CellLocator: Quick Start Guide

This guide provides a step-by-step walkthrough of analyzing your Incucyte® experiments using CellLocator.

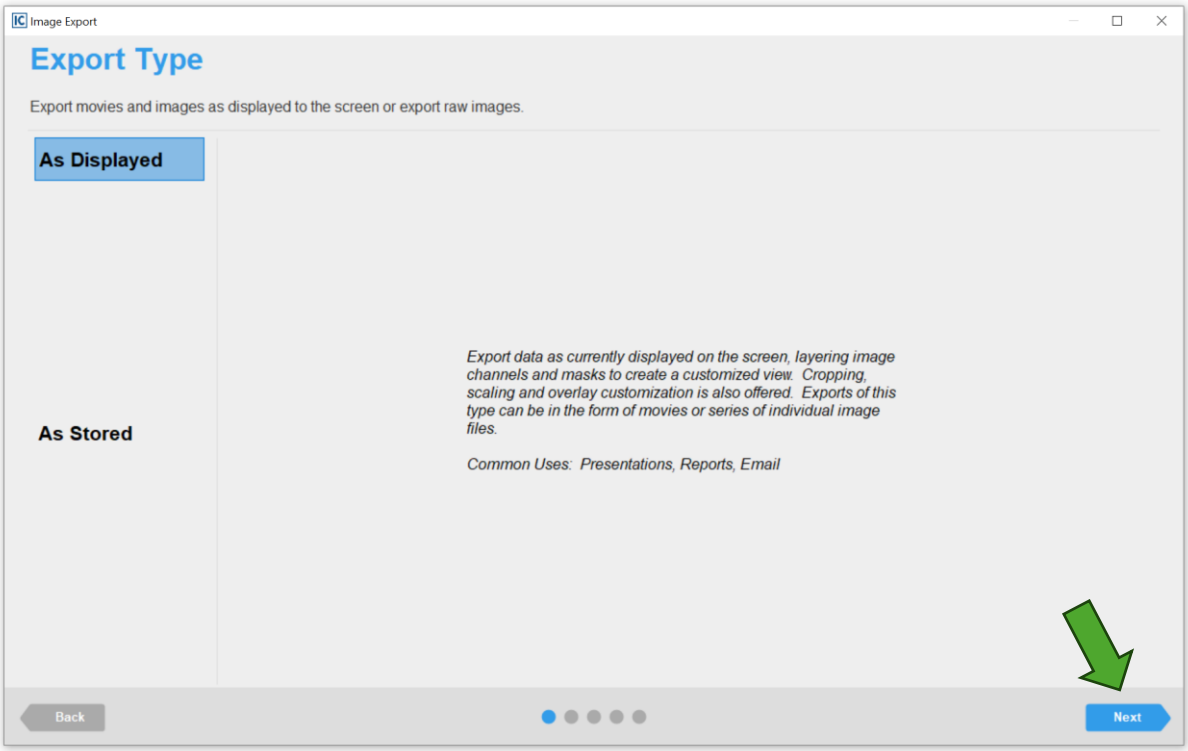
1. Experiment Preparation and Image Export

1.1 Export Images and Movies

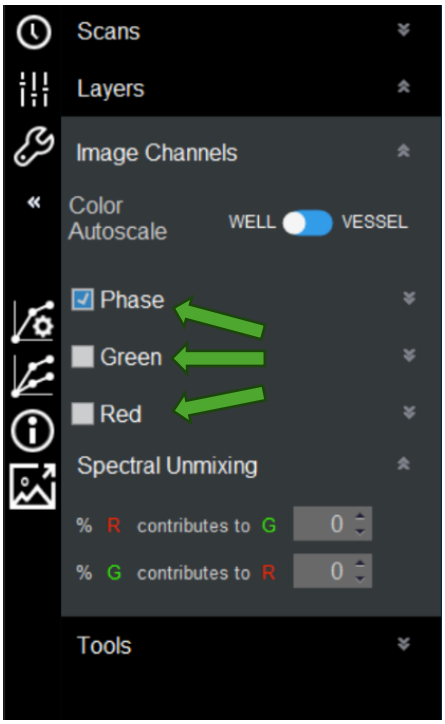
a. Open the desired experiment in your Incucyte® software.
b. Navigate to the "Export Images and Movies" function.
c. Select the Phase Images and all relevant fluorescence channels for export.

