Step 1: Launch Spectral-FLIM GUI

- Type *sFLIM* in MATLAB command line window

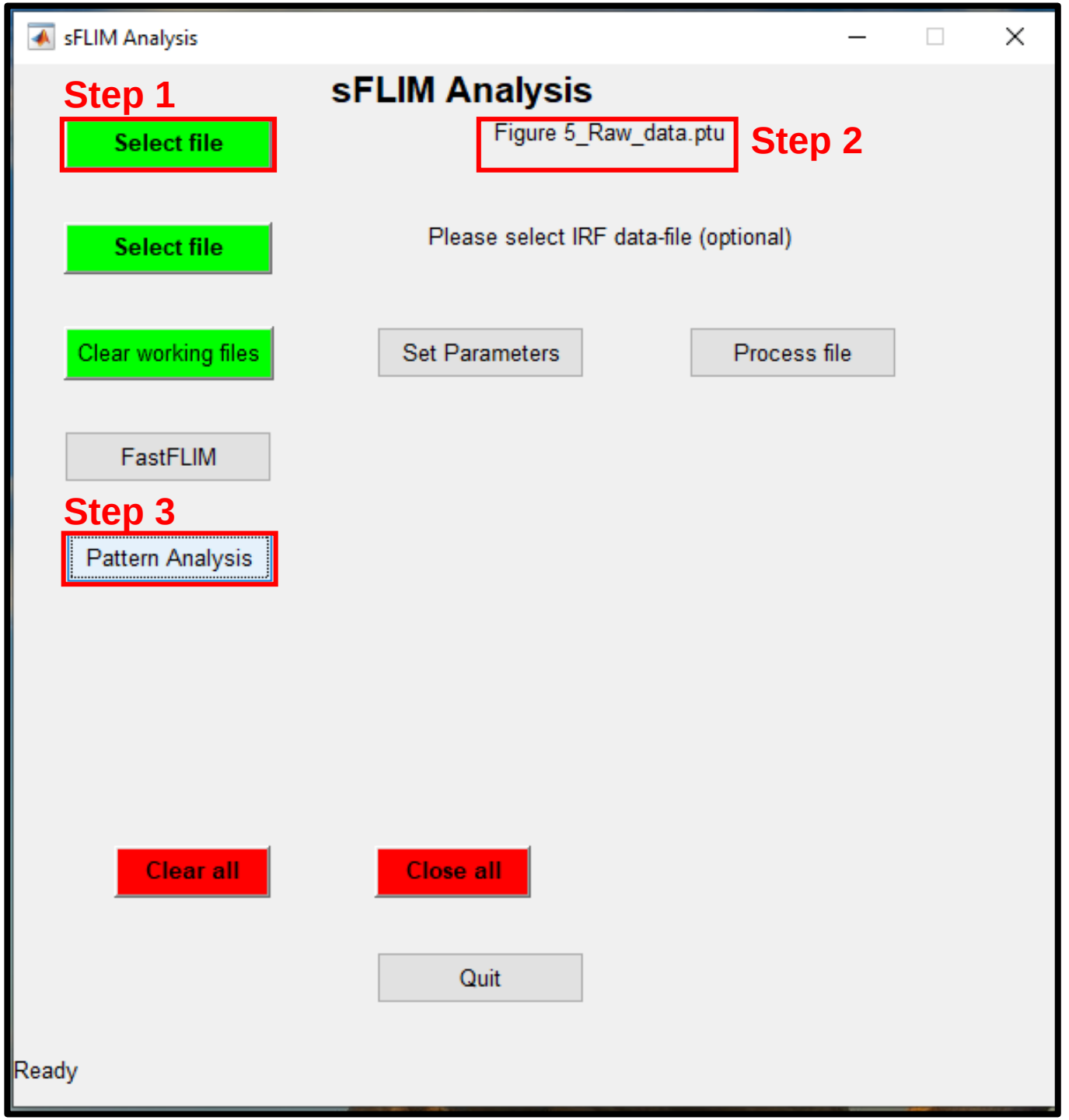
Step 2: Select a *.ptu* file for processing

- From Raw data folder choose file

  "Figure 5_Raw_data.ptu"

