guiForPrime User Manual

Dr. Weninger's Lab
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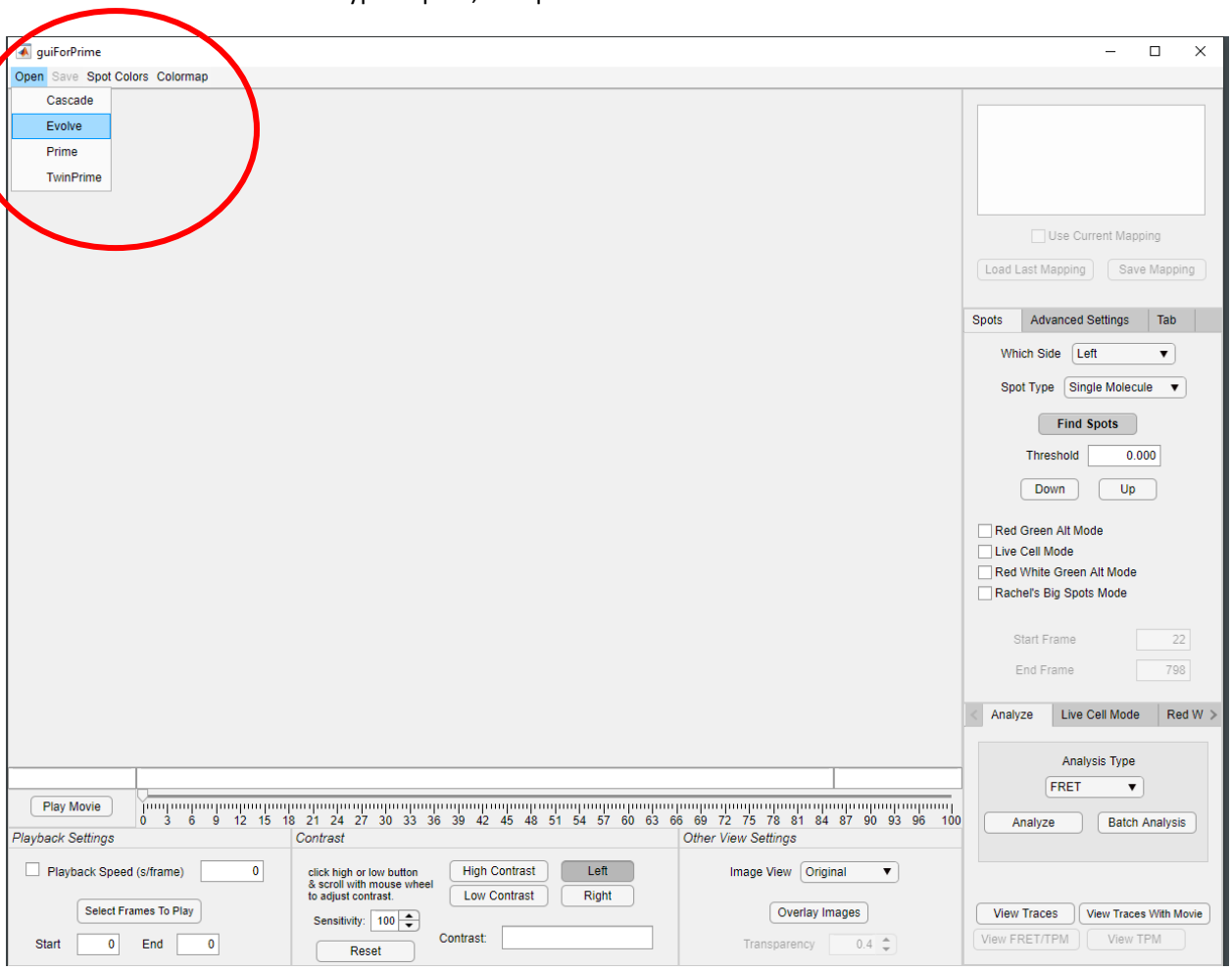
Contents

Opening Your File

Click “Open” in the menu on the top left. Select which camera you used to take your video.

