

- ☒ Images
- ☒ Metadata
- ☒ NamesAndTypes
- ☒ Groups
- ☒ **IdentifyPrimaryObjects**
- ☒ IdentifySecondaryObjects
- ☒ OverlayOutlines
- ☒ OverlayOutlines
- ☒ DisplayDataOnImage
- ☒ DisplayDataOnImage
- ☒ SaveImages
- ☒ SaveImages
- ☒ MeasureObjectIntensity
- ☒ ExportToSpreadsheet

Use advanced settings? ☒ Yes ☐ NoSelect the input image nuc (from NamesAndTypes)Name the primary objects to be identified IdentifyPrimaryObjectsTypical diameter of objects, in pixel units (Min,Max) 6 60Discard objects outside the diameter range? ☒ Yes ☐ NoDiscard objects touching the border of the image? ☒ Yes ☐ NoThreshold strategy GlobalThresholding method OtsuTwo-class or three-class thresholding? Two classesThreshold smoothing scale 1.3488Threshold correction factor 0.3Lower and upper bounds on threshold 0.0 1Log transform before thresholding? ☐ Yes ☒ NoMethod to distinguish clumped objects IntensityMethod to draw dividing lines between clumped objects IntensityAutomatically calculate size of smoothing filter for declumping? ☒ Yes ☐ NoAutomatically calculate minimum allowed distance between local maxima? ☒ Yes ☐ NoSpeed up by using lower-resolution image to find local maxima? ☒ Yes ☐ NoDisplay accepted local maxima? ☐ Yes ☒ NoFill holes in identified objects? After both thresholding and declumpingHandling of objects if excessive number of objects identified Continue