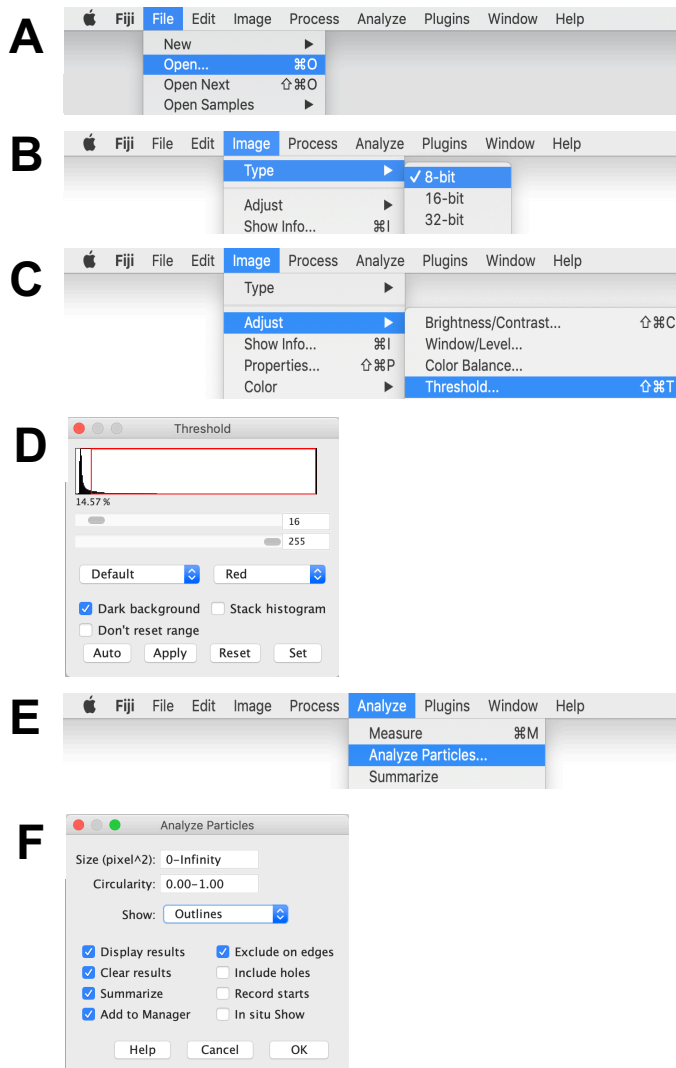


## Supplemental Files Index

File Name	Description
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Supplemental Figure 4	Determining the appropriate threshold correction factor
Supplemental Figure 5	Lipid droplet measurements analyzed with ImageJ and CellProfiler from the two images with low and high lipid density shown in Figure 3
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Supplemental File 2	CP_Pipeline Lipid Droplet Analysis

# Supplemental Figure 1



**Supplemental Figure 1. Using the “Analyze Particles” macro on ImageJ.** The ImageJ interface provided is for MacOS. **(A)** Image upload selecting “File” and “Open” options. **(B)** Conversion of color images to grayscale images selecting “Image”, “Type”, and “8-bit” options. **(C)** Conversion of grayscale images to binary images selecting “Image”, “Adjust”, and “Threshold” options. After all parameters from the “Threshold” option have been setup, “Set” must be selected. **(D)** The “Threshold” window allows for manual selection of an image threshold which is determined by selecting a range of minimum and maximum values of the RGB color (16 and 255 arbitrary units) based on the signal intensity of the image. **(E)** Selection of “Analyze” and “Analyze Particles” opens the “Analyze Particles” macro. **(F)** The “Analyze Particles” window allows user to choose the minimum and maximum particle sizes and circularity of objects to be identified.

## Supplemental Figure 2

**A**

To begin creating your project, use the Images module to compile a list of files and/or folders that you want to analyze. You can also specify a set of rules to include only the desired files in your selected folders.

Drop files and folders here

Filter images? Images only

Apply filters to the file Apply filters to the file list

View output settings

Adjust modules: + - < >

Start Test Mode Analyze Images

**B**

Search: Search

Module Categories

CreateBatchFiles

ExportToDatabase

ExportToSpreadsheet

LabelImages

LoadData

LoadImages

LoadSingleImage

SaveCroppedObjects

SaveImages

+ Add to Pipeline

? Module Help

Getting Started

Done

**C**

Pipeline for individual lipid droplet detection and quantification

To begin creating your project, use the Images module to compile a list of files and/or folders that you want to analyze. You can also specify a set of rules to include only the desired files in your selected folders.

