

Algorithm macro command sequence as used on ImageJ*Artifacts***Threshold artifacts**

```
run("Size...", "width=1024 height=1024 depth=1 constrain average interpolation=Bilinear");
run("Mean...", "radius=3");
setAutoThreshold("Minimum");
//setThreshold(0, x);*
setOption("BlackBackground", true);
run("Convert to Mask");
run("Analyze Particles...", "pixel add in_situ");
roiManager("Select", y, z);‡
roiManager("Delete");‡
roiManager("Select", newArray(0, 1, 2, n));
roiManager("Combine");
roiManager("Add");
roiManager("Select", 0);
roiManager("Rename", "Threshold artifacts");
*, involves manual thresholding to the average pixel value of obvious low-signal artifacts. ‡, select and delete foveal avascular zone (y) and optic nerve head (z) areas.
```

Segmentation artifacts

```
run("Size...", "width=1024 height=1024 depth=1 constrain average interpolation=Bilinear");
run("Variance...", "radius=9");
setAutoThreshold("Minimum");
//setThreshold(0, x);*
setOption("BlackBackground", true);
run("Convert to Mask");
run("Analyze Particles...", "pixel add in_situ");
roiManager("Select", y, z);‡
roiManager("Delete");‡
roiManager("Select", newArray(0, 1, 2, n));
roiManager("Combine");
roiManager("Add");
roiManager("Select", 0);
roiManager("Rename", "Segmentation artifacts");
*, involves manual thresholding to the average pixel value of obvious segmentation artifacts due to any cause. ‡, select and delete foveal avascular zone (y) and optic nerve head (z) areas.
```

Combine artifacts and inverse region of interest (ROI)

```
roiManager("Select", newArray(0,1));#
roiManager("OR");
roiManager("Rename", "low signal artifacts");
run("Make Inverse");
roiManager("Add");
roiManager("Rename", "inverse-low signal artifacts");
#, "0" denotes threshold artifact area, "1" denotes segmentation artifact area.
```

NPA calculation (mm²)

```
run("Set Scale...");  
run("Size...", "width=1024 height=1024 depth=1 constrain average interpolation=Bilinear");  
run("Set Scale...", "distance=1024 known=12 unit=mm");  
run("Subtract Background...", "rolling=50");  
run("Directional Filtering", "type=Max operation=Opening line=20 direction=32");
```

```
makeOval(x, y, 25, 25);    (Repeat three times)  
roiManager("Add");        (Repeat three times)  
roiManager("Select", newArray(a,b,c));  
roiManager("Measure");  
x = (a+b+c)/3 (Average of mean pixel value of circles a, b, c)  
setAutoThreshold("Huang");  
//setThreshold(0, x);*
```

```
run("Convert to Mask");  
run("Analyze Particles...", "size=250-Infinity pixel add in_situ");  
roiManager("Select", y, z);‡  
roiManager("Delete");‡  
roiManager("Select", newArray(1,x));  
roiManager("Combine");  
roiManager("Select", newArray(0,1));  
roiManager("AND");  
roiManager("Select", 1);  
roiManager("Select", newArray(0,1));#  
roiManager("AND");  
roiManager("Select", 1);  
roiManager("Rename", "True NPA");†  
roiManager("Measure");
```

*, involves manual thresholding to the average pixel value of three circles in the FAZ area. Each circle is drawn using the oval selection tool and should be of 25 pixels diameter. #, "0" denotes false NPA area, "1" denotes inverse ROI of low signal artifact area. †, rename the resultant ROI as the True NPA. ‡, select and delete foveal avascular zone (y) and optic nerve head (z) areas