Nile Red quant on Image J:

1. Open image in ImageJ

~~2. Under the Image menu, select Type, then 16-bit. Image should become black and white.~~

3. Now Image – Adjust - then Threshold. Set it to dark background.

4. Use the Brightness sliding bar in the Threshold Color window to highlight what you think is positive staining. This is subjective- slide it until you see decent separation of droplets with as much staining highlighted as possible. In high-sugar fat bodies, the lipid droplets run together into lobular structures. Hit "Apply."

(You can do this a few different ways. You can also threshold the B&W image by using the "saturation" sliding bar. Just do it the same way for all of your analyses.)

5. To quantify the droplets, go to the Analyze Menu, then Analyze Particles.

Make sure to show Outlines. I restrict size from 20 or 30 to infinity, allow any circularity, and select both Clear results and Display results. This produces measurements and a count of your lipid droplets in another window called "Results". This is your raw data.

There will be a third window, "Drawing" which outlines and counts your droplets. **Check this image to make sure it looks reasonable to you, then close it** Under the Image menu, you can scale the drawing to zoom in and look at each outlined droplet if you want. You don't need to select all droplets; it's more important to make sure that what's being selected is representative in terms of size and shape.

6. Select all in Results window, paste into Excel. Only need to keep 1 column of particle areas ("Area" 2nd column from left). Rename this column the name of the image you analyzed. ***Each worksheet should contain data from one date.***

Notes. I find it best to always compare microscope fields imaged in the same session in case the magnification gets messed up.