Files must have extensions of type: *.pma, or *.pmc

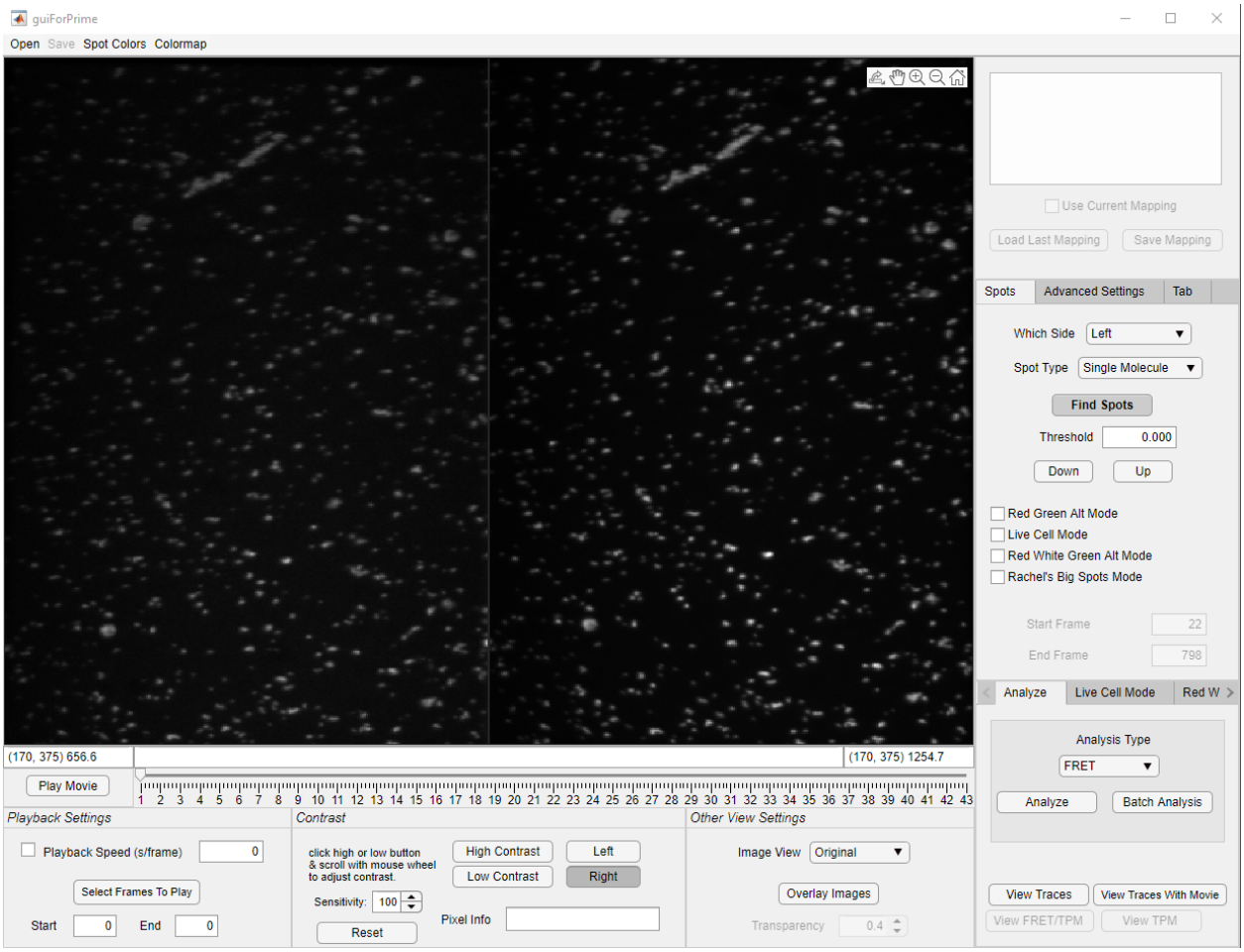


After clicking the open -> cameraType menu, the file explorer will open in to the last directory it was in. Navigate to the movie you want to load, select it, and press “open”.

