GalleryViewer v1.2.1

New features in v1.2.1

- Absorption mode (light mode), and a better color mixing algorithm
- Cell intensities from object data are displayed in the viewer
- Can import view settings from a *.viewsettings* file (exported from HALO)
- Can check image and object *csv* metadata before starting the program
- Can handle and filter data with multiple annotation layers. Can also filter by phenotype
- User control for interpolation
- Object data can have custom marker names (must include `Opal###` in the name somewhere)
- Optimized loading and saving code to minimize read/write operations
- Can reimport scoring decisions to HALO (unless the export already combined multiple jobs)

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 for most users. To avoid headaches, 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, circumventing 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.

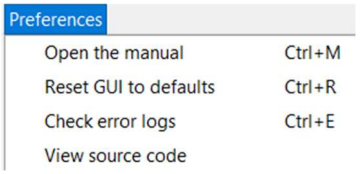
1. A multichannel TIF image. Testing was done on *.QPTIFF*s imaged on the Akoya Polaris, and on *.TIF*s created from these same images processed using inForm software. **Currently, the program is only guaranteed to function properly when given an image that was scanned using the MOTIF scan settings.** Users should be cautious when using images that do not fit this criteria. One way to check compatibility is by using the *Fetch image metadata* option

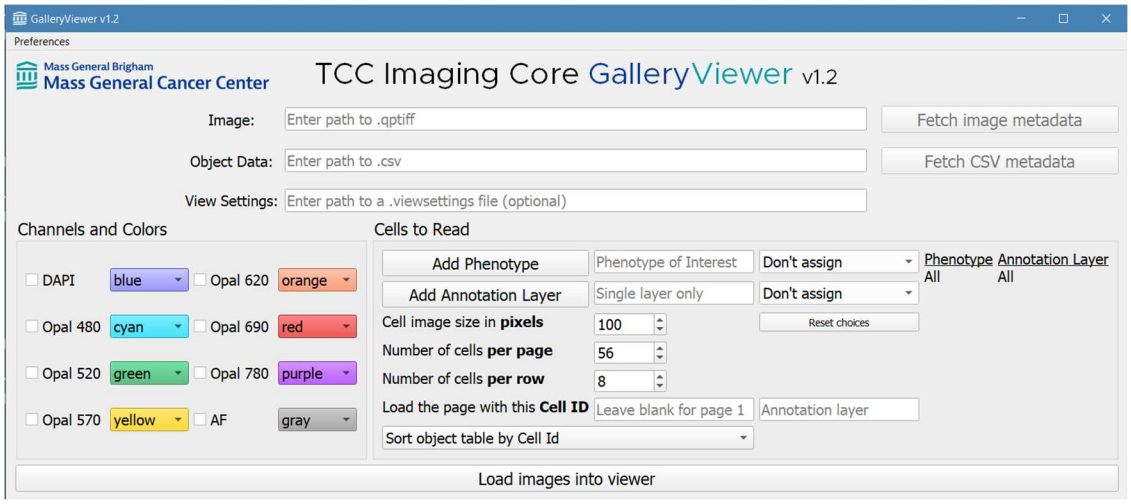
2. A data file in *.csv* format. This file may be easily exported from results generated in HALO. However there are only a couple true requirements. The file must be formatted such that each cell is represented as a row, and the following column headers are present: *Object ID, XMin, XMax, YMin, YMax*.

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

Parameter selection

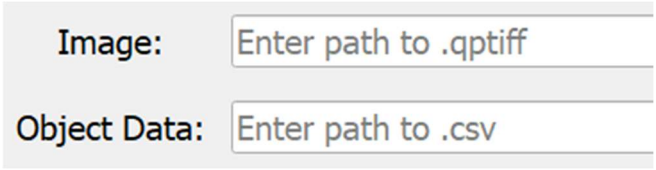
The program will open into the Parameter Selection GUI, through which you will start the main Napari-hosted application. Many of these settings are not currently modifiable after launching Napari, so you may have to restart the program if you wish to change something. Press the load button once you have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

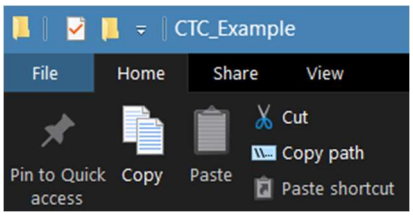




Entering paths to your data

As mentioned above, you will need to provide two files: an image, and a *csv*. The paths of these two files need to be entered into the appropriate data field. The easiest way to get the path to your image is to navigate to the containing folder in the Windows File Explorer, select your image/*csv*, and click the **copy path** button in the top left area of the window.



