**Yolk gland folder: SB\_cpeb1\_MX1 dataset. 10 z-stack images.**

This is a triple FISH experiment and I would like to quantify: 1) the total number SB+, cpeb1+, and MX1+ cells, and 2) measure the percentage cyan+ cells that are also red+ and/or green+ (in all combinations). In other words, there are single-positive cells, double- positive cells, and triple-positive cells present - what is the percentage of each of these categories?

4 channels present: channel 1: (Ch2-T1) cyan (these are SB+ cells), channel 2: (ChS2-T2) magenta (these are cpeb1+ cells), channel 3: (ChS1-T3) green, (these are MX1+ cells), channel 4: (Ch1-T4) gray (this is DAPI; all nuclei). 8 Bit. 1024x1024 pixels. 1 um z step.

There are no differences in these images, they all show different examples of the same thing.

I tried Imaris’s Spots function to count the cells. It would routinely miss cells (not count them) and would have trouble counting a clump of cells as multiple distinct cells. Imaris also does not report how many cells are expressing one of the markers vs both.