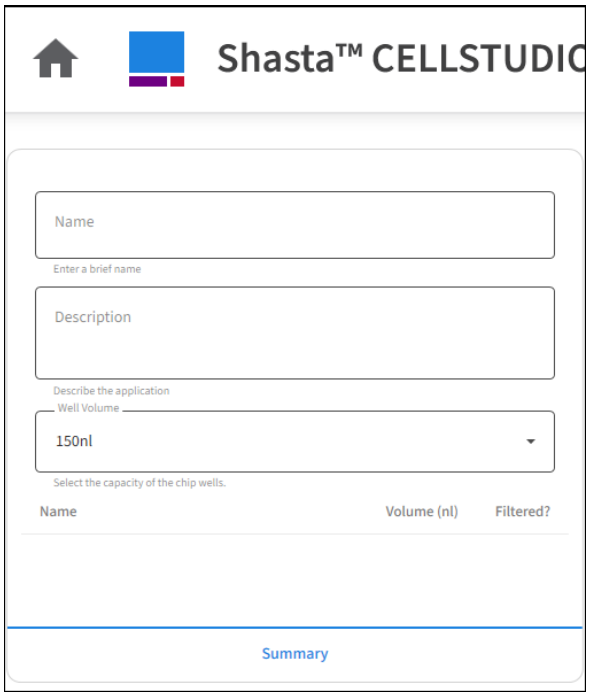

Figure 15. The experiment information section of the *App detail* page.

With the *Summary* tab selected at the bottom of the section, configure the experiment information on the left side of the page.

- Name—enter a succinct name to identify the new custom app you are developing.
- Description—(optional) if desired, you can enter more detailed information or a description of what the application is for into the text box.
- Well volume—from the drop-down menu, select the type of Single-Cell chip you plan to use for the application. Takara Bio sells compatible chips with two different nanowell volumes: 250 nl (Cat. No. 640183, also included in Cat. No. 640193) and 350 nl (recommended, Cat. No. 640019).

2. Step information

The right side of the page contains the fields for defining each dispense step of the experiment. More details about this section and the options can be found in Section III.B, "Details: Workflow Step".

3. Right-navigation menu bar

This menu contains icons for all the actions that can be enacted on the page. Each action is described in a section. The sections covering each action are listed below.

- [Add] —add a new workflow step to the application (Section III.C)
- [Remove] —delete an existing workflow step (Section III.D)
- [Move up] / [Move down] —move a selected workflow step up or down the order (Section III.E)

- [Save] —save edits made to the application configuration (Section III.F)
- [Cancel] —undo any edits made to the application configuration since the last save (Section III.G)
- [Exit] —exit the *App details* view (Section III.I)

B. Details: Workflow Step

NOTE: The following instructions are all required unless noted otherwise.

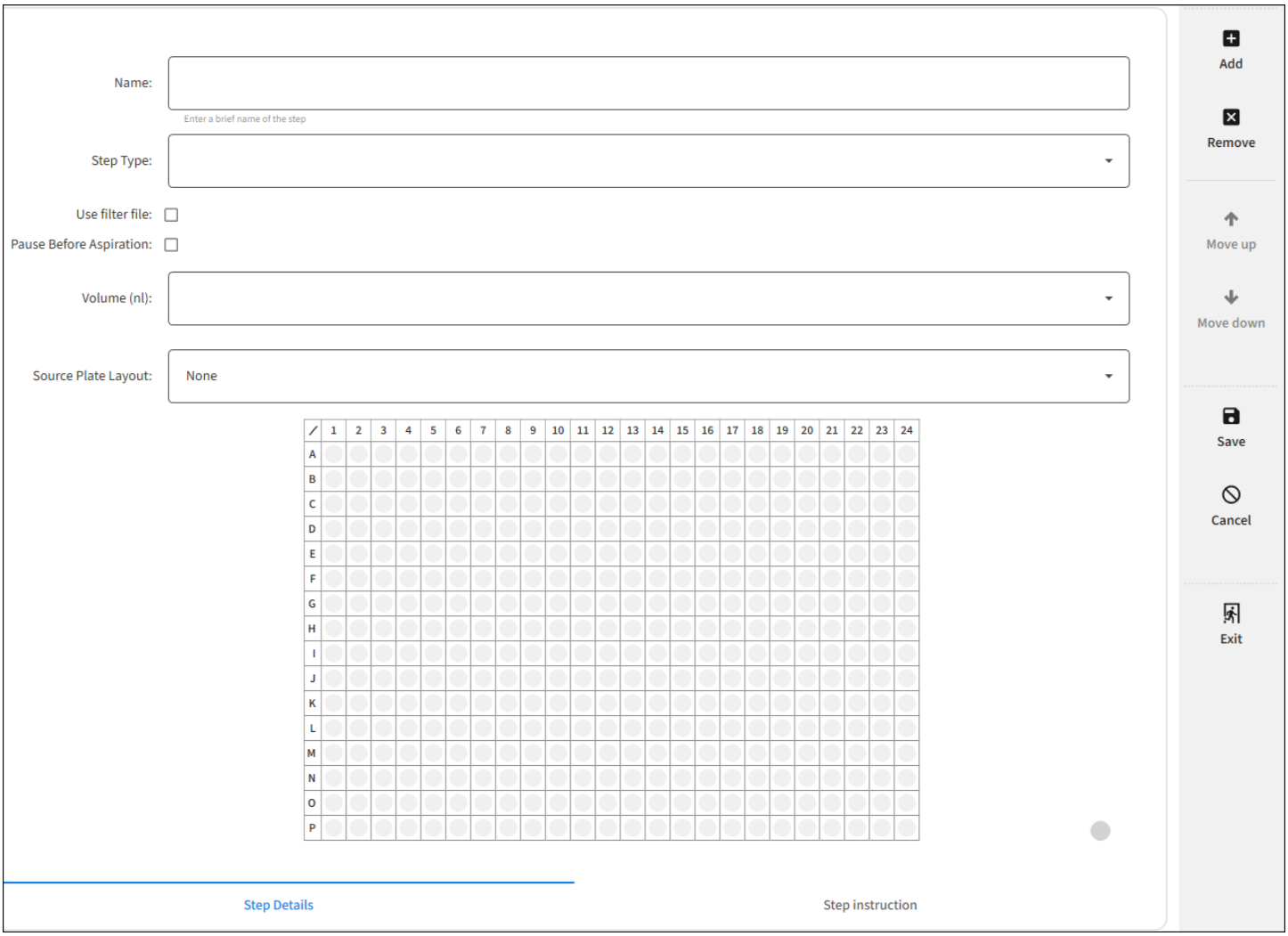

Figure 16. The workflow step information view of the *App details* page.

With "Step Details" selected at the bottom of the section, define the step information.

NOTE: As certain fields are filled in, the information will also be updated and display in the experiment details section of the page, in the space under the "Well volume" option.

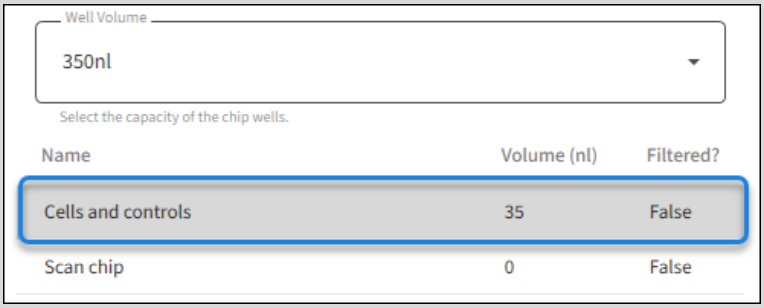

Figure 17. Experiment step overview in the experiment detail section of the *App details* page. The step name, dispense volume, and filter-file status are displayed. A "Filtered?" value of 'True' means the dispense will be restricted by a filter file; 'False' means the dispense has no restriction (i.e., dispense will be to all 5,184 wells of the chip).

1. Name

A brief name that describes the step, such as 'Cell' for a cell dispense step or a reagent name for a reagent dispense step, such as 'PCR mix'.

2. Step Type

The step type will be predefined by the choice made when the step is added (Section III.C), but it can also be configured from the drop-down list.

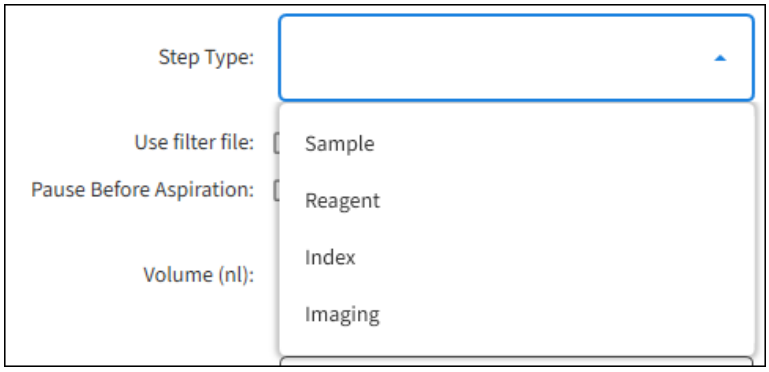

Figure 18. The step information section of the *App details* page.

- Sample—sample dispense
- Reagent—reagent dispense
- Index—index dispense
- Imaging—scanning the chip for candidates

3. Cell, Reagent, and Index Step Configuration Options

a) Use Filter File

(Optional) Check this box if the step should require use of a filter file for the dispense. For more information about filter files, see the Shasta Single Cell User Manual and the Shasta CellSelect® Software User Manual.

NOTE: It is unusual for sample dispenses to use a filter file, but there is no restriction on doing so.

b) Pause Before Aspiration

(Optional) Check this box if you want the system to pause during a dispense cycle and prompt the user to mix samples/reagents or refill the 384-Well Source Plate between aspirations.

c) Volume (nl)

Select the desired dispense volume from the drop-down list. Options range from 35–100 nl in 5 nl increments.

NOTE: Takara Bio has validated 35 nl, 50 nl, and 100 nl for general use. For any other volume choice, we recommend performing validation experiments. Refer to Appendix A, "Designing an Application", for more information about designing a non-standard application.

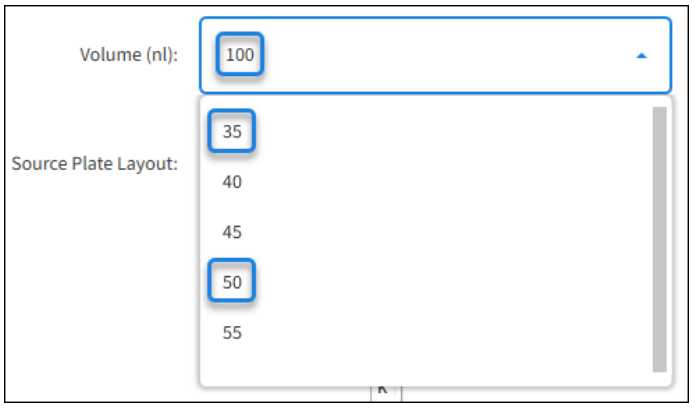

Figure 19. The "Volume (nl)" drop-down menu, expanded. The dispense volumes validated by Takara Bio (35, 50, and 100 nl) are highlighted by blue boxes.

d) Source Plate Layout

Select the desired 384-Well Source Plate layout pattern from the drop-down list. The drop-down feature will not be active until a "Step Type" is chosen (Section III.B.1, above); the selected step type will define the available options in the drop-down menu. For all selected layout types other than 'None', a preview of the plate layout will display under the option.

The layout options for each dispense type and an example of their correlated source plate maps are listed below.

- Sample dispense
  - Cells and controls—this layout will include eight wells that should be filled with your sample(s) and two wells (one each) for your positive and negative controls.
  - Cells—this layout will only include the eight wells designated for your experimental sample.
  - Cells and buffers—this layout is intended for situations where specific samples or reagents need to be dispensed into designated wells using a filter file, while the remaining wells are filled with buffer or other reagents.

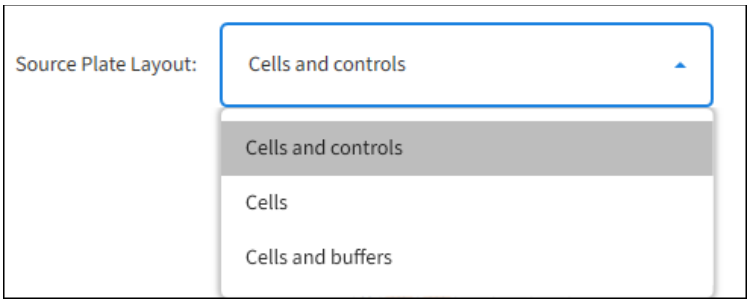

Figure 20. "Source Plate Layout" drop-down options for cell dispenses.

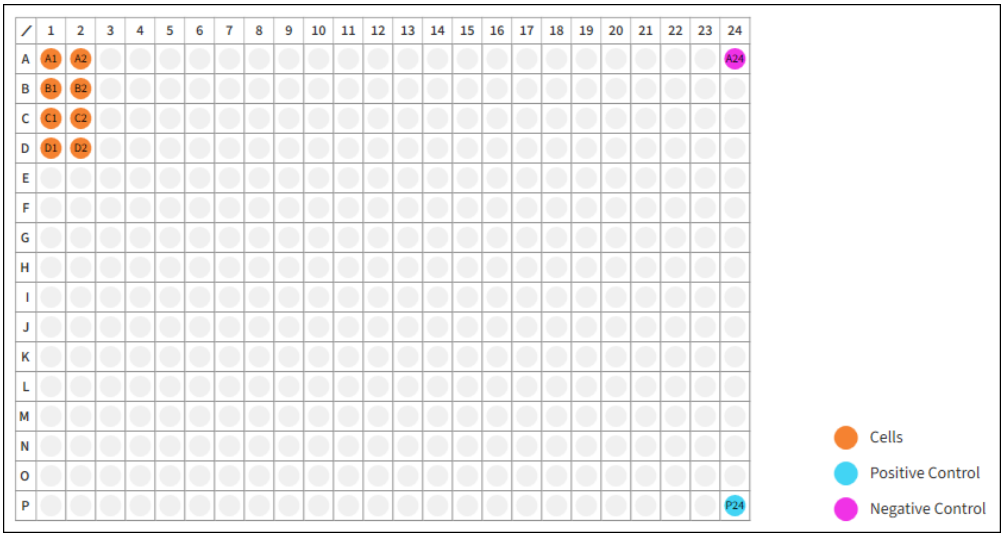

Figure 21. Source plate layout preview display. The 'Cells and controls' option of a sample dispense step is shown.

- Reagent dispense

  Reagent 1–6—Up to six different reagents can be configured to dispense; each selection from the drop-down menu represents a different source plate layout (Figure 23). Other than where the source wells for the reagents, there is no difference in these selections.

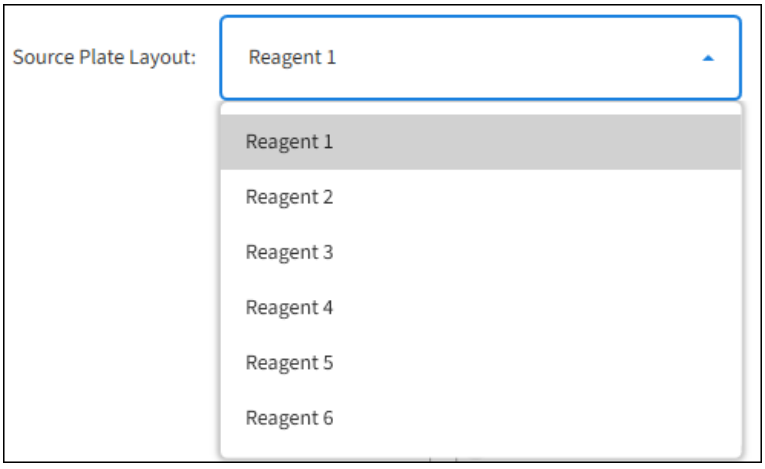

Figure 22. "Source Plate Layout" drop-down options for reagent dispenses.

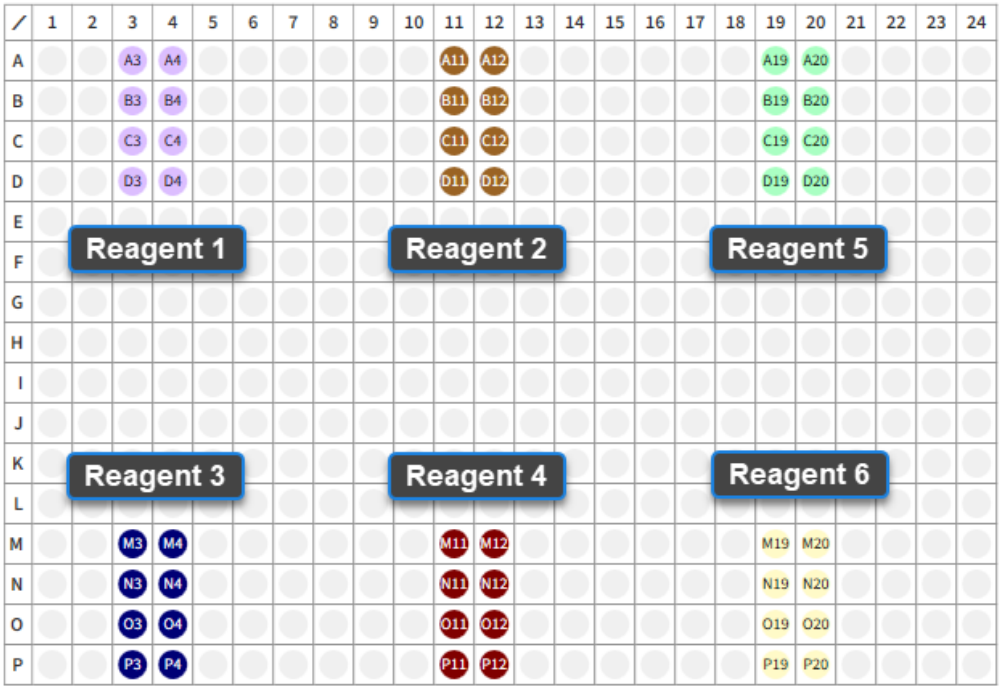

Figure 23. Source plate layout map, Reagents 1–6. This image is shown as a composite of the six layouts; when selecting one from the menu, only the associated eight wells will be highlighted.

- Index dispense

  Index1, Index2—Up to two index dispenses can be performed per application; the source plate layouts for each correlate to the forward and reverse indexes of the Shasta Long Indexing Primer Set - A (Cat. No. 640283) and the SMART-Seq® Pro Indexing Primer sets (Cat. No. 640258 & 640260) from Takara Bio.

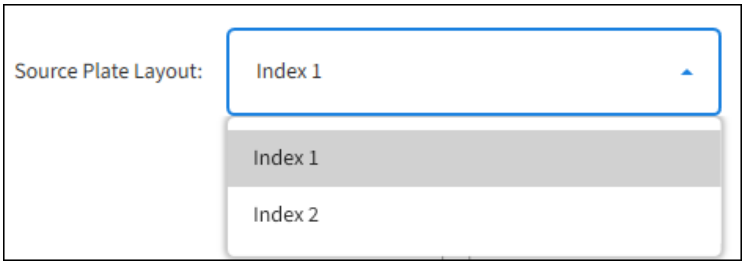

Figure 24. "Source Plate Layout" drop-down options for index dispenses.

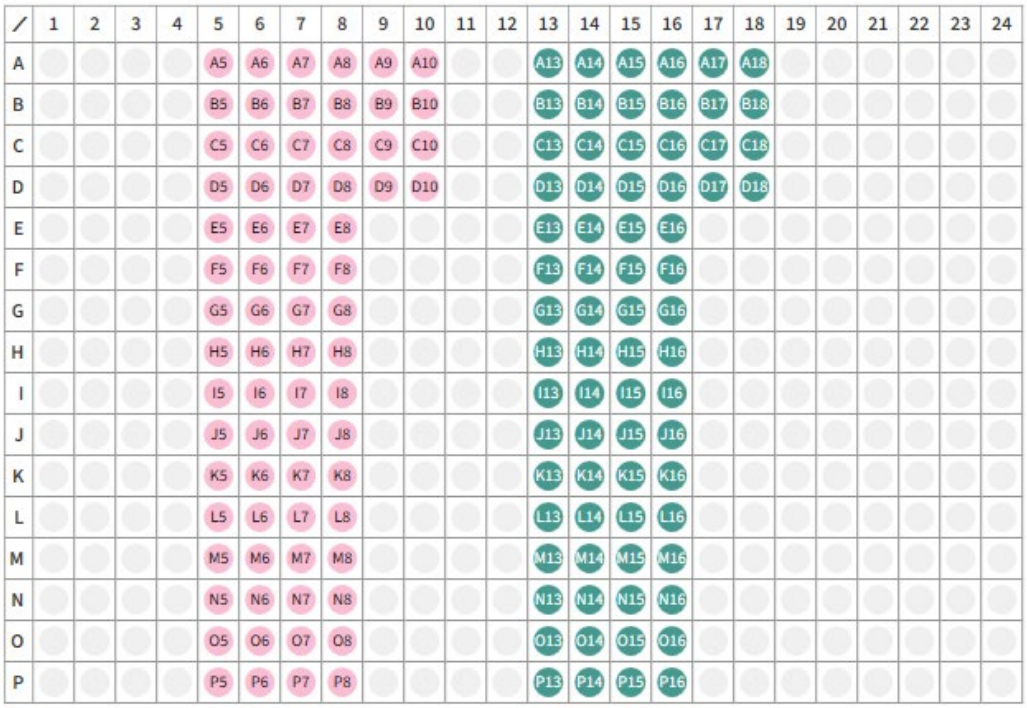

Figure 25. Source plate layout map, Index 1 & Index 2. This image is shown as a composite of the two layouts: pink (columns 5–10) indicates the source wells for Index1, teal (columns 13–18) indicates the source wells for Index2.

4. Imaging Step Configuration Option

If the step type selected is 'Imaging', the configuration options specific to cell, reagent, or index dispenses (Section III.B.3, above) are grayed out (i.e., cannot be modified). Instead, an [Add New Filter] section will display.