Drop files and folders here

Filter images? Custom

Select the rule criteria Match Any of the following rules

Extension Is "png"

Apply filters to the file list

View output settings

Adjust modules: + - < >

Start Test Mode Analyze Images

**D**

Pipeline for clustering of lipid droplets to quantify the total lipid content per individual adipocyte

To begin creating your project, use the Images module to compile a list of files and/or folders that you want to analyze. You can also specify a set of rules to include only the desired files in your selected folders.

Drop files and folders here

Filter images? Custom

Select the rule criteria Match Any of the following rules

Extension Is "png"

Apply filters to the file list

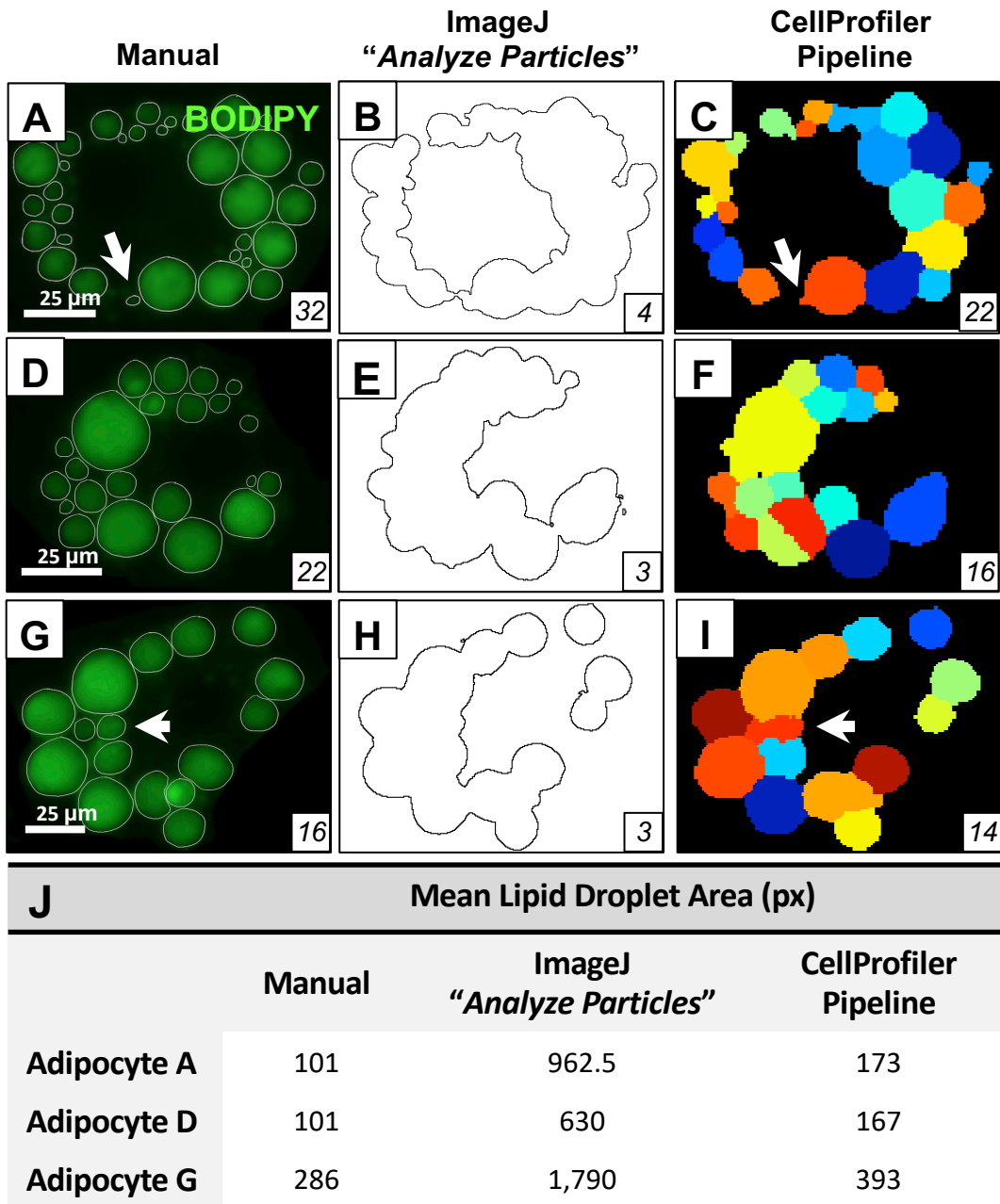
View output settings

Adjust modules: + - < >

Start Test Mode Analyze Images

**Supplemental Figure 2. Creating a CellProfiler Pipeline.** (A) CellProfiler v3.1.8 initial interface. *Left panel:* sequence of modules in a pipeline. In the initial software screen, the pipeline panel contains four basic modules shown (red box) related to the uploading and sorting of images (“Images”, “Metadata”, “NamesAndTypes”, and “Groups”). Images can be uploaded by dragging and dropping individual images or folders of images to the right side of the screen (see “Drop files and folders here”). Images can be sorted according to the type of image using the “NamesAndTypes” module. Modules may be added onto the pipeline using the “Adjust modules +” option (arrow) (B) **Modules menu:** The “Adjust modules +” will trigger a menu of potential modules to be dragged onto the pipeline. (C) **Pipeline for individual lipid droplet detection:** The