Things worth noting:

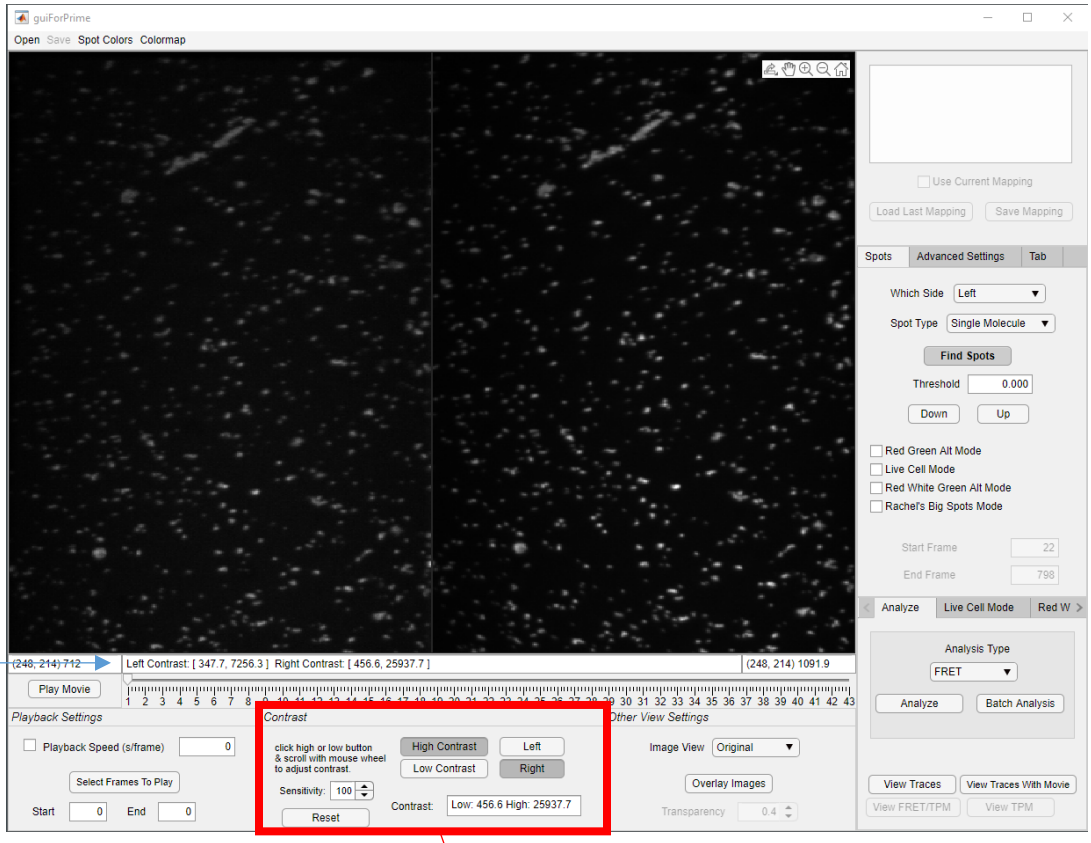
- The file explorer will only show you files with *.pma and *.pmc extensions
- as long as the extension is *.pma or *.pmc, the file will load.
    - For example, if you have a beads image and you re-named it beads1.pma, that is okay.
- The camera name that you choose determines how the analysis files are saved.
    - If using batch analysis, then it also uses the camera name to search for the rest of the movies in the folder.

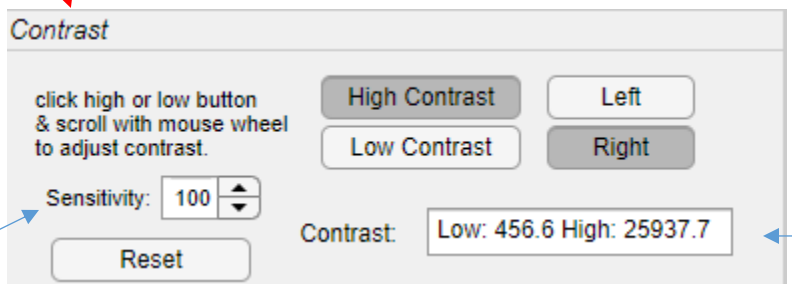
Your file should load and look something like this:



Adjusting The Contrast

Contrast is adjusted using the mouse scroll wheel. (If you have a laptop and it isn't working, let me know)