1.2 Export Options

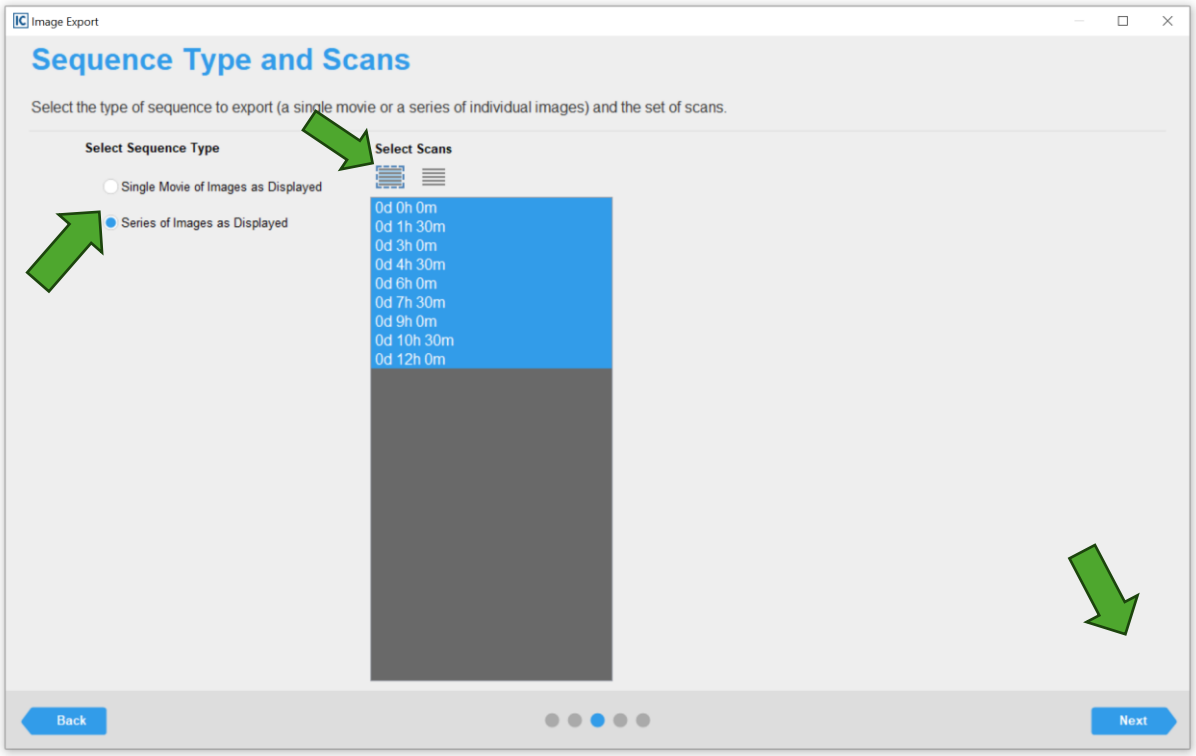
"As Displayed": This option exports images as they appear on your screen. Note: Older Incucyte® versions may require individual well selection. This limitation is addressed in newer software versions.



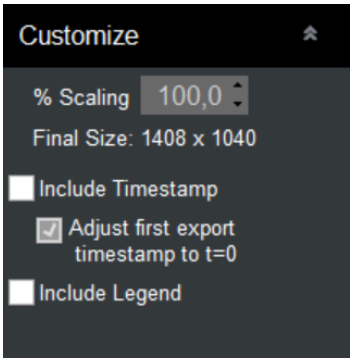
Ensure you select only the specific image you wish to export.



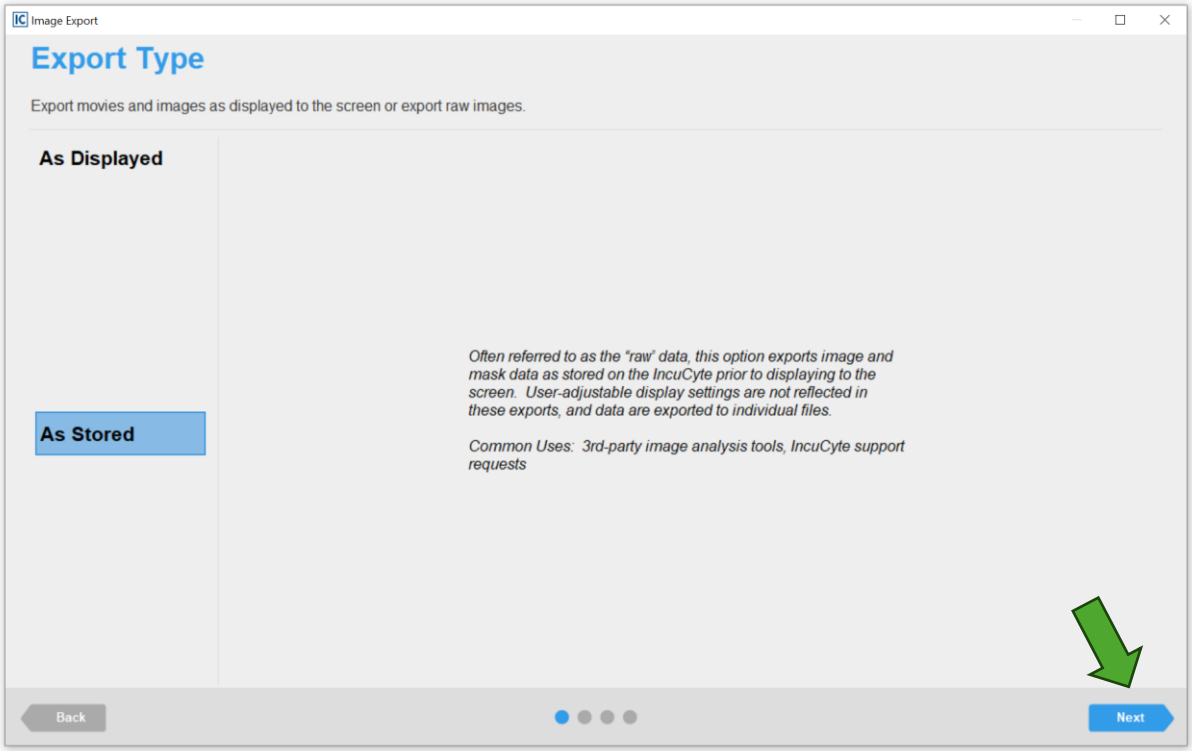
Choose "Series of Images as Displayed," click "Select all scans," and proceed to the next step.

