GalleryViewer v1.3

New features in v1.3

- Running Napari 0.4.18 and Python 3.11
- Context mode – full images are added to the viewer
- Smooth switching between gallery, multichannel, and full-size images
- Marker tool – fast scoring method in Context mode allows the user to mouse over cells to change the call
- Can filter cells by intensity thresholds
- Cell boxes – a visual aid for object detection can be toggled on/off
- Customization for scoring decisions, colors, and key mappings
- Images (single cell or full gallery) can be exported to PNG files or the clipboard
- Counts for each scoring type are tracked and displayed to the user

Dependencies / Build Info

GalleryViewer is written in Python and driven by the open-source Napari viewer. The entire codebase is publicly hosted at https://github.com/richierip/CTC-Gallery-Viewer. This project has many dependencies (see the readme on GitHub), so working with the developer version hosted here may be difficult. Instead, the project utilizes an application wrapper which creates a distributable zipped folder. Users can unzip this folder and click on the *exe* file within to start the application, avoiding the need to replicate the complex Python environment. The application was tested on Windows 10 and should work with any Windows version. There are no hardware requirements but performance may take a hit on underpowered computers.

Starting the application

Requirements

Before running the app, there are two required pieces of data that **must** be provided.

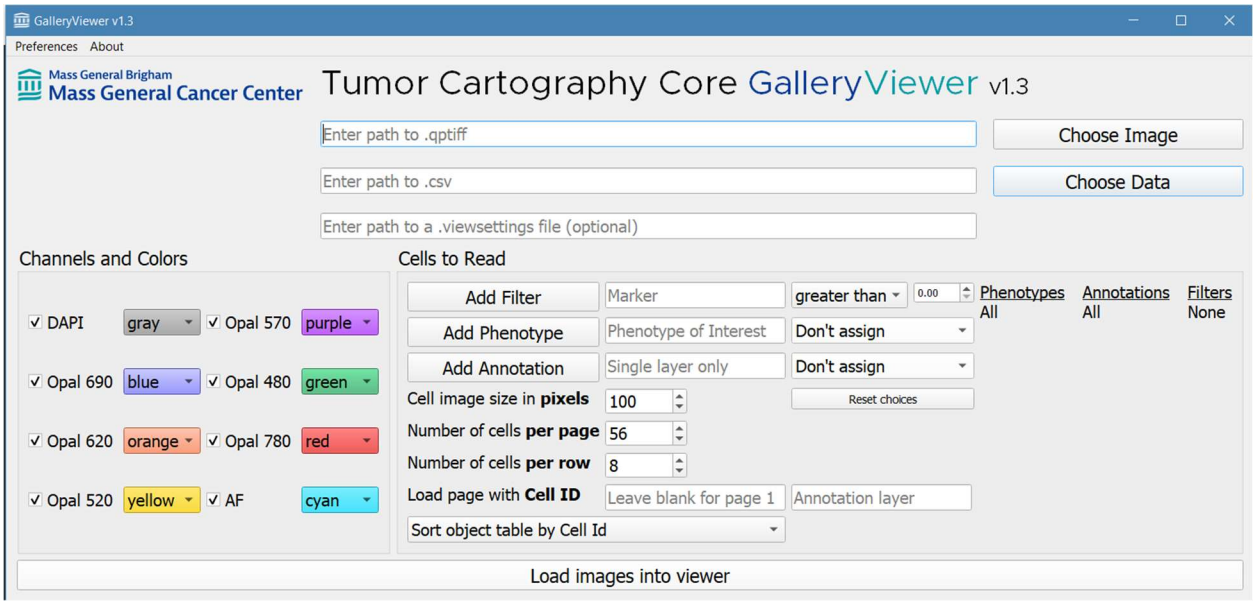
1. A multichannel TIF image. Testing was done on *.QPTIFF*s imaged on the Akoya Polaris, and on *.TIF*s created from these same images processed using inForm software. **Currently, the program is only guaranteed to function properly when given an image that was scanned using the MOTIF scan settings.** Users should be cautious when using images that do not fit these criteria. After selecting an image, the program will inform the user if there is a potential issue.

