hcat

v2.0.0.dev20240812

User manual

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1. INTRODUCTION

hcat is a platform solution for cochlear histopathology analysis, bringing deep learning analysis to auditory neuroscience. Right now, it is simply a graphical user interface which lets you: 1) import multiple images of a single cochleae, 2) predict cell locations and type with a deep-learning hair cell model, 3) assign them a best frequency, and crucially 4) verify and correct the predictions.

This software is in active development, with new features planned. Currently, hcat is limited to 2D hair cell detection and classification with a custom pre-trained model. In the future, we will allow users to fine tune custom models, enable automatic prediction of synapse puncta, and tools for automating ABR threshold analysis.

2. QUICK GUIDE

2.1 Installation

If not already available, install the anaconda distribution of python. Open a terminal on Mac or Linux, or the “Anaconda Navigator” on windows. Create a new conda environment with python 3.10. Copy and paste the following commands in order.

conda create -n hcat python=3.11

Once complete, activate the new conda environment

conda activate hcat

From there, install hcat and all dependencies with:

pip install hcat

To update, type the following:

pip install hcat --upgrade

2.2 Launch the software

In the command line, sure the conda environment is activated, if not, activate with:

conda activate hcat

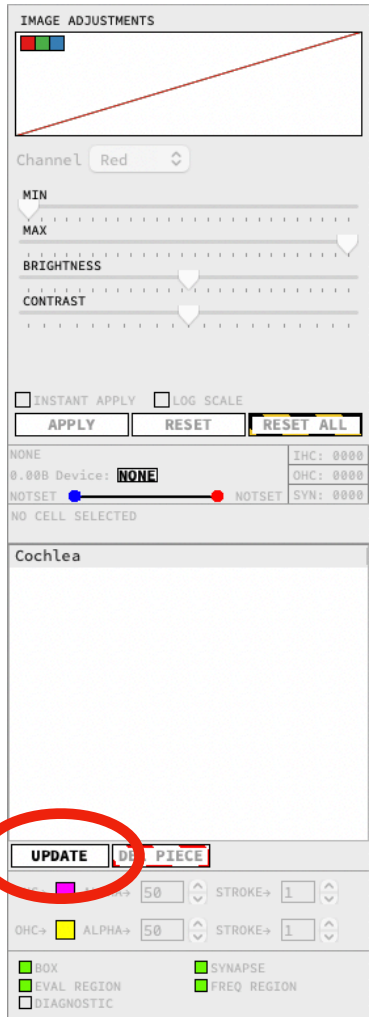
Type in the command line:

Hcat

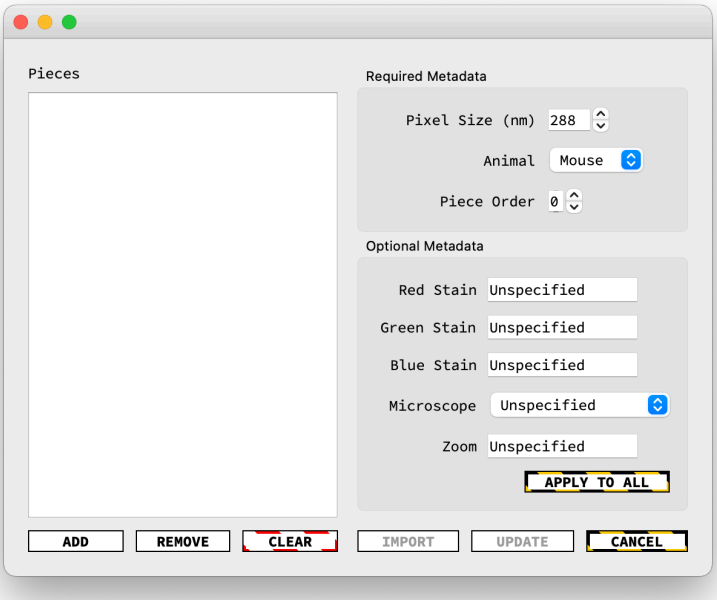
If this is the first launch, you may be prompted for a license key, enter it here.
Once validated, the software window will launch.

2.3 Import images of cochleae

Once launched, on the right sidebar, find, and click, the button labeled “**UPDATE**.”



