

After recording images with Labview, we will obtain **spe files** as a raw data set.

### For mapping (IDL)

1. Before making a mapping file, you need to make sure that a path of **C:\Users\IDL\TIR DATA** exists.
2. Put a mapping video in the path and rename it as **rough.spe** file.
3. Open the IDL file **mapSPE\_2** (an updated version of **mapSPE** by HLY), check that the input language of keyboards are English, and run the code.
4. You will be asked to choose three mapping pairs on both cy3& cy5 channel, which form a triangle and contain most of the dye pairs. If you did not double-check the input language and it is in Chinese mode, the IDL mostly comes into a crash; then you need to repeat running the code again after restarting IDL.
5. Follow the program instructions. After that, you will gain three more files in the path set before: **location.dat**, **rough.map**, and **rough\_average.tif**.
6. If you want to gain more dye-pair transformation information for a more precise mapping, delete the **rough.spe**, copy a second mapping video to the path, rename it again, and run **mapspe\_3.pro**. The process is actually the same as what we just done, but after that it will rewrite the **location.dat**, **rough.map**, combining all dye pairs I two videos and the transformation matrix.

### For finding AOI (IDL)

1. Open the file **find\_spepeaks\_2.pro**.
2. Before running the code, you need to check the input filename, map file path (2 lines below “開啟 polynomial mapping 檔”), and the current path which contain the file in which you want to find AOIs.
3. Run the code. At the end, it will pop out two image files of the AOI it mapped and chose, so you can check if the mapping works well with eyes.

### For analyzing AOI intensity (IDL)

1. Open the file **analyze\_SPEpeaks.pro**.
2. Before running the code, you need to check the input filename and the current path again.
3. Run the code. It may needs more time to finish that depends on the length of the image. When it finished, there will be a **hel1.traces** file be created. Within the trace file, we can do further analyze.

### For plotting time traces (MATLAB)

1. Open the MATLAB file **tirh\_SPE\_MovingAverage\_TingTzu\_reversed.m**.
2. Check that the current path contains both the code m-files and a **hel1.traces** file you want.
3. Run the code, and just follow the dialog box. The trace will pop out sequentially. If you unfortunately miss an important trace and skip it out of control, you can stop running the code and either revise the code or just re-run it again to retrieve it.
4. As long as you save the trace, it will also save the trace plot and the FRET values at each time point as an excel file, which is very useful for further analysis.