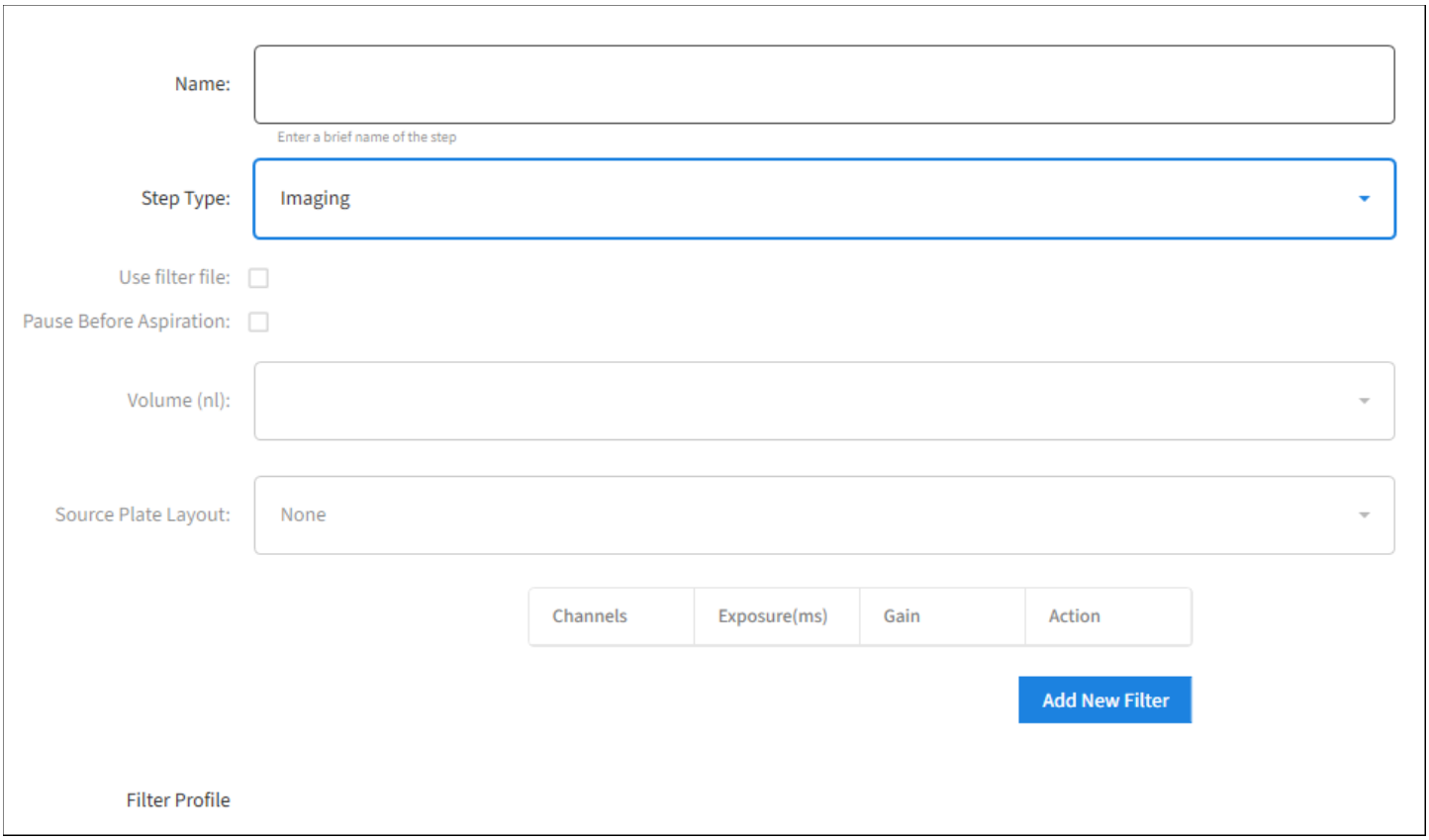

Figure 26. Add New Filter configuration option shown when the Imaging step type is selected.

This feature allows you to set up default parameters for your application relating to the three color channels available for imaging (red, blue, and green).

NOTE: Although this sets up the default imaging filter settings for your application, you can also modify the settings on an experiment-by-experiment basis in the chip scan step when running the custom workflow. Refer to the Shasta Single Cell System User Manual, Appendix B, for more details.

a) How to Add an Imaging Filter to the Imaging Step

1. Click [Add New Filter] to begin. This will set up an initial row in the table.

Figure 27. The configurable options of an imaging filter.

2. Configure the parameters of the color channel you wish to scan for. There are three values that can be set and one action that can be taken in this view:

   - Channels—selected by a drop-down menu, this is the color for which you want to scan.

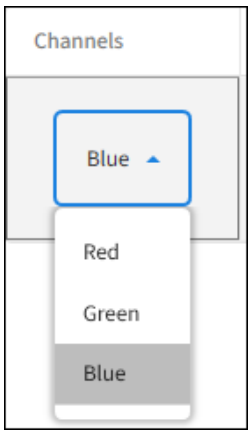

Figure 28. Drop-down menu options for the imaging filter "Channels" column.

Table 2. Excitation and emission information for the available color channels*.

Color channel	Excitation λ (nm)		Emission λ (nm)	
	Center	Width	Center	Width
Blue	377	50	447	60
Green	485	20	536	40
Red	544	24	641	75

*The values are the default hardware configuration; the hardware can be modified if desired. Contact your authorized Takara Bio service technician or field_support@takarabio.com for more information.

   - Exposure(ms)— the length of time in milliseconds the camera sensor spends capturing the image for each well. The exposure time for a given color channel should be in the range of 2–500 ms, although values up to 2,000 ms are possible.

     To set or edit the value, click on the cell to activate the input box (Figure 29, left) and type in the numerical value desired. Press **[Enter]** on your keyboard to commit the value to the configuration (Figure 29, right).

Figure 29. The imaging filter "Exposure(ms)" input box. (Left) The input box is shown (white background) after the cell in the column is clicked on, and a number can be typed into the field. **(Right)** The cell after committing the change.

- Gain—represents the amount of amplification applied to the signal coming from the camera sensor, i.e., the camera sensitivity.

  Select a value from the drop-down menu of either '5', '10', or '15'. We recommend that you start with a gain of 10 when setting up a new filter.

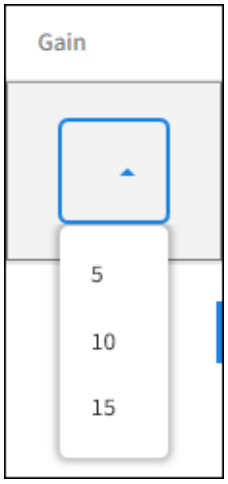

Figure 30. The imaging filter "Gain" drop-down menu.

- Action—clicking the ⊖ icon in this column will delete the row and its configuration information.

  IMPORTANT: There is no confirmation prompt and no method to undo the deletion. Please use it wisely.

3. Repeat Step 1 and Step 2 for any additional color channels you want to add to the imaging filter, up to the maximum of three.

Figure 31 shows an example of a two-color imaging filter set up for the red and blue channels.

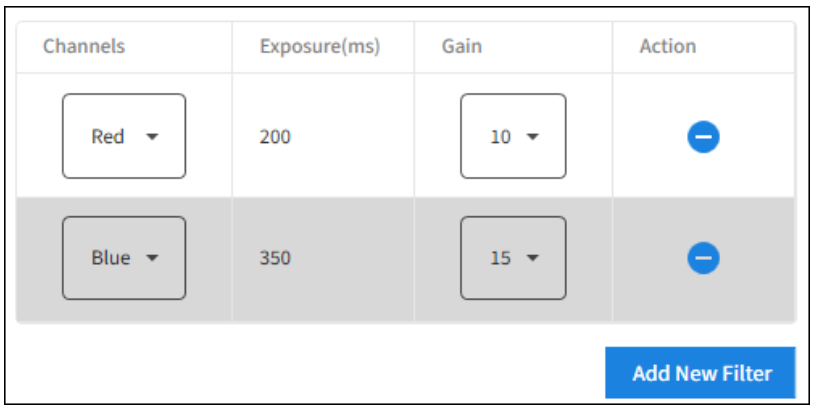

Figure 31. Example imaging filter configuration display.

b) Recommendations for the Imaging Filter Settings

When setting these values, keep the following in mind:

- Be sure that the "ID" selections reflect the candidate logic you wish to use in CellSelect software for experiments using your application. At least one dye color is required to identify the presence of cells in the nanowell; a second or third color may be used for further refinement to determine well candidacy. Refer to the Shasta CellSelect Software User Manual, Section II.C "Candidate Logic Selection" for more information.
- In general, you want to adjust the "Exposure" time and "Gain" so that all cells are clearly visible above the background, i.e., a high signal-to-noise ratio—but be careful to not overexpose the image.
  - Higher gain values produce brighter, but also noisier, images.
  - If the exposure time is too high, brighter pixels on the camera may become more visible. Also, longer exposure times also mean longer scan times.

For more assistance in determining the ideal settings for your novel stains or cell types, use the Microscope Utility. For more information about this feature, refer to Section VI.

5. Offline Instructions PDF File

(Optional) Associate an offline instructions file to display in the software after the step is completed. For more information about creating a custom instruction file, see Appendix B.

NOTE: For an example of how this is implemented in the workflow, refer to the Shasta Single Cell System User Manual, Section IX.E, "Start Dispense" in the note associated with Figure 59.

At the bottom of the page, select the *Step instruction* tab.

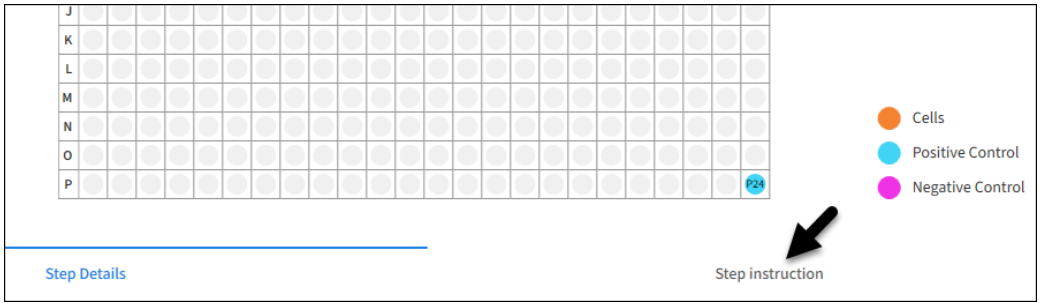

Figure 32. The *Step instruction* tab in the step details section of the *App details* page.

This will bring up the *Step instruction* page.

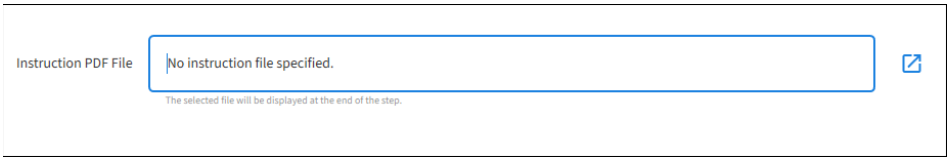

Figure 33. The *Step instruction* page.

a) Adding a New Instruction File or Replacing an Existing Instruction

1. Click on the [Select a PDF file] icon to the left of the box to bring up a File Explorer file selection window. By default, it will open in the "Documents" folder.
2. Navigate to the folder containing the instruction PDF file you want to associate with the step.

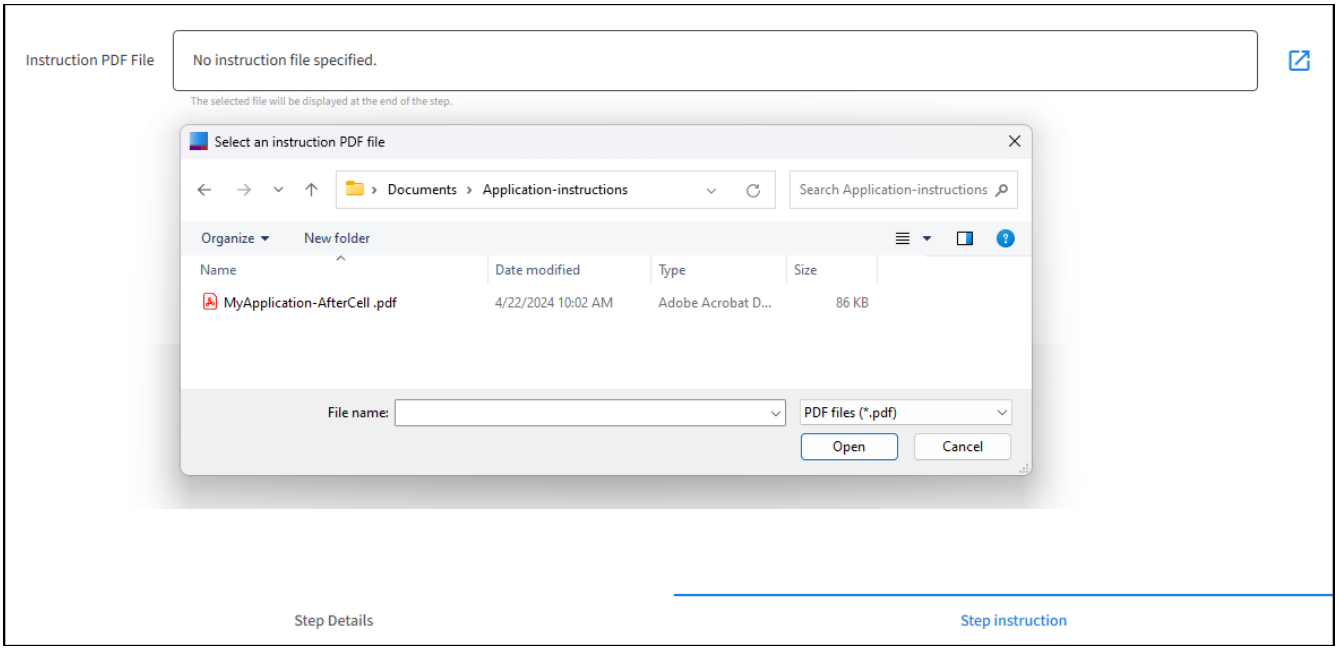

Figure 34. Selecting an instruction PDF file.

3. Select the PDF and click [Open]. The path where the instruction file is located will display in the "Instruction PDF file" input box, and the contents of the file will preview on the page.

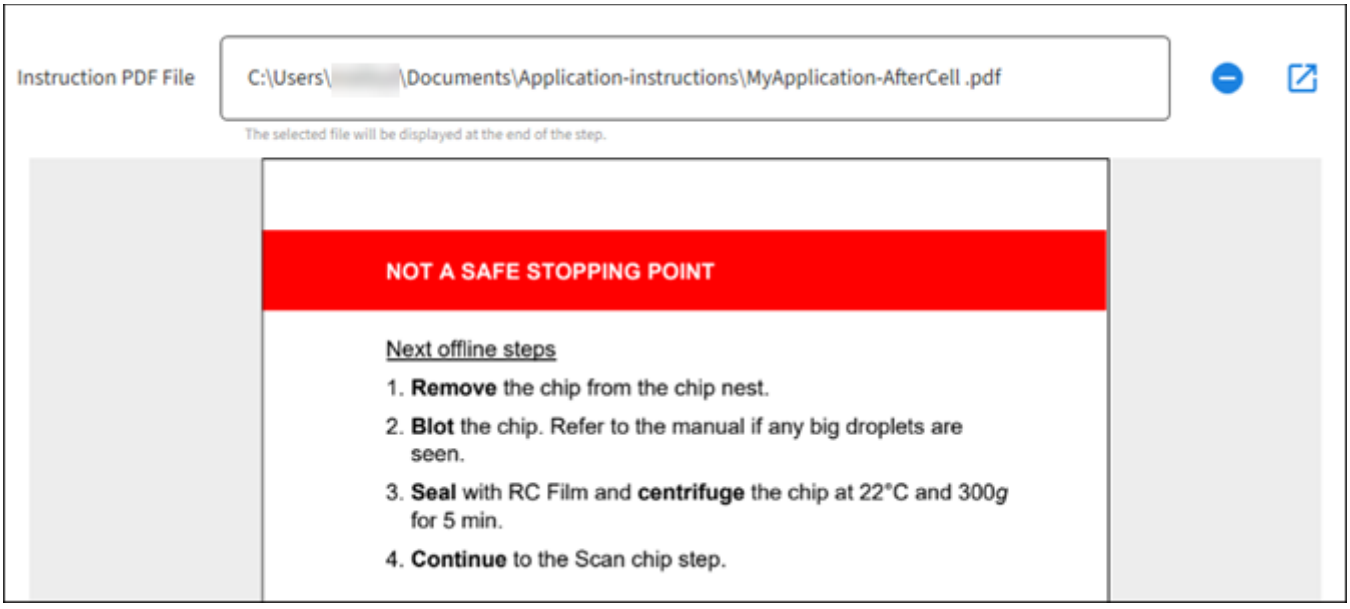

Figure 35. The *Step instruction* page after selecting a PDF.

b) Replace a Previously Defined Offline Instructions PDF

To replace an existing file, repeat Section III.B.5.a).

c) Removing the Association with an Offline Instruction File

To remove an instruction PDF file without replacing it, click on the [Remove] icon to the right of the file name.

Once the changes have been made, it is safe to navigate away from this page without further action.

C. Action: Add (a Workflow Step)

1. On the right-navigation menu bar, click [Add] to define a new workflow step.
2. A pop-up box will display, prompting you to choose the step-type to add (Figure 36). Click on the desired option from the list.

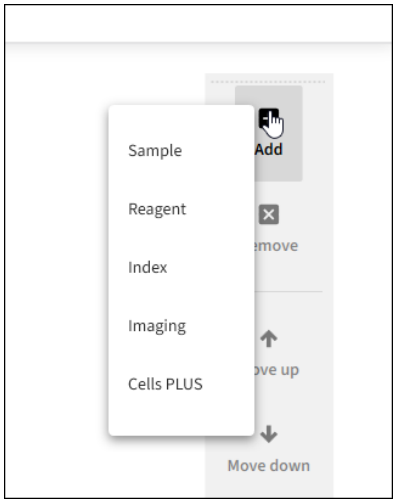

Figure 36. Selecting a step from the experiment detail section of the *App details* page.

3. The right-side section of the *App details* page (step information) will activate, as shown in Figure 16 (Section III.B, above).

Refer to Section III.B, "Details: Workflow Step" for more information about the option types.

NOTE: If the option selected is 'Sample', the add function will also automatically create an 'Imaging' step named "Scan chip", shown in Figure 37, below. It should be configured like an existing step (Section III.H) or deleted (Section III.D) if an imaging step is not required for the workflow.

It will not be automatically deleted if the related sample step is deleted; it should be deleted manually.

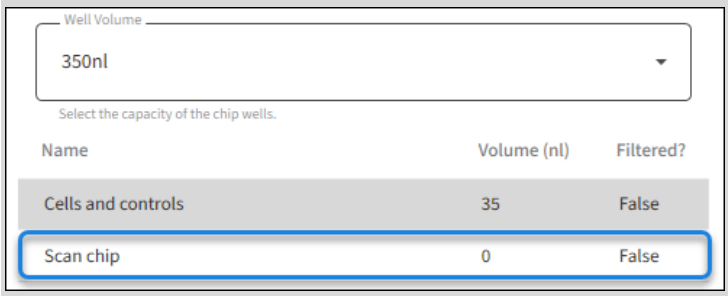

Figure 37. The automatically added "Scan chip" imaging step. This is added at the same time a 'Sample' workflow step is added to an application and should be configured if imaging is desired for the workflow.

D. Action: Remove (a Workflow Step)

1. On the list of steps in the experiment detail section, select the desired step that you want to remove. The selected step will have a gray background.

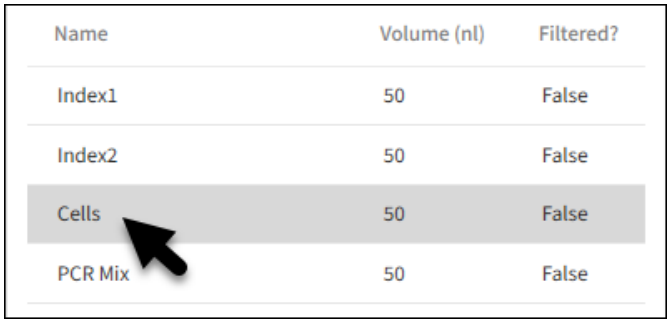

Figure 38. Selecting a step from the experiment detail section of the *App details* page.

2. From the right-navigation menu, click [Remove] to delete the step.

NOTE: There is no confirmation prompt for this deletion to take effect.

E. Action: Re-order the Steps in the Workflow

By default, workflow steps are listed in the experimental detail section in the order in which they are added.

The [Move up] and [Move down] arrows can be used to re-order the steps within the workflow.

1. Select the step from the workflow list that you would like to move (similar to Figure 38, above).
2. Click the [Move up] ↑ button to shift the step to be earlier in the workflow.

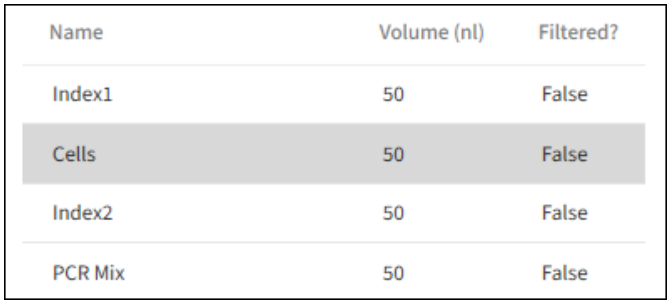

Figure 39. Moving a workflow step up in the list on the *App details* page. The image shows the step selected in Figure 38 moved up one step from the third to the second step in the workflow.

3. Click the [Move down] ↓ button to shift a step to occur later in the workflow.

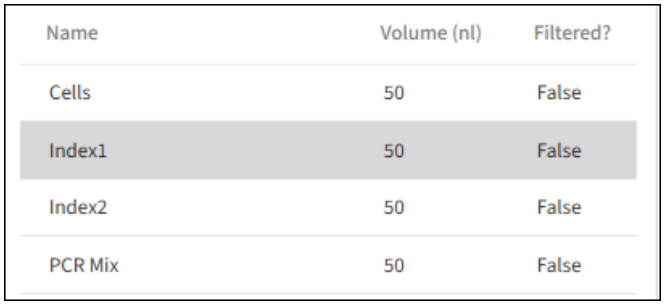

Figure 40. Moving a workflow step down in the list on the *App details* page. The first step in Figure 39 was moved down one step to become the second step. This has the effect of moving the second step up to be the first step.