listed modules (red box) allow for quantification of lipid droplet area and shape among other measurements (see main text for additional details on each of the modules included here). (D) **Pipeline for clustering of lipid droplets:** The additional modules listed in the pipeline panel (red box) were added to allow for the clustering of lipid droplets to quantify the total lipid content per adipocyte (see main text for additional details on each of the modules included here).

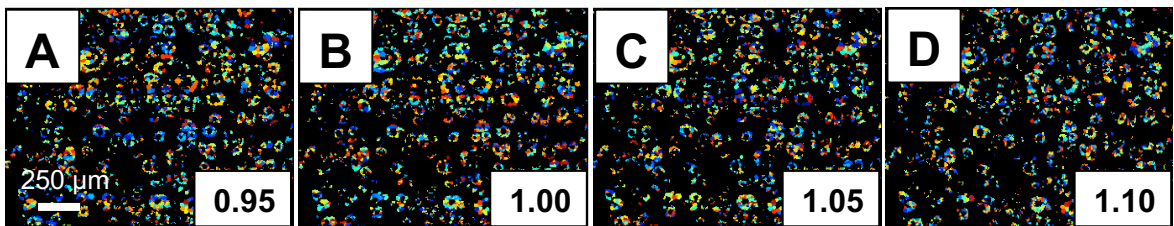
# Supplemental Figure 3



**Supplemental Figure 3: Comparing manual analysis of lipid droplets to ImageJ "Analyze Particles" and CellProfiler analyses with the total number of lipid droplets detected in the bottom right corner of each image. (A, D, G) BODIPY stained lipid droplets were manually outlined using the *GoodNotes* application for the iPad and thereafter measured using the "Freehand" option in ImageJ. (B, E, H) Lipid droplets identified and measured by ImageJ "Analyze Particles" macro. (C, F, I) Lipid droplets identified and measured by our CellProfiler pipeline. Number of lipid droplets identified are shown in the bottom right corner of each image. (J) Mean lipid droplet area per adipocyte by analysis type. White arrows denote failure to detect small lipid droplets by CellProfiler.**



## Supplemental Figure 4



**Supplemental Figure 4. Determining the appropriate threshold correction factor (0.95 to 1.10) in the “*IdentifyPrimaryObjects*” module for primary identification of lipid droplets. (A-D) Output with a threshold of 0.95, 1.00, 1.05, and 1.10 for a total of 3,841, 3,699, 3,562, and 3,409 lipid droplets identified, respectively.**