2. A data file in *.csv* format. This file may be easily exported from results generated in HALO. However, there are only a couple true requirements. The file must be formatted such that each cell is represented as a row, and the following column headers are present: *Object ID, XMin, XMax, YMin, YMax*.

This application **does not** perform any segmentation, classification, or phenotyping, so this work must be done beforehand (likely by HALO).

Parameter selection

The program will open into the Parameter Selection GUI, through which you will start the main Napari-hosted application. Many of these settings are not currently modifiable after launching Napari, so you may have to restart the program if you wish to change something. Press the ***Load images into viewer*** button once you have entered the desired settings to launch the viewer.



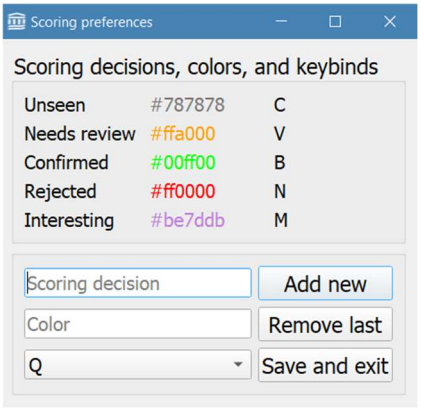
Selecting an image

Use the ***Choose Image*** at right to open a file explorer dialog, and navigate to your file. Alternatively, you may paste the file path into the entry both and then click the button. Selecting an image will automatically fill out the channel selection area with the names of the fluorophores present in your image. The viewer will also attempt to read any colors that are assigned to the channels in the data, otherwise assigning a random color by default.

Selecting an object data file

Use the ***Choose Data*** at right to open a file explorer dialog, and navigate to your *csv* file. Alternatively, paste the file path to the data in the entry box and click the button. This file's headers will be examined for compatibility, and will inform the user about the results. Files without *Object ID, XMin, XMax, YMin,* and *YMax* columns will be rejected.

Changing scoring decisions



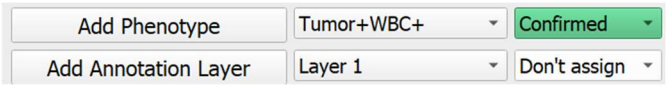
To open the dialog window, click on ***Preferences -> Modify scoring decisions and colors***. Write a name for a scoring decision and enter a color in the appropriate entry boxes and choose a keymapping for the keyboard shortcut. Then click on ***Add new***. The color can be in hexadecimal format (e.g. *#af01b7*), or as a human-readable name (e.g. *fuchsia* or *sky blue)* for most colors. All default scores may be removed, except for *Unseen*. Make sure to click ***Save and exit*** to lock in your changes.

Choosing channels and colors

Next, navigate to the lower left quadrant of the window. Check any boxes next to channels that you *want to add* to the viewer, and uncheck any that you do not. Also select corresponding colors for each channel using the dropdown menus associated with each channel. Duplicate colors are allowed but not recommended.

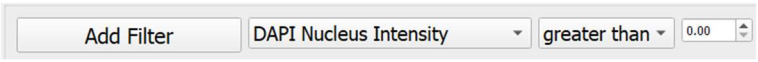
Filtering your list of cells before launching the viewer

You may want to look at cells from a subset of phenotypes or annotation layers in the data, or apply a threshold using average intensity. After selecting a valid Object Data file, the annotation layers (if any) and phenotypes present in the data will populate the dropdown menu, along with intensity columns. You are free to select which ones you wish to look at from the menu. Note that not adding any choices will cause the viewer to load the full list of cells into its pages.



You may also choose to immediately assign a scoring decision to a phenotype or annotation layer (leave the *Don't assign* selection alone to not modify these cells). Your choices will populate to the right of the dropdown menus. Use the *Reset choices* button to clear these settings. Note – cells belonging to *any* of the selected categories will be pulled into the Viewer. There is currently no option to bring in a phenotype only if it is also in a certain layer.

For intensity filtering, you can choose a fluorophore and region to filter on, set the numerical threshold, and choose whether the filter will be *greater than* or *less than.* Several filters can be applied at once



If you have applied several kinds of intensity filters and phenotype / annotation layer criteria, it is possible that there are no cells that satisfy all of them. If this happens, the Viewer will inform you and fail to open.