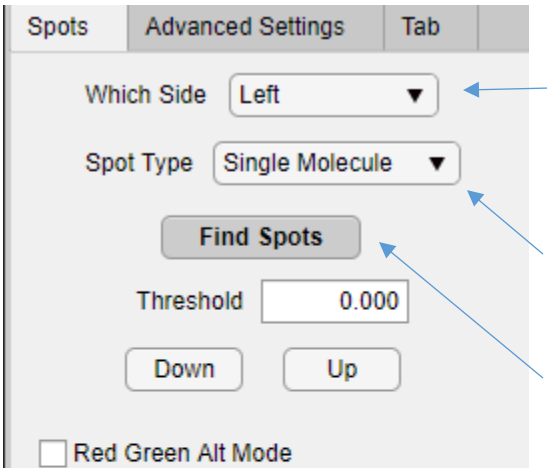


- Left and Right sides of the image are adjusted separately and the high/low limits are also separate.
- Scroll wheel sensitivity can be adjusted using the spinner labeled "Sensitivity".
- To adjust Contrast, select either the "High Contrast" or "Low Contrast" button, and choose which side you want to adjust with the "Left" and "Right" buttons. Clicking anywhere on the image will also select which side (left/right) you are adjusting.
- Scroll up or down with the mouse wheel to adjust the contrast.
- The numbers of the side you are adjusting are displayed here:
- The contrast levels of both images are also shown in the text area right below the image
- Press the "Reset" button to set the contrast back to matlab's original settings
- Note: Contrast adjustment only works when either the "High Contrast" or "Low Contrast" button is selected. If neither are selected, scrolling does nothing.

Bead Mapping

Creating/Saving Your Mapping

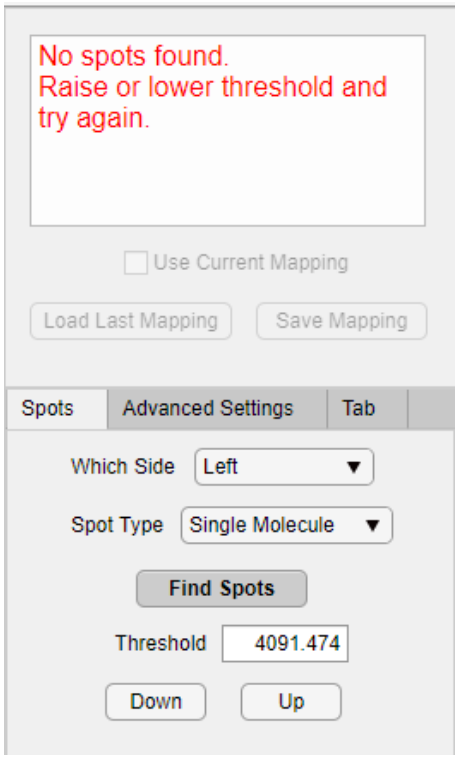
Usually with FRET data, we take a bead movie to map one side of the image to the other. Here is how that works:



1. First choose which side you are mapping from using the drop down menu. (Usually we use the left side, but you can also choose right side or both sides if you want to analyze the whole image)
2. Choose the spot type. Almost always choose "Single Molecule." The other option is "Random Spots", which selects spots that are in the background only.
3. Press the "Find Spots" button.

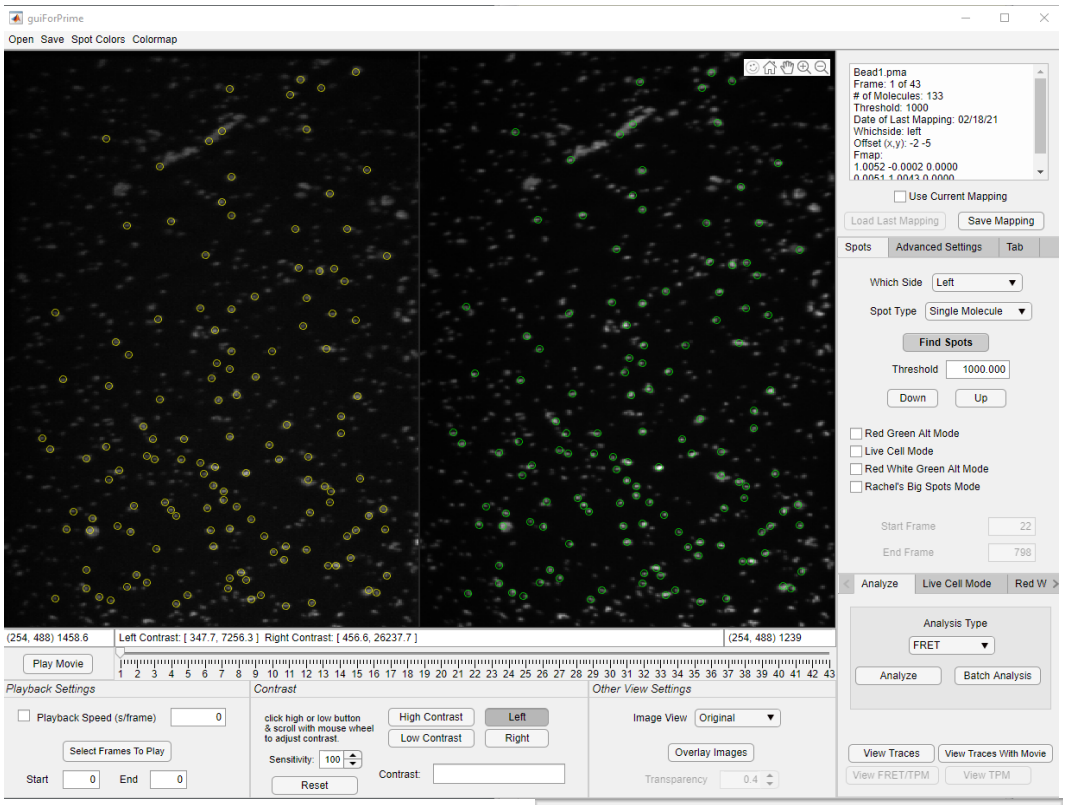
Notes:

- If the threshold is set to 0, the program picks a threshold based on the camera type and pixel data. This is a good place to start, but usually you will need to adjust the threshold afterwards.
- Sometimes the program will not find any spots with the threshold that is set. When that happens, a message will be displayed in the top right of the screen:

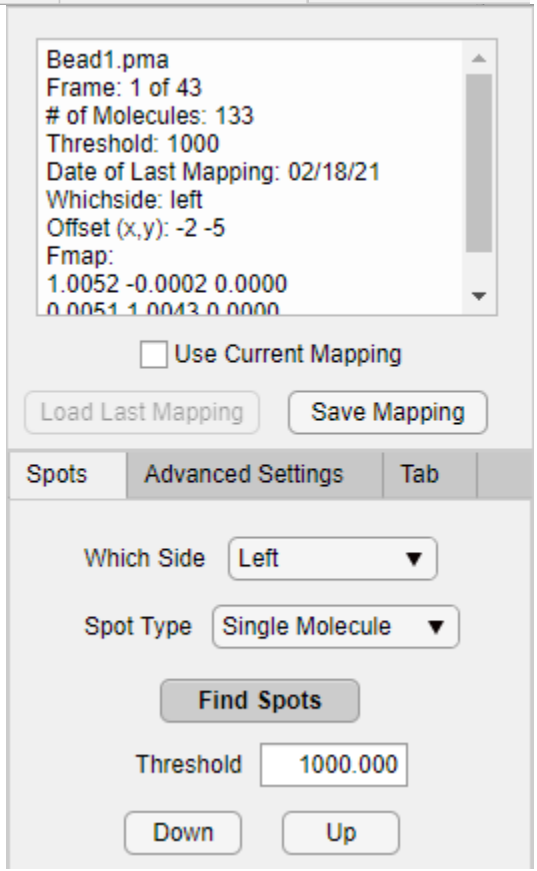


To adjust the threshold, either type in a number into the Threshold value field, or press the "up" or "down" buttons to adjust a small amount at a time.

Spots Found:



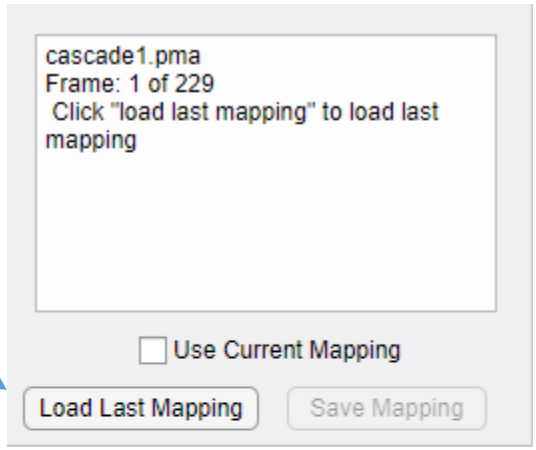
- When the program finds spots, it creates a mapping from one side to the other. It also calculates the offset of one side to the other. The information is displayed in the top right panel.
- If you are satisfied with the mapping, click the “save mapping” button.
- The mapping will be saved as a matlab file in the current folder under the name *lastmapping.m*
- This file needs to be in the same folder as the files you want to analyze. Otherwise the program won’t find it.



