Testes folder: pH3\_nanos\_klf4\_tes dataset. 14 z-stack images.

The goal is to count all the nanos+ (magenta) cells and all the klf4+ (green) cells. Then, to quantify the % of nanos+ cells that are also klf4+ and vice versa. Each of these files also has multiple testes. They are easily recognized as rounded lobes that have nanos+ cells around the periphery and mature sperm in the middle (lumen) of each testis. If it is possible to delineate the testis boundaries using these criteria (nanos+ cells along periphery and sperm with elongated nuclear morphology in the middle) that would be awesome! That way we could get the above counts and percentages as a PER TESTIS count instead of as a grand total within the whole file. Another marker in these files is the mitotic marker phosphohistone H3 (pH3, cyan). A final goal would be to get a mitotic index (number of nanos+ cells that are also pH3 positive). Finally, the male germ cells in the testes divide with incomplete cytokinesis. In other words, they remain connected as cysts which we can see with pH3 (they make doublets, 4-cell, 8-cell, and 16-cell cysts.) Is it possible to recognize these cysts (based on proximity of pH3+ nuclei) and count how many there are per testis, and whether or not they are also nanos- or klf4-positive?

4 channels present: channel 1: (Ch2-T1) cyan (these are pH3+ mitotic cells), channel 2: (ChS2-T2) magenta (these are nanos+ cells), channel 3: (ChS1-T3) green, (these are klf4+ cells), channel 4: (Ch1-T4) gray (this is DAPI; all nuclei). 8 Bit. 1024x1024 pixels. 1 um z step.