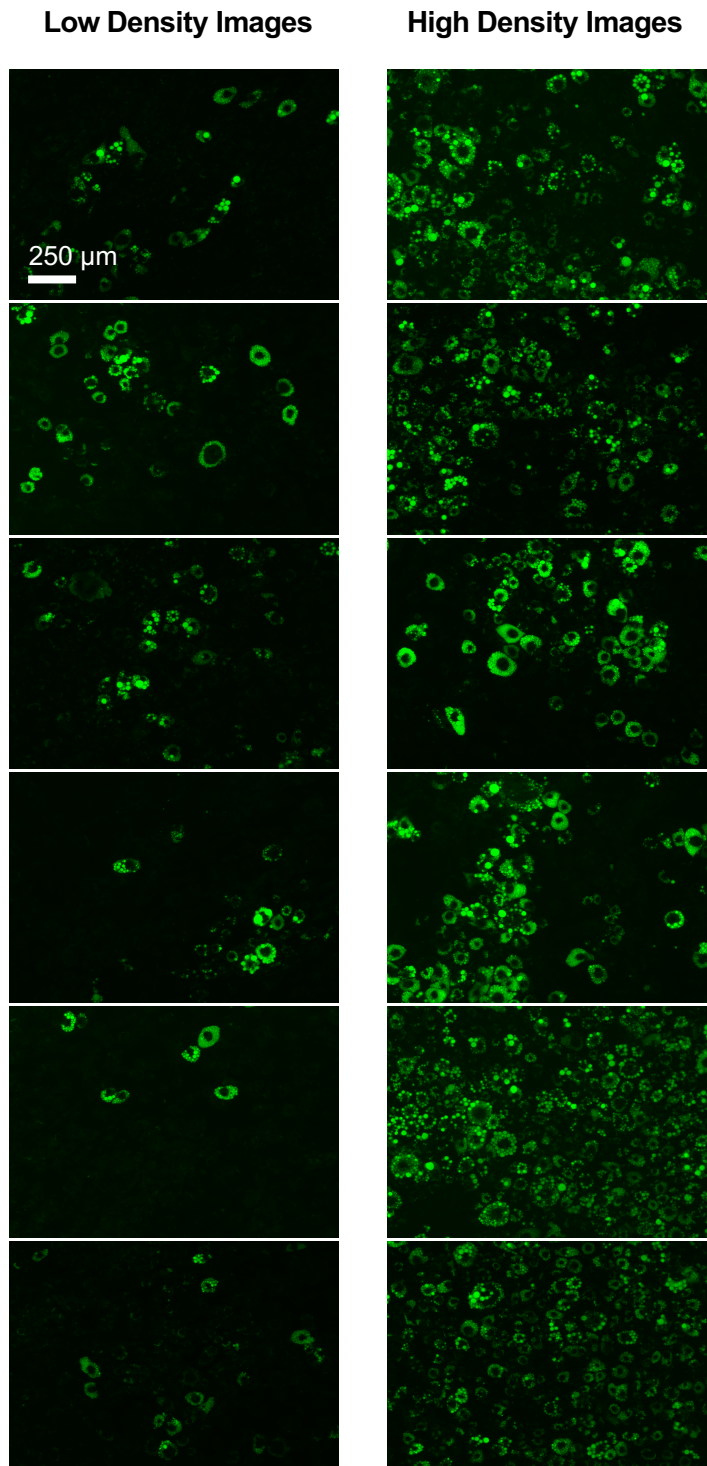
## Supplemental Figure 5

**Supplemental Figure 5.** Lipid droplet measurements analyzed with ImageJ and CellProfiler from the two images with low and high lipid density shown in Figure 3.

Lipid Droplets Parameters		Low Density Image		High Density Image	
		ImageJ	CellProfiler	ImageJ	CellProfiler
<b>Total Number</b>		395	603	2,239	3,409
<b>Lipid Droplet Area (px)</b>	Mean ( $\pm$ SEM)	143 $\pm$ 6 <sup>a</sup>	148 $\pm$ 12 <sup>b</sup>	171 $\pm$ 15 <sup>a</sup>	144 $\pm$ 2 <sup>b</sup>
	Ln Mean ( $\pm$ SEM)	2.1 $\pm$ 0.09 <sup>a</sup>	4.7 $\pm$ 0.02 <sup>b</sup>	2.7 $\pm$ 0.04 <sup>a</sup>	4.7 $\pm$ 0.01 <sup>b</sup>
	Median	6	109	11	106
	Mode	1	84	1	51
	Minimum	1	29	1	29
	Maximum	7,276	713	13,426	1,065
	25 <sup>th</sup> Percentile	2	73	3	64
	50 <sup>th</sup> Percentile	6	109	11	106
	75 <sup>th</sup> Percentile	19	173	45	173
<b>Mean Radius (px)</b>		ND	2.12	ND	2.19
<b>Eccentricity</b>		ND	0.68	ND	0.64

Mean radius (pixels) and eccentricity were measured by CellProfiler, but could not be determined using the “*Analyze Particles*” macro on ImageJ. Analysis of mean lipid droplet area was log transformed prior to Independent T-test analysis. a  $\neq$  b denotes  $P < 0.05$  within image densities and between analysis programs. ND: not detectable, and n/a: not available.

# Supplemental Figure 6



**Supplemental Figure 6.** Additional BODIPY stained images containing high- and low-density lipid droplets used to compare differences between ImageJ and CellProfiler.