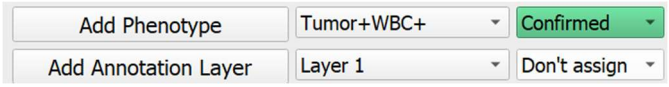


Choosing channels and colors

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.

Choosing phenotypes or annotation layers

You may want to look at cells from a subset of phenotypes or annotation layers in the data. Use the *Fetch CSV metadata* button to load your data's phenotypes, and then select which ones you wish to look at from the menu. 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.



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.

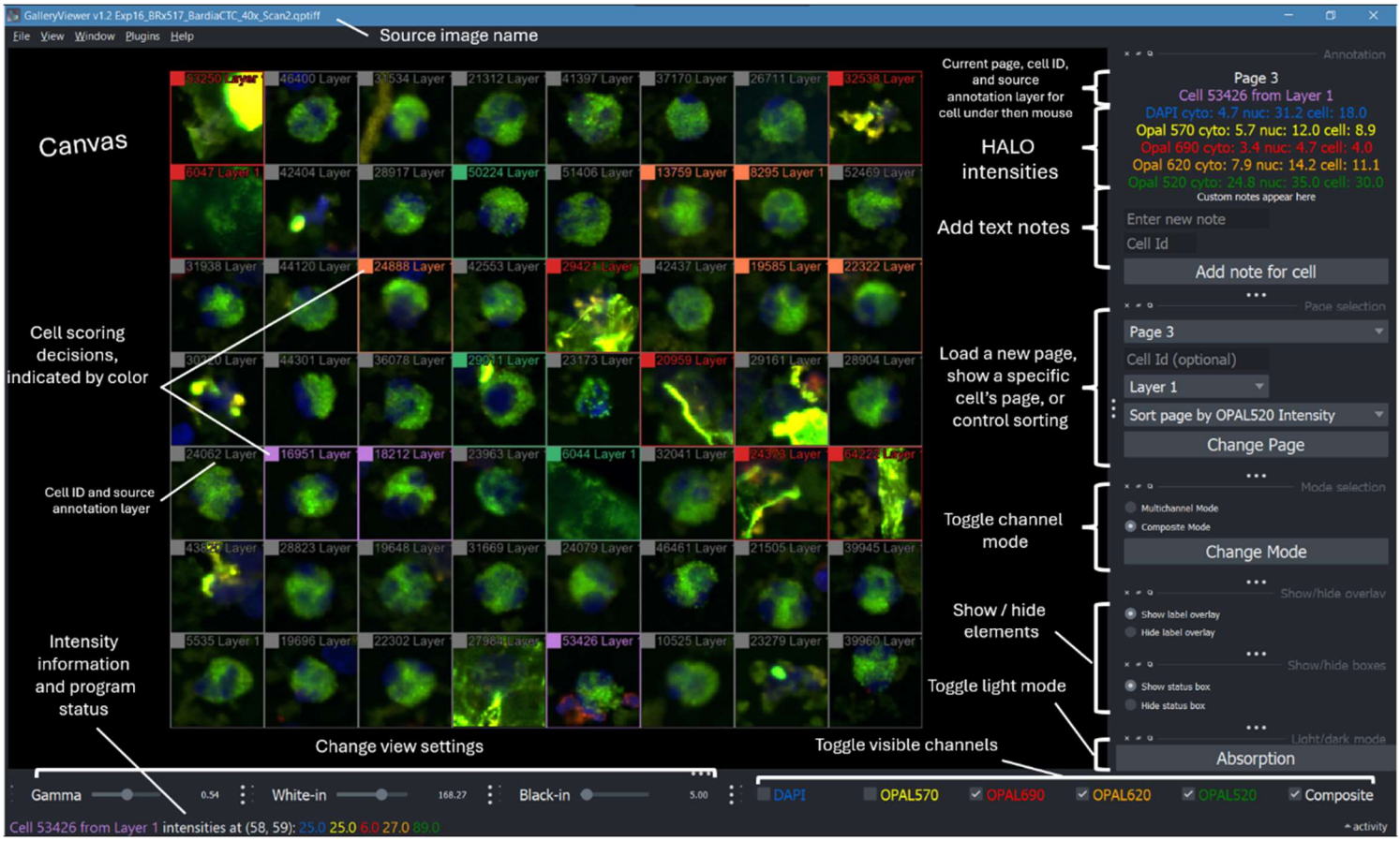
Working inside the Napari GUI

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 **Ctrl** 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 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.