Other parameters

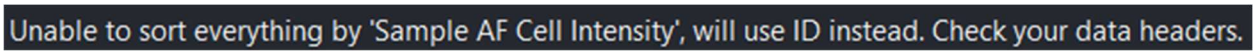
- ***View Settings*** (optional) – enter a path to a *.viewsettings* file (exported from HALO) to use as the default in the viewer. This is highly recommended.
- ***Image size in pixels*** – this adjusts the size length of the square image punched out around each cell in the viewer
- ***Load page with this Cell ID***: use this field to enter the *ID number* and *annotation layer* (if present in the data) of a specific cell. The viewer will load the page containing this cell.
- ***Number of cells per page*** – adjust this to control how many cells appear at once
- ***Number of cells per row*** – how many cells will load in a row before wrapping to the next
- ***Sort object table*** – *global sorting* of the *csv*. The first page will have cells with the strongest signal in the chosen channel, while the last page has the weakest signal.

Working inside the Napari GUI

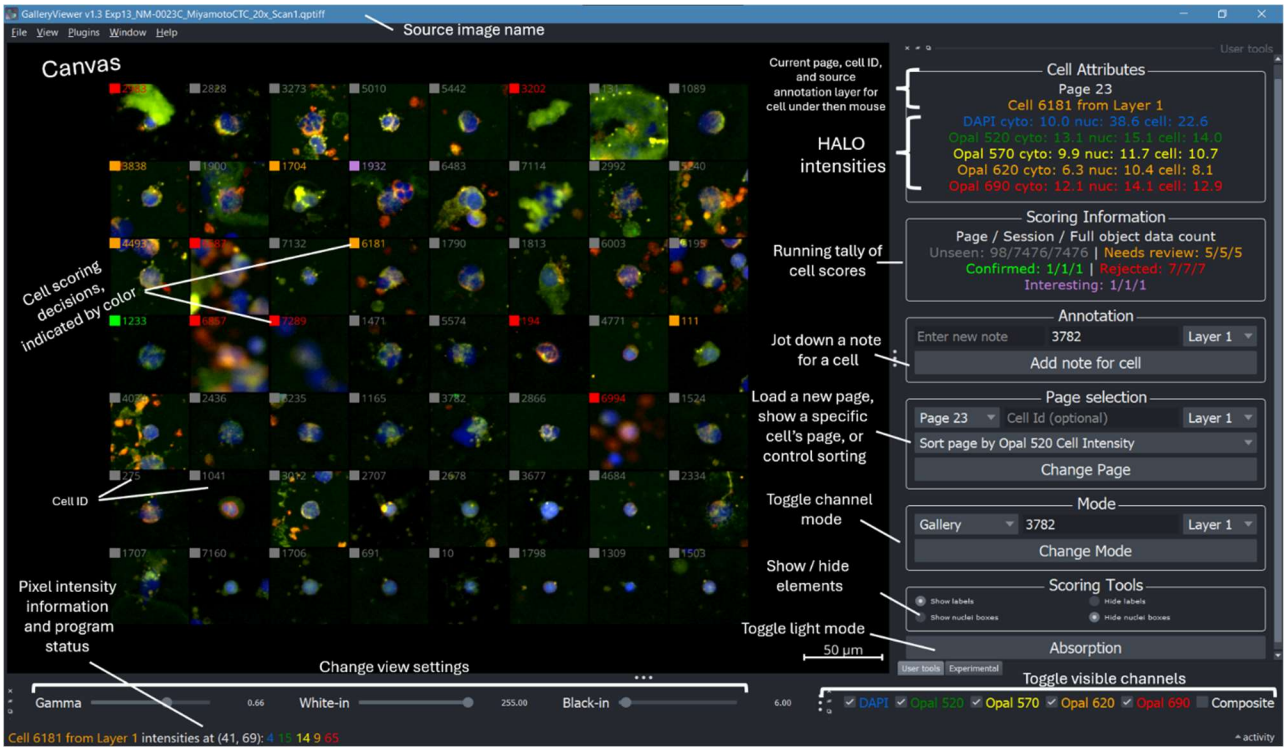
Canvas controls

In Napari, the canvas is the black space on top of which your images are rendered. You are free to move around in the X and Y directions by using the arrow keys. Left-clicking and dragging will also move the canvas. Holding ***Control*** or ***Shift*** when using the ***arrow keys*** will zoom in or out. Using the mouse wheel, or right-clicking and dragging the canvas, will also zoom in and out. The bottom of the canvas contains docked controls for view settings and channel settings, while the right-hand dock contains controls for annotation, page selection, viewing mode, and overlay toggling. All docked controls are movable and may be manipulated by clicking and dragging the three vertical dots for any parameter set (not recommended – it is mostly just annoying).