This will open the tissue piece update window. Here you can import new images, set and update metadata, and define the order (base to apex) of any tissue.



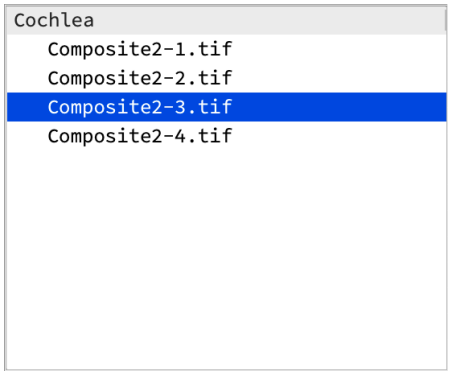
Click “**ADD**” to open a file-picker dialog, and choose a single, or multiple, image(s). Currently we only support “.png,” “.jpeg,” and “.tif” file extensions.

You can adjust metadata by first clicking on the filename of the image, then typing in the fields on the right. It is important to properly set the pixel size of each image, and the order from base to apex. Order and pixel size are critical for optimal neural network accuracy, and are therefore required.

When all appropriate image metadata has been set, click import to close the window and load the image from the file. This action may take a while.

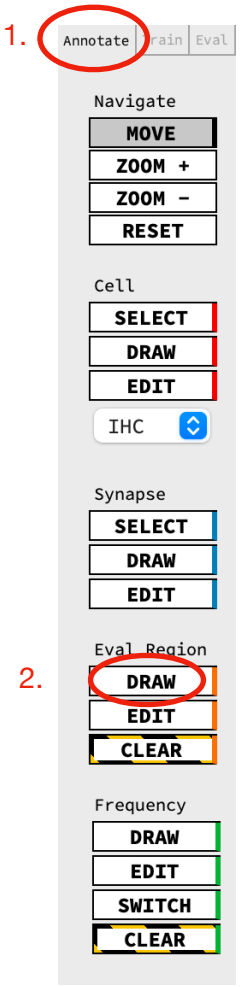
2.3 Navigation

To pan around an imported image, right click, hold, and drag on the image. Zoom in and out with the scroll wheel. To show another image, in the right sidebar, click the image name of the piece you wish to view.



2.3 Define an evaluation region

To analyze a specific region of a cochlea, click the “Annotate” tab in the left sidebar to bring up the annotate sidebar. From here, under “**Eval Region**” click “**DRAW**”.



Now, draw a shape around the sensory epithelium by clicking to set a corner. To finish drawing, double click. To edit the vertex of a shape, click “**EDIT**”, then click and drag a corner of the eval region. To clear your annotation, click “**CLEAR**.”

When you are done defining an evaluation region, you will see tiled regions which define where the detection model will evaluate.

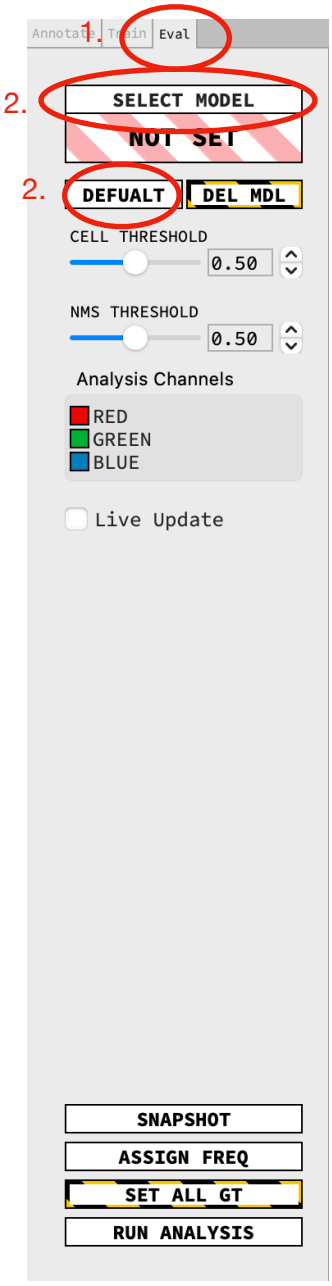
2.4 Label tonotopic axis

To assign a best frequency to a cell, you must first define the tonotopic path for each piece of a cochlea. To do this, navigate to the Annotate tab in the left sidebar. From here, under “**Frequency**” click “**DRAW**.” In a similar manner as the eval region, starting at the one end of your piece of tissue, draw a path following the tonotopic axis by clicking. To finish drawing, double click. This line defines the directional tonotopic axis, base to apex. To switch direction, under “**Frequency**” click “**SWITCH**.” To edit the vertex of a