F. Action: Save (Changes)

After any field is populated with a value in the experiment detail section of the *App details* page, the [Save] icon on the right-side menu can be clicked to save the configuration to-date.

It is recommended, but not necessary, for the experiment "Name" field to be filled in, to more easily identify it in the list of applications in the app manager.

G. Action: Cancel (Undo Changes)

The [Cancel] button can be used to undo all edits made to an application's configuration in the *App details* view since the last time it was saved (Section III.E).

NOTE: There is no confirmation prompt for this rollback to take effect.

H. Action: Edit an Existing Workflow Step

On the list of workflow steps in the experiment detail section, select the desired step that you want to change. The selected step will have a gray background (Figure 38).

The configured options for the step can then be modified. Refer to Section III.B ("Details: Workflow Step") above for the information that can be changed.

I. Action: Exit (*App Details*)

At any point, you can click the [Exit] icon to leave the *App details* view and return to the *App manager* page.

If any changes have been made to the application configuration while in App details, you will be reminded to save before exiting. Click [No] to return to the App details view to save (Section III.E, above) or [Yes] to exit without saving the changes (Figure 41, next page).

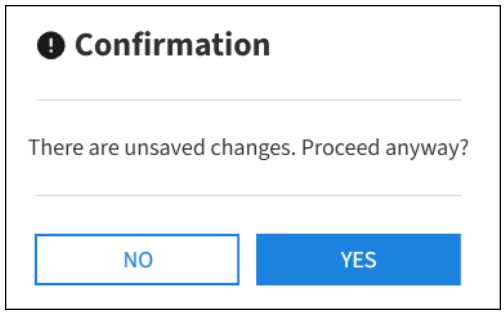

Figure 41. Confirmation dialog when attempting to exit the *App details* page. The prompt will only pop up if edits were made to the application configuration and were not saved.

IV. Barcode Manager

The barcode manager functionality can be accessed via drop-down from the Options menu (hamburger ☰ icon) (Figure 42) in the top right corner of CELLSTUDIO software.

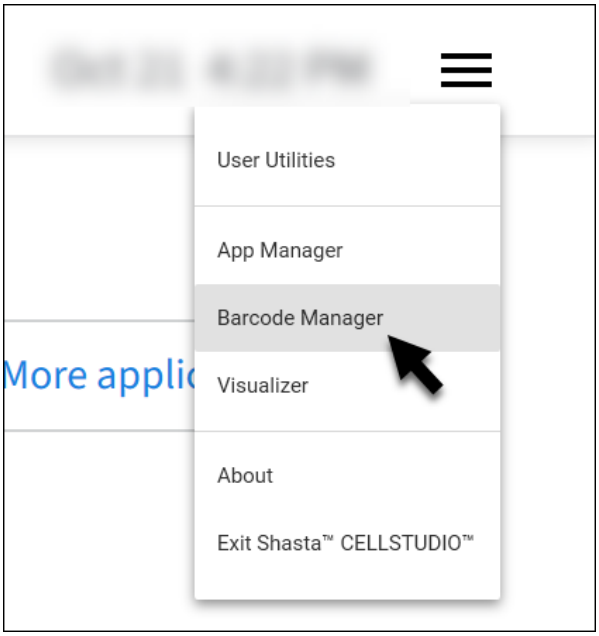

Figure 42. How to bring up the barcode manager.

This will bring up the *Barcode manager* view. The sections below describe the functions available in the barcode manager and correspond to the letters labeling areas of the *Barcode manager* view in Figure 43.

Figure 43. *Barcode manager* elements. For more information about the labeled options, see Sections A–G below.

A. Installed Barcodes

The list in the box on the left of the screen displays the list of the barcodes currently installed on the system.

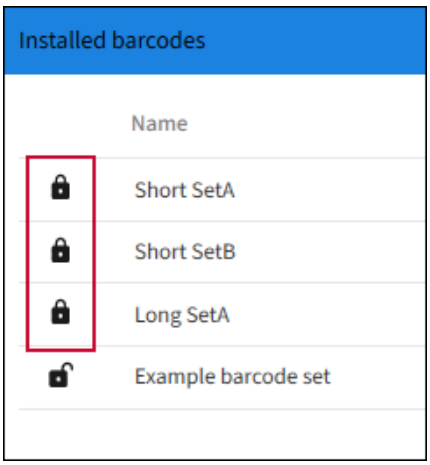

Figure 44. Example list of installed barcodes. The barcodes for index sets sold by Takara Bio have a lock icon next to their name (enclosed in the red box) and come preinstalled with the software.

CELLSTUDIO software comes preinstalled with the barcode information for index sets sold by Takara Bio for use with the Shasta system (Table 3); these sets are denoted by the lock icon next to their name (highlighted by the red box in Figure 44). Custom barcode files will be listed with the name given to the file set during barcode definition (Section IV.C) or if renamed (Section IV.D); they are indicated as custom by the unlocked icon next to the name.

Table 3. Preinstalled barcode file association with Takara Bio index sets.

CELLSTUDIO barcode name	Product name	Cat. No.
Short SetA	SMART-Seq Pro Indexing Primer Set - A	640258
Short SetB	SMART-Seq Pro Indexing Primer Set - B	640260
Long SetA	Shasta Long Indexing Primer Set - A	640283

B. Barcode Details

Selecting one of the installed barcodes (Figure 44) will display information about the index set in the *Selected Barcode Details* view on the page (Figure 45).

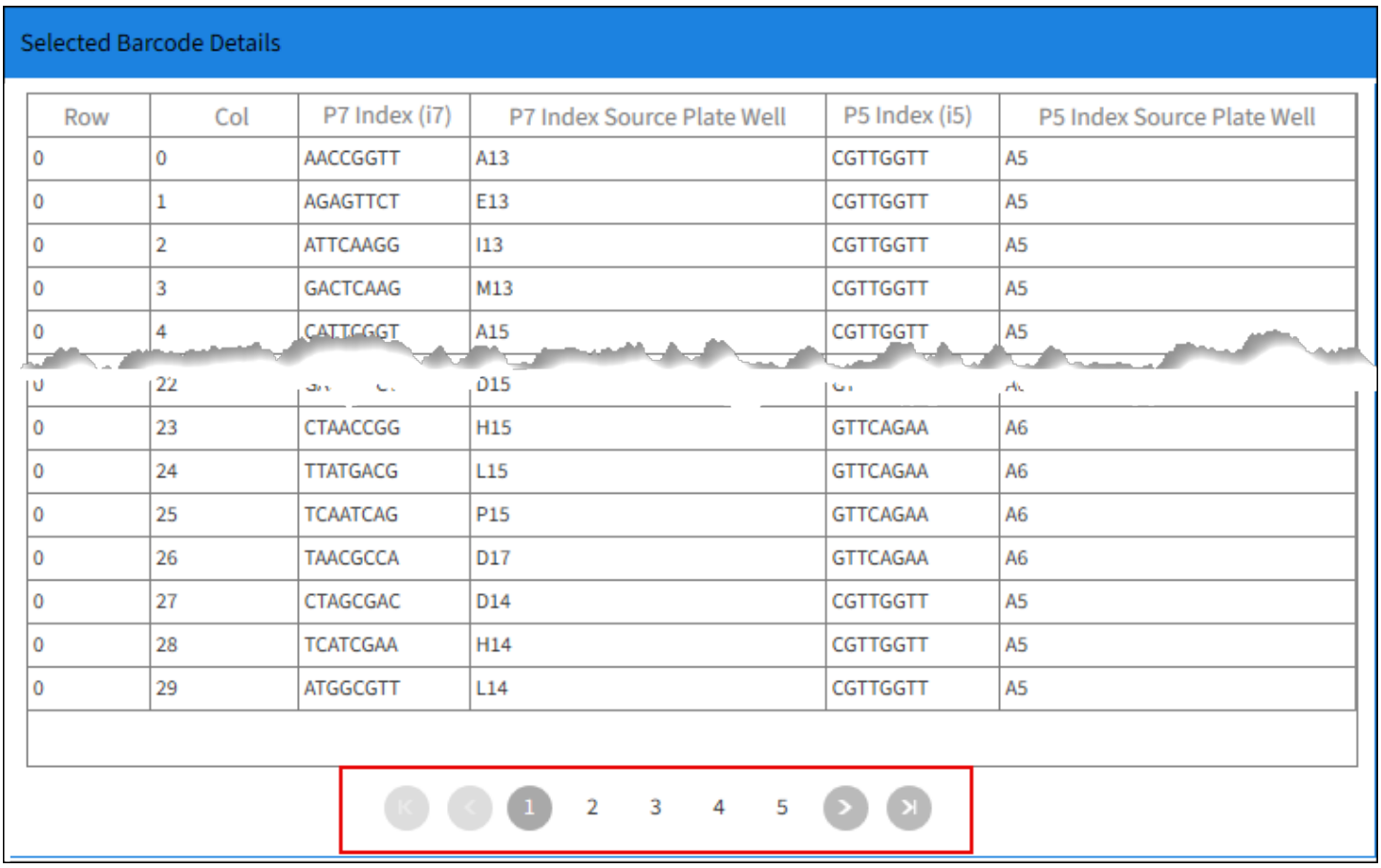

Figure 45. Example *Selected Barcode Details* view of the Short SetA option. The display is shown truncated in the screenshot in order to include both the headers and navigation button footer, outlined by the red box.

By default, the table will span 5,184 rows across 173 pages—one row for every well on the Single-Cell chip. Page navigation can be performed in one of three ways, using the buttons in the footer of the view:

- Click the single forward or backward arrow to go one page forward or one page back, respectively.
- Click a number from the bar to jump to that numbered page (i.e., Page 5).
- Click the "go to the end" button to jump to the last page of the table (page 173). Similarly, click the "go to the beginning" button to jump to the first page from anywhere in the table.

The column information for each row (well) is described in Table 4.

Table 4. Column definitions for the *Selected Barcode Details* table.

Column header	Definition
Row	The row location of the well on the Single-Cell chip. Rows are numbered 0–71 (72 rows).
Col	The column location of the well on the chip. Columns are also numbered 0–71 (72 columns).
P7 Index (i7)	The set of bases that represent the i7 barcode
P7 Index Source Plate Well	The well location on the 384-well plate where the i7 barcode is drawn for the dispense.
P5 Index (i5)	The set of bases that represent the i5 barcode
P5 Index Source Plate Well	The well location on the 384-well plate where the i5 barcode is drawn from for the dispense.

C. Define a New Barcode Set

To create a new set of barcodes, select the [New] icon, which will take you to the *Barcode creation* view.

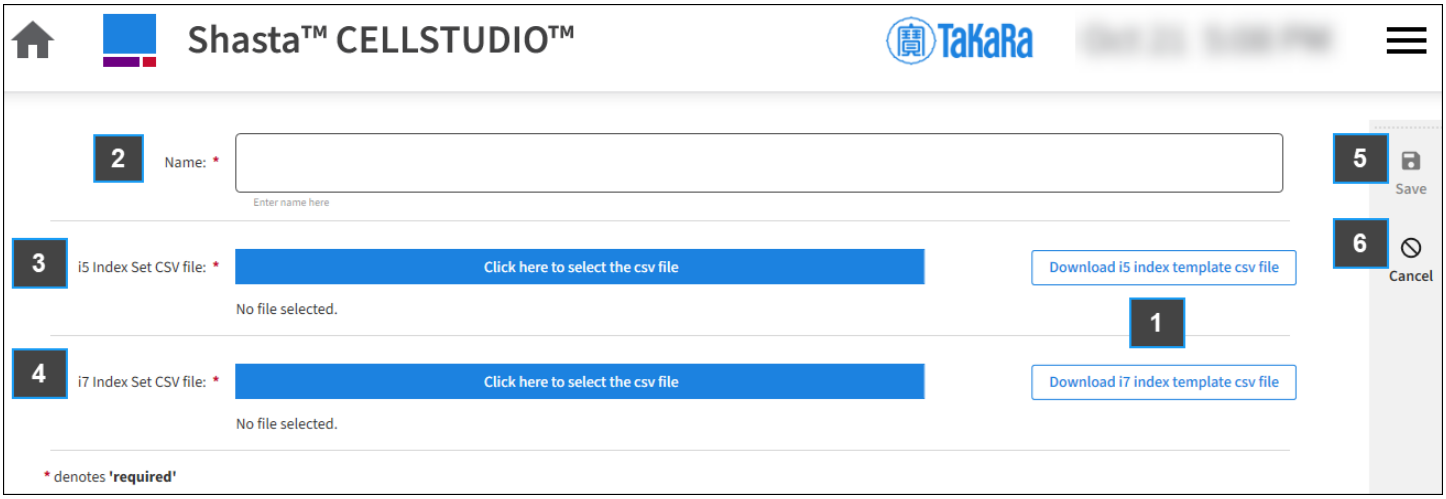

Figure 46. *Barcode creation* view.

The parts of the interface identified by the numbered callouts—and the list of steps to define the new barcode option—are listed below:

1. Download the i5 and i7 index template files from the buttons on the right. It is recommended to use the template file as the basis for populating your index CSV files to ensure the correct format is followed.

   Once both CSV files are set up with your indexes of choice, save them somewhere where they can be located by CELLSTUDIO software (locally or on a network drive).
2. Name—give a unique name for the new barcode you are creating. Special characters are allowed.
3. i5 Index Set CSV file—select the CSV file you created for the i5 barcodes in Step 1 by clicking the [Click here to select the csv file] button.
4. i7 Index Set CSV file—select the CSV file you created for i7 barcodes in Step 1 by clicking the [Click here to select the csv file] button.

5. Save —once Steps 2–4 are completed, the save icon will darken to indicate it is active to select. Click it to save the new barcode options.
6. Cancel —at any time, click the cancel button to quit out of the view and return to *Barcode manager* view.

D. Rename a Barcode Set

NOTE: Predefined barcode sets cannot be renamed.

Custom barcode sets can be renamed directly from the *Barcode manager* view.

1. From the list of installed barcodes (Figure 44), select the barcode set you want to rename.
2. Click the [Rename] icon. This will bring up the *Renaming* dialog.

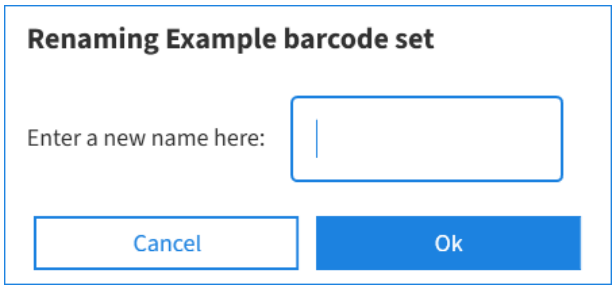

Figure 47. *Renaming* pop-up dialog.

3. Enter the new name in the box.
4. Click [OK] to apply the new name to the set or [Cancel] to exit the dialog with no changes.

E. Delete a Custom Barcode Set

NOTE: Predefined barcode sets cannot be deleted.

To delete a custom barcode set:

1. From the list of installed barcodes (Figure 44), select the custom barcode set you want to delete.
2. Click the [Delete] icon. A confirmation window will pop up.

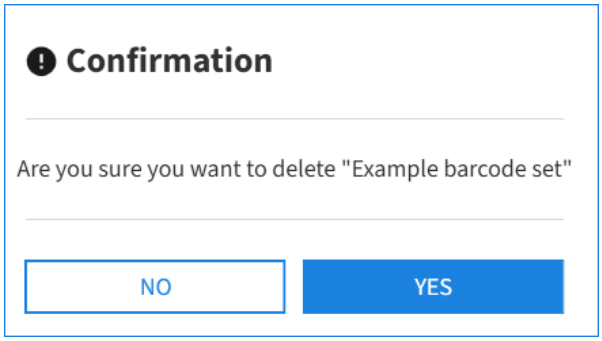

Figure 48. *Confirmation* pop-up dialog to delete a custom barcode set.

3. Click [Yes] to delete the set or [No] to exit the dialog with no change.

F. Export Barcode Set

Any barcode set in CELLSTUDIO software can be exported as an XML file, which can be useful for reanalyzing a scan output with CellSelect software specifying a custom barcode set.

1. From the list of installed barcodes (Figure 44), select the custom barcode set you want to delete.
2. Click the [Export] icon. A *Save As* dialog window will pop up.

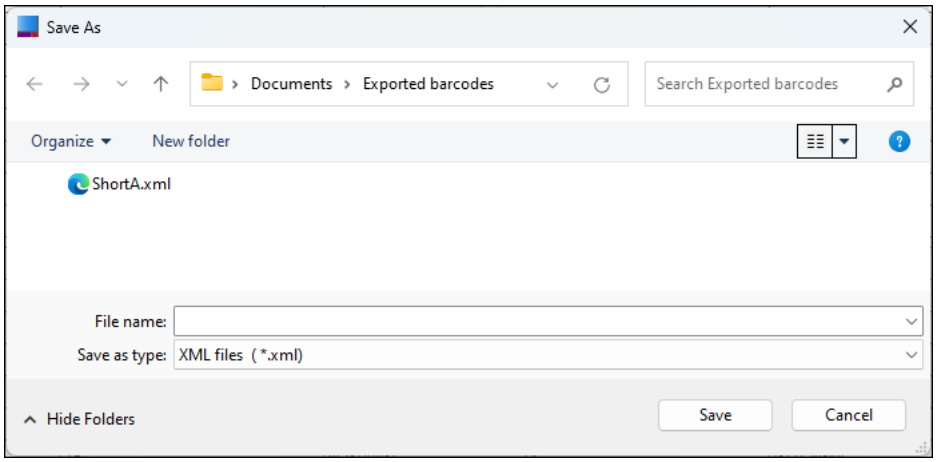

Figure 49. *Save As* window pop-up when exporting a barcode set.

3. Navigate within the window to the directory where you want to save the file.
4. Type the name with which to save the file as in the "File name" field.
5. Click [Save] to complete the export or [Cancel] to quit with no change.

G. Barcode Search

If you have a long list of barcode sets and you want to find one or a group of sets using name pattern matching, you can use the search input box to filter on the list.

To begin a search, click on the box and begin typing. The search is case-insensitive and will do partial matching on any part of the application name. Figure 50 shows an example of searching the list for the phrase 'ORT; the two results, 'Short SetA' and 'Short SetB', demonstrate the pattern matching does not require that the search phrase start at the beginning of the name, nor does it need to match the case (lower or upper case) of the phrase.

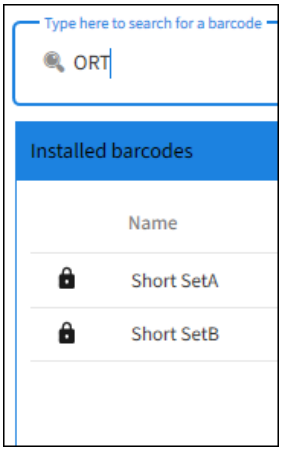

Figure 50. Example of the search bar results in the barcode manager.

H. Exit Barcode Manager

To exit the *Barcode manager*, either click on the home icon or select another option from the hamburger menu.

V. Visualizer

The visualizer tool can be accessed through the Options menu (hamburger icon) by selecting **Visualizer**.

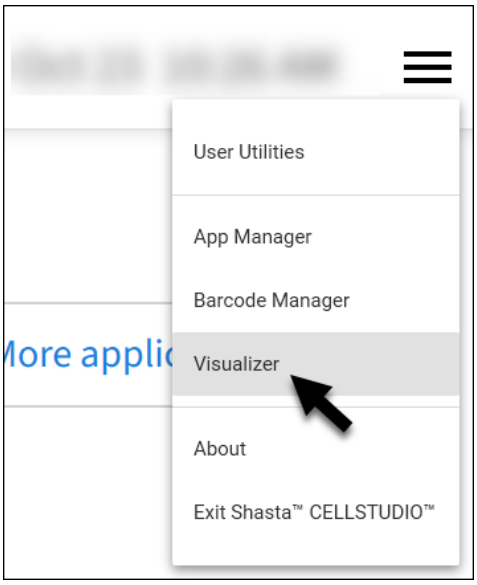

Figure 51. How to bring up the visualizer tool.

This will bring up the *Visualizer* view (Figure 52). The sections below describe the functions available in the tool.

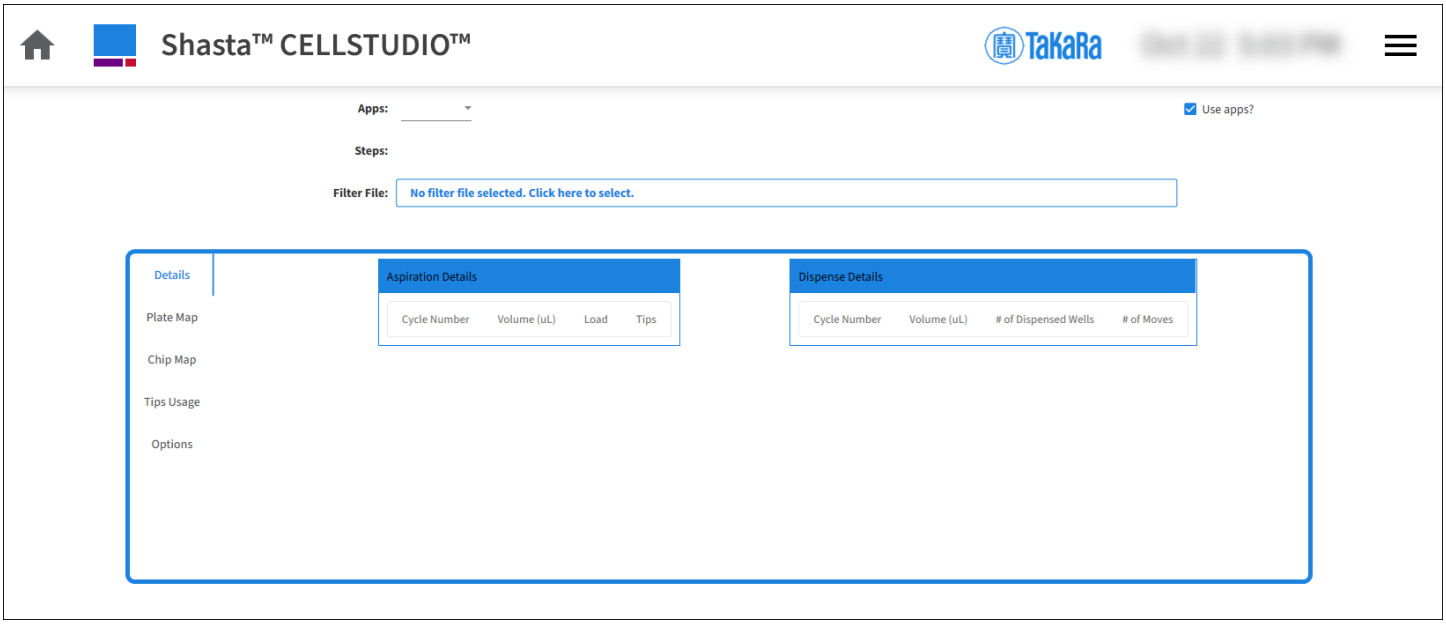

Figure 52. The *Visualizer* page.