Make sure to pay attention to the lower left corner where the program occasionally outputs some information regarding a user action, like saving or loading a page. Below is one such example.



Evaluating cells



The developer team has provided several tools to assist in decision making. Users can make use of view settings and channel toggles to adjust the appearance of all cells. **Multichannel mode** (see the *View all channels at once* section) allows for the side-by-side viewing of all channels separately. **Context mode** allows the user to browse the original image, centered at a chosen cell, to inspect a larger spatial context. **Global and local page sorting** can be used to control which cells appear together on a page.

Users can see raw intensity data read out in the lower left corner when hovering over image pixels in the canvas. These numbers *are not affected by changes to view settings*.

Scoring cells and saving to file

Scoring is performed by hovering the mouse over a cell and pressing the space bar to cycle through choices. By default, there are five scoring decisions: *unseen, needs review, confirmed, rejected,* and *interesting*. The **C/V/B/N/M** letter keys may also be used to immediately select one of these options. These selections have the corresponding colors displayed around the composite image: gray, orange,

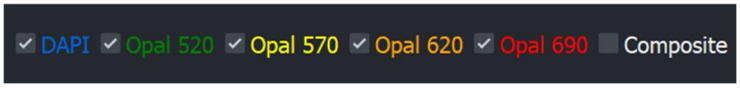
green, red, and purple, respectively. The user is free to change all of these scoring decisions, hotkeys, and colors - except for *unseen*, which is applied to every cell initially.

After you are satisfied with the scoring performed on the current page, pressing the **S key** will save your decisions back to the object data file. The program will create new mutually exclusive phenotype columns in the *csv* for each scoring decision and assign all cells to *unseen* as a default. Saving will also happen automatically when the program closes. The source *csv* file **must not be currently opened by the user** in order to save. If the file is open, you will see the message picture below in the lower left corner status label.

Toggling visible channels

The check boxes arranged in the bottom right of the application are used to toggle which channels are added to the **composite image**. Keyboard shortcuts will be automatically generated to toggle these checkboxes: the **1 key** will toggle the first channel, **2** will toggle the second, and so on. The *Composite* checkbox is used to display all channels at once, ignoring which of the other channel boxes are checked.

The check boxes are *also* used to determine which channels should be affected by changes to the view settings sliders

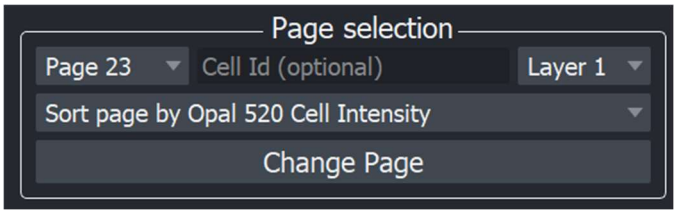


Changing view settings

View settings can be adjusted by clicking and dragging three sliders at the bottom of the page. Exact values may also be entered by clicking and typing a valid number into the field directly. The valid range for ***gamma*** is 0.0-1.0, and the valid range for contrast limits (***White-in*** and ***Black-in***) is 0-255. Note that changing these values does not affect the underlying image data, but rather how it is displayed.

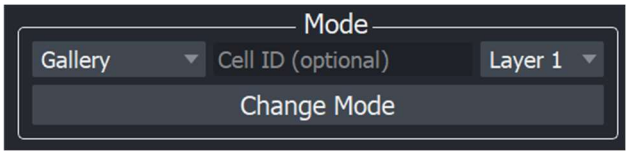
The changes you make using the sliders or associated entry boxes will only affect channels that are currently checked. Changes will affect ***all checked channels***, so if you want to apply different settings for each channel you will have to cycle through them. The ***Composite*** check box is not taken into account for this process. Click ***Restore defaults*** to reset the view settings to what the Viewer loaded at the start. This will be whatever your custom *.viewsettings* file specifies, or value of gamma = 0.5 and contrast limits = 0-255 if no *.viewsettings* file was given.

