1. Image segmentation
2. Background subtraction
3. Autofluorescence subtraction
4. BiFC signal and cell properties quantification

Figure 2

Raw images
• BiFC channel
• Autofluorescence channel(s)
• Subcellular compartment channel

Binary images
• Background mask
• Cell mask
• Subcellular compartment masks (e.g. nucleus and cytoplasm)

Background subtracted images
• BiFC channel
• Autofluorescence channel(s)

Autofluorescence subtracted BiFC images
• BiFC fluorescence

Single cell measurements
• BiFC intensities
• Cell autofluorescence
• Cell properties (size, shape, ...)

Cell and subcellular compartment masks
BG mask