Step 3: Launch sFLIM pattern-matching based data analysis

- Push button – *Pattern Analysis*



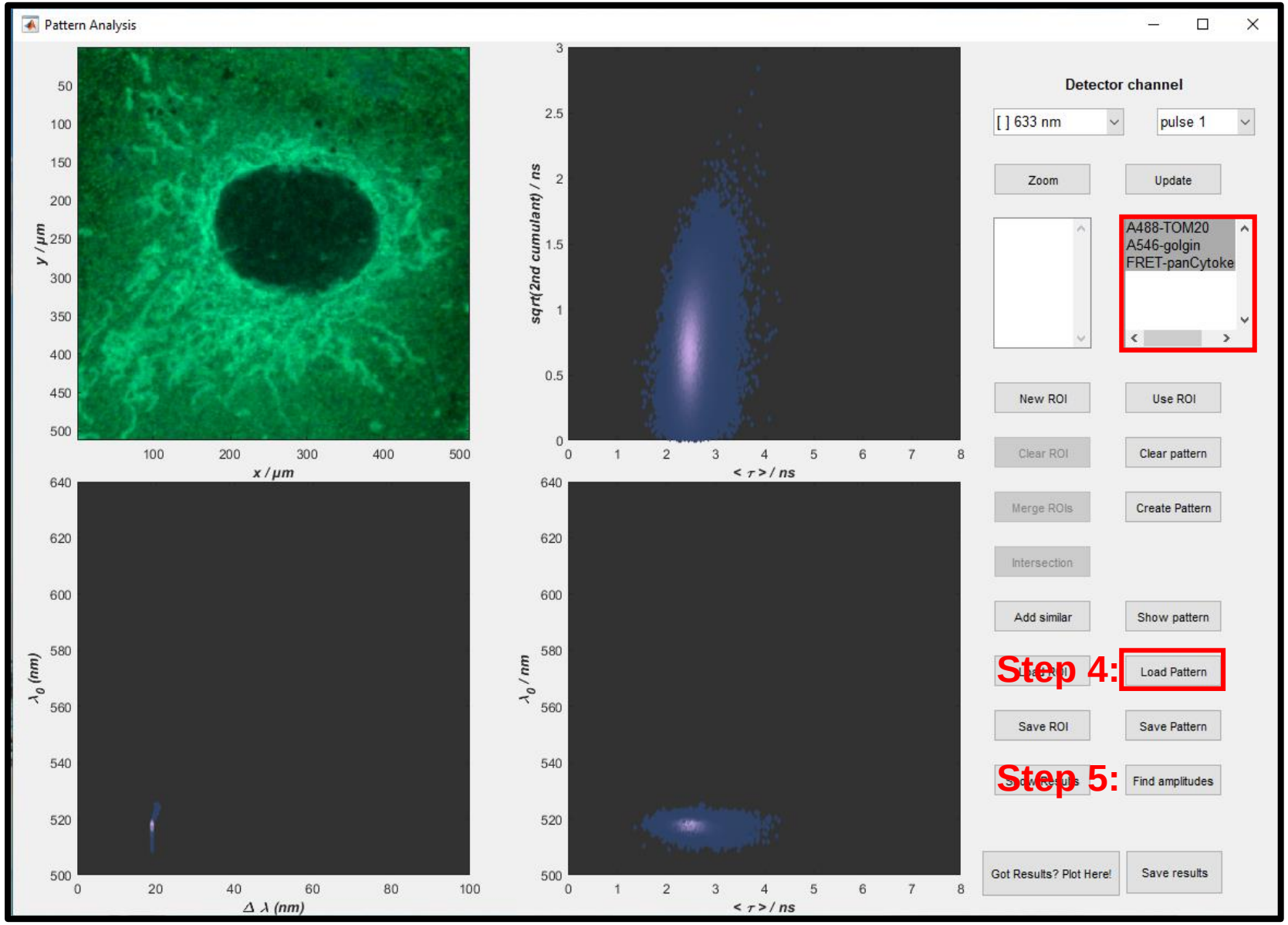
Step 4: Load reference patterns

- Load reference patterns from folder - Figure 5 Reference Patterns and Results

- Select patterns one-by-one
  - A488-TOM20
  - A546-golgin
  - FRET-panCytokeratin