shape, click “EDIT”, then click and drag a corner of the tonotopic path. To clear your annotation, click “**CLEAR**.”

2.5 Load an machine learning model

To load a machine learning model for evaluation, first navigate to the “**Eval**” tab in the left sidebar. From there, click 1. “**SELECT MODEL**” to launch a file-picker dialog and choose a pre-trained neural network model. To load the default model instead, simply press “**DEFAULT**”



Upon successful load, the region labeled “**NOT SET**” will change to the filename of the chosen model.

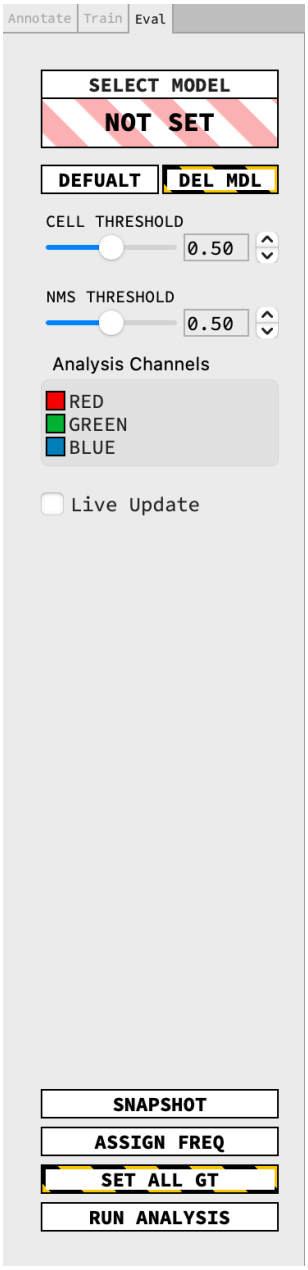
2.6 Image adjustments

To adjust the image, use the “**IMAGE ADJUSTMENTS**” tool in the right sidebar. Choose a channel to edit, then adjust sliders based on the pixel brightness histogram. To apply your adjustments, press “APPLY.” To have the image dynamically update based on slider change, click “**INSTANT APPLY**.”

To reset a single channels’s adjustments, click “**RESET**.” To reset all channels, click “**RESET ALL**.”

2.6 Eval the machine learning model

Many factors affect a neural network’s performance, such as image brightness and contrast, threshold values, or the number of included channels. Adjust the brightness and contrast sliders such that the hair cell seterocillia bundles are clearly visible.



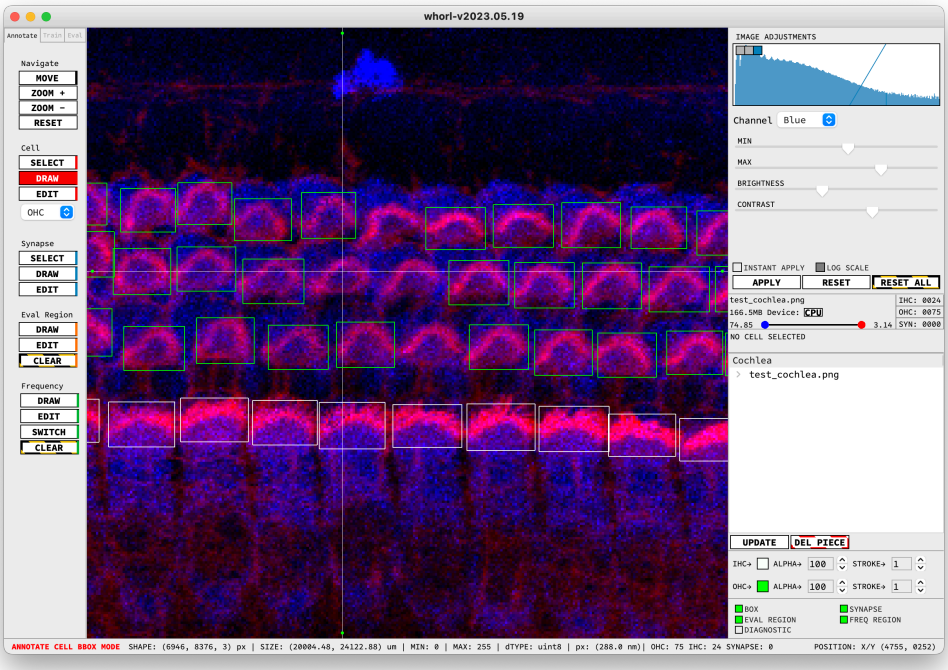
Verify each piece has a correctly annotated tonotopic path, and press “RUN ANALYSIS.” This will evaluate the deep learning model on each eval region for all pieces, predicting a bounding box (box around the cell) and automatically assign a best frequency. This may take a while.

