Using the cell phasor in a time sequence

Purpose: analysis of a region of an image, generally a single cell, as a function of time. The program calculates the average phasor as a function of time in the region of analysis. It produces a table of the G and S coordinates of the phasor and if the intensity.

Input: a stack of referenced (.REF) files. The stack is limited to 102 frames.

Instructions: Open the ref stack sequence. In the following example we loaded 60 FLIM Images. Each FLIM image contains 5 sub-images, so that the maximum amount of frames leaded is 512. Each frame is 256x256 and each entry is a 32bit floating. The total memory used by the stack is then 512x256x256*4=128Mb, which is the total allowed limit for a stack in SimFCS.

In the tools menu use “show image in chart1”, select scan all images, center the cursor in chart1 on the cell you want to analyze and press “add to series”.

![Cell Phasor Image](image.png)
Now select the columns you will like to plot. You can also smooth the data.

All data can be exported to excel. The table can also be copied and pasted.

More graphs can be superimposed if you continue to select other cells.
Although all cells have a similar trend, there is a difference between cells