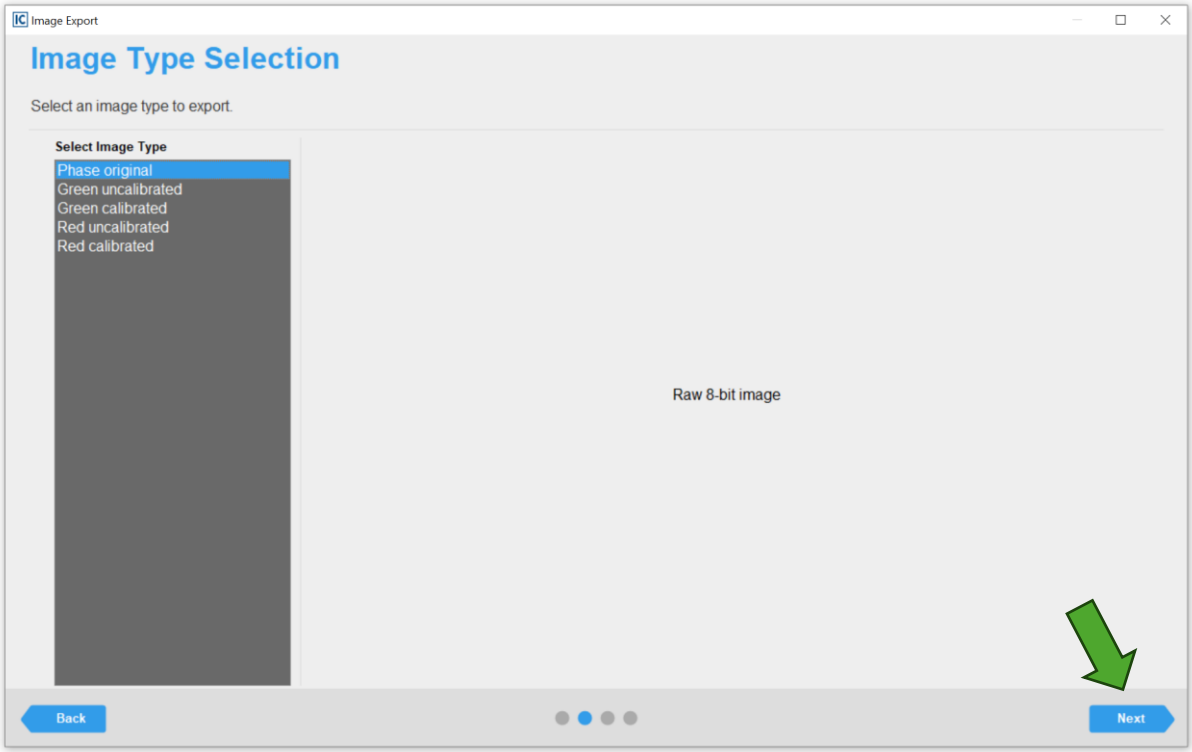


Important: Deselect "Legend" and "Timestamps" for export.