Page Selection



Use the appropriate controls on the right-hand dock to load a new page of images. Click on the ***Page*** menu item to expand the context menu, and then select another page. The sorting option menu can be used to *locally* sort the page, i.e. this sorting only applies to the current page you are viewing and does not affect the global sorting of all pages. Lastly, if you enter an *ID number* into the ***Cell Id*** field, the program will ignore the page you have selected with the menu item above and load the page containing the cell you are looking for. A cell's ID number will autofill this field if you left click on it, which can be useful when you are in Context Mode.

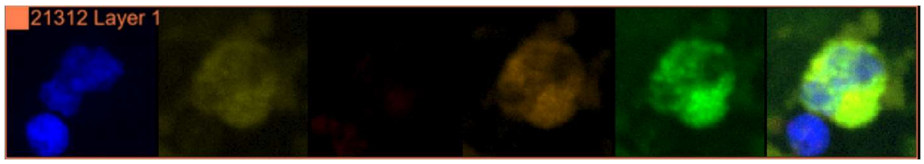
Channel modes



The application has three modes that you can switch between by selecting a mode from the dropdown menu and pressing the ***Change Mode*** button. Choosing a *Cell ID* is optional for switching between Gallery and Multichannel, but required for Context. Left clicking on a cell will autofill the *Cell ID* field for you. There are also keyboard shortcuts for switching to and from **Multichannel mode** and **Context mode**, listed below.

Composite mode displays just one combined image for each cell, although you may use the channel controls at the bottom right of the application (see the ***Toggling visible channels*** section) to modify which channels appear. The Viewer loads into Composite mode by default.

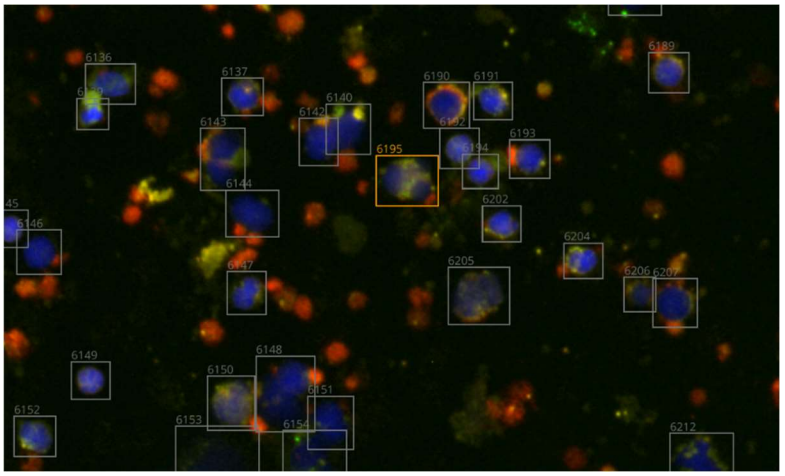
Multichannel mode displays each image channel side by side, so that they can be compared simultaneously. In this mode, all images in a row correspond to different channels of the same cell – the last image on the right will show the composite. ***Shift+click*** on a cell in Gallery mode to shift to Multichannel mode centered on that cell. You may only do this if the cell is in the current page, which is often not the case when in Context mode. If that happens, change to that page and try again.



Context mode shows the spatial context around a cell by showing the original full-size image centered at a chosen cell. ***Control+click*** on a cell to show that cell in spatial context.

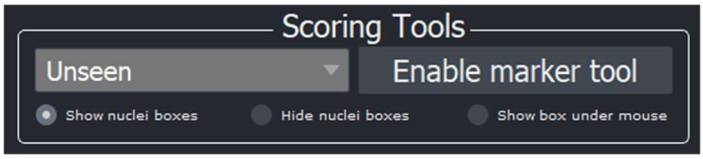
You can change scores for all cells in any of these three modes.

