1. Draw around the acini using the freehand selection tool

Cntrl+M to measure

Cntrl+T to record ROI

1. Press Edit 🡪 Clear Outside to isolate your selection
2. Image🡪 Adjust 🡪 color Threshold to open the color threshold window
3. Change Hue, Saturation, and Brightness to measure the following:

* Lumen – the pink area inside the acini
* Spermatozoa - dark blue sperm cells found close to the lumen
* Spermatocytes and spermatozoa – all blue area including the sperm cells near the lumen and the circular spermatocytes (lighter blue shade) that cover the majority of the acini area

After each…

* Cntrl+M to measure
* Cntrl+T to record ROI

1. Add the area values for total area, lumen, spermatozoa, and spermatozoa+spermatocytes to a cumulative spreadsheet.