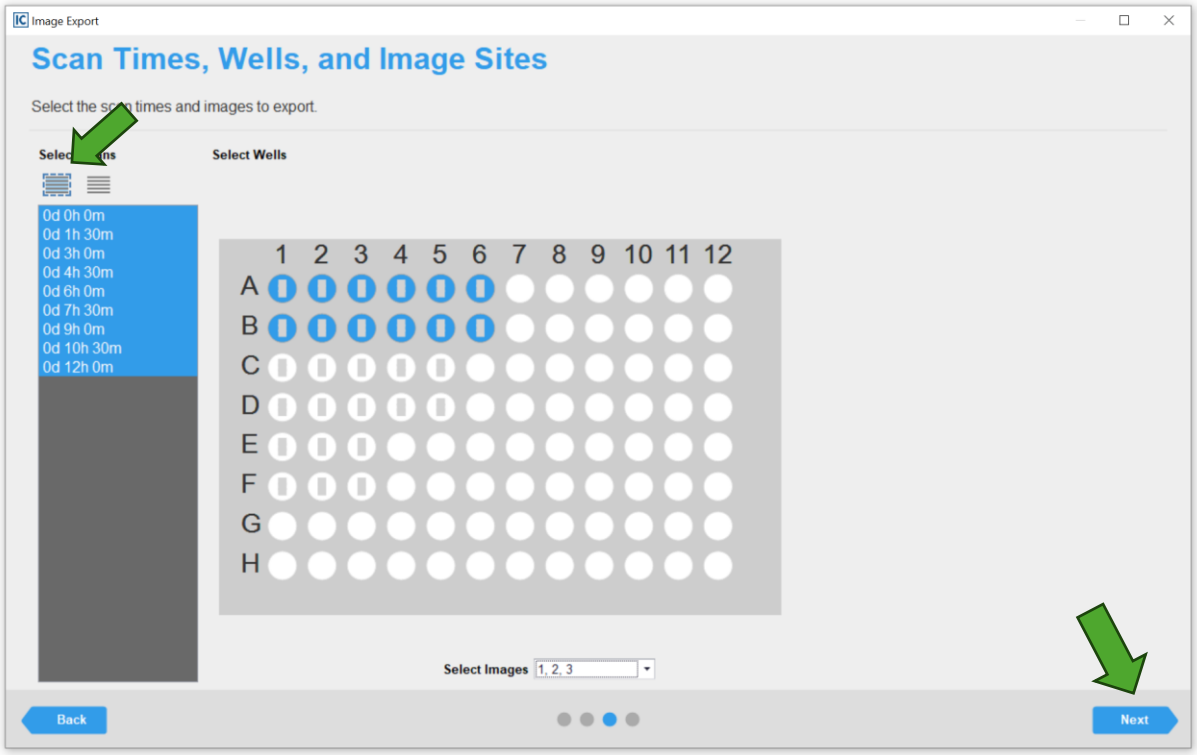


"As Stored": This option exports the raw image data.





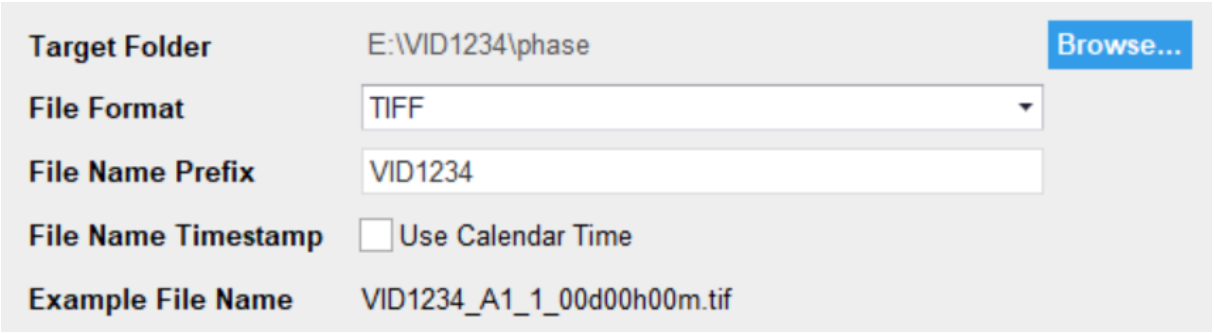
Phase images are exported in their original format, while fluorescence channels are exported as grayscale (16-bit or 32-bit) images. CellLocator automatically converts these to 8-bit. Note: Internally, minimal differences were observed between uncalibrated (16-bit) and calibrated (32-bit) images. However, calibrated images require more storage space.



You can select specific wells of interest by marking them.

1.3. Folder Structure

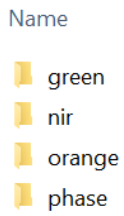
- **Target Folder:** Create a dedicated folder for your exported images, ideally named after your experiment (e.g., "VID1234").
- **Channel Subfolders:** Within the target folder, create subfolders for each exported channel, using lowercase names (e.g., "phase," "red," "green," "nir").
- **File Format:** CellLocator expects images in TIFF format.



Click "Export" to begin the export process. You can export multiple experiments and channels simultaneously.

1.4. Export Verification

After export, verify that your folder structure matches the example below:

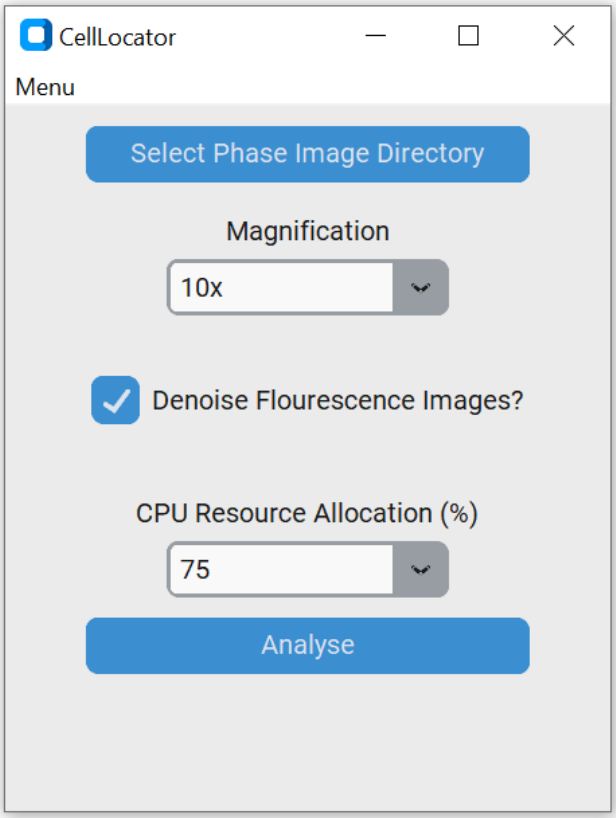


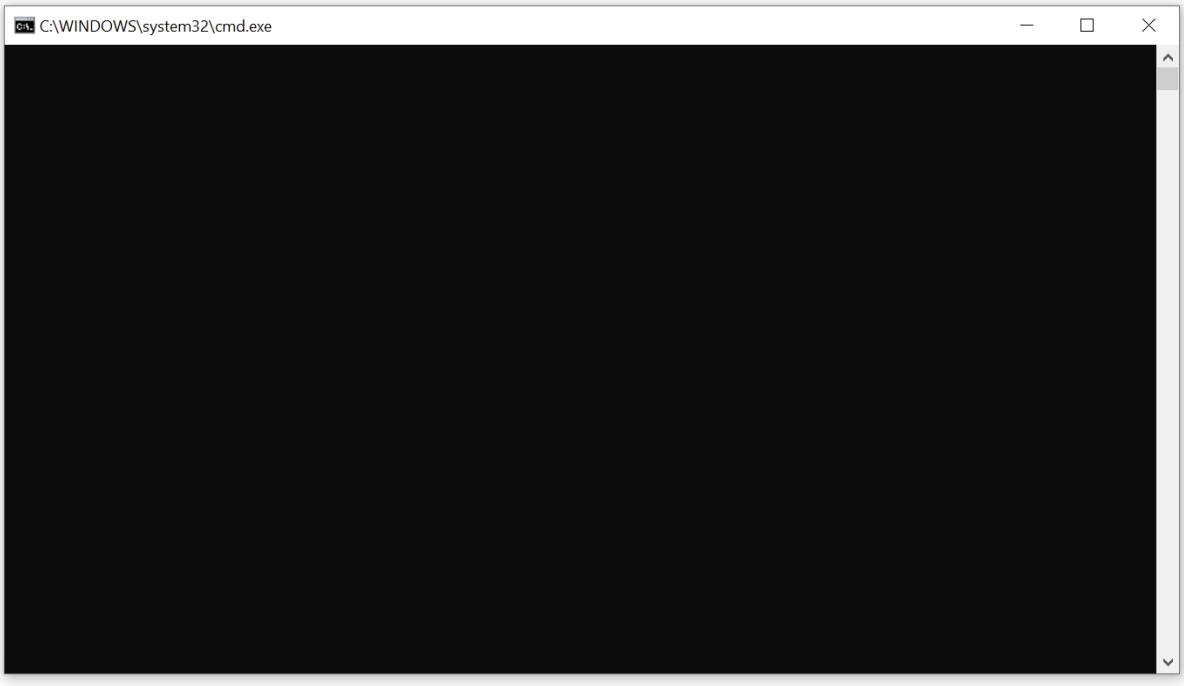
Also all Folders should contain the same number of images. CellLocator will give you a warning if the number or images names don‘t match, but will still try to run the analysis. Missing fluorescence channel images will be replaced with blank placeholder images.

2. Initiating Analysis with CellLocator

2.1. Launching the Application

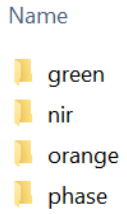
a. Double-click the CellLocator application icon.
b. Two windows will appear: the main application window and a console window displaying progress information during analysis.



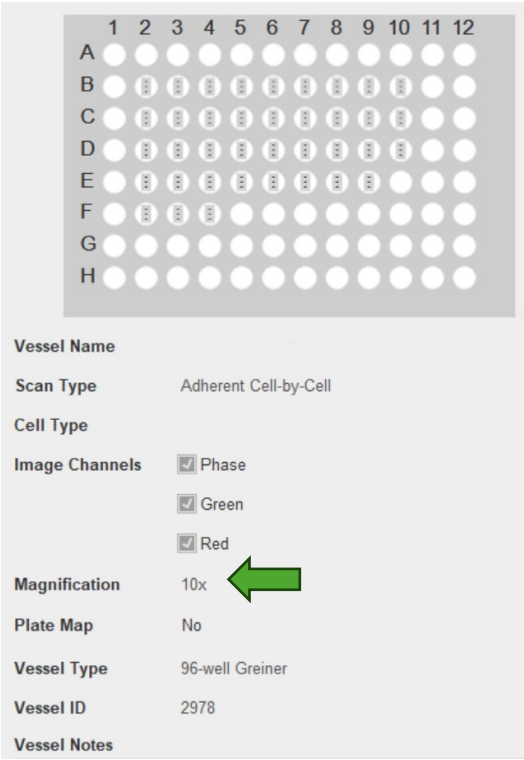


2.2. Analysis Settings

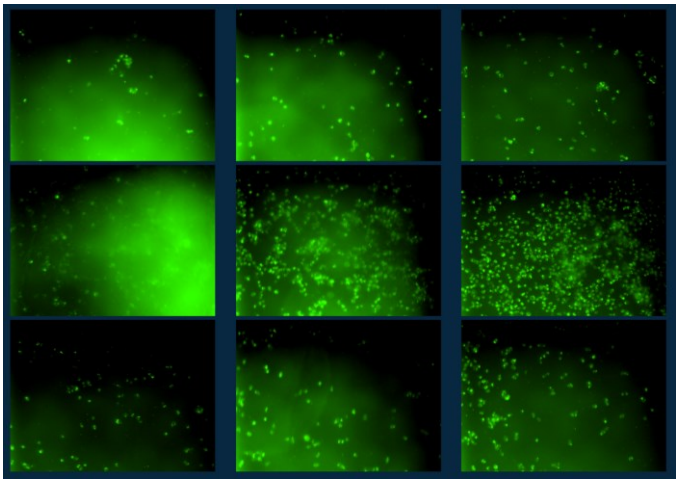
- **Select Experiment Directory:** Choose the main folder containing all folders related to your experiment



- **Magnification:** Select the magnification level used in your experiment.