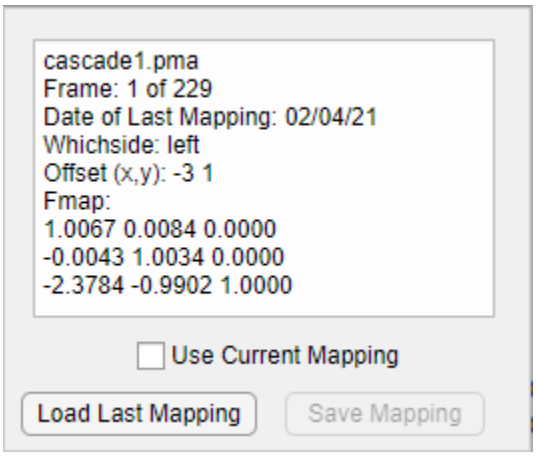
Loading Your Mapping:

When you open a movie to analyze, if there is a saved mapping in the folder, you will have the option to load that mapping:

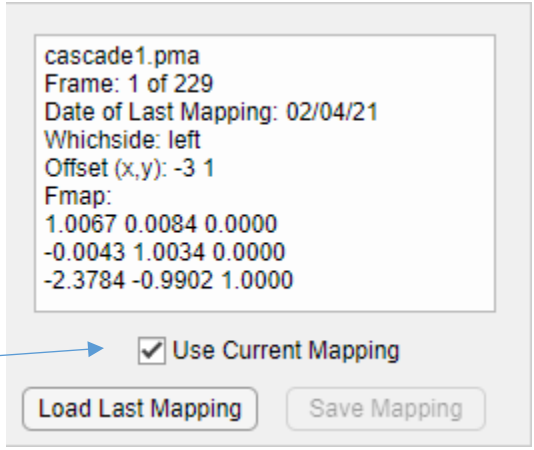
Just click “Load Last Mapping”



Mapping will load and display:

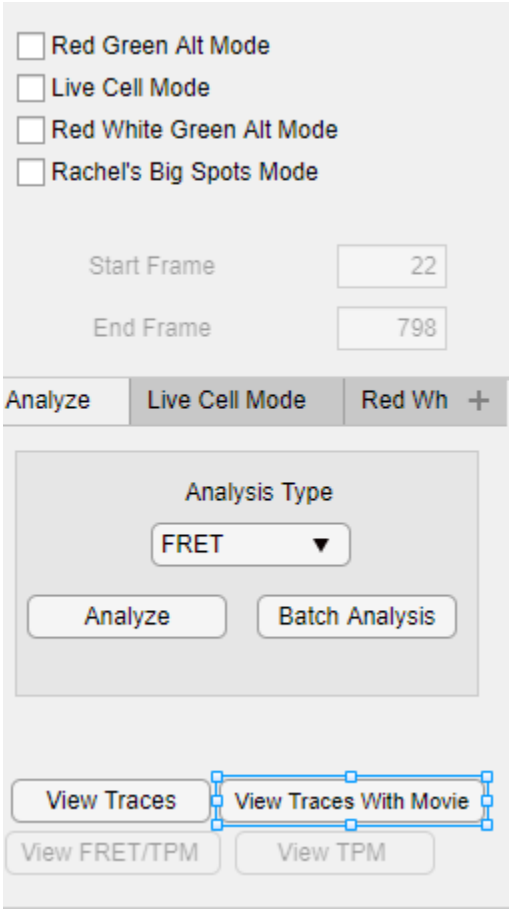


For the mapping to be used in your spot finding, you need to check the “Use Current Mapping” checkbox.



As long as the “Use Current Mapping” checkbox is checked, the program will use that mapping for the spot locations.

Analyzing Your Data



General Steps:

1. Create mapping with bead image (described above)
2. Find spots on movie you want to analyze
3. Press “Analyze” button and it will save the .traces files in the folder with your movies
    a. If in Red green alt mode, make sure you check that box.
4. You can press “Batch Analysis” if you want to analyze all the movies in your folder.

Viewing Your Data

Press “View Traces With Movie” to view the traces you just saved. (That app is still a work in progress though).

If you press “View Traces”, right now that takes you to the app I made for analyzing the traces for the kinsoft data with a k-means code. You can view your traces with it, but it’s got a bunch of options all over and I haven’t written an instruction manual for it yet.