How to Use the Visualizer

1. Select the app you are interested in visualizing.
2. Select a step of the selected app to visualize.
3. Use the available tabs on the left panel to visualize information about the aspirations and dispenses.
   a. Use the options and filters on the right panel on the Chip Map tab to refine what data is displayed. You can select specific tips, aspiration numbers, and drop indexes.
   b. Query Specific Wells: Enter the row and column of a specific well to get detailed information about it in the "Query Well details" section.

A. Dispense Type Selection

The fields at the top of the visualizer (everything above the box) allow you to select the dispense type you want to obtain more in-depth information for. The parameters available display based on whether the "Use apps?" box is checked or unchecked. Sections 1 and 2 cover these two options.

1. With "Use Apps?" Checked

When the "Use Apps?" button is selected, the top bar offers view selections based on the applications defined within CELLSTUDIO software, both predefined and custom workflows.

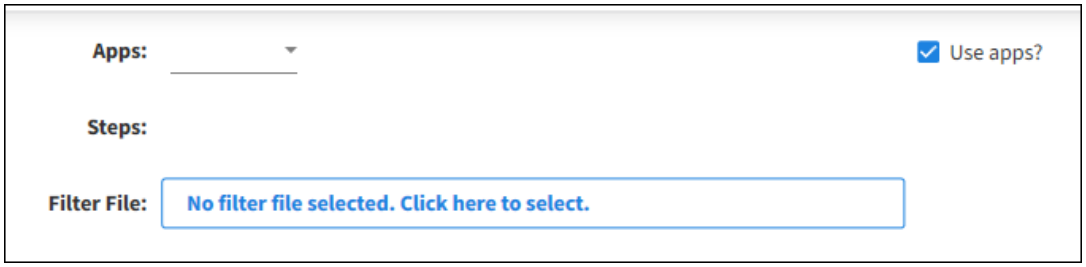

Figure 53. The top bar of the *Visualizer* page, using apps.

- Apps—select a defined application from the dropdown menu.

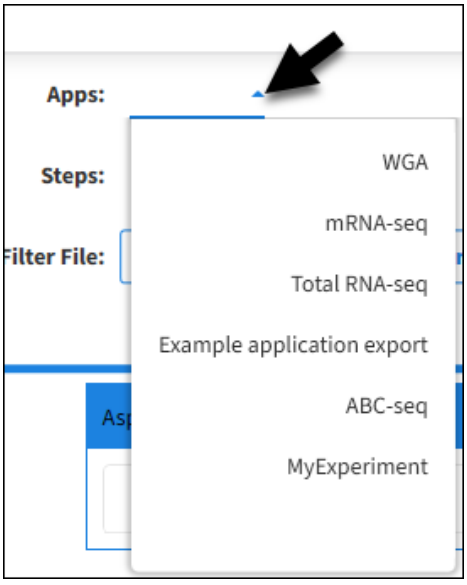

Figure 54. Example "Apps" dropdown menu on the *Visualizer* page.

- Steps—once an app is selected, an individual step of the workflow can be selected. This allows you to view detailed information on the mechanics of the dispense.

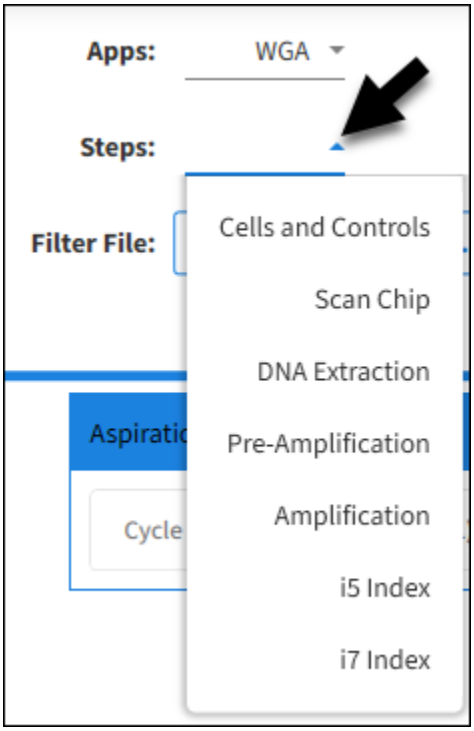

Figure 55. Example "Steps" dropdown menu on the *Visualizer* page. The workflow steps of the prevalidated Shasta WGA application is shown here.

- Filter file—(optional) if the step selected is for a reagent or index, this third option will be available. Selecting a filter file (CSV), such as one used for a dispense, will restrict the information details by the effects of the filter file. For sample dispense steps or leaving this blank, the information displayed will assume dispense to all wells on the Single-Cell chip.

2. With "Use Apps?" Unchecked

NOTE: This option is only recommended if you want to explore different dispense volumes prior to building a new application (i.e., design mode). You must configure the parameters under the *Options* tab (Section V.B.5) to make use of the unchecked "Use Apps?" option.

When the "Use Apps?" button is not selected, the main option of the top bar is based on the source plate layouts available when defining a custom application.

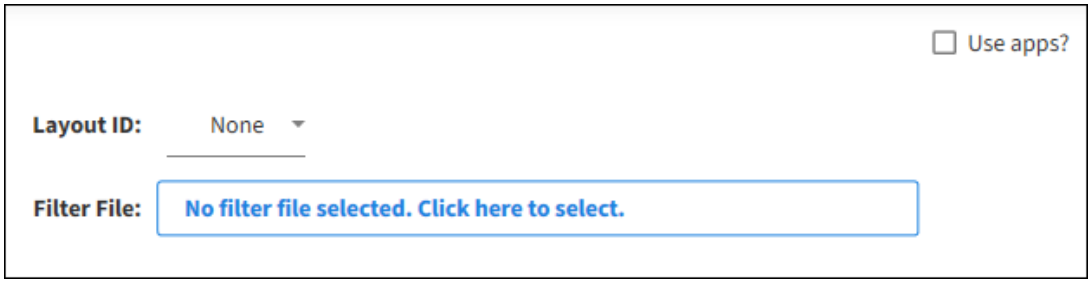

Figure 56. The top bar of the *Visualizer* page, "use apps?" is unchecked.

- Layout ID—select one of the source plate layouts from the dropdown menu. The scrollbar on the right of the list can be used to access layouts not displayed in the first seven options. For more information on the layouts, refer to Section III.B.3.d, "Source Plate Layout".

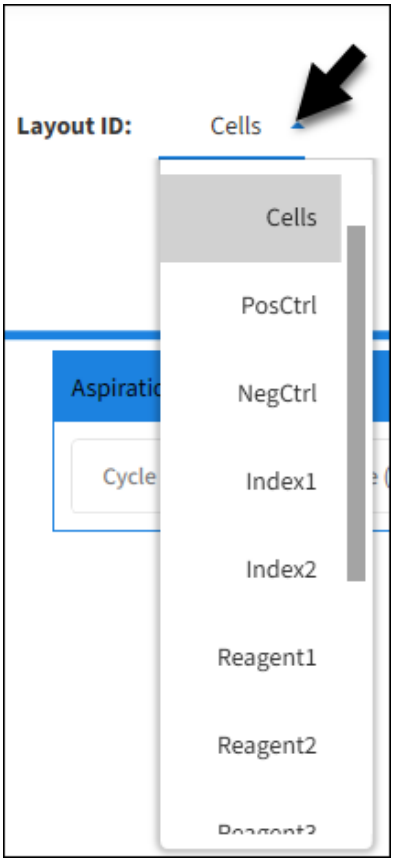

Figure 57. Example "Apps" dropdown menu on the *Visualizer* page.

- Filter file—(optional) if the step selected is for a reagent or index and you want to see what the dispense would be like for the layout selected based on a filter file, select the file here. This will restrict the information details displayed by the parameters defined by the filter file. For sample dispense steps or leaving this blank, the information displayed will assume dispense to all wells on the Single-Cell chip.

B. Dispense Details

Once the parameters for the dispense type you want to view are selected (Section A), detailed information about the dispense will display in the box on the main part of the visualizer page. The information displayed is based on the menu on the left side of the box.

Click on any of the tab names to view the information on that tab page. A brief overview of each tab option is listed below; see their corresponding sections for more details.

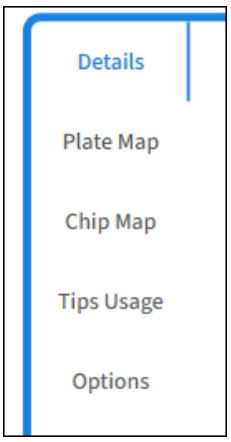

Figure 58. The tab options in the visualizer dispense details section.

1. *Details*—provides the overall detailed information about the required aspiration and dispense information
2. *Plate Map*—displays the layout of samples on a 384-Well Source Plate
3. *Chip Map*—displays the chip map visualization
4. *Tips Usage*—displays how the tips are used to dispense in a selected step
5. *Options*—the settings available to configure for visualization purposes

NOTE: The 'DNA Extraction' step of the Shasta WGA application is used as an example to illustrate the concepts below.

1. Details Tab

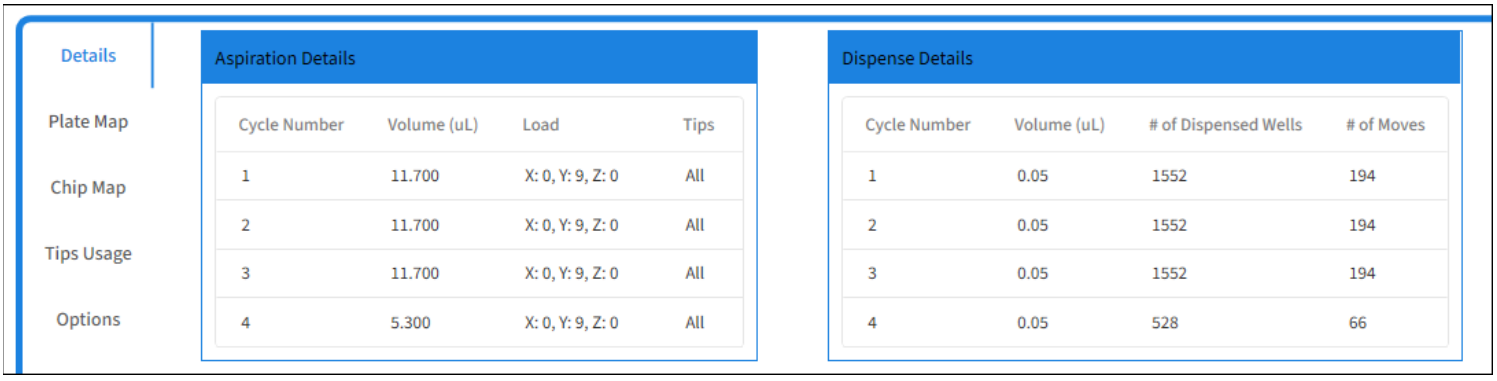

Figure 59. The visualizer *Details* tab contents.

Two tables are displayed on the *Details* tab:

- Aspiration Details

  This table summarizes the necessary aspirations from a 384-well plate to perform the complete dispense step into the chip. Each row represents a cycle of aspirating from a source plate—i.e., when the syringes load the sample, reagent, or index; the totality of cycles equals the dispense.

Each row details for the specified cycle:

- The volume (per tip) aspirated for each cycle
- The loading position of the tip nozzle assembly (the XYZ location in space according to the control software)
- The number of tips employed (1–7, or 'All' when all eight tips are utilized)

This information can be used to calculate the loading volumes for each well of the 384-Well Source Plate but requires that the *Options* parameters (Section V.B.5) be set; refer to that section, if necessary.

- Dispense Details

  This table provides a summary of the dispenses into the chip corresponding to each cycle of aspirations from the source plate. It outlines:

  - Volume of solution dispensed per chip well
  - Number of wells that will be dispensed to (total value, from all tips employed)
  - Number of movements by the tip nozzle assembly necessary to cover the number of wells (previous column)

2. Plate Map Tab

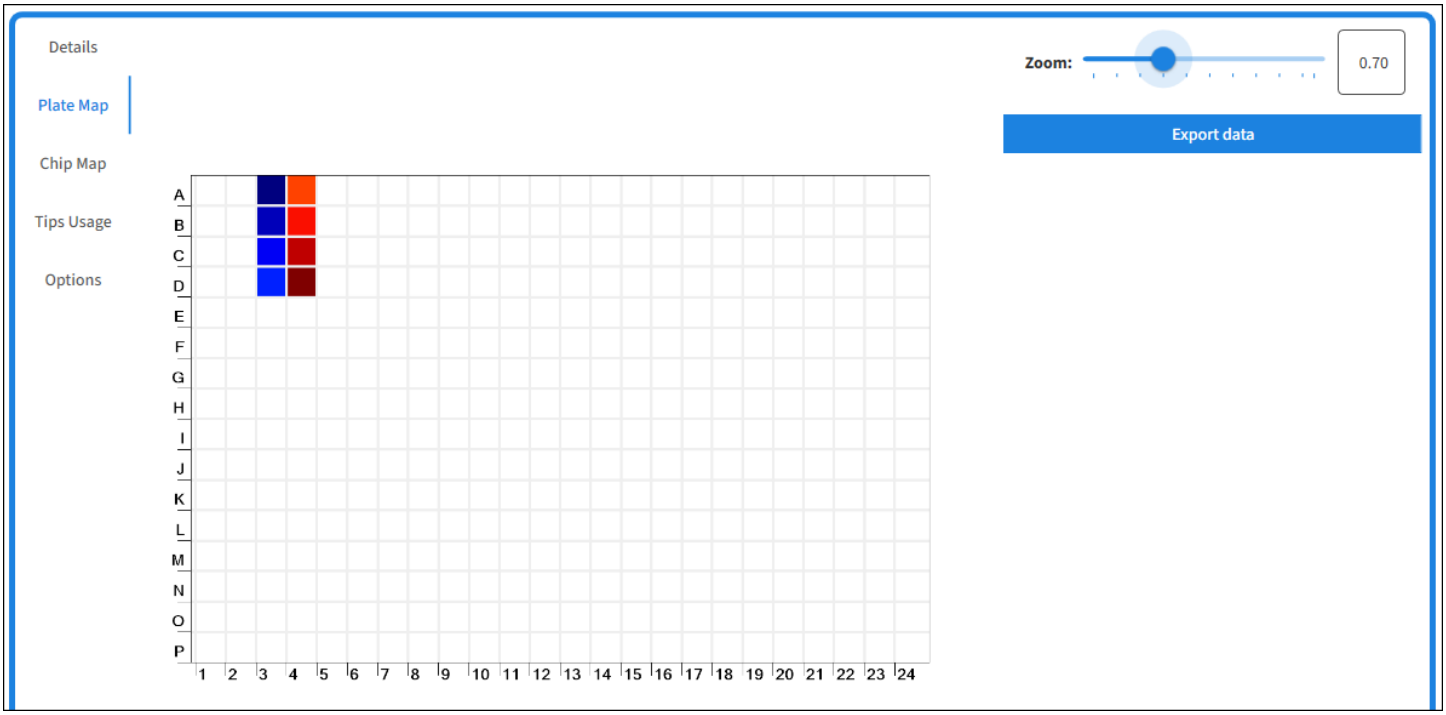

Figure 60. The visualizer *Plate Map* tab contents.

This tab displays the 384-well source plate layout map representing the selected dispense step, shading the wells where the reagents should be aliquoted.

- The "Zoom" slider in the top-right corner can be used to enlarge or shrink how the map is displayed on the screen.

NOTE: There is no functionality to move the view of the plate map around while zoomed in, which could roll wells off the screen.

- The [Export data] button can be used to export the data of the layout map to a TSV file.

3. Chip Map Tab

This tab displays a heat map of the Single-Cell chip and represents dispense data for each well. The color gradient indicates the values of the selected parameters under "Options" and "Filters".

The heatmap is shown rotated 90° counterclockwise from the orientation of the chip in the chip nest, with the chamfered corner on the upper right (heatmap) rather than the lower right (chip nest). Refer to Figure 61 for a comparative view.

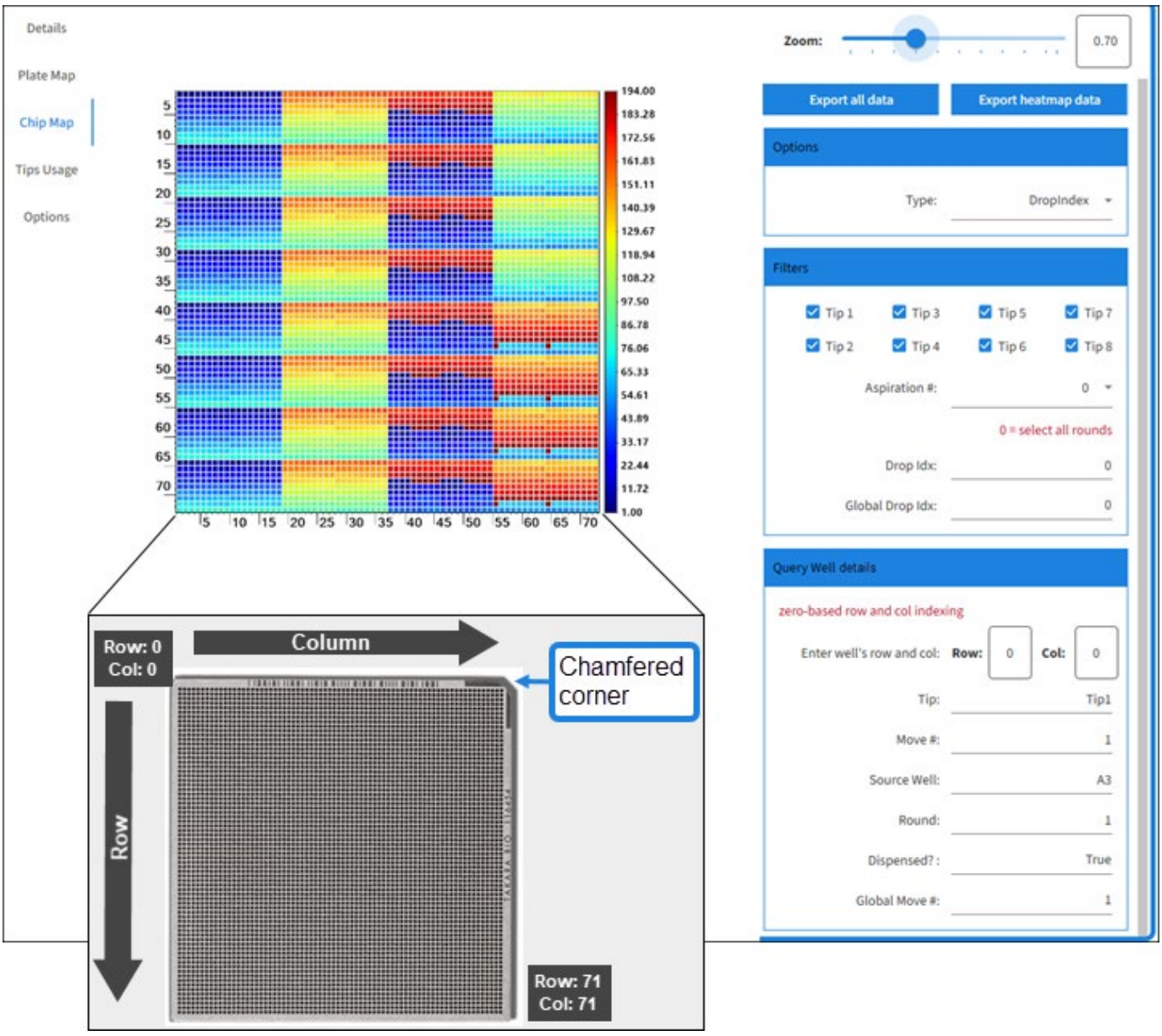

Figure 61. The visualizer *Chip Map* tab, aggregate view, with chip comparison. By default, the boxes on the right-side of the image are part of a scroll-bar menu and not fully displayed. The inset image illustrates the orientation of the heatmap as compared to the actual chip, which is rotated 90° counterclockwise from the orientation in the chip nest.

This tab displays a heat map of the Single-Cell chip and represents dispense data for each well. The color gradient indicates the values of the selected parameters under "Options" and "Filters".