Unable to sort everything by 'Sample AF Cell Intensity', will use ID instead. Check your data headers.



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. **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

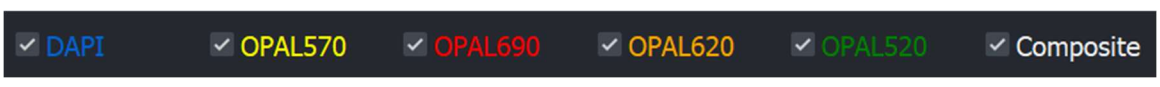
Scoring is performed by hovering the mouse over a cell and pressing the space bar to cycle through choices. The **C/V/B/N/M** letter keys may also be used to immediately select one of the four options (see the *Shortcuts* section). There are five choices: *unseen, needs review, confirmed, rejected,* and *interesting*. These selections have the corresponding colors displayed around the composite image: gray, orange, green, red, and purple, respectively.

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 you switch pages, or change modes. 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 check boxes are *also* used to determine which channels should be affected by changes to the view settings sliders



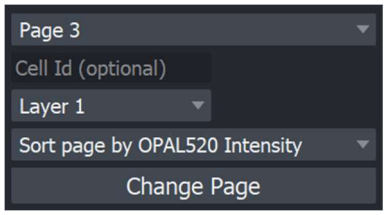
Changing view settings

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 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 channels you will have to cycle through them. The *Composite* check box is not taken into account for this process.

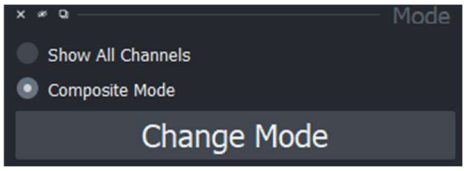
Page Selection

Use the appropriate controls on the right-hand dock to load a new page of images. Click on the "<Phenotype> page <page number>" 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.



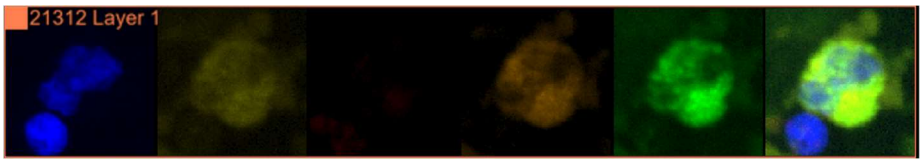
Channel modes

The application has two modes that you can switch between by selecting the appropriate radio button and pressing the *Change Mode* button.



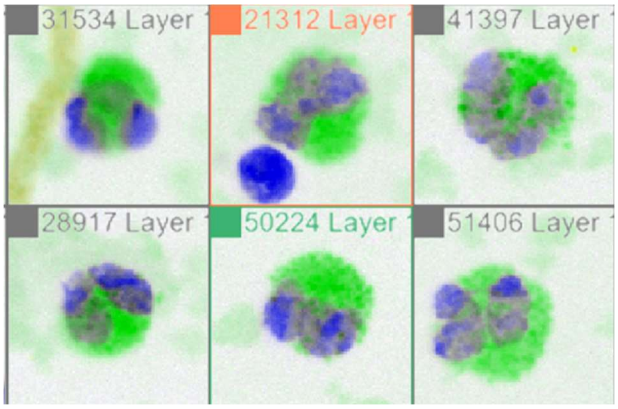
Composite mode displays just one combined image for each cell, although you may use the channel controls at the bottom right of the application (see the *Toggling visible channels* section) to modify which channels appear.

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 will show the composite.



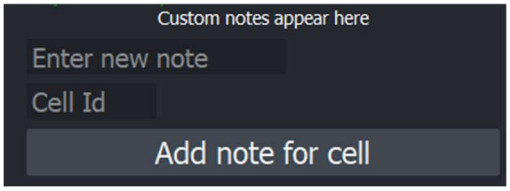
Toggle light mode

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. 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.