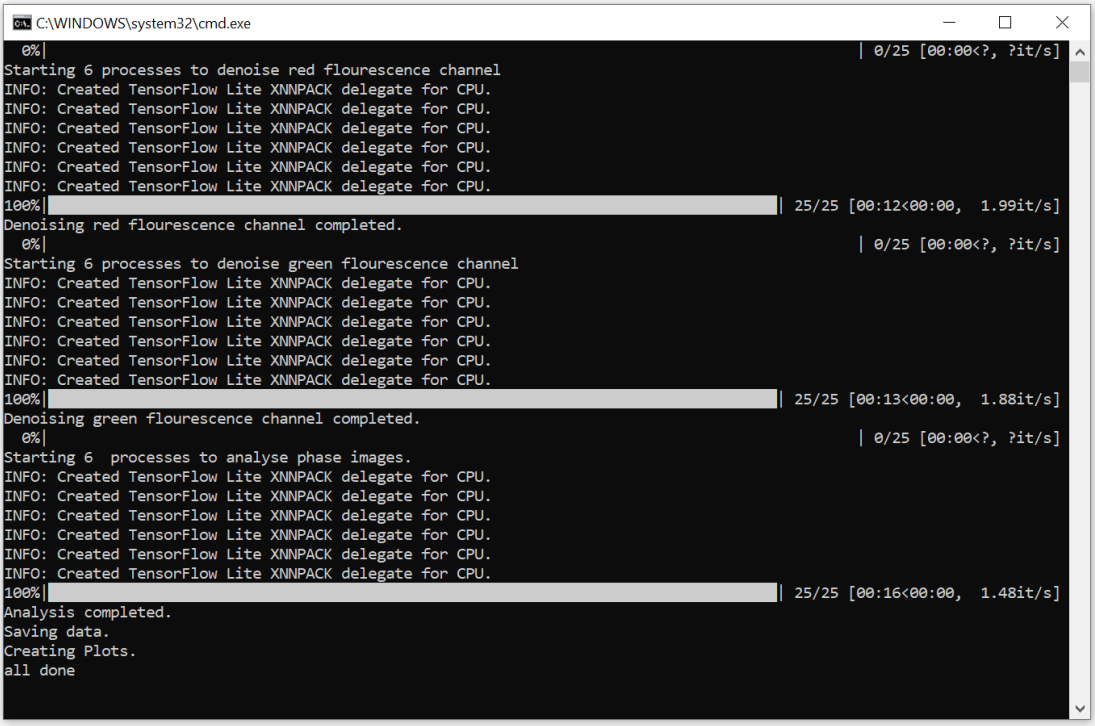
- **Denoise Fluorescence Images:** Enable this option to apply a denoising algorithm (U-Net) to your fluorescence images, particularly if they appear noisy like this.



- **CPU Resource Allocation:** Specify the percentage of CPU threads CellLocator can utilize. A setting of 25-50% allows for background analysis while using your computer normally. 75% enables light web browsing and other tasks, while 100% may cause system lag.

2.3. Running the Analysis

- Click the "Analyze" button to start the analysis.
- An informational message, "INFO: Created TensorFlow Lite XNNPACK delegate for CPU," may appear in the console window. This is normal.
- If "Denoise Fluorescence Images" is enabled, denoising occurs before analysis.
- A progress bar tracks the analysis progress.

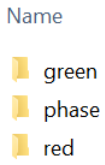


3. Results

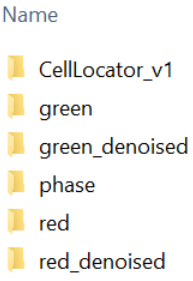
3. Exploring the Results

- During analysis, CellLocator creates several folders. Locate the folder named after the analysis model used (e.g., "CellLocator_v1").