Context mode tools



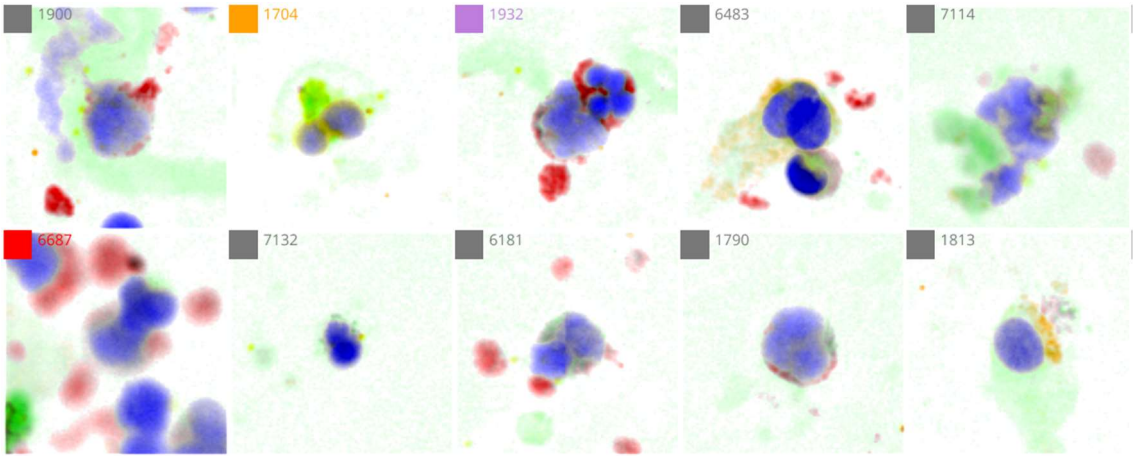
You are free to assign scoring decisions in Context mode with the same keyboard shortcuts, or with the spacebar to loop through all options. You can change the ID overlay with the radio button options in the *Scoring Tools* section of your toolbar. *Show nuclei boxes* and *hide nuclei boxes* will toggle this overlay on and off. *Show box under mouse* will display the ID overlay for the nearest cell to the mouse only.

Use the ***marker tool*** to score cells faster. When enabled, the marker tool will change cell scores to a chosen decision to cells as you move your mouse over them. Use the dropdown menu so select a scoring decision, and then enable the tool to start. The shortcut for enabling this tool is ***Shift+[x]***, where ***[x]*** can be any scoring decision keyboard shortcut button.



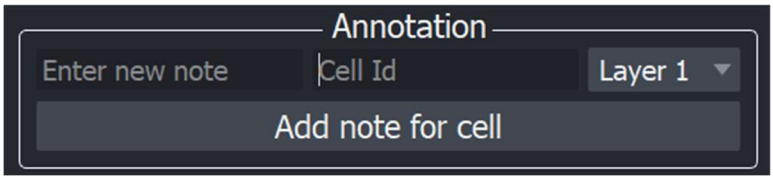
Toggle light mode

Absorption (light) mode may be toggled with the button at bottom right, or with the ***A key***. Dark mode (default) mimics HALO's display very well when using the same view settings, but light mode does not seem to match HALO's color mixing. Use caution with light mode.



Adding a custom note

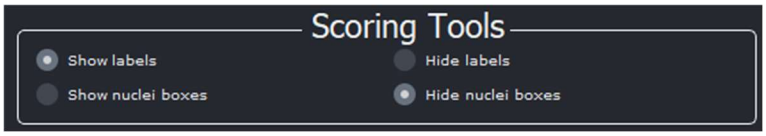
You can add a note for any cell that will appear in near the cell ID readout label in the top right area. Notes will appear under the *CID* label, as pictured at right, when you hover over the corresponding cell. These noted will be saved to your *csv* alongside your scoring decisions.



To add a note, enter some text into the first entry box, enter the number of the cell you wish to annotate into the second box, select an annotation layer (if applicable) and click the button. ***You can autofill the Cell ID entry box*** by left clicking on any cell in the Viewer.