Step 5: Launch Pattern-matching analysis*

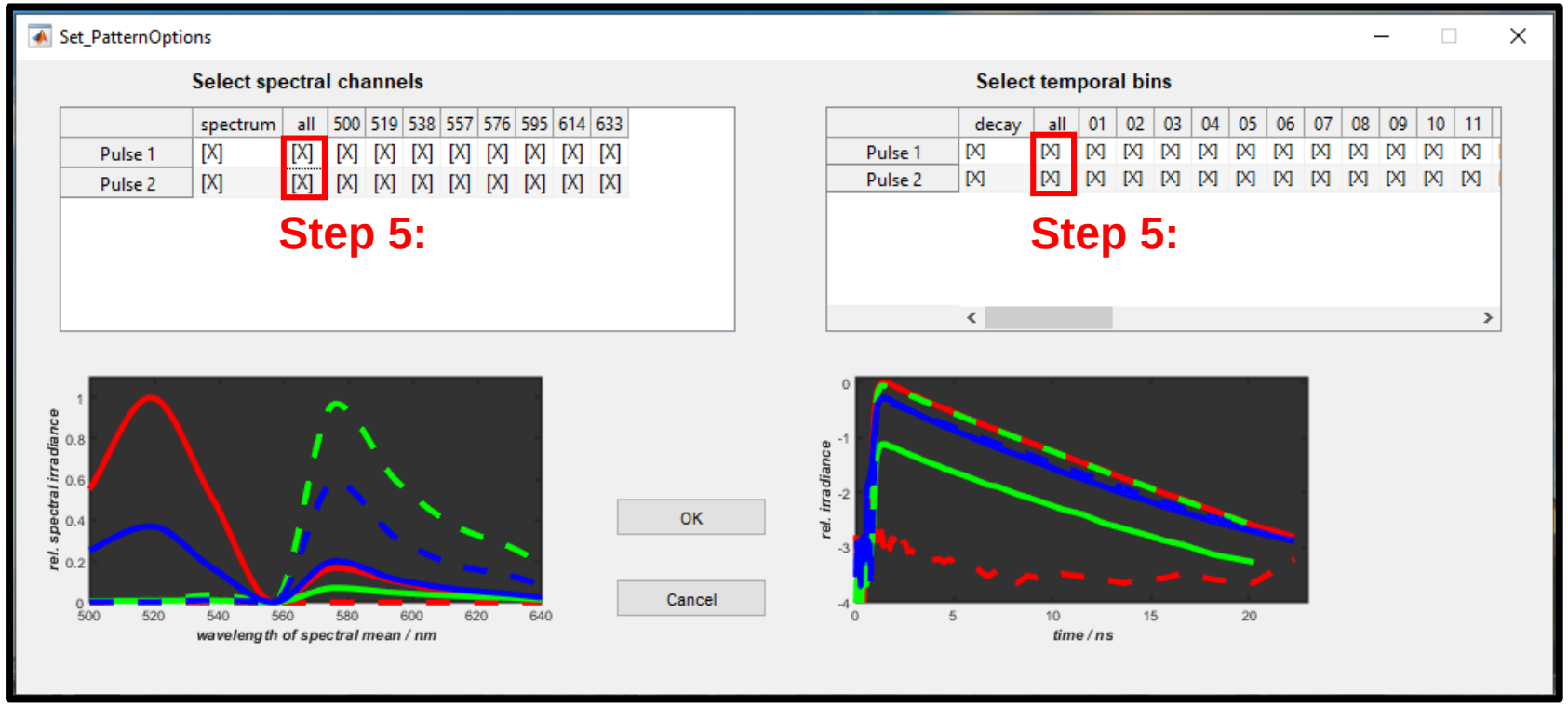
- Push button – *Find amplitudes*



*Results for Figure 5 are already available and can be visualized directly – goto step 9

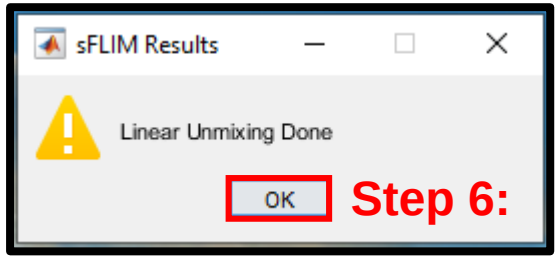
Step 5: Select all information channels

- Click twice (not a double click) on the red marked buttons in *Set_PatternOptions* gui



Step 6: Wait for analysis to be finished

- Once the unmixing is done, sFLIM Results message will pop up.
- Press *ok* and proceed to results visualization



Step 8: Make composite image

- After unmixing we get three unmixed image corresponding to 3 reference patterns
- Press *Show Results* button to generate a composite image

Step 9: Results for Figure 5 are already available and can be visualized directly

- After unmixing we get three unmixed image corresponding to 3 reference patterns
- Press *Show Results* button to generate a composite image
- Select file *Figure 5_Raw_data_results.mat*