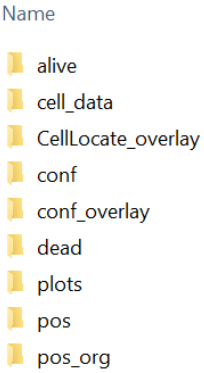
Befor the analysis



After the analysis

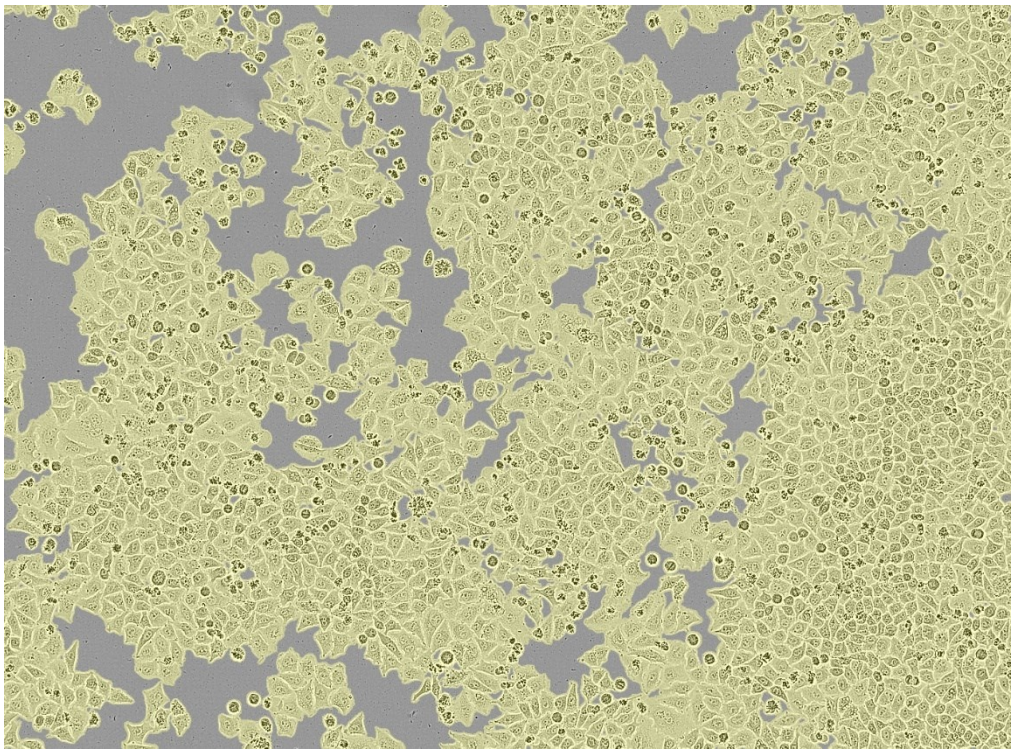


Inside CellLocator_v1

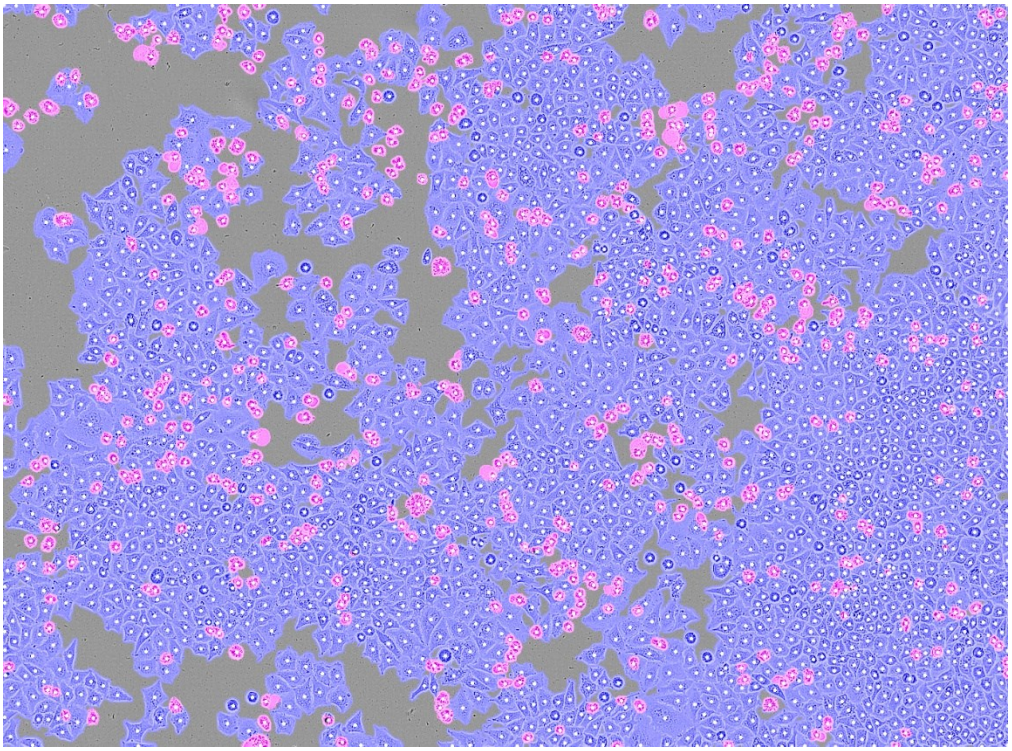


- **Mask Folders:**
  - **"Conf," "alive," "dead," "pos_org," and "pos":** These folders contain the generated masks for confluence, live cells, dead cells, and cell positions, respectively.

