**Imaging binary-FRET software instructions**

1. **Create a folder name “Igor”. Save the “SSA-FLIM Images.pxp” Igor file in this folder.**
2. **Create a folder name “Image processing” inside the “Igor” folder.**
3. **Create another folder name “UrVV” inside the “Image processing” folder.**
4. **Save all ASCII .asc files (10 files) to the “UrVV” folder.** Note that there are 10 files, not 6, since there are three parallel amplitude files (a1, a2. & a3) generated in *SPC-Image* instead of one, and three perpendicular amplitude files (a1, a2. & a3) instead of one since each component of the triple exponential fit used has its own amplitude. Our software combines these three files to generate one parallel amplitude file and one perpendicular amplitude file.
5. **Run the Igor program. From the control panel, click “Load Data (Bundle)” button**
6. **Select “Image processing” folder and click OK.**
7. **Select the SSA image generated by clicking on it.**
8. **In the *Image* menu select *Modify Image Appearance*.**
9. **Select SpectrumBlack LUT, set first color at Z=0.3, last color at Z=0.5, press Do It button at bottom.**
10. **Select the *FLIMImage* image generated by clicking on it.**
11. **In the *Image* menu select *Modify Image Appearance*.**
12. **Select SpectrumBlack LUT, set first color at Z=0, last color at Z=3500, press Do It button at bottom.**