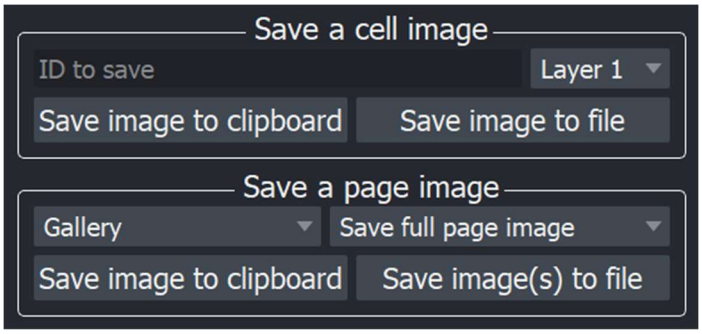
Show or hide the scoring overlay

The colored decision overlay that is rendered on the canvas may be hidden by toggling the appropriate radio buttons on the right tool pane. The ***H*** key can be pressed as a shortcut for this action as well.



Exporting images

First, display the export tools by clicking on the *Export data* tab in the lower part of the toolbar.

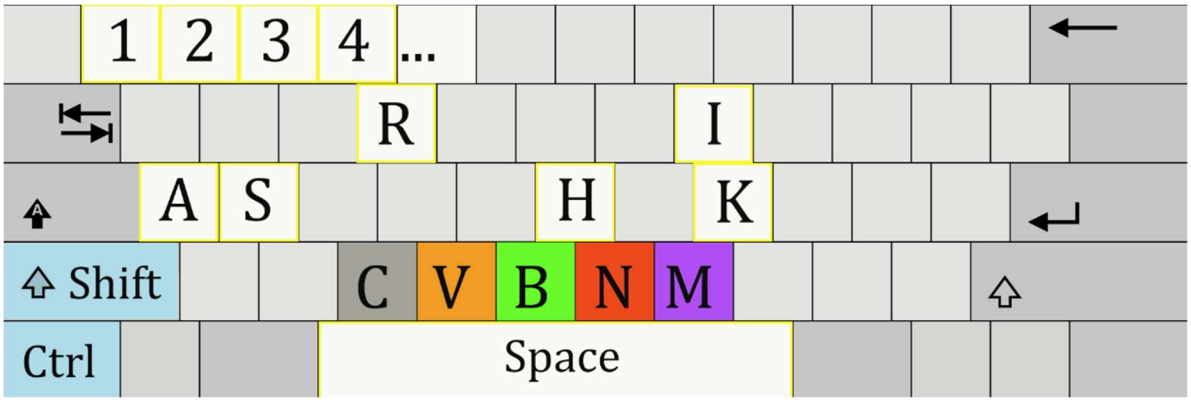


Save a cell image – export an image for just one cell in the current page. Left click on the cell image to autofill the *ID* and *annotation layer* field (if present), and then either save the image to the clipboard or to a file. View settings and currently visible channels will be taken into account when exporting. If if Multichannel mode, the exported image will also have it's channels split. Saving to clipboard means that the image can be pasted into a slide deck or document while saving to file means that a *.png* image will be written to the physical location of your choosing.

Save a page image – export all the cells in the current page to an image. First choose Gallery or Multichannel to pick whether each cell will have all channels merged or split. If *Save full page image* is selected, the full gallery of cells will appear in one image. When saving to file, you will save just one *.png*. If *Save each cell separately* is selected, you will be prompted to choose a destination folder, and each cell will be saved as a separate *.png*. This option cannot be used with *Save image to clipboard*. View settings and currently visible channels will be taken into account when exporting.

Keyboard shortcuts

There are several built-in keyboard shortcuts that user can employ to speed up their work. The diagram below shows which keys are assigned a function.

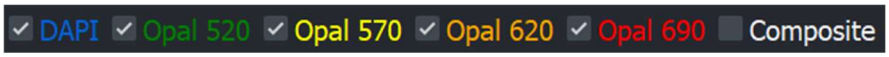


Arrow Keys

Use the arrow keys for panning around the canvas. Hold **Ctrl** or **Shift** to zoom in (right/up arrow) or out (left/down arrow).

Numbers

The number keys are assigned dynamically depending on the number of channels the user has requested to view, and may not refer to the same channel each time. Pressing a number will toggle the corresponding channel on or off, depending on the current state. If the user has selected the channels that appear in the image below, ***1*** would toggle DAPI, and ***4*** would toggle autofluorescence, for example.