The sections below provide more information about each of the right-side menu elements.

a) Zoom

Use the slider to zoom in and out of the displayed chip heat map.

b) Options

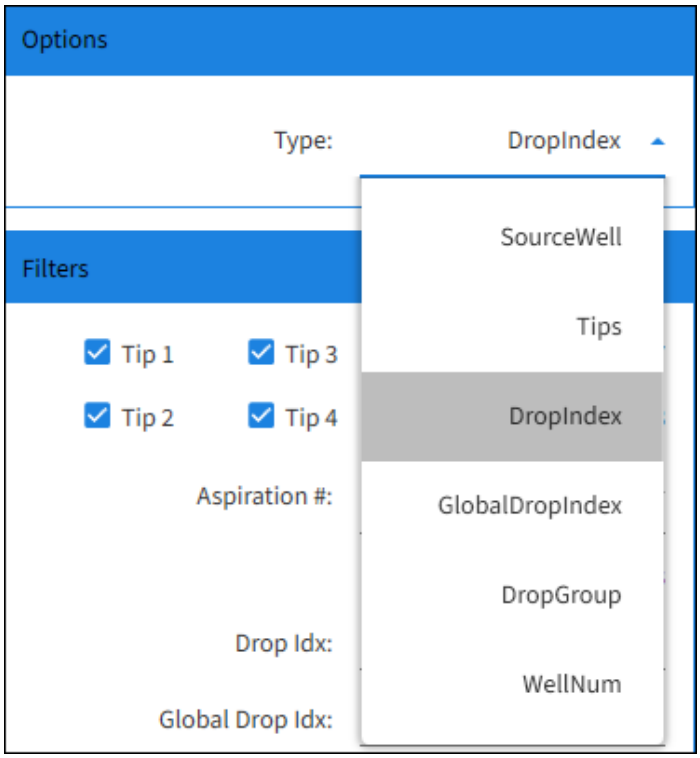

Figure 62. The Options dropdown menu in the visualizer *Chip Map* tab.

Use the "Type" dropdown menu to select the type of heat map data to be displayed as a relationship of the selection and the chip wells shown. By default, "DropIndex" is currently selected.

- SourceWell—relationship with the 384-Well Source Plate wells.
- Tips—relationship with which tips were employed to dispense.
- DropIndex—relationship with the movements of the tip nozzle assembly for each aspirate-dispense to the chip. The value starts with '1' at the beginning of each cycle.
- GlobalDropIndex—the relationship with the movements of the tip nozzle assembly for the entire dispense (aggregation of the DropIndex data); i.e., this value will increment for every time the assembly moves and is not reset when a new cycle commences.

- DropGroup—the relationship with the aspirate/dispense cycle, i.e., which cycle the dispense occurs. The wells will be color coded depending on which cycle it is dispensed in.
- WellNum—the sequential well numbering of the chip wells.

c) *Filters*

The filter parameters can be used in conjunction with the options to view only the portion of the data on the chip heat map.

- Tip 1–8—the checkboxes enable or disable the display of data from a specific tip nozzles. Figure 63 shows a view of the tip nozzle assembly as seen from the open door of the Shasta instrument with two of the tips in the foreground labeled (Tip 2 and Tip 8). Table 5 shows a map of the tip nozzle assembly if viewed from the top down, with the tip numbers identified by their position within the assembly. Figure 64 shows an example heatmap when filtering by tip number.

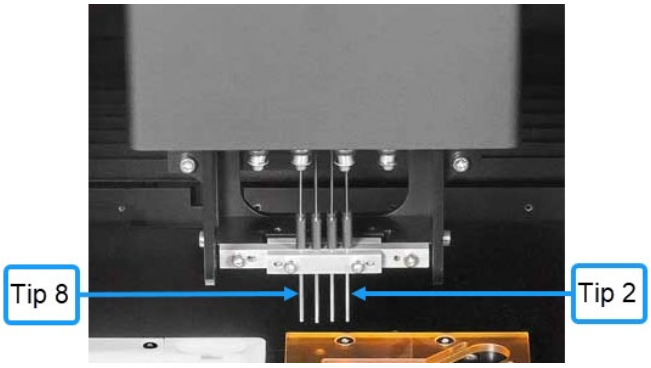

Figure 63. Tip numbering identification visual guide. When looking into the Shasta instrument from the open door, Tips 2, 4, 6, and 8 are the ones visible (in the front, right to left) of the tip nozzle assembly. See Table 5 for the top-down map.

Table 5. Tip numbering map in the tip nozzle assembly, top down-view.

Back row			
7	5	3	1
8	6	4	2
Front row			
[DOOR]			

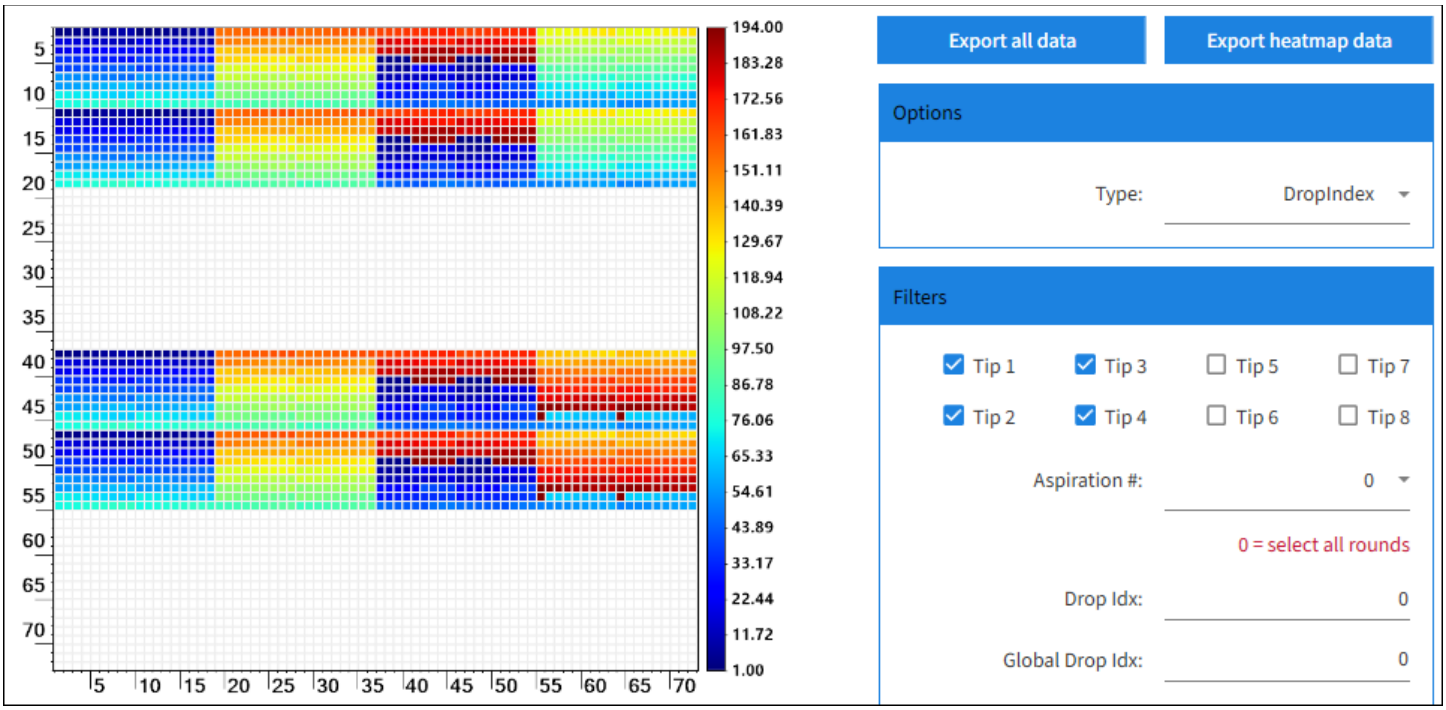

Figure 64. Example of filtering by tip number, visualizer *Chip Map* tab. The chip heat map is displaying only the wells dispensed to by Tips 1–4.

- Aspiration #—allows you to view the heat map for a specific cycle (round of aspiration); the cycle number correlates to the values in the *Details* tab tables. The default value, '0', is an aggregate of all cycles.

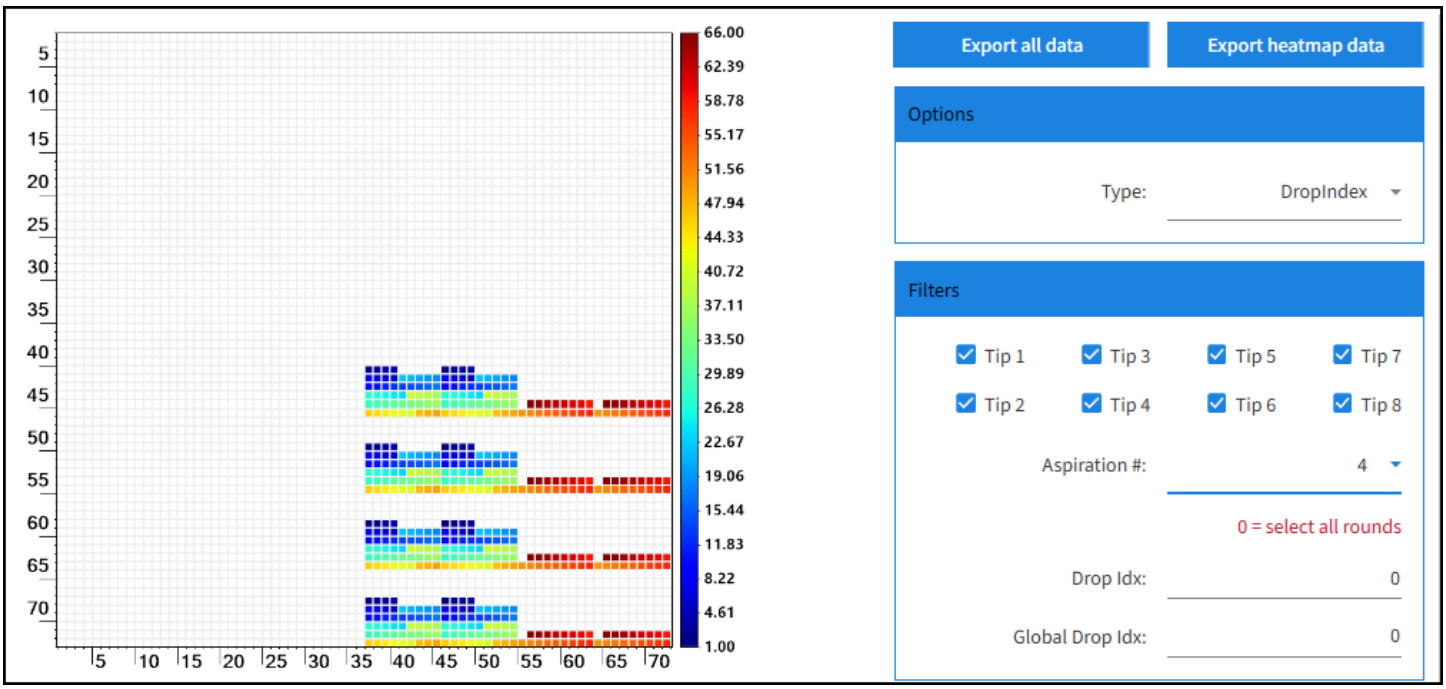

Figure 65. Example of filtering by Aspiration #, visualizer *Chip Map* tab. The chip heat map is displaying only cycle #4.

- Drop Idx— filters the data by specific drop indexes. This value indicates the movement number of the tip nozzle assembly when performing a dispense within a cycle. Because, as described above in Options, this index value resets at the

beginning of each cycle/aspiration within the dispense, specifying a "Drop Idx" value with no additional filters will display every well dispensed on the 10th position within each cycle. For a four-cycle dispense, this might be up to 32 wells (8 tips * 4 cycles).

Example (shown in Figure 66):

Putting a value of '10' in this field will no other filters will display all 32 wells dispensed to on the 10th position of the tip nozzle assembly.

However, if also restricted by the "Aspiration #" value of '1', it will show only the 10th position within the first cycle and therefore only eight wells (one for each tip).

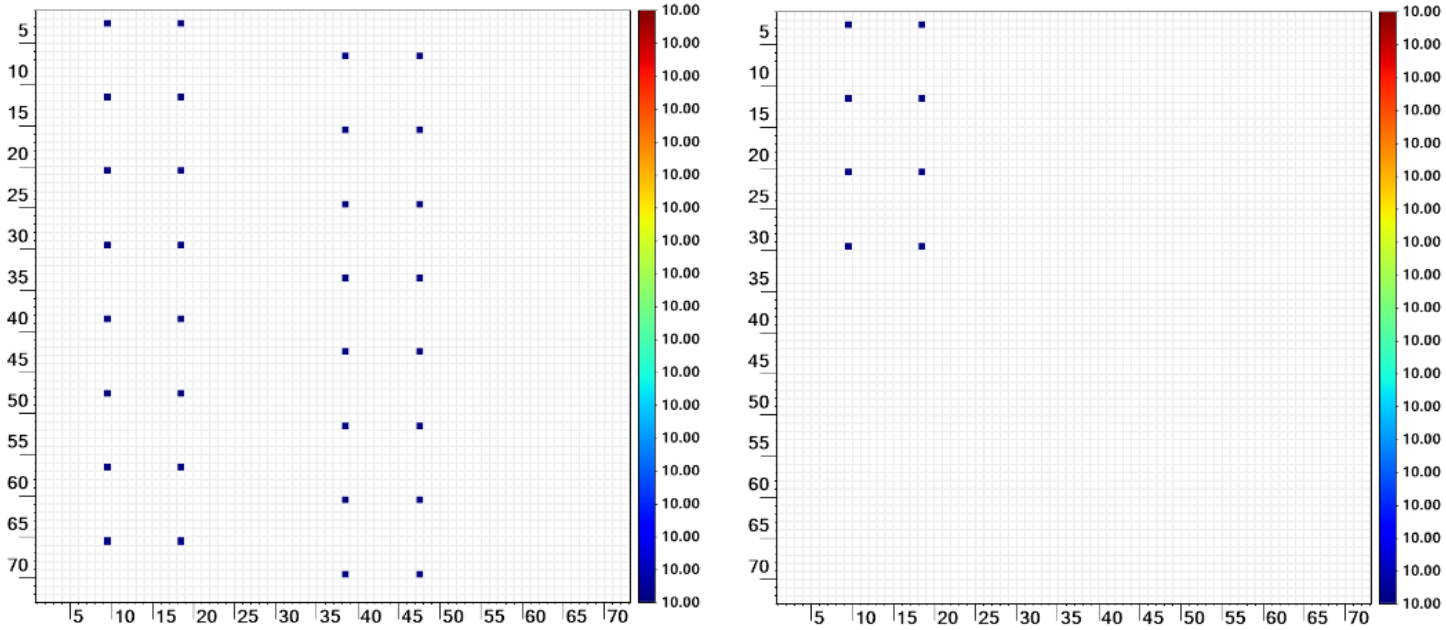

Figure 66. Example of filtering by "Drop Idx", visualizer *Chip Map* tab. The "Drop Idx" value for both heat maps is '10' in the example. **(Left)** Drop Idx filter, with no other filters. **(Right)** "Drop Idx" value '10' of "Aspiration #" '1'.

- Global Drop Idx—filter the data by a drop index aggregated across all cycles.

  Example:

  If viewing the heat map through the option 'GlobalDropIndex', it can be determined that the total number of drops (tip nozzle assembly movements to dispense eight wells per drop) is 648 (circled in Figure 67).

  The total number of drops (648) defines the upper bound of the input value range for the "Global Drop Idx" filter; in this case, a value from 1–648 can be selected.

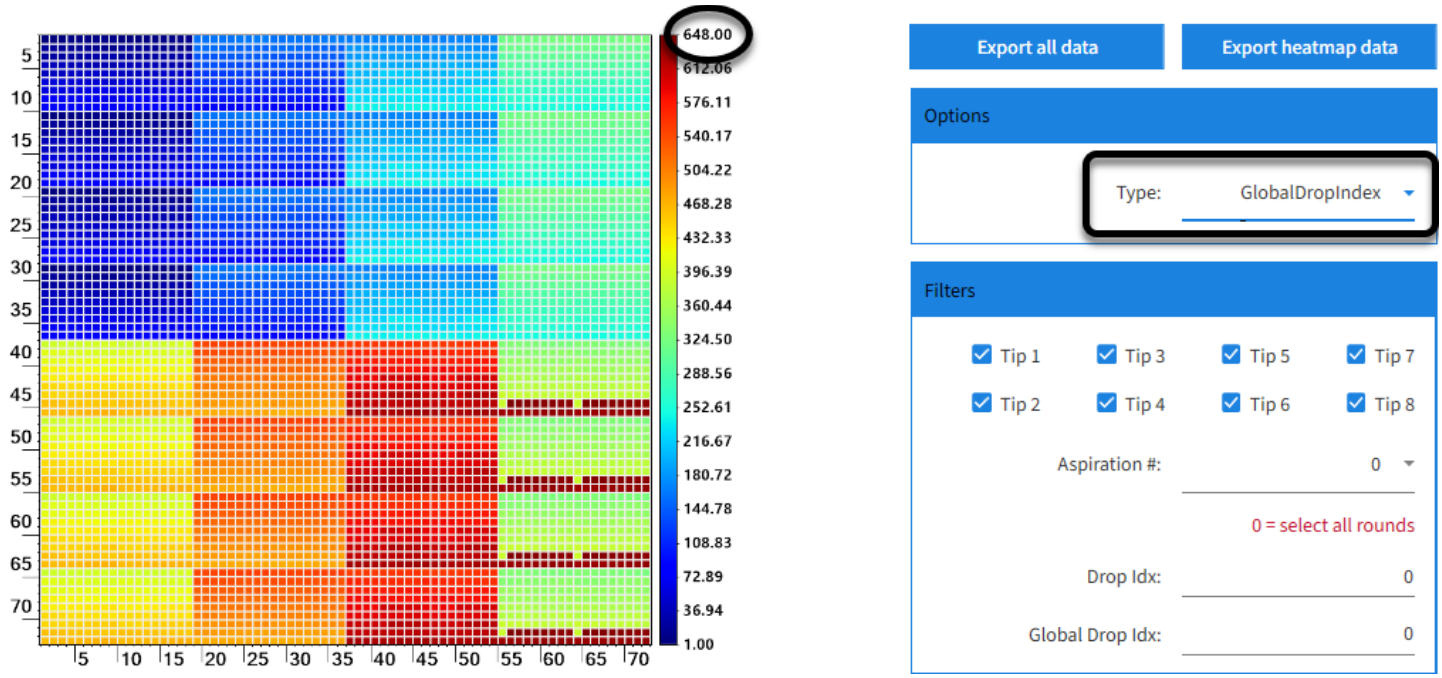

Figure 67. Example of the 'GlobalDropIndex' option in the visualizer *Chip Map* tab.

In this example, the number of drops in cycle #1 is '194'. This means that the "Global Drop Idx" equal to '195' is the equivalent of cycle #2, drop value '1'.

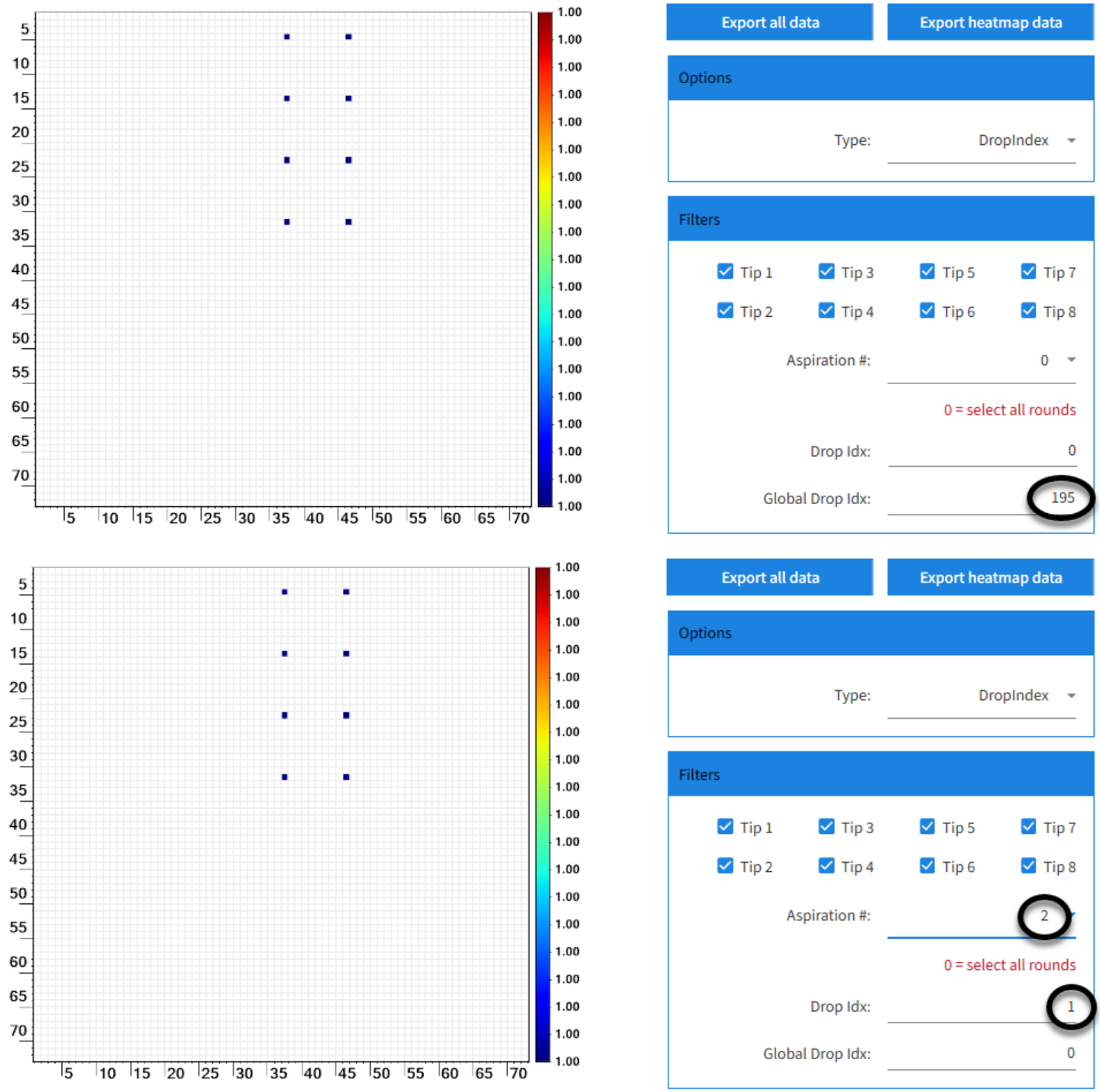

Figure 68. Comparison of "Global Drop Idx" versus "Drop Idx" for equivalent tip nozzle assembly positions. **(Top)** The 195th position in the overall dispense run. **(Bottom)** The first position of cycle #2. The heatmap illustrates that these are the same dispense.

d) Query Well Details

This section of the menu allows you to enter a specific Row (0–71) and Column (0–71) location for a well (well address) on the chip.