2.7 Correct predictions

After a whole cochlear analysis. Some errors may occur: 1) missing cell, 2) falsely predicted cell, and 3) a classification error. These each may be corrected in the software.

1) Missing Cell

To annotate a missing cell, navigate to the “**Annotate**” tab in the left sidebar and under “**Cell**” click the dropdown selector to choose cell type (OHC vs IHC) then click “**DRAW**”.



You will see a cross follow your curser, indicating you are ready to draw a cell bounding box. Click and drag a box around a cell. To select a cell which has already been drawn, in the “Annotate” tab in the left sidebar, under “Cell” click select. From here, either draw a selection region by clicking and drawing, or simply click a cell bounding box.

2) Falsely Predicted Cells

To delete falsely predicted cells, in the “**Annotate**” tab in the left sidebar, under “**Cell**” click “**Select**.” From here, draw a box around falsely predicted cells and hit the “**Delete**” key on your keyboard to delete the cells. Use caution, this action cannot be undone.

3) Classification Error

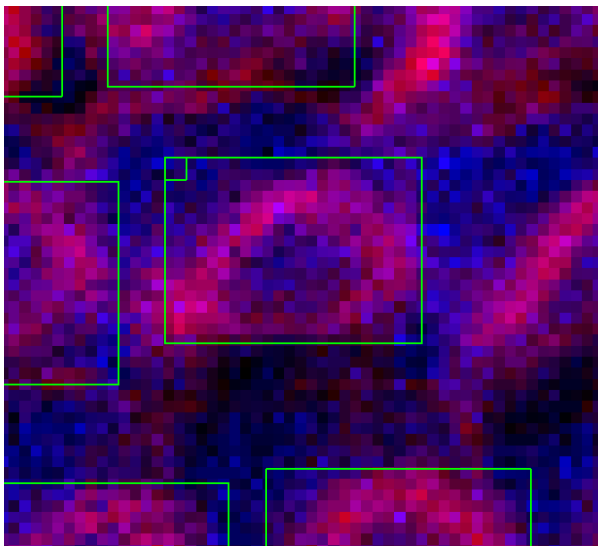
In the right sidebar, you will find a “Cell Info” tool, showing the information of the currently selected cell.

This displays information about the cell, including its unique ID, likelihood score, best frequency, cell type, and bounding box. To toggle cell type, press the colored button with IHC or OHC.

When multiple cells are selected, the tool displays new buttons allowing mass assignment of selected cells to a cell type.

2.8 Save

To signal that all cells are correct and have been human validated, in the “Eval” tab in the left sidebar, near the bottom, click “**SET ALL GT.**” This signals to the software that this cell has been human verified. You can verify if a cell has been verified by the presence of a small additional square in the top left of the bounding box.



To save, press “Ctrl + S” on your keyboard, or navigate to the file menu, and click “Save”. This will launch a file-save dialog. Image data, annotations, evaluation regions, and tonotopy paths are saved in a file with the extension “.hcat”

2.9 Export as CSV

To export annotations for further analysis, press “Ctrl + E” or in the file menu, click “Export as CSV.” This will launch a file-save dialog.