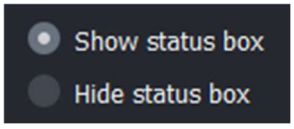


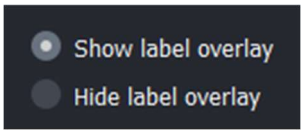
To add a note, enter some text into the first entry box, enter the number of the cell you wish to annotate into the second box, and click the button. You can autofill the Cell ID entry box by left clicking on any cell in the Viewer.

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.

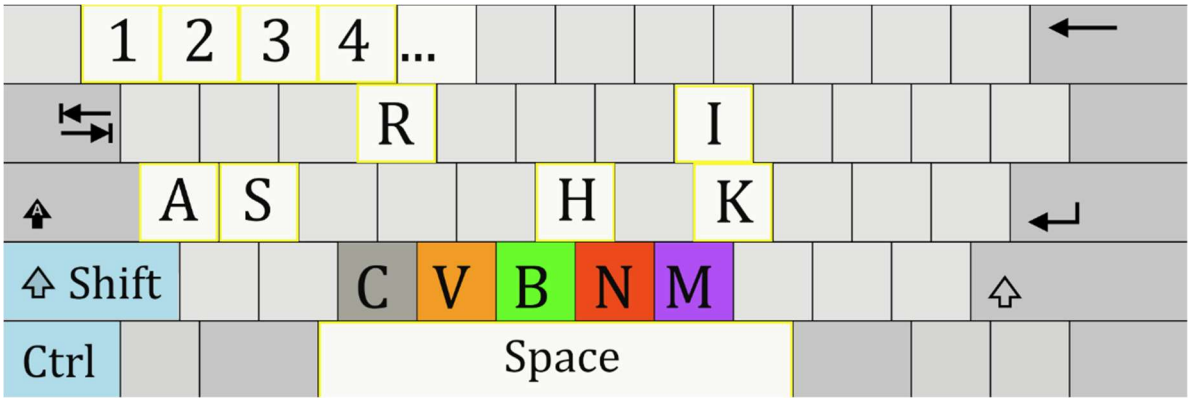
The color boxes can be hidden with a different set of buttons, leaving the cell ID display alone. The shortcut for this toggle is **Ctrl+H**.





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

When the mouse is hovered over a cell, pressing the **space bar** will cycle through the different scoring choices, represented by five colors. The five letter keys, described below, assign a specific 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. Holding the **Shift or** Ctrl keys before pressing one of these keys will assign its value to *every cell in the current page*. Scoring decisions will be written to the appropriate column in the object data *csv* file in the row of the corresponding cell.

R KEY

The **R** key is used to 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

The **I** key is used to toggle interpolation settings for the displayed images. Interpolation can be used to create a smoother image, simulating finer detail. HALO uses interpolation by default, but GalleryViewer does not.

K KEY

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

A KEY

The **A** key is used to 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

The **S** key is used to 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 saved automatically when changing modes, loading a new page, and upon exiting the program.

H KEY

The **H** key is a shortcut used to show or hide the colored scoring overlay. **Ctrl+H** can be used to *only* show or hide the colored borders of each cell's image, leaving the cell ID text alone.

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. Make use of the *Fetch image metadata* and *fetch csv metadata* buttons, which can provide a warning message with details

Resetting saved parameters

The program will remember the choices you have made in the selection GUI. Occasionally, something can go wrong here, and there is currently not a GUI option to reset this. If the program fails to start correctly on input that it **used to handle,** this may be of use. 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 by pressing **Ctrl+E** in the Parameter Selection GUI.

Known Bugs

- View setting sliders (gamma, e.g.) sometimes do not expand properly on startup. The developer team has found that toggling the window resize key  found in the upper right of the program window fixes this issue. You may use the three dots on the side to expand them manually otherwise.
- Images not scanned with MOTIF settings may not work
- Saving your scores while the *csv* is open will not work
- Absorption mode in the viewer does not exactly match the appearance in HALO for the same view settings

Features to come, possibly

- Upgrade the image reading code to create compatibility for more images
- User added scoring labels and colors, with choice of hotkey
- Color selector in docked viewer controls
- GUI elements – clean up to avoid confusion
- 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
- Display a box around the HALO-detected nucleus for each cell, with a *hide* toggle