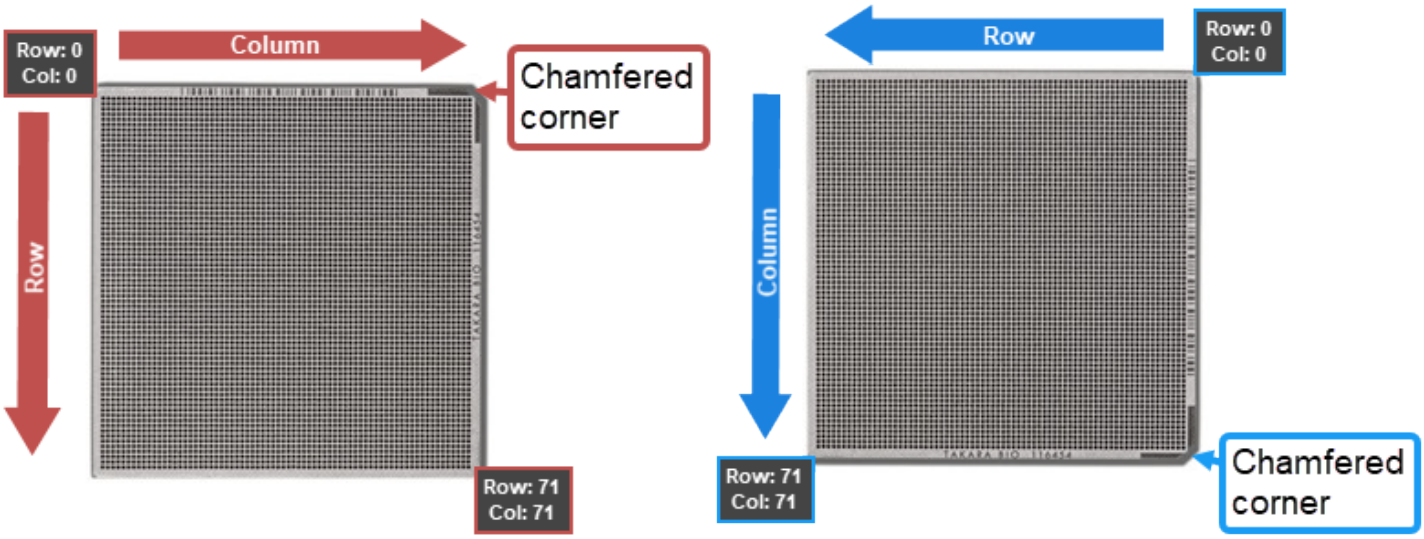

Figure 69. Row and column number identification on a Single-Cell chip. The chamfered corner of the chip is indicated to aid with visualization. **(Left)** The chip in the same orientation on the heat map. **(Right)** The top-down view of the chip as it is oriented in the chip nest. Note that the image on the left, which matches the orientation of the heat map, is rotated 90° counterclockwise from how the chip is oriented in the nest.

After entering the row and column index values, the following information is displayed for the specified well:

- Tip—the number of the tip used to dispense to it
- Drop idx—the drop index value for the dispense; this is the numbered position of the tip nozzle assembly within a cycle
- Source well—the letter and number identifier of the well on the 384-well plate that was the source of the aspiration dispensed to this location
- Round—the cycle number when this well was dispensed to
- Dispensed?—a boolean (True/False) value that indicates whether a dispense occurs in this well

e) Export all data/Export heatmap data

The two export buttons, [Export all data] and [Export heatmap data], can be used to translate the data populating the heatmap into one of two formats.

- [Export all data]—repackages the data into a TSV file with seven columns of data a row for each of the 5,184 wells on the chip across the following seven data columns:
  - Chip row number (Figure 69)
  - Chip column number (Figure 69)
  - Dispense tip number (Figure 63 and Table 5)
  - Drop index value (see c) Filters)
  - Global drop index value (see c) Filters)

- Dispense round (i.e., the cycle number)
- Source well ID—of the 384-Well Source Plate

	A	B	C	D	E	F	G
1	Row	Col	Dispense tip	Drop index	Global drop index	Round	Source well
2	0	0	1	1	1	1	A3
3	0	1	1	2	2	1	A3
4	0	2	1	3	3	1	A3
5	0	3	1	4	4	1	A3
6	0	4	1	5	5	1	A3
7	0	5	1	6	6	1	A3
8	0	6	1	7	7	1	A3
9	0	7	1	8	8	1	A3
10	0	8	1	9	9	1	A3
11	0	9	2	1	1	1	A4
12	0	10	2	2	2	1	A4
13	0	11	2	3	3	1	A4
14	0	12	2	4	4	1	A4
15	0	13	2	5	5	1	A4

Figure 70. Partial view of an example [Export all data] TSV file for the *Chip Map* view. Values reflect the Shasta WGA > DNA extraction dispense step.

- [Export heatmap data]—translates the heatmap into a numerically equivalent TSV file of 72 columns x 72 rows, where each cell of the file is a well on the plate. The file is read like the heatmap, with Row 0, Col 0 in cell A1 of the file and Row 71, Col 71 in cell BT72; the cell values correspond to the number of the well on the 384-well plate (1–384), where plate well A1 is '1' and plate well P1 is '16'.

	H	I	J	K
8	33	33	49	49
9	33	33	49	49
10	34	34	50	50
11	34	34	50	50
12	34	34	50	50
13	34	34	50	50
14	34	34	50	50
15	34	34	50	50
16	34	34	50	50
17	34	34	50	50
18	34	34	50	50
19	35	35	51	51
20	35	35	51	51

Figure 71. Partial view of an example [Export heatmap] TSV file for the *Chip Map* view. Values reflect the Shasta WGA > DNA extraction dispense step: '33' is the source well "A3", '34' is source well "B3", '49' is source well "A4", etc.

4. Tips Usage tab

This tab displays heat maps that show the relationship between each tip nozzle and the drop index for each aspiration and dispense cycle for the selected steps. Refer to the *Chip map* "Options" (Section OptionsV.B.3.b)) for the definition of "drop index".

Interpreting the charts

Figure 72 shows aspirations #3 and #4 of the Shasta WGA DNA extraction dispense step. The X-axis is the drop index—i.e., the total number of drops distributed on the chip during the cycle. As can be seen by the numbered ticks, aspiration #3 performed 194 drops, but aspiration #4 only performed around 67.

The Y-axis is divided across all eight tip nozzles, with each horizontal bar capturing an aggregate of whether or not the nozzle distributed fluid for the given drop index (black, Used) or not (white, Not used)—see the legend to the right of each chart.

In this instance, all eight tip nozzles are employed to dispense on every drop index, but the individual stripes on the chart display differently if a filter file was implemented depending on the distribution of candidate wells on the chip (Figure 73).

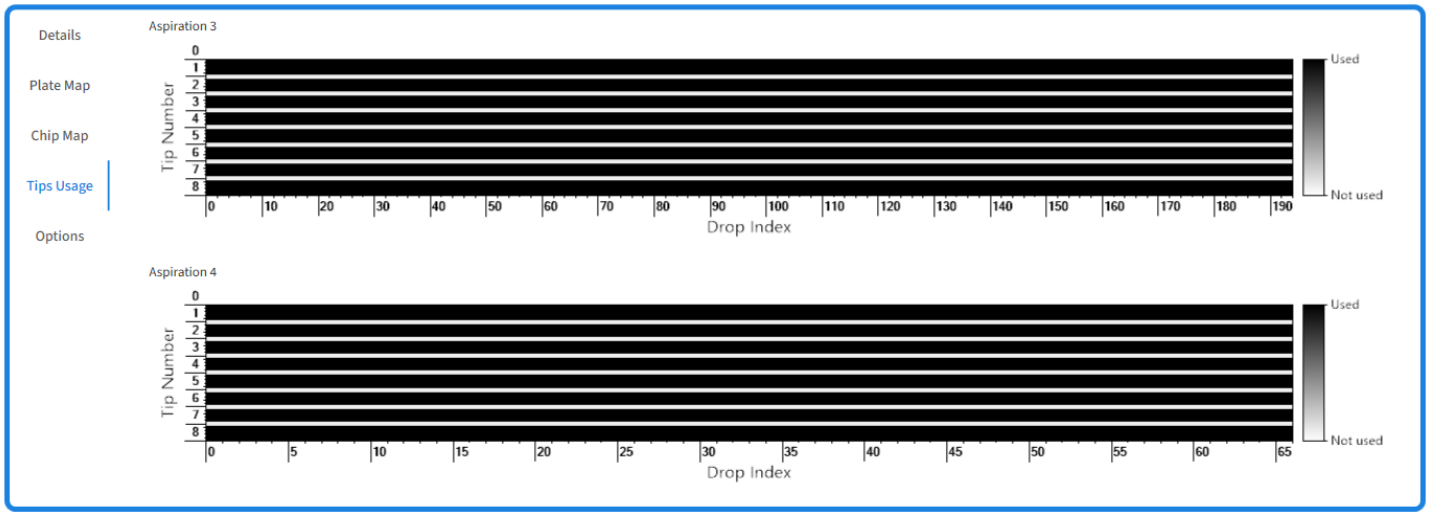

Figure 72. Example of the *Tips usage* view in the visualizer, with no filter file applied.

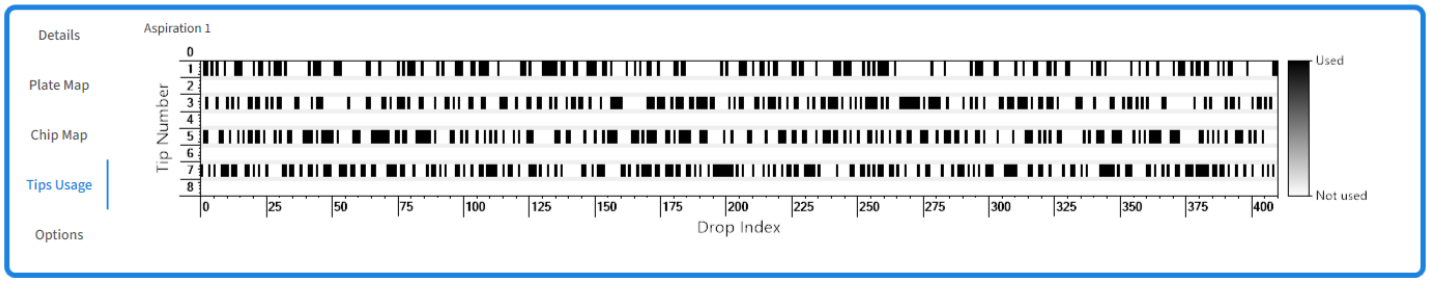

Figure 73. Example of a *Tips usage* chart in the visualizer with a filter file applied.

5. Options tab

This tab displays the available configurations for the visualized data. Modification of this is not generally recommended. Please contact your authorized Takara Bio service technician or field_support@takarabio.com for how to properly modify this.

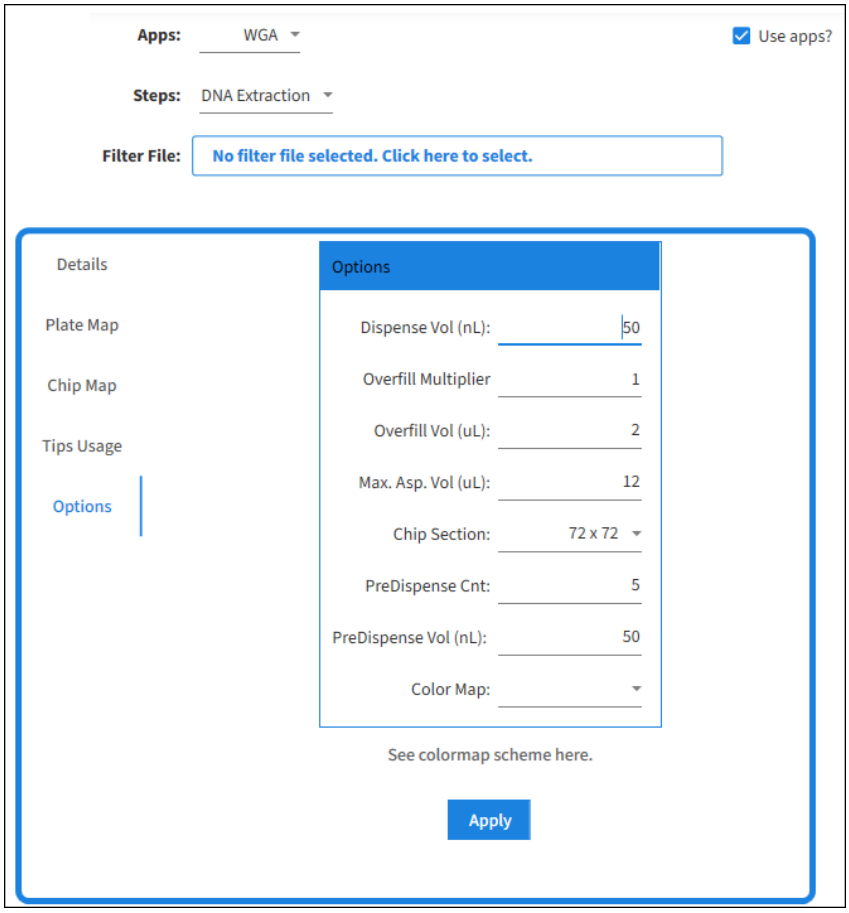

Figure 74. *Options* tab example.

VI. Microscope Utility

A. Overview

This option from the *User Utility* view allows you to visually inspect individual wells on the chip.

The feature is intended to assist with optimizing the imaging settings needed during the cell scan step (Section III.B.4.b, "Recommendations for the Imaging Filter Settings"), either because of novel sample cell types or when using custom cell stains.

The Microscope utility can be accessed under the Options menu (hamburger ☰ icon) in the title bar and selecting the **User Utilities** menu option.

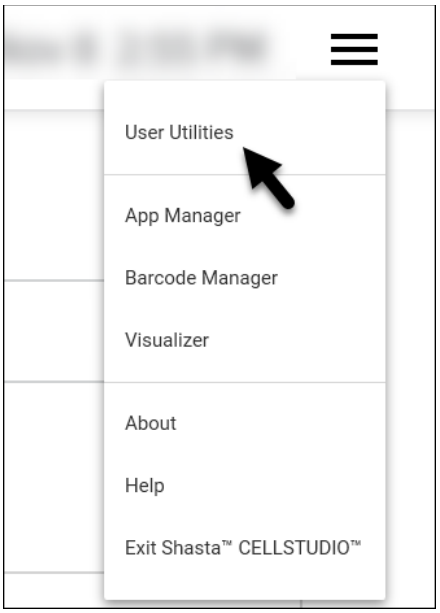

Figure 75. Accessing the User Utilities menu item.

From the *User Utilities* view, click on the [Microscope] icon.

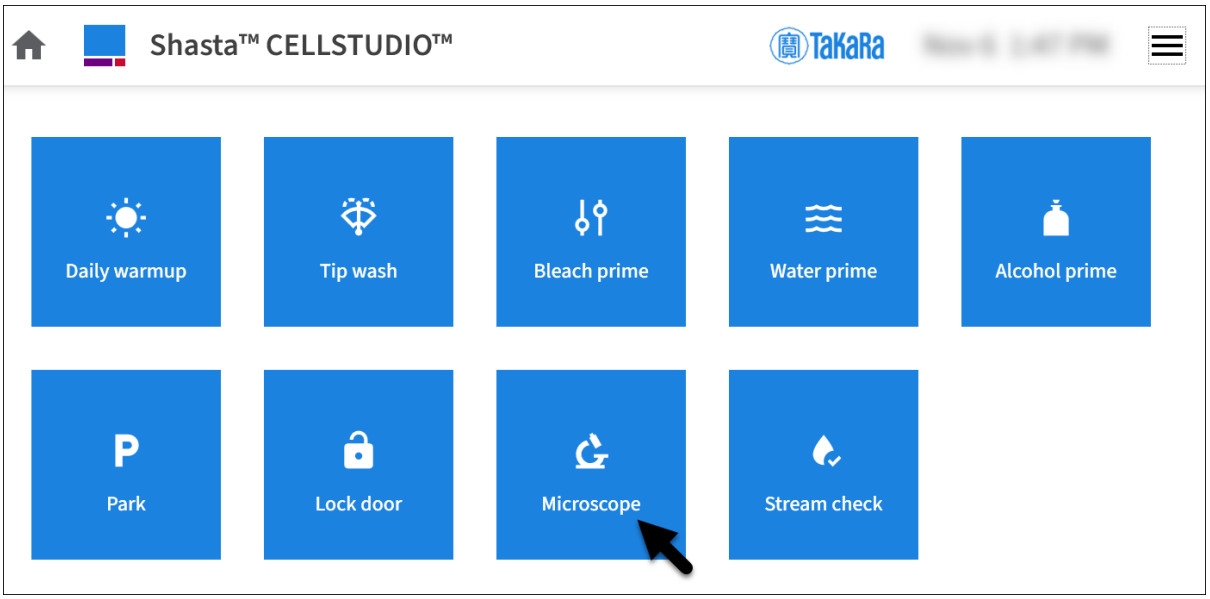

Figure 76. The [Microscope] icon on the *User Utilities* view.

B. Microscope View

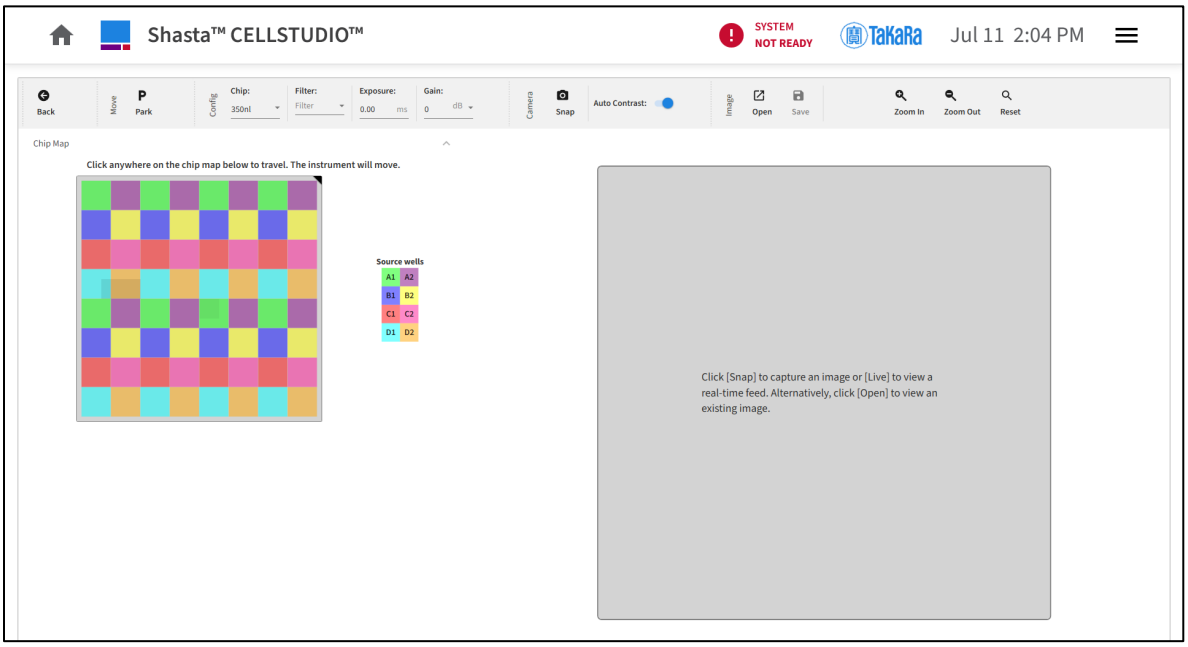

Figure 77. The *Microscope* utility view.

The parts of the view (Figure 77) numbered (1–6) correspond to the list below.

1. Place the chip

If the chip of interest is not already loaded into the Shasta instrument chip nest, insert it first. The two methods here are used to move the camera to set positions:

- Clicking the [Park] button moves the camera to the Y and Z position of '0' and the X position to be centered behind the chip nest. This position has the tip nozzle assembly (which includes the camera) away from the chip nest area.
- Clicking anywhere on the chip map moves the camera to the selected position on the chip. The chip map is color coded to display the source wells.

2. Select chip type

From the dropdown menu, select the well volume for the chip to be examined. This will either be '250nl' for the Single-Cell 250f Chip or '350nl' for the Single-Cell 350v Chip. Do not select the '150nl' option.

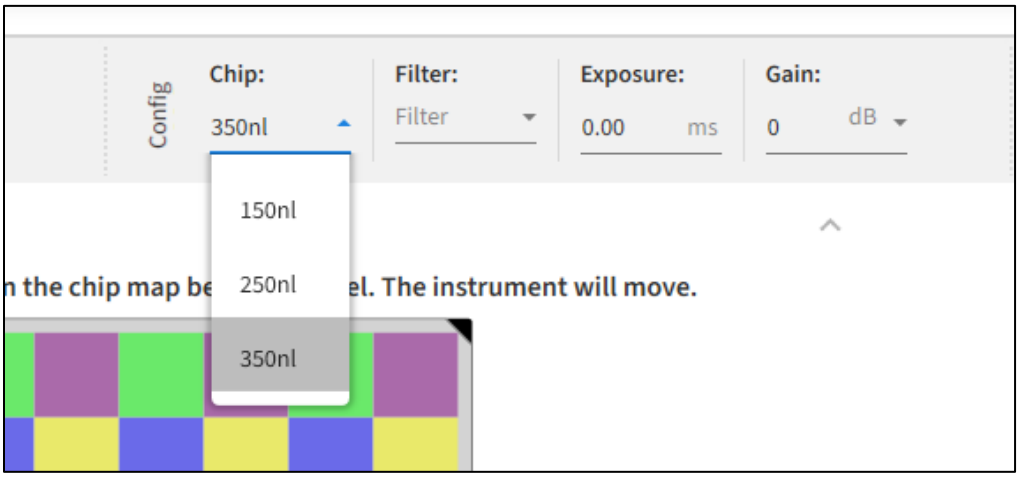

Figure 78. *Microscope* "Select chip type" dropdown menu.

3. Select filter

Filter, in this case, refers to the color channel suitable for the stains, such as blue, red, etc. White is also an option here.