Space bar and C/V/B/N/M keys (default scoring)

When the mouse is hovered over a cell, pressing the **space bar** will cycle through the different scoring choices, represented by their assigned colors. In the default scoring scheme, the five letter keys described below assign a value directly without cycling.

The **C** key assigns a value of *unseen*, represented by a gray color. The **V** key assigns a value of *needs review*, represented by an orange color. The **B** key assigns a value of *confirmed*, represented by a green color. The **N** key assigns a value of *rejected*, represented by a red color. Finally, The **M** key assigns a value of *interesting,* represented by purple. Scoring decisions will be written to the appropriate column in the object data *csv* file in the row of the corresponding cell. To modify the default scoring values, see the ***Changing scoring decisions*** section.

Shift + Any scoring key

In Gallery / Multichannel mode – assigns the chosen scoring decision to all cells in the page

In Context mode – Activates the marker tool with the chosen scoring decision assigned. For a more detailed description, see ***Context mode tools section***

Shift + Click
Hold *Shift* and click on a cell in Gallery mode to quickly change to Multichannel mode

Control + click
Hold *Control* and click on a cell in Gallery mode to quickly change to Context Mode

R key
Reload the initial view settings. If you imported view settings from a file before launching the viewer, these will be reloaded. Otherwise, default values of 0.5, and [0,255] will be chosen for gamma and the contrast limits respectively.

I key
Toggle interpolation settings for the displayed images. Interpolation smooths pixels in the image, simulating finer detail. HALO uses interpolation by default, but GalleryViewer does not.

Control + I
Toggle the pixel intensity tooltip on or off, allowing values to be shown close to the mouse's current position on the canvas

K key
The **K** key is used to return the viewer to the initial x, y coordinates with a neutral zoom value.

A key
Toggle light/dark mode. Dark mode (default) mimics HALO's display very well when using the same view settings, but light mode does not. Use caution with light mode.

S key
Manually save the scoring information to your object data *csv* for the cells on the current page. For large object data files, this may take some time. Your scores are also saved automatically upon exiting the program by default.

H key
Show or hide the colored scoring overlay.

Shift + H
Show or hide boxes around the Cell Object of interest

Troubleshooting

Check your input

Make sure that your image and your data file have the correct properties as specified in the ***Starting the application*** section. Check that your parameters are spelled correctly, i.e. congruent with the column header of your *csv,* and that the other required columns are present. Please make the

developer team aware of any images that fail to open. Pay attention to any warnings that the Viewer gives after selecting an image or object data as well.

Resetting saved parameters

The program will remember the choices you have made in the selection GUI. Occasionally, something might go wrong. If the program fails to start correctly on input that it used to handle, this may be of use. Find the ***Reset*** option under the ***Preferences*** tab, or press ***Ctrl+R*** in the Parameter Selection GUI, and then restart the program.

Logging system

The program will automatically record a log if there is an unexpected error that occurs. If the developer team does not have access to the computer that hosts your copy of the program, you will need to send over the file yourself. Note that errors may or may not result in a crash, e.g. images may fail to load but Napari stays open. The parameters passed into the application will be recorded in this log, along with other relevant information that the developer team should be able to use to create a patch. The logs will be placed into the '*runtime logs*' folder inside the main application folder, and the date and time of the error will be in the title of the file. Users can access this folder under the ***Preferences*** tab or by pressing ***Ctrl+E*** in the Parameter Selection GUI.

Known Bugs

- Images not scanned with MOTIF settings may not work
- Absorption mode in the viewer does not exactly match the appearance in HALO for the same view settings
- Boxes draw around Halo detected cell objects may be *slightly* different than appears in HALO due to a scaling issue. Inform the dev team if this is an issue
- The toolbar does not always expand enough horizontally when the Viewer loads. You can do this manually by clicking and dragging the three vertical dots

Features to come, possibly

- Upgrade the image reading code to create compatibility for more images
- Custom colormaps for channels (e.g. can pass any hex code)
- Color selector in docked viewer controls
- Set scroll limits (don't allow X axis movement)
- GraphQL integration for direct communication with a HALO instance
- Allow user to load cells from multiple images at once
- Dynamic user control for image size per cell after Viewer has started