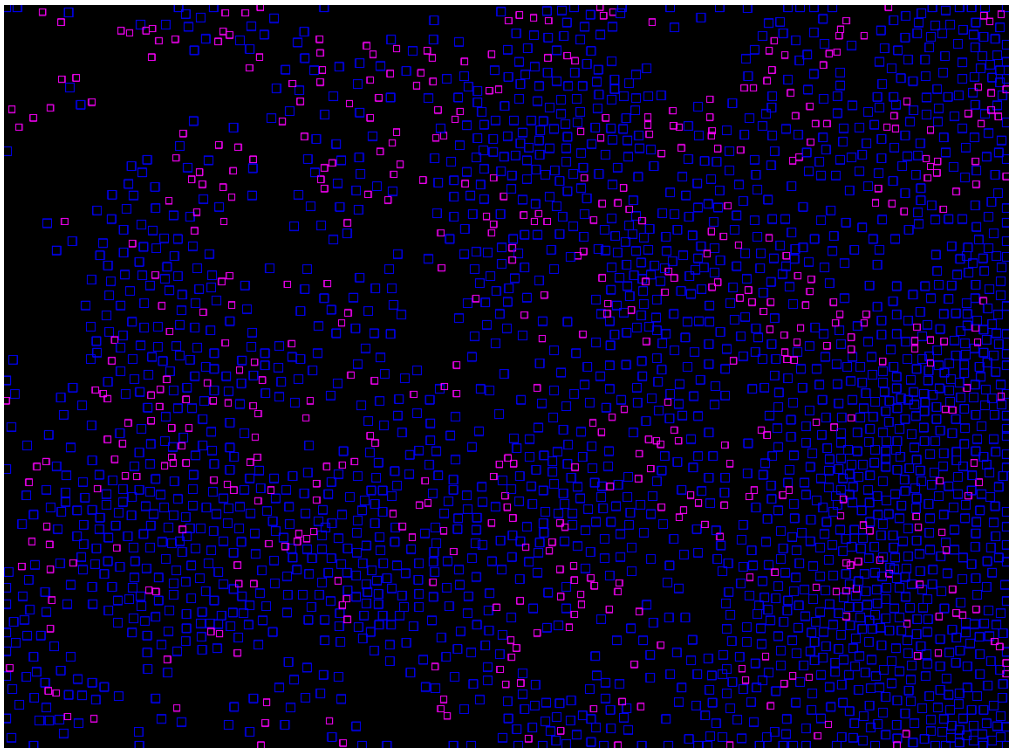
- **Overlay Folders:**
  - **"Conf_overlay":** Contains phase images overlaid with predicted confluence.



- **"CellLocate_overlay":** Contains phase images overlaid with predicted areas of live (blue) and dead (magenta) cells, along with individual cell locations.



- **"measurement_overlay":** Visualizes the area around the cells used for measuring fluorescence intensities.

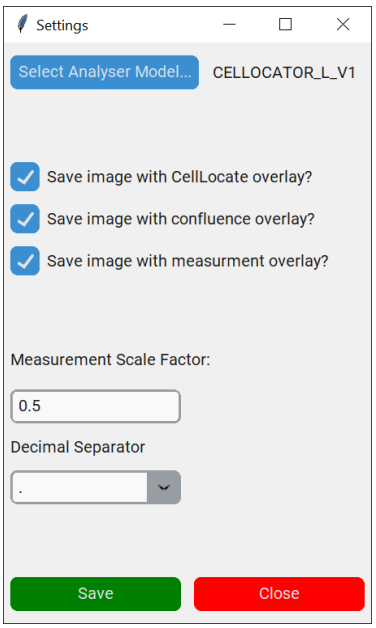


Note: The overlay folders can consume significant storage space. You can disable their creation within the application settings.

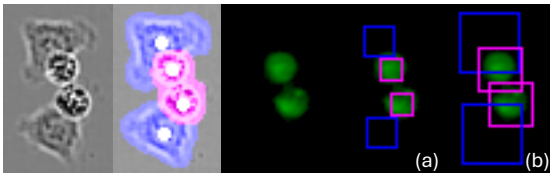
- **"Plots":** This folder contains various generated plots.
    - **Denoised plots:** Based on denoised fluorescence channels.
    - **Normalized plots:** Based on the original fluorescence channels with average pixel intensity subtracted for normalization.

4. Settings

To open the Settings click Menu -> Settings.



- **Select Analyser Model:** Choose the desired analysis model.
- **Save image with CellLocate overlay:** Disable these options to prevent the creation of corresponding overlay images.
- **Save image with confluence overlay:** Disable these options to prevent the creation of corresponding overlay images.
- **Save image with measurement overlay:** Disable these options to prevent the creation of corresponding overlay images.
- **Measurement Scale Factor:** Scales the measurement area around each cell position. Increasing this value expands the area, while decreasing it shrinks the area. A value of 1 corresponds to the average cell size, but the default is set to 0.5 to prevent overlap (b) between measurement areas.



- **Decimal Separator:** Define the decimal separator used in CSV file output.

Click "Save" to apply any changes and close the settings window.

4.1 Config.ini

4.1.1 Advanced Configuration (config.ini)

- CellLocator automatically detects fluorescence channel names defined in the "config.ini" file.
- **Adding New Channels:**
  a. Open "config.ini" with a text editor.
  b. Search for „fluorescence_channel_names“
  c. Add new channel names separated by commas without spaces.
  Example:

```
fluorescence_channel_names: red,green,orange,nir
fluorescence_channel_names: red,green,orange,nir,yellow
```