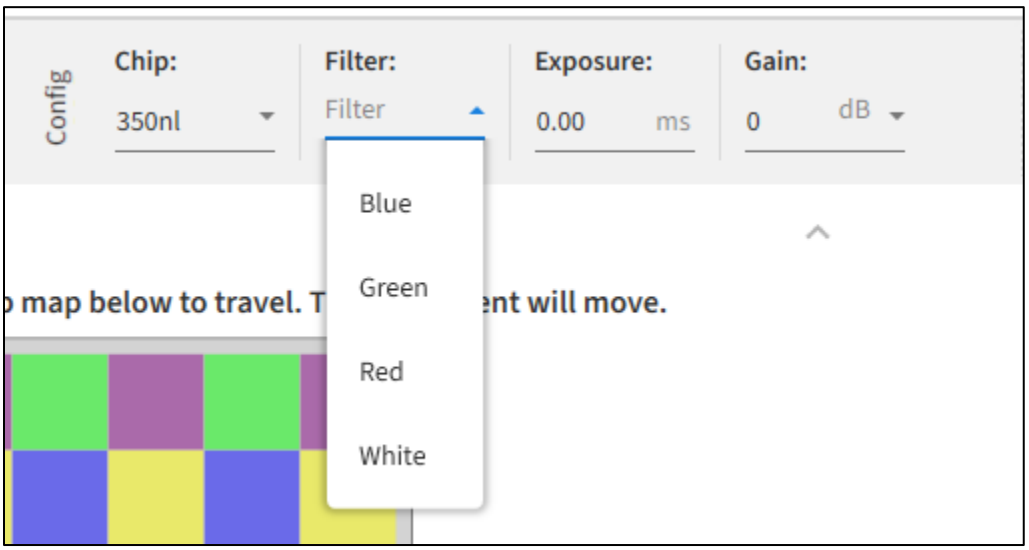

Figure 79. *Microscope* "Select filter" dropdown menu.

4. Select exposure and gain

Type in a numerical value for the exposure (in milliseconds) and select a gain value from the drop-down for how you would like to review your images. For more information about what these parameters mean, refer to Section III.B.4.a), "How to Add an Imaging Filter to the Imaging Step".

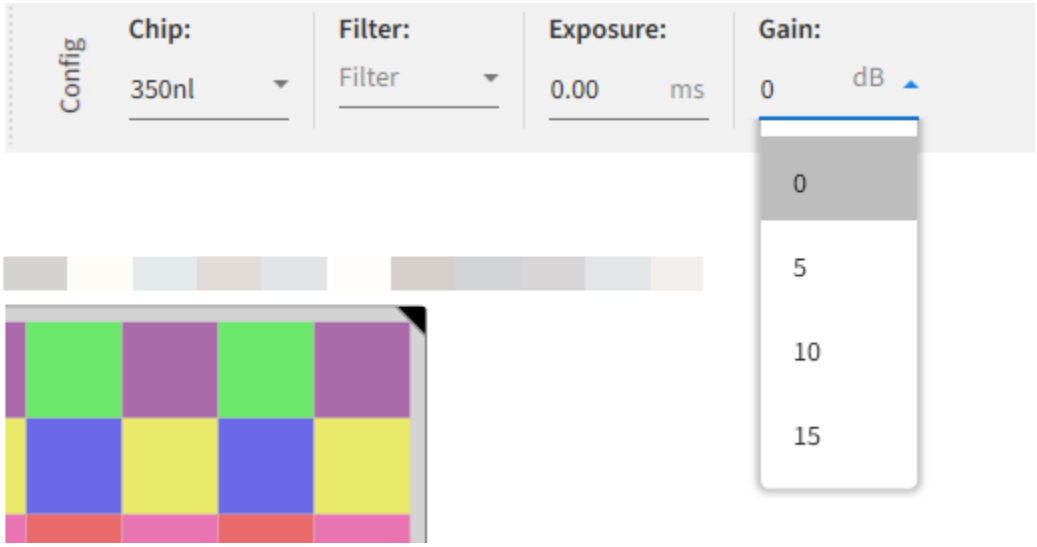

Figure 80. *Microscope* "Select exposure and gain" step.

5. Select a chip pos

'Pos' refers to the position of the chip you want to visualize. The position value refers to a specific 6 x 6 well area of the single-cell chip assigned by CELLSTUDIO software; there are 144 total positions (Pos0–Pos143) mapped on the chip. Select any area on the chip to travel to the desired position.

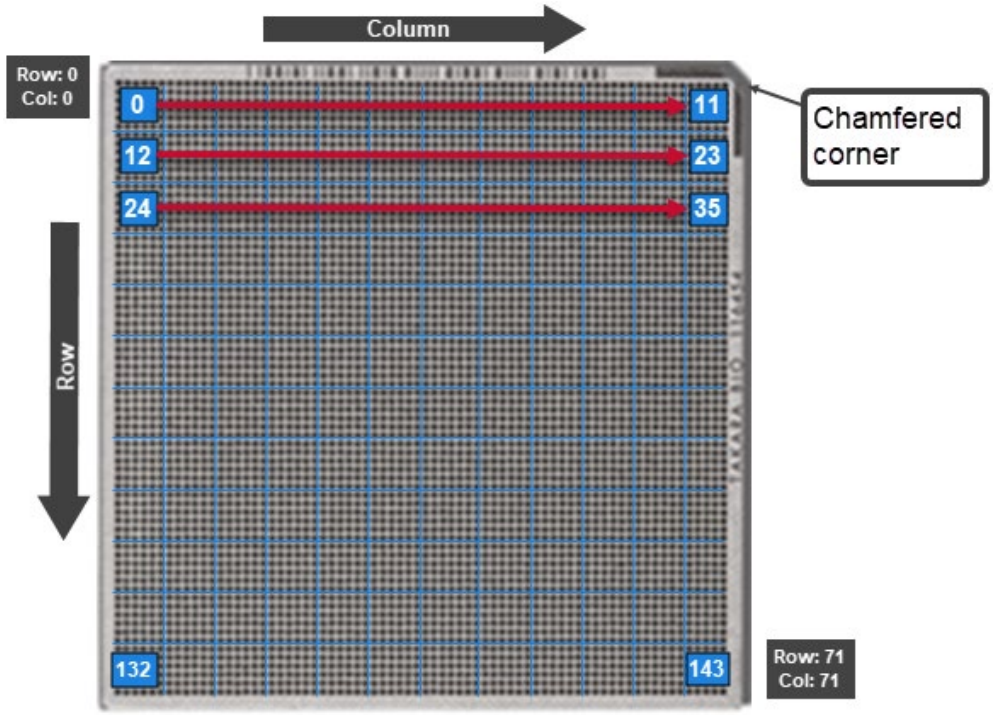

Figure 81. How to map the Pos values to areas on the Single-Cell Chip. 'Pos0' is assigned to the 6 x 6 grid of wells starting at Row: 0/Col: 0; 'Pos143' is at Row: 71/Column: 71. The numbering increments from left to right, then starts at the far left on the next row. The figure reflects the orientation of the chip as it is displayed in CellSelect software and in the visualizer chip heatmaps (Section V.B.3).

NOTE: As a reminder, the chip orientation shown above is rotated 90° counterclockwise from the orientation of the chip as it presents in the chip nest (Figure 69).

C. Image Viewer Tools

The *Image Viewer* portion of the microscope utility offers a number of tools to assist you with evaluating the images. The toolbar is shown in Figure 82, with a description of each tool listed below it.

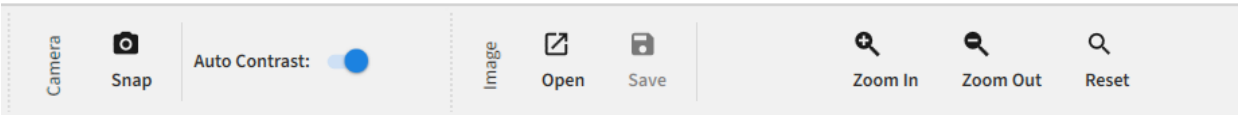

Figure 82. Microscope *Image Viewer* toolbar.

- [Snap] – takes a snapshot of the current wells displayed in the *Image Viewer* field.
- [Auto contrast] – applies automatic contrast tool automatically if toggled on. This tool uses algorithmic logic to brighten the lighter areas of the image and deepen the darker areas to better visually distinguish detected objects in the well. This may make it easier to detect the presence of objects if the image has poor contrast without the enhancement, but it may also introduce noise to an image that is already of good quality.
- [Open] – opens an image file taken with the [Snap] tool.
- [Save] – saves an image taken by the [Snap] tool to a file on the computer. The icon is shown grayed out here, but will darken to black (meaning it is active and can be interacted with) when a snapshot is present in the viewer.
- [Zoom In] – zooms in on the image file taken with the [Snap] tool.
- [Zoom Out] – zooms out of the image file taken with the [Snap] tool.
- [Reset] – resets the image file to the default zoom setting.

D. Using the Image Viewer

As mentioned in the overview section, the microscope feature is intended to assist with optimizing the imaging settings needed during the cell scan step. In general, you want to adjust the "Exposure" time and "Gain" so that all cells are clearly visible above the background, i.e., a high signal-to-noise ratio—but be careful to not overexpose the image. Higher gain values produce brighter, but also noisier, images. If the exposure time is too high, brighter pixels on the camera may become more visible. Also, longer exposure times also mean longer scan times.

Example:

If using a novel stain for your cells or nuclei samples, and the CellSelect assessment of candidate wells is very low, this might be due to the filter settings. By using the microscope features, you could:

- Experiment with different color channels, exposure duration, and gain on the same set of 36 wells (one Pos area) until one set of parameters seems like the best fit for your experimental needs.
- You could then move to another Pos set to see if those parameters are effective there as well.
- For comparison purposes, you could use [Snap] to take screenshots of the two areas and compare them

- You could also use [Snap] to image the same area with different settings to compare those (for example Blue and Red to identify live/dead cells).

E. Exit the *Microscope* View

The *Microscope* view can be exited in a few ways:

- Clicking on the [Home] icon.
- Clicking on the back arrow (Figure 83). This will return you to the *User Utilities* view.

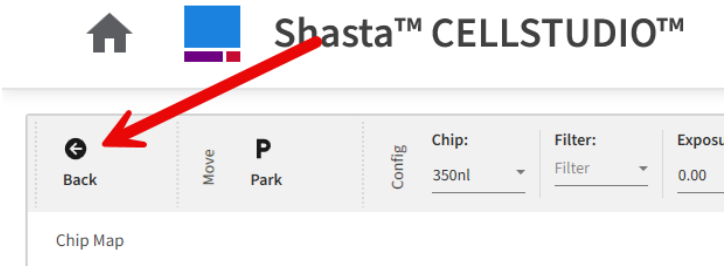

Figure 83. Where to locate the back arrow in the *Microscope* view.

- Navigating to a different selection in the Options menu (hamburger icon).
- Via timeout after a certain amount of inactivity.

For all options, when exiting the view, a *Move to Teachpoint* progress window will pop up (Figure 84). This is an expected, routine action indicating the tip nozzle assembly (camera) is returning to 'home' (park, 0,0,0) to reset to default. It is recommended to allow the procedure to complete without stopping, so the tip nozzle assembly is in a safe position with good access to the dispense platform.

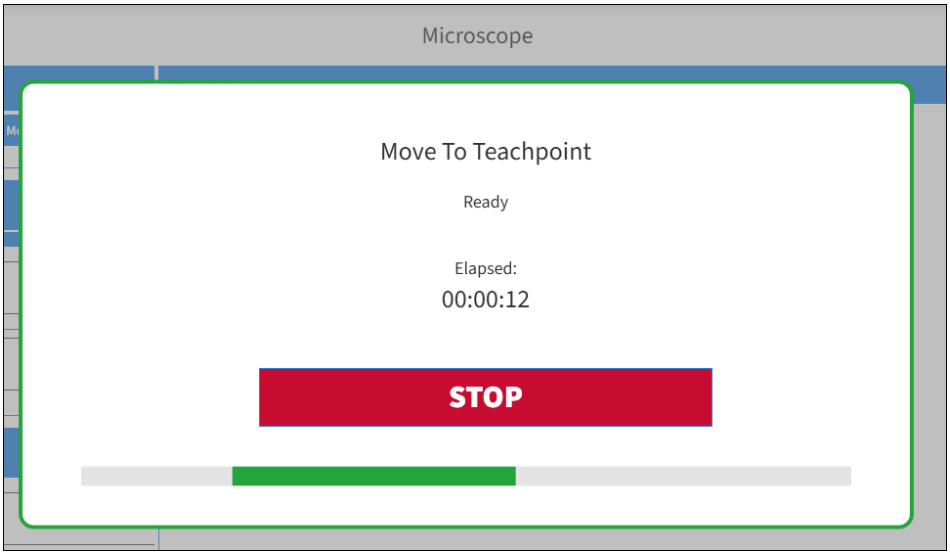

Figure 84. The *Move To Teachpoint* window displayed when exiting the *Microscope* view.

Appendix A. Designing an Application: Advice from Our Scientists

A. Overview: Application Workflow

The graphic below depicts the overall workflow of a typical application performed on the Shasta Single Cell System. For additional information on any of the steps, refer to the associated section number.

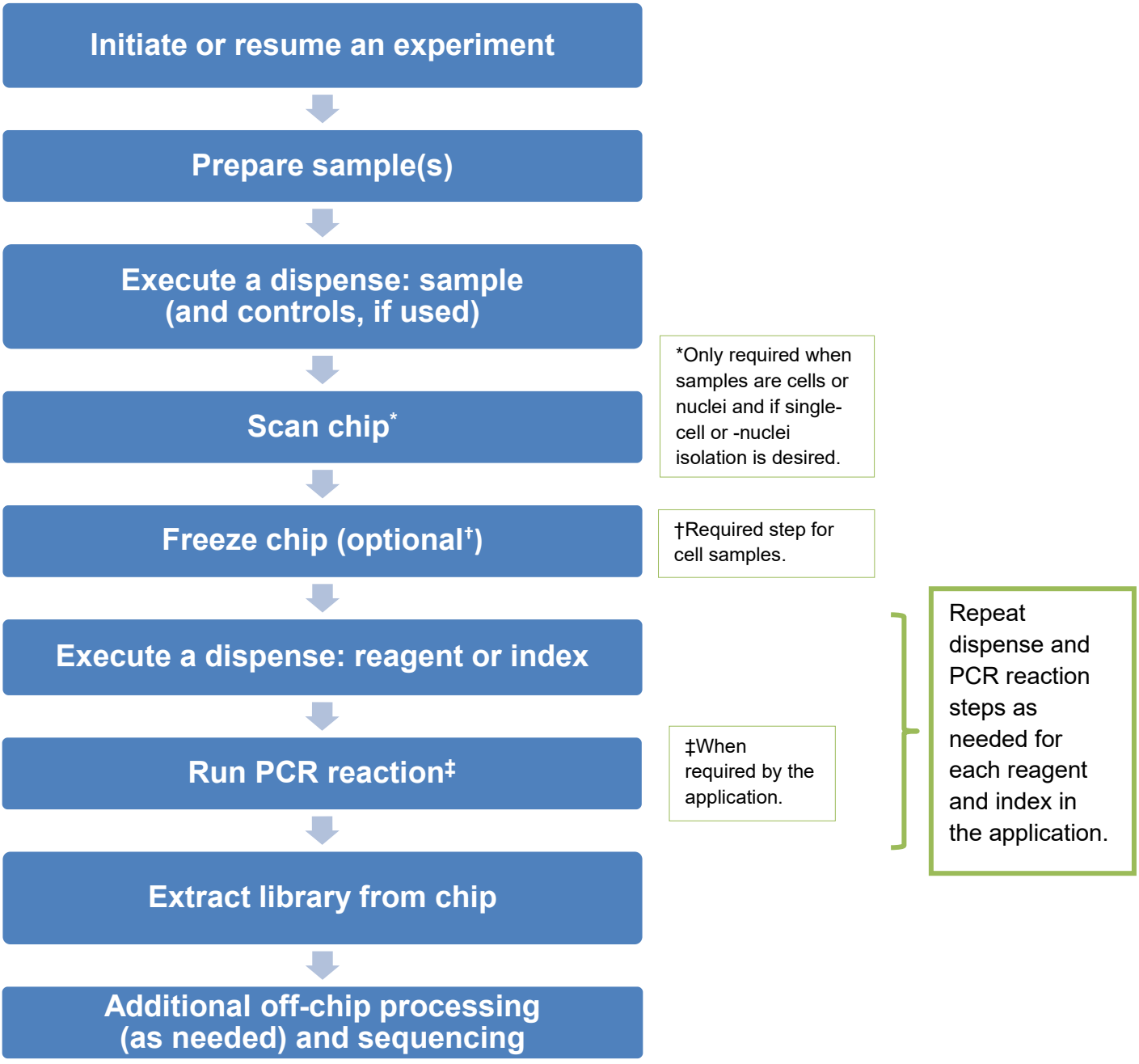

Figure 85. Application workflow overview. This image is also available in the Shasta Single Cell User Manual.

B. How Should I Begin?

You can think of transitioning to the Shasta system as miniaturization of an existing protocol that has already been tested at larger volumes. A protocol that is a good candidate for an application on the Shasta system is one in which consecutive reactions are additive (i.e., only addition of reagents, no purification required between steps).

It may be a good idea to perform in-tube experiments to figure out the initial concentrations and volumes for each reagent and reaction conditions for each step, then scale down to apply them to the first experiment in chip. However, conditions that work in tube do not necessarily work in chip without some further optimization.

C. How Do I Choose What Chip to Use?

Currently, two Single-Cell chips with volumes of 250 nl (Cat. No. 640183) and 350 nl (Cat. No. 640019), are available from Takara Bio for the Shasta system. The recommended target volume per well for these chips are 235 nl and 300 nl, respectively. Choose the correct chip according to the total volume being dispensed per well.

As all samples will be extracted and pooled at the end of the application, each well needs to be identifiable by dispensing unique barcodes (or combinatorial index pairs) as part of the application.

D. What Volume Should I Dispense?

Calculate and choose the right dispense volume according to the number of steps and the concentration of reagents and indexes.

- The predefined volume options for cell, RNA, and DNA dispenses are 35 nl or 50 nl per well.
- The predefined volume options for reagents and index dispenses are 35 nl, 50 nl, or 100 nl per well.

We have guidelines for testing the dispense quality of reagents and acceptable concentrations of some common buffer components; see the tech note at takarabio.com. In cases where a larger volume of a less concentrated component is needed, the dispense volume for cells or other reagents may need to be reduced to accommodate the maximum allowable volume in the nanowell.

The number of steps available for your application depends on the dispense volume of each step and the Single-Cell chip type. Table 6 shows the maximum steps allowed for a 250 nl and a 350 nl chip at equivalent volumes for each step.

Table 6. Recommended volume fill of sample, control, reagent, or indexes added to the sample source plate

Dispense volume per step	250 nl chip (Max. vol.: 235 nl)	350 nl chip (Max. vol.: 300 nl)
35 nl	6	8
50 nl	4	6
100 nl	2	3

The dispense volume for each step can be different. An example is shown in Table 7 (next page).

Table 7. Example application workflow for a 350 nl chip with 7 steps and differing dispense volumes in the steps

Application workflow	350 nl chip
Step 1: Cell dispense	35 nl
Step 2: RT-PCR mix dispense	50 nl
Step 3: Master mix 1 dispense	50 nl
Step 4: Master mix 2 dispense	35 nl
Step 5: Index 1 dispense	35 nl
Step 6: Index 2 dispense	35 nl
Step 7: PCR mix dispense	50 nl
TOTAL: 7 steps	290 nl

E. What Centrifugation Conditions Should I Use for my Chip?

Table 8 lists our recommendations for initial centrifuge parameters after a sample, reagent, or index dispense into the Single-Cell chip.

Table 8. Initial recommendations for centrifuge guidelines by dispense type for custom applications.

Dispense type	Centrifuge temperature	Time	Centrifuge speed
Sample	22°C	5 min	300*g*
Reagent or index	4˚C	3 min	3,220*g*

While developing your custom application, experiment, if needed, to determine the parameters best for your conditions.

F. Tips for Developing a New Protocol for the Shasta System

- If your application allows it, you should consider using nucleic acids to mimic single cells (or nuclei) as your starting material. For example, you could use purified total RNA (at an estimated 2–15 pg of total RNA per cell) or genomic DNA (at an estimated 6 pg of genomic DNA per cell).
- Nucleic acids should be diluted in whatever the cells will be dispensed in (i.e., PBS). We suggest confirming that the reagent used to dispense the sample dispenses without issue.
- We have observed that Second Diluent improves dispense quality of cells, thereby increasing the candidate count, and suggest adding Second Diluent 100X to cells, nucleic acids, and all controls. During initial application development, test that the addition of Second Diluent will not interfere with your reaction, so this recommendation can be implemented. If you cannot use Second Diluent, you may see more doublets than expected from a standard Poisson distribution.
- Recombinant RNase Inhibitor (Takara Bio, Cat. No. 2313A or 2313B) should be included for RNA dispenses, and it should also be included with a cell dispense for any application that will use RNA.
- During the development of an application, you should also monitor your application at various steps. For example, if RT-PCR is the first step, you may want to extract the product off the chip after this stage to look at the profile on a fragment analyzer (e.g., Agilent2100 Bioanalyzer used with the Agilent High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626), the Fragment Analyzer and High

Sensitivity Large Fragment Analysis Kit (Advanced Analytical Technologies, Inc., Cat. No. DNF-493), or an equivalent microfluidic device/kit to see if it looks as expected.

- You can also use an intermediate product to mimic being partway through protocol. To continue with the example above, if you use RT-PCR upstream, you could dispense cDNA you have generated in-tube to the chip, and then test downstream steps, like PCR or ligation.

G. Considerations for Special Types of Cells

If your cells settle quickly, such as cardiomyocytes, you should consider taking advantage of the CELLSTUDIO software "Ask user to remix source plate before aspirating" (pause) option within the application workflow configuration.

Refer to Section III.B.3.b, "Pause Before Aspiration", for how to configure this in the application and Shasta Single Cell User Manual, Section IX.E, "Start Dispense", for an example of how the function would affect the sample dispense step in the workflow.

H. Positive and Negative Control Recommendations

CELLSTUDIO software designates specific wells in the source plate for positive and negative controls. At early stages of application development, we recommend including a negative control (PBS or the dispense reagent your cells will be in are good choices). Water does not dispense well and is therefore not a good negative control.

Once you move to testing your protocol with cells, we recommend including a positive control in addition to the negative control. The purified nucleic acid that you used to develop your application is a good choice for a positive control. Positive control wells can be left empty if there is no proper positive control for an application.

I. Indexing

We recommend combinatorial dual indexing, as CELLSTUDIO software allows for up to 72 forward and reverse indexes. Single indexing would require 5,184 unique indexes, each to be dispensed to the correct location, which is beyond the capabilities of the software.

J. Testing Different Conditions

- When using nucleic acids prior to using cells, you will most likely be dispensing reagents to every nanowell of the dispense pattern.
- For unfiltered dispenses, up to eight different reagent compositions for each reagent can be added to their corresponding source wells for reagent dispensing.
- For filtered dispenses, up to four different reagent compositions for each reagent can be added to their corresponding source wells for reagent dispensing. This can be used to simultaneously test more than one experimental condition in a chip: different reagents or reagent compositions can be added to their corresponding source well for dispensing.

  Example:
  If you wanted to test four different magnesium concentrations in a PCR master mix, and the master mix is Reagent 4, then each test concentration can be added to wells M11, N11, O11, and P11 in the source well plate. CELLSTUDIO software will track which source well was dispensed to each chip nanowell so you can later review the effect of different magnesium concentrations on the results.

- If there is more than one reagent source well, CELLSTUDIO software has been designed to dispense approximately equal numbers of nanowells from each of the source wells.
- Different filter files can be manually created (Shasta Single Cell User Manual, Appendix C) to guide the instrument to dispense different reagent compositions into specific wells. This approach also allows selection of which positive and negative controls receive which reagent composition. Make sure to add reagent to the same location of a new source plate for each filter file using the same step.

  Example:
  One magnesium concentration can be used in each of the source wells, but a filter file can be used to define exactly which nanowells receive that reagent. Reagent4 would be selected for each of the dispenses, but a different filter file selected.
- After library generation and sequencing, the `*_WellList.txt` file (Shasta Single Cell User Manual, Section XI) should be used as the `WellList.txt` for input into Cogent™ NGS Analysis Pipeline (CogentAP).
- After the data is analyzed by CogentAP, you can generate a chip map source well data file (Section V.B.3.e, "Export all data/Export heatmap data") to determine which nanowells (identified by their barcodes) received which reagent.

K. Improving Cell Reactivity

Including molecular biology-grade BSA in the cell dispense may improve cell viability. For RNA-based assays, we recommend Recombinant RNase Inhibitor (Takara Bio, Cat. No. 2313A or 2313B).

Once you move to cells, you will have to be sure that your cells are sufficiently lysed prior to moving to the next step. For many cells, a simple freeze/thaw of the chip—freezing at –80°C for at least 30 min—is sufficient.

L. Filtering Dispenses with Nucleic Acid Samples or Samples that are not Imaged

A filter file is automatically generated by CellSelect software after image processing (Shasta Single Cell User Manual, Section X and Shasta CellSelect Software User Manual, Section III.D). The filter file is commonly used to dispense reagents into the specific wells, although you may also choose not to use one.

There is no filter file generated after dispensing nucleic acids or in cases where no cell scan (and therefore no image processing) is performed. In these situations, if you want to dispense reagents or indexes to only a subset of the nanowells, you will need to manually create your own filter file to target specific wells in the chip and choose to have those dispense steps require your custom filter file when you set up the application.

For more information about creating or editing to customize an existing filter file, see the Shasta Single Cell User Manual, Appendix C.B.

M. Cell Labeling Options

CELLSTUDIO software allows up to three combinations of the three filters (blue, red, and green) for taking cell images. Cells may be stained with dyes such as Hoechst and propidium iodide, labeled with fluorescent antibodies, or by expression of a fluorescent protein.

CellSelect software identifies wells containing signal and generates a filter file based on rules the user chooses to guide the Shasta instrument to dispense reagents into those wells. For example, you may only want cells positive for Hoechst but negative for propidium iodide, but you may use a FITC-labelled CD4 antibody and an RFP-labeled CD8 antibody, use CellSelect software to identify your cell populations, and then select the cells you wish to process.

See the Shasta Single Cell User Manual, Appendix C.A for more details about the structure of filter files, or the Shasta CellSelect Software User Manual (Section III.D and III.I) for more information about how the CellSelect software creates the files.

N. Reagent Parameters

In general, the Shasta instrument can dispense a wide variety of reagents. Takara Bio has established guidelines for common reagent components, including those that are viscous or have low surface tension (see the tech note at takarabio.com), and we will continue to expand testing.

For reagents or combinations not in the tech note, we provide guidelines for testing the dispense quality. One simple test is to dispense a reagent and then use blotting paper on the chip. If the blotting paper has a large damp area (see Figure 86, below), that could indicate the dispense was not successful. If necessary, you may need to make the reagent more or less concentrated and/or dispense a different volume to accommodate for the final concentration in the reaction.

Pure water does not dispense well on the instrument. If you wish to dilute your reactions, we recommend dispensing 10 mM Tris or PBS into the chip. Additionally, you can try adding 100X Second Diluent (Takara Bio, Cat. No. 640016) or a low percentage of PEG8000 to a reagent that does not pass the blot test.

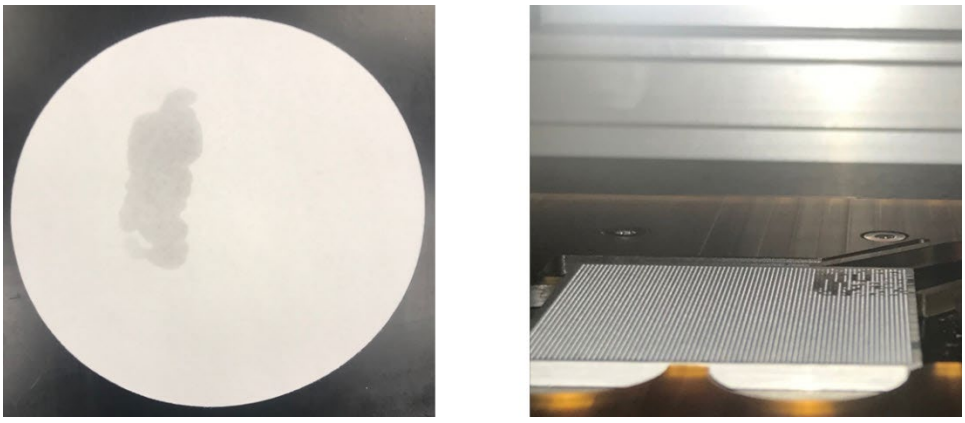

Figure 86. Images from failed dispenses. **(Left)** Blotting paper shows large damp area. **(Right)** Chip shows liquid on top of chip.

O. Maximum Potential Volume of Eluate Extracted from the Chip

The volume of the eluate collected will be less than the calculated maximum volume (formula below). Evaporation, which may occur when the chip is not sealed, decreases the volume. Having more dispense steps will increase the chances of evaporation, further decreasing the final volume.

To calculate the maximum potential volume (in µl) from the chip, use the formula:

$$\frac{(Total\ sample\ volume)\ +\ (Number\ of\ candidates\ x\ (Well\ dispense\ volume))}{1{,}000\ nl/\mu l}$$

where:

Total sample volume = *Number of wells** x *Sample dispense volume per well*
Well dispense volume = *Total volume of reagent dispenses per well* + *Total volume of index dispenses per well*

*Number of wells should include both positive and negative controls in addition to the number of candidate wells.

Example

When doing Shasta WGA:

- The entire chip is used (72 x 72 dispense pattern), so *Number of wells* = 5,184
- *Sample dispense volume per well* is configured as: 50 nl/well
- There are three reagent dispenses (DNA extraction, preamplification, and amplification) of 50 nl each

  (50 nl/well + 50 nl/well + 50 nl/well) = 150 nl/well
- There are two index dispenses (Index1 and Index2) of 50 nl each

  (50 nl/well + 50 nl/well) = 100 nl/well

If 1,000 nanowells are selected as candidate wells (*Number of candidates*), the formula would be:

$$\text{Maximum potential volume (µl)} = \frac{(5{,}184\ \text{x}\ 50\ \frac{\text{nl}}{\text{well}})\ +\ (1{,}000\ \text{x}\ (150\ \frac{\text{nl}}{\text{well}} + 100\ \frac{\text{nl}}{\text{well}}))}{1{,}000\ \text{nl/µl}}$$

and the maximum potential value would be: 509 µl

P. Designing Programs for the Single-Cell Thermal Cycler

To achieve the temperatures in the Single-Cell chip that are required by your protocol (as determined by in-tube reactions), the Single-Cell Thermal Cycler needs to be programmed appropriately. Table 9 is provided to aid in the selection of the temperature setpoints.

- The first column is the desired protocol set point.
- The second column is the actual set point of the cycler.

To reach the desired temperature, the thermal cycler must be programmed with a temporary over (or under) temperature value for the duration indicated in the table.

Example:

If the cycler is currently set at 72°C and you wish to go down to 40°C, the next 2 steps in your protocol would be:

- 34.5°C for 6 sec
- 39.2°C for the duration required by your protocol.

If the cycler is at 20°C and you wish to go up to 40°C, the next 2 steps in your protocol would be:

- 45.2°C for 5 sec
- 39.2°C for the duration required by your protocol

NOTES:

– The initial setpoint and its duration depend on the chip target temperature.

– Table 9 is only valid if the lid temperature is set to 72°C.

Table 9. Thermal cycler lookup table. Table continues over the next three pages.

		Starting temperature lower than target		Starting temperature higher than target	
Chip target (°C)	Thermal cycler set point (°C)	Initial set point (°C)	Duration (sec)	Initial setpoint (°C)	Duration (sec)
16	13.3	NA	NA	12.9	8
17	14.5	NA	NA	13.7	8
18	15.7	NA	NA	14.6	8
19	16.8	NA	NA	15.5	8
20	17.9	NA	NA	16.3	8
21	19.1	NA	NA	17.2	8
22	20.2	NA	NA	18.1	8
23	21.3	NA	NA	19.0	10
24	22.4	NA	NA	19.8	10
25	23.5	NA	NA	20.7	10
26	24.6	NA	NA	21.6	10
27	25.7	NA	NA	22.5	10

		Starting temperature lower than target		Starting temperature higher than target	
Chip target (°C)	Thermal cycler set point (°C)	Initial set point (°C)	Duration (sec)	Initial setpoint (°C)	Duration (sec)
28	26.7	NA	NA	23.4	10
29	27.8	NA	NA	24.3	10
30	28.8	NA	NA	25.2	10
31	29.9	36.6	5	26.2	10
32	30.9	37.5	5	27.1	10
33	32.0	38.5	5	28.0	10
34	33.0	39.4	5	28.9	10
35	34.1	40.4	5	29.8	6
36	35.1	41.4	5	30.8	6
37	36.1	42.3	5	31.7	6
38	37.2	43.3	5	32.7	6
39	38.2	44.3	5	33.6	6
40	39.2	45.2	5	34.5	6
41	40.2	46.2	5	35.5	6
42	41.2	47.2	5	36.5	6
43	42.3	48.1	5	37.4	6
44	43.3	49.1	5	38.4	6
45	44.3	50.0	5	39.4	6
46	45.3	51.0	5	40.3	6
47	46.3	52.0	5	41.3	6
48	47.3	52.9	5	42.3	6
49	48.4	53.9	5	43.3	6
50	49.4	54.9	5	44.3	6
51	50.4	55.8	5	45.3	5
52	51.4	56.8	5	46.2	5
53	52.4	57.8	5	47.2	5
54	53.5	58.7	5	48.3	5
55	54.5	59.7	5	49.3	5
56	55.5	60.6	5	50.3	5
57	56.5	61.6	5	51.3	5
58	57.5	62.6	6	52.3	5
59	58.6	63.5	6	53.3	5
60	59.6	64.5	6	54.4	5
61	60.6	65.5	7	55.4	5
62	61.7	66.4	7	56.4	5

		Starting temperature lower than target		Starting temperature higher than target	
Chip target (°C)	Thermal cycler set point (°C)	Initial set point (°C)	Duration (sec)	Initial setpoint (°C)	Duration (sec)
63	62.7	67.4	7	57.5	5
64	63.7	68.4	8	58.5	5
65	64.8	69.3	8	59.6	5
66	65.8	70.3	8	60.6	5
67	66.9	71.2	9	61.7	5
68	67.9	72.2	9	62.7	5
69	69.0	73.2	9	63.8	5
70	70.0	74.1	10	64.9	5
71	71.0	75.1	10	66.0	5
72	72.1	76.1	10	67.0	5
73	73.2	77.0	11	68.1	5
74	74.2	78.0	11	69.2	5
75	75.3	79.0	11	70.3	5
76	76.3	79.9	12	71.4	5
77	77.4	80.9	12	72.5	5
78	78.4	81.8	12	73.6	5
79	79.5	82.8	12	74.7	5
80	80.6	83.8	13	75.8	5
81	81.6	84.7	13	76.9	5
82	82.7	85.7	13	78.0	5
83	83.8	86.7	14	79.1	5
84	84.8	87.6	14	80.3	5
85	85.9	88.6	14	81.4	5
86	86.9	89.6	15	82.5	5
87	88.0	90.5	15	83.7	5
88	89.1	91.5	15	84.8	5
89	90.1	92.4	16	NA	NA
90	91.2	93.4	16	NA	NA
91	92.2	94.4	16	NA	NA
92	93.3	95.3	17	NA	NA
93	94.4	96.3	17	NA	NA
94	95.4	97.3	17	NA	NA
95	96.5	98.2	18	NA	NA
96	97.5	99.2	18	NA	NA
97	98.5	100.0	18	NA	NA

		Starting temperature lower than target		Starting temperature higher than target	
Chip target (°C)	Thermal cycler set point (°C)	Initial set point (°C)	Duration (sec)	Initial setpoint (°C)	Duration (sec)
98	99.6	100.0	18	NA	NA
99	100.0	100.0	18	NA	NA
100	100.0	100.0	18	NA	NA

Appendix B. Creating an Offline Instructions PDF

The offline instructions file can be a PDF in any format. However, for your convenience, a template file in Microsoft Word DOTX format can be downloaded from takarabio.com; this template is identical to what was used to generate the offline instruction files for the Takara Bio prevalidated applications.

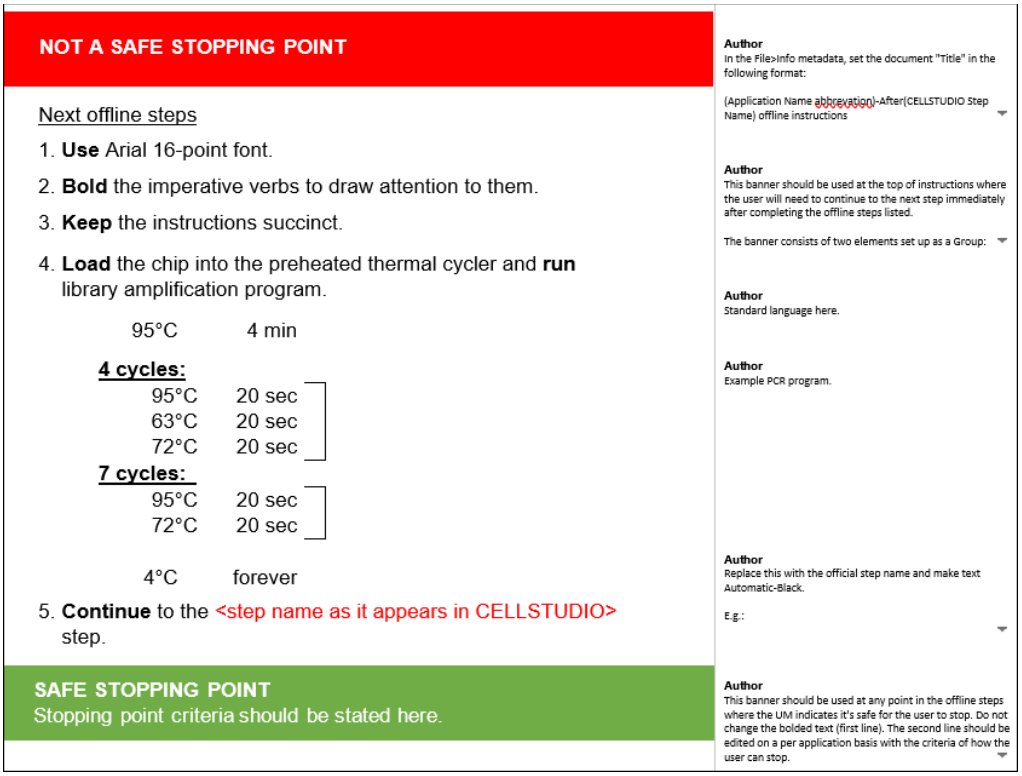

Figure 87. Offline instructions template file snapshot.

The text in the body of the document and embedded comments provide guidelines for how to synthesize a new instructions document following the same style.

Procedure

To generate an offline instructions file, using either the template provided or your own format:

1. Create a file of a type that can be converted to a PDF.
   - Examples of this include MS-Word, Excel, PowerPoint, Rich Text Format, TXT, etc.
   - The file can have any name valid for Windows.

NOTE: The file name will be visible when the offline instructions display in the workflow, so it is recommended to give the file a meaningful or at minimum a work-safe name.

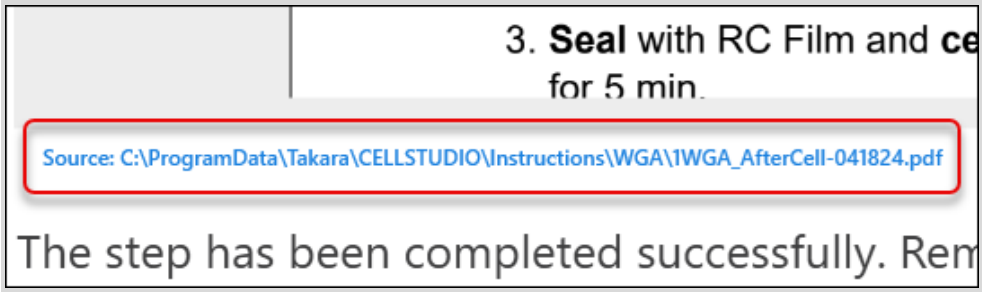

Figure 88. Offline instruction file name, shown on the *Instruction* view after a dispense. The file name is outlined by the red box.

- If using the template (DOTX) file, download it from the website and open it. This will create a new DOCX file without affecting the original file downloaded. Save the new file with your preferred file name somewhere on your computer where you can easily find it later.

2. In your file, write up the directions for users of your custom application.

   While writing the instructions, keep in mind that they will display AFTER the dispense step it will be associated with, e.g., to blot, seal, centrifuge, and freeze the chip after imaging cells. This is an opportunity to communicate any steps in the process you want a user to take prior to the next dispense step.

   - If you used the downloaded DOTX file as a starting point, before saving the finalized instructions, go into the **Review>Comments>Delete** dropdown menu and select **Delete All Comments in Document**. This is recommended so the PDF file created does not contain information unnecessary to executing the instructions you're providing.

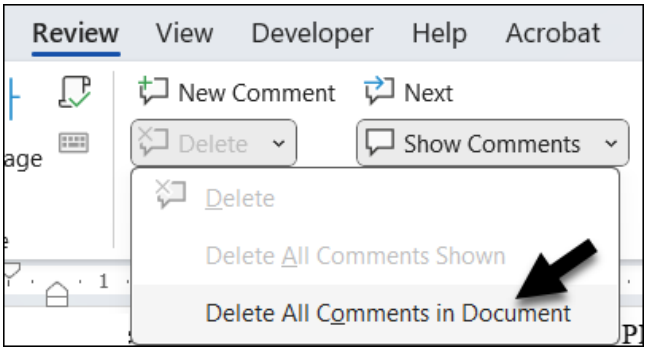

Figure 89. How to delete all comments from an instruction file created from the template.

3. Save the file and convert to a PDF. For more information on how to convert your file to a PDF, please reference the instructions for your original file type.

   - If using the template file in MS-Word, generate the PDF by going into the **File>Save As** menu option and selecting PDF as the file type from the dropdown box (Figure 90, next page).

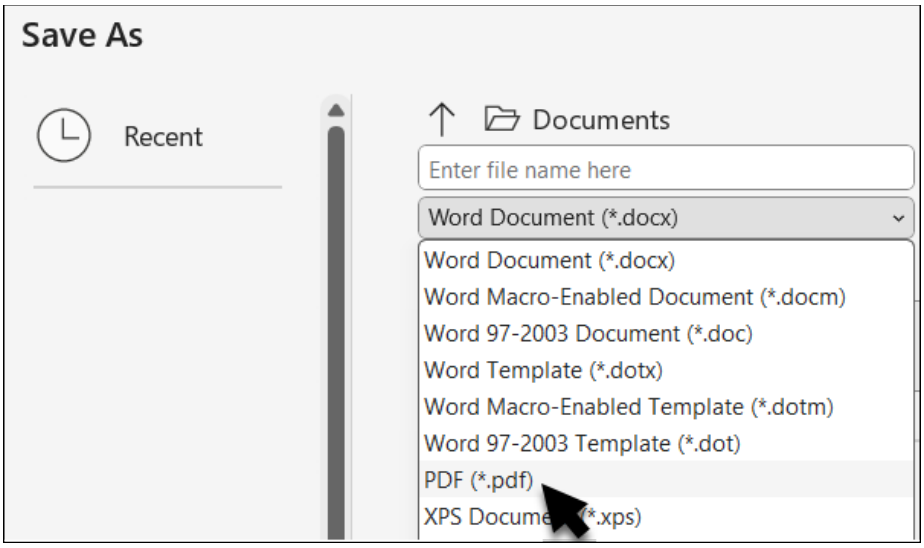

Figure 90. How to save an offline instruction file written in MS-Word as a PDF.

4. Use the resulting PDF file as input when defining the offline instruction for the associated dispense step (Section III.B.5, "Offline Instructions PDF File").

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